RESEARCH PAPER

SOME PHARMACOLOGICAL PROPERTIES OF 5-CHLORO-2,4-DISULPHAMYLTOLUENE "DISAMIDE" AN ORALLY ACTIVE DIURETIC AGENT

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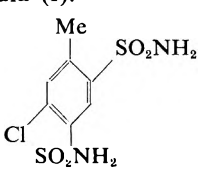
Received November 17, 1959

5-Chloro-2,4-disulphamyl toluene, "Disamide", is a non toxic orally active diuretic agent which has been compared with acetazolamide and chlorothiazide. Disamide at half the dose of chlorothiazide caused a greater diuresis and Na^+ excretion but had a slightly less Cl^- excretion. The carbonic anhydrase inhibitory activity was 0·4 times that of acetazolamide but unlike the latter Disamide still caused diuresis after prolonged administration. Disamide and chlorothiazide were found to have no effect on blood pressure or respiration. The anti-convulsant activity in mice is similar to acetazolamide. The new diuretic agent has no antibacterial, hypoglycaemic or analgesic properties.

5-CHLORO-2,4-disulphamyltoluene was synthetised in our Chemical Research Laboratories. It is a non-hygroscopic white crystalline powder with a molecular weight of 284·6. It is odourless and practically tasteless, melting at about 260°. It is insoluble in water and dilute hydrochloric acid, slightly soluble in ethanol and soluble in cold sodium hydroxide solution.

It has the structural formula (I).



(I)

METHODS

Acute Oral Toxicity

Male albino mice weighing approximately 20 g. each were fasted overnight. Three groups of 20 animals were given a suspension of Disamide by stomach tube in an aqueous suspending medium[1]. The volumes were adjusted to 0·5 ml./20 g. body weight. The mice were kept for 7 days.

A group of ten male albino rats was given a single large dose, by stomach tube, of Disamide suspended in 2·5 ml. of the aqueous suspending medium per 100 g. body weight, a second group of 10 rats was given the vehicle alone. They were carefully observed for 8 hours following administration and on the following day. The rats were killed on the seventh day.

Chronic Oral Toxicity

Sixty immature female albino rats weighing between 74 and 112 g. were divided into four groups of 15 animals with mean weights not differing by more than 3·4 g. Three groups were given 75, 150 and 300 mg./kg. respectively of Disamide in 1 ml. of aqueous suspending medium per 100 g. and the fourth group was given the vehicle alone. The animals were dosed daily by stomach tube 5 days a week for 13 weeks. Weekly weights were recorded and the doses adjusted accordingly. The rats were kept under identical conditions and given water and diet 41 *ad libitum*, except when the diuretic responses were being investigated. Haematological studies were made on the 300 mg./kg. and control groups at the end of the fourth and tenth weeks and on all groups at the end of the thirteenth week. Twenty-four hours after the last dose 5 rats from each group were killed and sections of lung, liver, kidney, spleen, stomach, heart, thyroid, pituitary, adrenal and ovary were prepared for histological examination. The remaining animals were kept under observation for a further 9 weeks.

Urine, Na^+, K^+ and Cl^- Excretion

Oral diuretic activity was investigated in mature male albino rats using a modification of Lipschitz, Hadidian and Kerpezar's[2] method. Groups of 8, 10 or 20 rats were deprived of food and water overnight. The following morning a 0·9 per cent saline load, 25 ml./kg., was given by stomach tube together with varying amounts of Disamide, chlorothiazide or acetazolamide suspended in 5 per cent gum acacia. The control animals were given the saline and 5 per cent gum acacia only. Each group was placed in wire meshed cages over a large funnel and the urine collected for 5 or 8 hours following administration. Urinary sodium and potassium were estimated with a flame photometer and the chloride content by King and Wooton's[3] method. At 4, 9 and 13 weeks the diuretic responses of the rats in the chronic toxicity experiment were determined. The usual daily dose was administered in a saline load of 25 ml./kg. and the urine collected. A similar procedure was followed for acetazolamide except that the diuretic responses were recorded initially, after the first dose, and at 3, 5, 8 and 13 weeks. The mean urinary excretion was expressed as ml. or m-equiv./kg. for 5 or 8 hours and the diuretic activities were expressed relative to the controls taken as unity.

Carbonic Anhydrase Inhibition

The carbonic anhydrase inhibitory activity was estimated by a method adapted from Roughton and Booth[4].

Anticonvulsant Properties

The anticonvulsant activities of Disamide and acetazolamide were determined against electroshock and leptazol-induced convulsions in unstarved male albino mice.

Varying doses of Disamide or acetazolamide in aqueous suspending medium, 0·5 ml./20 g., were given by stomach tube to groups of 20 mice.

Two hours later the mice were challenged either by a current of 15 mA applied for 0·2 seconds through ear electrodes or by an intravenous injection of 50 mg./kg. of leptazol. The absence of the hindleg tonic extensor component of the convulsion was regarded as evidence of protection. The ED50 values and confidence limits were calculated by the method of Litchfield and Wilcoxon[5].

Effect on Blood Sugar

This was investigated in three groups of four rabbits fasted overnight. Initial and hourly blood values for 5 hours following the administration of 50, 100 and 200 mg./kg. were estimated by Somogyi's[6] modification of Nelson's method.

Effect on Blood Pressure and Respiration

The carotid blood pressures of urethane anaesthetised male albino rats were recorded following the intraperitoneal injection of Disamide or chlorothiazide. The blood pressure and respiratory responses to Disamide given by stomach tube was investigated in three urethane-chloralosed cats.

Hypnotic Properties

The possible hypnotic properties of Disamide and chlorothiazide were investigated by observing their effects on sodium pentobarbitone treated mice. Chlorpromazine was used as the reference compound. Disamide and chlorothiazide were given in aqueous suspending medium and sodium pentobarbitone and chlorpromazine in water. The control mice were given the aqueous suspending medium only. All volumes were adjusted to 0·5 ml./20 g. body weight. Groups of 8 or 10 mice were given 25 or 50 mg./kg. of sodium pentobarbitone intraperitoneally 30 minutes after the administration by stomach tube of Disamide or chlorothiazide, 10, 50 or 250 mg./kg., chlorpromazine, 5 mg./kg. or aqueous suspending medium.

Analgesic Properties

The mouse tail pinch method of Bianchi and Franceschini[7] was used. Groups of ten mice fasted overnight were given 10, 40, 160 or 640 mg./kg. of Disamide and two other groups 40 and 80 mg./kg. of pethidine hydrochloride. The compounds were given by stomach tube in aqueous suspending medium, the volumes being adjusted to 0·5 ml./20 g. At 30, 60, 120 and 240 minutes the rubber covered bull dog clip was applied to the base of the tail for 30 seconds, and if no attempt was made to remove the clip a positive analgesic response was recorded.

Antibacterial Properties

The antibacterial activity was investigated using a double dilution tube assay against the following organisms which had been incubated for 24 hours in double strength Lemco broth. *Pseudomonas aeruginosa* (*Pseudomonas pyocyanea*) NCTC 8058, *Streptococcus faecalis* NCTC 775,

Proteus vulgaris NCTC 401, *Staphylococcus lactis* NCTC 189, *Escherichia coli* NCTC 86.

A solution of the disodium salt of Disamide was made by dissolving 0·71 g. in distilled water containing 0·165 g. NaOH and making up to a final volume of 100 ml. to give a 0·825 per cent clear solution. Serial dilutions were made and the 24 hours growth determined after incubation at 37°.

Results

Acute Oral Toxicity

No deaths occurred in mice given 12 g./kg. In rats 9 g./kg. caused no deaths or neuromuscular disturbances, but there was an impression that the treated rats were slightly lethargic compared with the controls.

Chronic Oral Toxicity

There was no significant difference, by Student's *t* test, between the weight increases of the treated and control groups. Red, white and differential counts and haemoglobin values of treated and controls were similar. Histological examination of lung, liver, kidney, spleen, stomach, heart, thyroid, pituitary, adrenal and ovary, by Professor T. Crawford, did not reveal any abnormality.

Urine, Na^+, K^+ and Cl^- Excretion

The diuretic responses in groups of twenty rats to oral administration of three doses of Disamide and chlorothiazide were investigated and the results recorded in Table I. The increased excretion of urine over the

TABLE I

THE MEAN ORAL DIURETIC RESPONSE AND RELATIVE ACTIVITIES OF DISAMIDE AND CHLOROTHIAZIDE, THE Na^+, K^+, AND Cl^- CONTENT AND IMBALANCE IN SALINE LOADED RATS

Compound	Dose mg./kg.	Urine excreted ml./kg./8 hrs.	Relative Activity	m-equiv./kg. 8 hrs. Na^+	K^+	Cl^-	Imbalance $Na^+ + K^+$ minus Cl^-
Disamide	7·5	9·6	2·4	2·0	1·0	1·9	1·1
	15	13·3	3·3	2·9	1·1	2·3	1·7
	30	19·0	4·7	4·1	1·3	3·2	2·2
Chlorothiazide	15	9·4	2·3	1·9	0·9	2·6	0·2
	30	12·2	3·0	2·6	1·0	3·3	0·3
	60	14·5	3·6	3·0	1·1	3·7	0·4
Controls	—	4·1	1·0	0·7	0·6	1·1	0·2

control value for both compounds was linear to log dose but not parallel, (Fig. 1). The doses required to give an increase of 6 ml./kg./8 hours are 8·3 mg./kg. for Disamide and 17·5 mg./kg. for chlorothiazide. The greater activity of Disamide was confirmed in a number of experiments which were combined to give mean responses for 600 rats; Table II records the results. Disamide 10 mg./kg. caused a slightly greater diuresis and Na^+ excretion than 20 mg./kg. of chlorothiazide, but the Cl^- excretion was

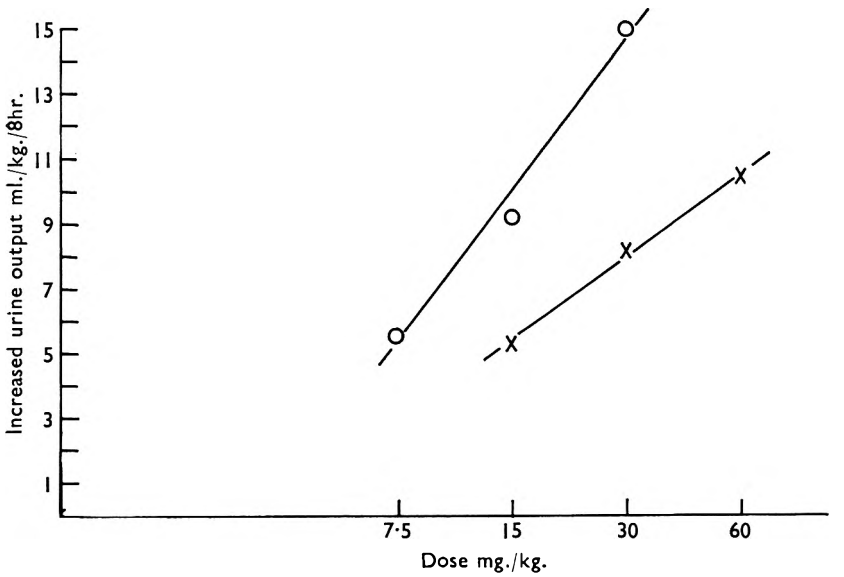


FIG. 1. The log dose/response for Disamide (○——○) and chlorothiazide (×——×), twenty rats per group.

TABLE II

THE MEAN ORAL DIURETIC RESPONSE AND RELATIVE ACTIVITIES OF DISAMIDE AND CHLOROTHIAZIDE, THE Na^+, K^+ AND Cl^- CONTENT AND IMBALANCE IN SALINE LOADED RATS

Compound	Dose mg./kg.	No. of rats	Urine excreted ml./kg./5 hrs.	Relative Activity	m-eqiv./kg./5 hrs. Na^+	K^+	Cl^-	Imbalance $Na^+ + K^+$ minus Cl^-
Disamide	10	600	12·0	3·2	2·6	1·0	2·1	1·5
Chlorothiazide	20	600	10·3	2·8	2·2	0·8	2·9	0·1
Controls	—	600	3·7	1·0	0·7	0·5	1·0	0·2

TABLE III

THE MEAN ORAL DIURETIC RESPONSE AND RELATIVE ACTIVITIES OF DISAMIDE, CHLOROTHIAZIDE AND ACETAZOLAMIDE, THE Na^+, K^+ AND Cl^- CONTENT AND IMBALANCE IN SALINE LOADED RATS

Compound	Dose mg./kg.	Urine Excreted ml./kg./5 hrs.	Relative Activity	m-equiv./kg./5 hrs. Na^+	K^+	Cl^-	Imbalance $Na^+ + K^+$ minus Cl^-
Disamide	1	3·6	0·8	1·1	0·5	1·3	0·3
	3	6·0	1·4	1·5	0·8	1·5	0·8
	9	12·0	2·8	3·4	1·0	2·4	2·0
Chlorothiazide ..	9	7·1	1·7	1·7	0·8	2·4	0·1
	27	8·9	2·1	2·5	0·8	3·0	0·3
	81	12·4	2·9	3·7	1·2	4·2	0·7
Acetazolamide ..	1	6·6	1·6	1·6	0·5	1·1	1·0
	3	7·0	1·6	2·0	0·8	1·2	1·6
	9	13·3	3·1	3·1	1·0	1·5	2·6
Controls	—	4·3	1·0	0·9	0·7	1·5	0·1

slightly less. Tables I and II and Figure 2 show that the diuretic activities are accompanied by similar well marked increases in Na^+ and smaller but similar excretions in K^+. The Cl^- excretion for Disamide is lower and

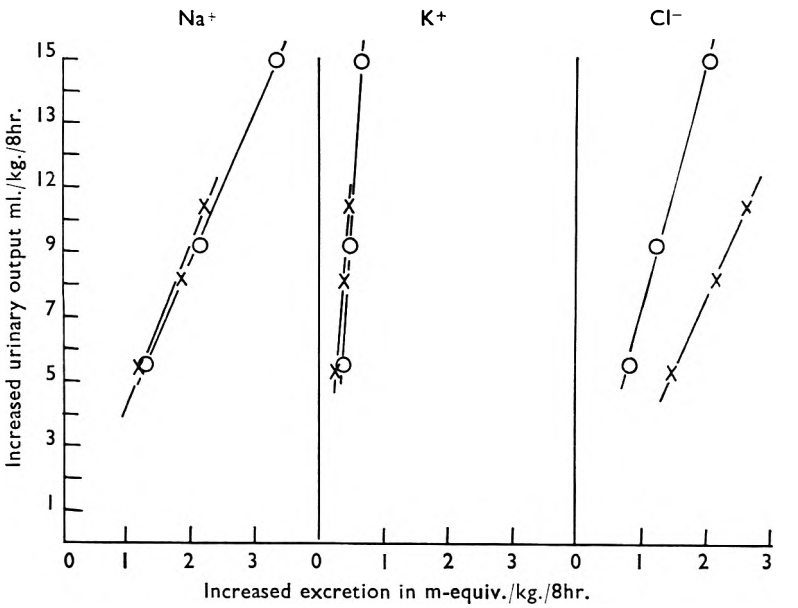


FIG. 2. The increase in Na^+, K^+ and Cl^- excretion with diuresis to Disamide (○——○) and chlorothiazide (×——×), twenty rats per group.

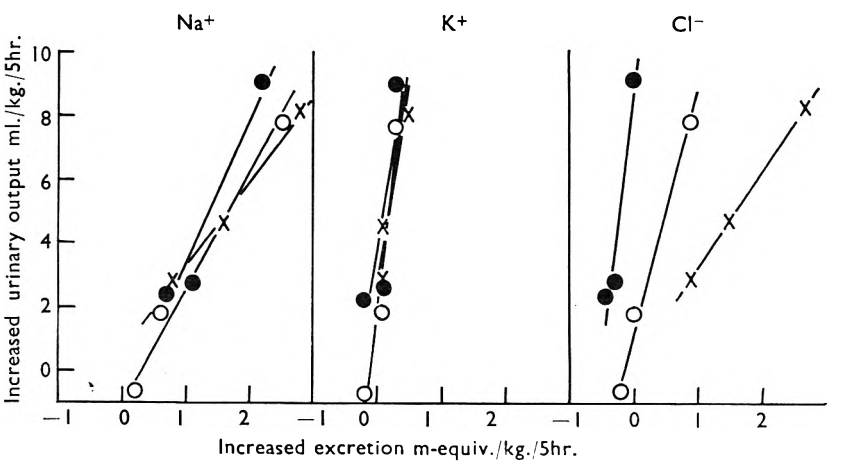


FIG. 3. The increase in Na^+, K^+ and Cl^- excretion with diuresis to Disamide (○——○), chlorothiazide (×——×) and acetazolamide (●——●), eight rats per group.

consequently the imbalance between Na^+ plus K^+ and Cl^- is higher. This can be explained by a greater inhibition of carbonic anhydrase by

Disamide resulting in a greater excretion of bicarbonate. As this enzyme-inhibiting activity of Disamide is lower than that of acetazolamide the diuretic activity of Disamide was directly compared with chlorothiazide and acetazolamide, Table III and Figure 3. This experiment again confirmed the greater diuretic activity of Disamide compared with chlorothiazide and showed that it has about the same activity as acetazolamide. Acetazolamide had no effect on Cl^- excretion and consequently the electrolyte imbalance with acetazolamide was considerably higher than Disamide.

The rapid development of tolerance to acetazolamide is due to its carbonic anhydrase inhibitory properties[8]. As Disamide has some carbonic anhydrase inhibitory activity the diuretic responses in the chronic toxicity experiment were investigated and compared with the responses to repeated daily doses of acetazolamide. Table IV records the results.

TABLE IV

THE MEAN INCREASED URINARY EXCRETION IN RATS AT INTERVALS FOLLOWING REPEATED ORAL ADMINISTRATION OF DISAMIDE AND ACETAZOLAMIDE

Time in weeks	Dose mg./kg.	Urine ml./kg./5 hrs.	
		Disamide	Acetazolamide
Initial	75	—	16·3
"	150	—	20·6
"	300	—	23·0
3	75	—	3·9
"	150	—	2·0
"	300	—	0·8
4	75	9·9	—
"	150	9·6	—
"	300	9·1	—
5	75	—	5·8
"	150	—	5·0
"	300	—	4·5
8	75	—	5·6
"	150	—	4·6
"	300	—	5·0
9	75	9·5	—
"	150	11·6	—
"	300	7·5	—
13	75	9·7	3·6
"	150	11·4	3·8
"	300	11·6	5·0

Acetazolamide showed a marked diminution in diuretic activity following 3 weeks daily treatment with 75, 150 and 300 mg./kg. and this persisted throughout the 13 weeks observation period. In contrast the diuretic activity of Disamide was considerably greater.

Carbonic Anhydrase Inhibition

The relative *in vitro* activities of Disamide and chlorothiazide taking acetazolamide as unity were: Disamide 0·40; chlorothiazide 0·009.

Anticonvulsant Properties

Disamide has similar anticonvulsant properties to acetazolamide against leptazol and electroshock induced convulsions. Table V records the results.

Effect on Blood Sugar

Disamide has no hypo- or hyperglycaemic effect on rabbits after the administration of 200 mg./kg. by stomach tube.

TABLE V

THE NUMBER OF MICE PROTECTED AGAINST ELECTROSHOCK AND LEPTAZOL INDUCED CONVULSIONS TWO HOURS AFTER ORAL ADMINISTRATION OF DISAMIDE AND ACETAZOLAMIDE

Challenge	Dose mg./kg.	Number protected		ED 50 mg./kg. (95 per cent confidence limits)	
		Disamide	Acetazolamide	Disamide	Acetazolamide
Leptazol	75	7/20	5/20	174	143
	150	8/20	11/20	(93–325)	(100–204)
	300	13/20	15/20		
Controls	—	0/20	0/20	—	—
Electro-Shock	5	—	0/20		
	10	1/20	4/20		
	20	3/20	4/20	38·5	29·2
	40	10/20	14/20	(27·9–53·1)	(20·0–42·6)
	80	17/20	17/20		
	160	20/20	20/20		
Controls	—	0/20	0/20	—	—

Effect on Blood Pressure and Respiration

Disamide and chlorothiazide 500 mg./kg. intraperitoneally each had no effect on the carotid blood pressure of male rats anaesthetised with urethane.

The carotid blood pressure and respiration of three urethane-chloralosed cats was unaffected after the administration by stomach tube of 200, 400 and 1000 mg./kg. respectively of Disamide given in divided doses.

Hypnotic Properties

Disamide or chlorothiazide, 250 mg./kg., had no effect on mice given 25 mg. or 50 mg./kg. of sodium pentobarbitone intraperitoneally, whereas chlorpromazine 5 mg./kg. had a potentiating effect on the loss of righting reflex and sleeping time.

Analgesic Properties

Using the tail pinch technique in mice 640 mg./kg. of Disamide orally was inactive up to 4 hours after administration. Pethidine hydrochloride used as the standard gave satisfactory analgesic responses at 40 mg./kg.

Antibacterial Properties

Disamide has only slight antibacterial properties. There was some inhibition of bacterial growth at a dilution of 1 in 250, but at the other dilutions varying from 1 in 500 to 1 in 250,000 a profuse growth occurred in 24 hours incubation at 37° for all the organisms tested.

Discussion

Disamide is more than twice as active as chlorothiazide and has approximately the same activity as acetazolamide as a diuretic agent.

It has been shown that the diuretic activities of acetazolamide, Disamide and chlorothiazide are not parallel to their *in vitro* carbonic anhydrase inhibitory activities. Acetazolamide-like compounds exert their diuretic activity by inhibiting the carbonic anhydrase of the cells of the renal tubules thus preventing the re-absorption of bicarbonate from the glomerular filtrate causing an osmotic diuresis with a high urinary bicarbonate content[9]. Although chlorothiazide possesses some carbonic anhydrase inhibitory properties these play a minor role in its diuretic action which is characterised by a low bicarbonate and a high Cl^- excretion compared with acetazolamide; the Na^+ excreted is approximately equimolar to the Cl^-.[10,11]

In our experiments the Cl^- excretion after chlorothiazide administration is approximately equivalent to the combined Na^+ and K^+ output. This is not so with acetazolamide the Cl^- excretion being similar to the controls at all dose levels and the high electrolyte imbalance is due to the bicarbonate content. Disamide, like chlorothiazide, shows an increased Cl^- excretion with increased dosage and in the combined experiment the mean Cl^- excretion of 600 rats after 10 mg./kg. of Disamide is 2·1 times that of the controls. The electrolyte imbalance of Disamide is considerably less than acetazolamide and can be explained by its chloruretic and lower carbonic anhydrase inhibitory activities. Further evidence to support this dual action is seen in the diuretic responses following prolonged administration where it is reasonable to assume that if the diuretic activity of Disamide is due entirely to its enzyme-inhibiting properties a similar reduction in diuretic activity as observed with acetazolamide would have occurred.

The possible dual mechanism of Disamide distinguishes it from other known diuretics and may be of considerable clinical importance particularly in refractory cases.

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POTENTIAL RESERPINE ANALOGUES

PART I. DERIVATIVES OF TRYPTAMINE

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Received November 6, 1959

The six amides, *cis*-*N*-(3-indolylethyl)-3-methoxycyclohexanecarboxylic acid amide (II), *cis*-*N*-(3-indolylethyl)-*N*-3-methoxycyclohexylmethylacetamide (IV), *N*-(3-indolylethyl)-3,4,5-trimethoxybenzamide (V), *N*-(3-indolylethyl)-3,4-dimethoxyphenylacetamide (VII), 3-indolyl-*N*-(3,4-dimethoxyphenethyl)acetamide (VIII) and 4-methoxycyclohexylacetotryptamide (X), have been prepared. II has been reduced to *cis*-*N*-(3-methoxycyclohexylmethyl)tryptamine (III); VII and VIII have both been reduced to *N*-(3,4-dimethoxyphenethyl)tryptamine (IX), X has been reduced to *N*-(4-methoxycyclohexylethyl)tryptamine (XI) and V to *N*-(3,4,5-trimethoxybenzyl)tryptamine (VI). Nine of the compounds, II–VI, VIII–XI, were compared with reserpine for their power to potentiate barbiturate hypnosis in mice, and to deplete the 5-hydroxytryptamine content of rat brain. Only compound (IV) was active in both tests, having about one-thirteenth the activity of reserpine. Compound (V) was about one-eighth as active as reserpine in producing potentiation of barbiturate hypnosis.

SINCE the elucidation of the structure of reserpine (I) by Mueller, Schlittler and others[1], many analogues have been synthesised in attempts to reproduce the pharmacologically-active fragments of the molecule[2–29]. The most promising compounds differ from reserpine only in the nature of the acyl group on carbon atom 18[26–28]. These have been subjected to thorough pharmacological tests by Garattini and colleagues[30], and some appear to be more effective in certain tests than reserpine itself. The evaluation of such analogues is difficult, however, as the pharmacological actions of reserpine are complex.

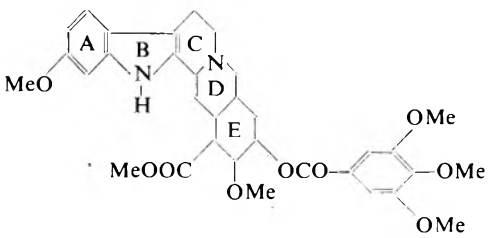
The present work describes the preparation of six amides, *cis*-*N*-(3-indolylethyl)-3-methoxycyclohexanecarboxyamide, (II); *cis*-*N*-(3-indolylethyl)-*N*-3-methoxycyclohexylmethylacetamide (IV); *N*-(3-indolylethyl)-3,4,5-trimethoxybenzamide, (V); *N*-(3-indolylethyl)-3,4-dimethoxyphenylacetamide (VII); 3-indolyl-*N*-(3,4-dimethoxyphenethyl)acetamide (VIII) and 4-methoxycyclohexylacetotryptamide (X).

The insolubility of the amides in dilute acid and the evidence available from the preparation of 3-β-(*o*-tolylacetamido)ethylindole by Clemo and Swan[31], 3,4-dimethoxyphenylacetotryptamide by Onda and colleagues[5] and 4-methoxycylohexylacetotryptamide by Protiva and colleages[22] prove that the acylation has taken place at the β-*N*, and not at the α-*N* of the tryptamine molecule.

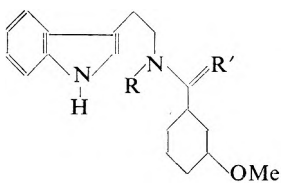
The amides were then converted to their corresponding amines by lithium aluminium hydride reduction; (III) was obtained from (II); (IX) from both (VII) and (VIII); (VI) from (V) and (XI) from (X).

The amine (III) was also obtained by the condensation of tryptamine with *cis*-3-methoxycyclohexylmethyl chloride.

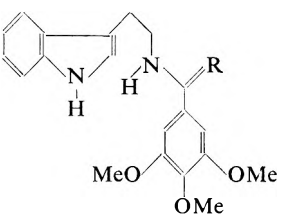
The structural relations between these compounds to reserpine may be seen in Fig. 1.



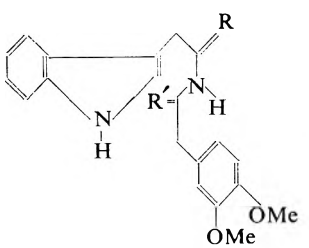
I Reserpine.



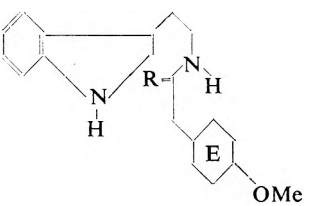
II, R = H; R′ = O
III, R = H; R′ = H_2
IV, R = COMe; R′ = H_2



V, R = O
VI, R = H_2



VII, R = H_2; R′ = O
VIII, R = O; R′ = H_2
IX, R = R′ = H_2



X, R = O
XI, R = H_2

FIG. 1.

Pharmacological Data

The compounds were compared with reserpine for their power (i) to potentiate the hypnosis in mice produced by intravenous doses of hexobarbitone (50 mg./kg.) and (ii) to deplete the 5-hydroxytryptamine (5-HT) content of rat brain. In the first test, the standard intraperitoneal dose of reserpine was 5 mg./kg.; in the second, reserpine was given intraperitoneally at a dose of 1 mg./kg. The doses of the new compounds used in the present experiments are shown in Table I, together with their pharmacological activities.

We should like to express our thanks to Miss S. A. P. Price and Dr. G. B. West of the Department of Pharmacology of this School for carrying out the pharmacological tests.

Discussion of Pharmacological Results

Of the nine compounds tested, only one (IV, Table I) produced a depletion of the 5-HT content of rat brain. This compound differed from the remainder in having its aliphatic secondary amine group acetylated (i.e. there is no free –NH– group in the carbon chain joining the ring systems). This compound also showed slight barbiturate potentiation at the dose level used.

TABLE I

COMPARISON OF THE PHARMACOLOGICAL ACTIVITIES OF VARIOUS TRYPTAMINE DERIVATIVES (RESERPINE ACTIVITY IS TAKEN AS 100 FOR EACH TEST)

	Potentiation of barbiturate hypnosis in mice		Depletion of 5-HT in rat brain	
Tryptamine derivative	Max. dose used (mg./kg.)	Relative activity	Max. dose used (mg./kg.)	Relative activity
II	400*	0	100	0
III	200*	0	50	0
IV	40	2·5	20	5
V	80	12·5–15	20	0
VI	100	0	—	–
VIII	100*	5	20	0
IX	100*	0	—	–
X	100*	5	40	0
XI	100*	5–6	40	0

* Some mice died at this dose level.

Compound (V) was more active than (IV) in potentiating barbiturate hypnosis, and other compounds were active in this test but the doses required were lethal to some of the mice.

Compounds with an aromatic ring E (V and VIII) possess a more powerful action in potentiating barbiturate hypnosis than those in which ring E is hydrogenated (IV and X).

Only compound IV possessed the power to deplete brain 5–HT and potentiate barbiturate hypnosis, and this is now being subjected to further tests (e.g. hypotensive activity). The other compounds do not justify further testing.

EXPERIMENTAL

Expt. 1. cis-3-*Methoxycyclohexylmethanol.* Methyl *cis*-3-methoxycyclohexanecarboxylate (as prepared by Noyce and others[32]) (13 g.) in ether (100 ml.) was added dropwise to a suspension of $LiAlH_4$ (6·5 g.), in ether (100 ml.), in a three-necked flask (fitted with a condenser and a stirrer) at such a rate that the reaction mixture was just boiling. The mixture was refluxed and stirred for 33 hours. The excess $LiAlH_4$ was destroyed by dropwise addition of ice-cold water and the precipitated inorganic hydroxide was dissolved by dropwise addition of ice-cold 10 per cent sulphuric acid. The acidified mixture was extracted with

ether and the ether removed by distillation. Yield: 9·5 g. (88 per cent), b.p. 110° 18 mm., n_D^{22} 1·4670. Found: C, 65·97; H, 11·48 per cent. $C_8H_{16}O_2$, requires C, 66·66; H, 11·20 per cent.

Expt. 2. cis-3-*Methoxycyclohexylmethyl chloride.* *cis*-3-Methoxycyclohexylmethanol (7 g.) was treated with excess thionyl chloride (25 ml.) and was allowed to reflux for 5 hours. The excess thionyl chloride was removed by distillation with the aid of benzene, and the product distilled *in vacuo*. Yield: 7·32 g. (93 per cent), b.p. 45 to 46°/0·1 mm., n_D^{21} 1·4686. Found: C, 59·0; H, 9·24; Cl, 21·82 per cent. $C_8H_{15}OCl$ requires C, 59·07; H, 9·18; Cl, 22·39 per cent.

Expt. 3. cis-N-(2-*Indol*-3′-*ylethyl*)-*methoxycyclohexanecarboxyamide* (II). Tryptamine, (technical) (0·08 g.) was intimately mixed with methyl *cis*-3-methoxycyclohexanecarboxylate (0·84 g.) and heated at 180 to 190° for 2 hours. The product was dissolved in warm methanol and treated with charcoal. After removal of the charcoal the solution was concentrated. The cold solution was treated with water dropwise until tubid and allowed to stand overnight in the refrigerator. The colourless crystals were separated by filtration. Yield: 0·95 g. (63·3 per cent), m.p. 115 to 117°. Found: C, 71·68; H, 7·82; N, 9·20 per cent. $C_{18}H_{24}O_2N$ requires C, 72·00; H, 8·06; N, 9·33 per cent.

Expt. 4. N-(cis-3-*Methoxycyclohexylmethyl*) *tryptamine* (*III*). *Method* (1). The above amide (200 mg.) was placed in the thimble of a Soxhlet extractor fitted to a flask (250 ml.) containing $LiAlH_4$ (0·5 g.) in ether (200 ml.), the flask also being provided with a mercury-sealed stirrer. The mixture was refluxed for 96 hours under nitrogen and then cooled. The excess $LiAlH_4$ was destroyed by the dropwise addition of ice-cold water and, after inorganic hydroxides had been dissolved by dropwise addition of 10 per cent sulphuric acid, the aqueous layer was extracted thoroughly with ether to remove unchanged amide. The solution was strongly basified with 20 per cent sodium hydroxide solution and then extracted with ether. After drying over anhydrous sodium sulphate, the ether was removed and the residue distilled. Yield: 140 mg. (73·6 per cent), b.p. 190 to 196°/0·1 mm. Found: C, 75·07; H, 9·39; N, 9·99 per cent. $C_{18}H_{26}ON_2$ requires C, 75·45; H, 9·15; N, 9·78 per cent.

Hydrochloride. The above base (5 g.) was dissolved in dry ether and dry hydrogen chloride was passed in. The precipitated hydrochloride was recrystallised from ethanol and ether. Yield: 5·15 g. (92 per cent), m.p. 167 to 168°. Found: C, 67·34; H, 8·52; N, 8·69; Cl, 10·67 per cent. $C_{18}H_{28}ON_2$,HCl requires C, 66·95; H, 8·43; N, 8·68; Cl, 10·97 per cent.

Method (2). Tryptamine (1·6 g.) in tetrahydrofuran (25 ml.) was added drop by drop to a solution of *cis*-3-methoxycyclohexylmethyl chloride (0·85 g.) in tetrahydrofuran (15 ml.) with constant shaking in an atmosphere of nitrogen. Immediate formation of tryptamine hydrochloride was observed. The reaction mixture was then allowed to stand at room temperature for 48 hours and the tryptamine hydrochloride was separated by filtration (0·88 g.). After the solvent had been removed

by distillation under reduced pressure, the residue was distilled and the fraction (200 mg.) boiling at 190 to 196°/0·1 mm. was dissolved in ether, cooled in a refrigerator and then filtered. The filtrate was treated with dry hydrogen chloride The precipitated hydrochloride, after five crystallisations from ethanol and ether, had m.p. 166 to 168° (mixed m.p. with product from Method (1). 166 to 167°). Found: C, 66·65; H, 8·16; N, 8·83; Cl, 10·66 per cent. $C_{18}H_{28}ON_2$,HCl requires C, 66·95; H, 8·43; N, 8·68; Cl, 10·97 per cent.

Expt. 5. *Indol*-3-*yl*-N-(3,4-*dimethoxyphenethyl*)*acetamide* (*VIII*). *Method* (1). Homoveratrylamine (0·9 g.) and 3-indolylacetic acid (0·8 g.) were dissolved in methanol (2 ml.) and the methanol removed by distillation. The mixture was heated at 210 ± 5° for 45 minutes and then dissolved in warm methanol and treated with charcoal; after removal of the charcoal, the solution was concentrated and water added dropwise until a turbidity appeared. On cooling in the refrigerator, colourless crystals were obtained. Yield: 1·2 g. (78 per cent), m.p. 122 to 123°. Found: C, 17·13; H, 6·76; N, 8·35 per cent. $C_{20}H_{22}O_3N_2$ requires C, 70·97; H, 6·56; N, 8·28 per cent.

Method (2). 3-Indolylacetic acid was esterified with freshly prepared diazomethane and the ester (1 g.) dissolved in methanol (2 ml.) together with homoveratrylamine (1 g.). After removal of the methanol the mixture was heated for 3 hours at 170 to 180° and the product worked up as before. Yield: 1·5 g. (84 per cent), m.p. and mixed m.p. with the product from the previous experiment 122 to 123°.

Expt. 6. N-(2-*Indol*-3′-*ylethyl*)-3,4-*dimethoxyphenylacetamide* (*VII*). Homoveratric acid was esterified with freshly prepared diazomethane and the methyl ester (190 mg.) was dissolved in methanol (2 ml.) with tryptamine (160 mg.). After removal of the methanol by distillation the mixture was heated at 180 to 190° for 2½ hours and the product worked up as described in Expt. 5 (1). Yield: 196 mg. (65 per cent), m.p. 65 to 66°. Found: C, 70·44; H, 6·74; N, 8·15 per cent. $C_{20}H_{22}O_2N_2$ requires C, 70·97; H, 6·55; N, 8·28 per cent. It was later found that this amide had been previously prepared by Onda and others[5] by condensing homoveratric acid with tryptamine.

Expt. 7. N-(3,4-*Dimethoxyphenethyl*)*tryptamine* (*IX*). *Method* (1). *N*-(3,4-Dimethoxyphenethyl)-3-indolylacetamide (0·5 g.) dissolved in tetrahydrofuran (25 ml.) was added dropwise to a suspension of $LiAlH_4$ (0·7 g.) in ether (200 ml.) in a 250 ml. flask fitted with a stirrer and a condenser. The mixture was refluxed with stirring in an atmosphere of nitrogen for 72 hours. The excess $LiAlH_4$ was destroyed by the dropwise addition of aqueous tetrahydrofuran with vigorous shaking and the solution filtered. The residue was washed thoroughly with warm tetrahydrofuran and the mixed tetrahydrofuran extracts dried over anhydrous sodium sulphate; after removal of the solvent the residue was distilled under reduced pressure. Yield: 0·35 g. (73 per cent), b.p. 210 to 212°/0·1 mm. Found: C, 74·30; H, 7·49; N, 8·40 per cent. $C_{20}H_{20}O_2N$ requires C, 74·09; H, 7·46; N, 8·63 per cent.

Picrate. The above base (0·35 g.) in ethanol (20 ml.) was treated with a saturated ethanolic solution of picric acid at room temperature and heated on a water bath for 5 minutes; the solid deposited on cooling was recrystallised from ethanol. Yield: 0·45 g. (75 per cent), m.p. 158 to 158·5°. Found: C, 56·03; H, 4·97; N, 12·40 per cent. $C_{26}H_{27}O_9N_5$ requires C, 56·14; H, 4·88; N, 12·65 per cent.

Method (2). *N*-(3-Indolylethyl)-3,4-dimethoxyphenylacetamide (0·5g). was reduced in exactly the same way as the isomeric amide in *Method* (1). Yield: 0·32 g. (66 per cent), b.p. 210 to 212°/0·1 mm., m.p. picrate 158 to 159·5°, mixed m.p. with picrate from *Method* (1) 158 to 159·5°.

Hydrochloride. The hydrochloride was prepared as described in Expt. 4 and recrystallised from acetone; m.p. 175 to 176°. Found: C, 68·54; H, 7·32; N, 7·60 per cent. $C_{20}H_{24}O_2N_2$, HCl requires C, 66·54; H, 6·98; N, 7·76 per cent.

Expt. 8. cis-N-(2-*Indol*-3′-*ylethyl*)-N-3-*methoxycyclohexylmethylacetamide* (*IV*). *cis-N*-(3-Methoxycyclohexylmethyl)tryptamine (425 mg.) was dissolved in pyridine (45 ml.) and acetyl chloride (200 mg.) was added dropwise. Spontaneous formation of a coloured precipitate was observed, which was removed by filtration. The filtrate was allowed to stand overnight (the colour changed to dark yellow) and after distillation of the solvent the residue was dissolved in methanol and treated with charcoal. After removal of the charcoal, the solution was concentrated and allowed to stand in the refrigerator for 4 days, when a white precipitate was deposited. This was recrystallised from aqueous methanol (also from aqueous ethanol). Yield: 200 mg. (41 per cent), m.p. 88 to 90°. Found: C, 73·72; H, 8·65; N, 8·53 per cent. $C_{20}H_{28}O_2N_2$ requires C, 73·10; H, 8·58; N, 8·53 per cent.

Expt. 9. 3,4,5-*Trimethoxybenzoyl chloride.* 3,4,5-Trimethoxybenzoic acid (10 g.) and thionyl chloride (20 g.) were mixed together and allowed to stand at room temperature for 3 hours and then refluxed for 1 hour. The excess thionyl chloride was removed with the aid of dry benzene. Yield: 10·31 g. (95 per cent), m.p. 77 to 78°. (lit. m.p. 77 to 78°).

Expt. 10. N-(2-*Indol*-3′-*ylethyl*)-3,4,5-*trimethoxybenzamide* (*V*). Tryptamine (1·9 g.) in tetrahydrofuran (25 ml.) was added dropwise to a cooled well-stirred solution of 3,4,5-trimethoxybenzoyl chloride (1·4 g.) in tetrahydrofuran (30 ml.) and the stirring continued for 30 minutes. The mixture was allowed to stand overnight under dry nitrogen.

The precipitated tryptamine hydrochloride (0·98 g.) was removed by filtration and the solvent by distillation under reduced pressure. The residue was dissolved in absolute methanol and treated with charcoal. After removal of the charcoal the solution was concentrated to about 20 ml. and allowed to stand in the refrigerator. The colourless crystals deposited were recrystallised from methanol. Yield: 1·70 g. (65 per cent), m.p. 200 to 202°. Found: C, 68·00; H, 6·44; N, 7·89 per cent. $C_{20}H_{22}O_4N_2$ requires C, 67·79; H, 6·24; N, 7·90 per cent.

Expt. 11. N-(3,4,5-*Trimethoxybenzyl*)*tryptamine* (*VI*). The above amide (0·5 g.) was placed in the thimble of a Soxhlet extractor, fitted to a flask (250 ml.) containing $LiAlH_4$ (1·0 g.) in tetrahydrofuran (200 ml.) and provided with a mercury-sealed stirrer. The mixture was then refluxed for 72 hours in an atmosphere of nitrogen and then the procedure described in Expt. 7 (1) was followed. Yield: 0·3 g. (66 per cent), b.p. 215 to 217°/0·2 mm. Found: C, 71·04; H, 7·08; N, 8·20 per cent. $C_{20}H_{24}O_3N$ requires C, 70·58; H, 7·08; N, 8·23 per cent.

Picrate. M.p. 147·5 to 149°. Found: N, 12·77 per cent. $C_{26}H_{27}O_{10}N_5$ requires N, 12·32 per cent.

Hydrochloride. M.p. 229 to 231°. Found: C, 64·01; H, 6·43; N, 7·42; Cl, 9·39 per cent. $C_{20}H_{24}O_3N_2$,HCl requires C, 63·72; H, 6·66; N, 7·43; Cl, 9·42 per cent.

Expt. 12. 4-*Methoxycyclohexylacetotryptamide* (*X*). *Method* (1). Tryptamine (technical) (1·6 g.) in tetrahydrofuran (20 ml.) was cooled to below 10° and to this cooled and stirred mixture, 4-methoxycyclohexylacetyl chloride (0·95 g.) in tetrahydrofuran (15 ml.) was added dropwise. The mixture was stirred at room temperature for 30 minutes and then allowed to stand overnight. The precipitated tryptamine hydrochloride was removed by filtration and the solvent by distillation under reduced pressure. The residue was dissolved in excess benzene and washed with 5 per cent sodium hydroxide solution followed by 3N hydrochloric acid. The benzene extract was dried over anhydrous sodium sulphate and the solvent removed. The residue solidified on standing in the refrigerator and was then recrystallised from benzene. Yield: 1·0 g. (69 per cent), m.p. 96 to 101°, b.p. 300°/0·2 mm. Found: C, 73·11; H, 8·36; N, 8·89 per cent. $C_{19}H_{26}O_2N_2$ requires C, 72·65; H, 8·35; N, 8·92 per cent.

Method (2). Tryptamine (1·0 g.) in benzene (50 ml.) and 4 per cent sodium hydroxide solution (40 ml.) were mixed. To the mixture, 4-methoxycyclohexylacetyl chloride (1·3 g.) in benzene (10 ml.) was added dropwise. Stirring was continued at room temperature for about an hour, after which the benzene layer was washed with 5 per cent. caustic soda solution followed by 3N hydrochloric acid. The benzene extract was dried over anhydrous sodium sulphate and the benzene removed. The rest of the procedure follows that described in Method (1). Yield: 0·98 g. (46 per cent), m.p. and mixed m.p. with the product of Method (1) 95·5 to 100·5° (mixture of stereoisomers).

Method (3). Methyl 4-methoxycyclohexylacetate (1·0 g.) and tryptamine (0·8 g.) were mixed together and heated at 180 to 190° for 3 hours. The reacted product was dissolved in warm benzene and washed with 5 per cent sodium hydroxide solution followed by 3N hydrochloric acid. The benzene layer was separated and dried over anhydrous sodium sulphate and the benzene removed. The residue was allowed to stand in the refrigerator for about three months and then recrystallised from benzene.

Yield: 0·25 g., m.p. and mixed m.p. with the product of Method (1) 95·5 to 100·5°.

Expt. 13. *N-[2-(4-Methoxycyclohexyl)ethyl]tryptamine (XI).* 4-Methoxycyclohexylacetotryptamide (0·5 g.) in tetrahydrofuran (20 ml.) was added dropwise to a boiling suspension of $LiAlH_4$ (1·0 g.) in ether (100 ml.) in a flask (250 ml.) fitted with a mercury-sealed stirrer and a condenser (calcium chloride guard tube). The mixture was refluxed with stirring in an atmosphere of nitrogen for about 56 hours. The rest of the procedure followed that described in Expt. 7 (1). Yield: 0·305 g. (65 per cent), b.p. 180 to 182°/0·05 mm. Found: C, 75·82; H, 9·63; N, 9·57 per cent. $C_{19}H_{28}ON$ requires C, 75·97; H, 9·36; N, 9·33 per cent.

Hydrochloride. The above base (about 250 mg.) was dissolved in tetrahydrofuran (3 ml.) and ether (25 ml.) was added. The solution was treated with dry hydrogen chloride The precipitated hydrochloride was recrystallised from ethanol, m.p. 205 to 210°. Found: C, 67·75; H, 8·40; N, 8·34; Cl, 10·55 per cent. $C_{19}H_{28}ON,HCl$ requires C, 68·36; H, 8·38; N, 8·32; Cl, 10·38 per cent.

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POTENTIAL RESERPINE ANALOGUES

PART II. 3,4,5-TRIMETHOXYBENZOIC ACID DERIVATIVES

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Received November 6, 1959

Seven derivatives of 3,4,5-trimethoxybenzoic acid have been prepared and tested for their ability to potentiate barbiturate hypnosis in mice, to deplete the 5-hydroxytryptamine content of rat brain, and to exert a hypotensive action on the cat blood pressure. The only derivative to exert all three actions is the simple anilide, 3,4,5-trimethoxybenzanilide, which is about 8 times less active than reserpine. The anilide, *N*-(3,4-dimethoxyphenethyl)-3,4,5-trimethoxybenzamide, is also about 8 times less active than reserpine in the first two tests but it raises the blood pressure.

THE report of Miller and Weinberg[1] that 3-diethylaminopropyl 3,4,5-trimethoxybenzoate has reserpine-like activity (one-third that of the potency of reserpine) raises the question of the necessity of any of the ring systems of reserpine. A number of similar compounds have recently been prepared by Zdenek and colleagues[3]; Sastry and Lasslo[2] and Perron[4,5] but complete pharmacological tests on the products have not been carried out.

The present work describes the preparation of trimethoxybenzoyl derivatives which retain only the ring A of reserpine, the other rings being represented only by fragments. To this end the compounds indicated in Figure 1 were synthesised. Finally ring A was dispensed with in the *N*-pentyl derivative of trimethoxybenzamide (VII).

Pharmacological Data

The compounds were compared with reserpine for their power (i) to potentiate the hypnosis in mice produced by an intravenous dose of hexobarbitone (50 mg./kg.), (ii) to deplete the 5-hydroxytryptamine (5-HT) content of rat brain, and (iii) to exert a hypotensive action on the arterial blood pressure of a cat anaesthetised with nembutal and chloralose. The standard doses of reserpine were 5 mg./kg. intraperitoneally in test (*a*), 1 mg./kg. intraperitoneally in test (*b*) and 1 mg./kg. subcutaneously in test (*c*). The doses of the new compounds used and their relative activities are shown in Table I.

We should like to express our thanks to Miss S. A. P. Price and Dr. G. B. West of the Department of Pharmacology of this School for carrying out the pharmacological tests.

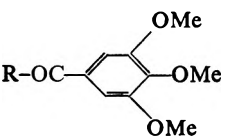
Discussion of Pharmacology

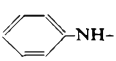
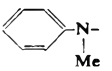
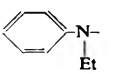
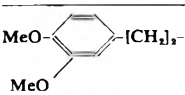
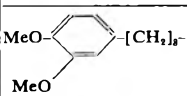
These comparatively simple components are pharmacologically more promising than the tryptamine derivatives discussed in Part I[6]. The

anilide (II) is the only compound so far tested which simulates three of the properties of reserpine (potentiation of barbiturate hypnosis, depletion of brain 5-HT, and hypotensive action). Compound II nevertheless does not appear to act like reserpine even in these three tests, for although

TABLE I

COMPARISON OF THE PHARMACOLOGICAL ACTIVITIES OF VARIOUS DERIVATIVES OF 3,4,5-TRIMETHOXYBENZOIC ACID. (RESERPINE ACTIVITY IS TAKEN AS 100 FOR EACH TEST)



Trimethoxybenzoic acid derivative	R	Potentiation of barbiturate hypnosis in mice		Depletion of 5-HT in rat brain		Action on cat blood pressure	
		Max. dose used (mg./kg.)	Relative activity	Max. dose (mg./kg.)	Relative activity	Dose (mg./kg.)	Effect
I	NH_2-	150	1	40	0	—	—
II		40*	10–16	40	10–16	20	Hypotension + adrenergic block
III		80	0	—	—	50	Adrenergic block only
IV		80	0	20	0	50	Adrenergic block
V		100	12	20	10	25	Hypertension
VI		80	1	20	3·5	22	Hypertension
VII	$Me-[CH_2]_4-NH-$	100	1–3	—	—	—	—

* Some of the mice died at this dose level.

it is a hypotensive agent it also possesses marked anti-adrenaline activity. Alkylation of the N atom in compound II results in compounds which have no activity in tests (i) and (ii) and only adrenergic block activity in test (iii).

When the N atom in compound II does not form part of the aromatic ring system but forms benzamides (as in V, VI and VII), the compounds potentiate barbiturate hypnosis and deplete the brain of 5-HT but exert *hyper*tensive effects on the blood pressure.

The results indicate that the actions of comparatively simple derivatives of 3,4,5-trimethoxybenzoic acid mimic those of reserpine in a few tests, and the indole nucleus is not so important for reserpine-like activity as has been suggested by other authors.

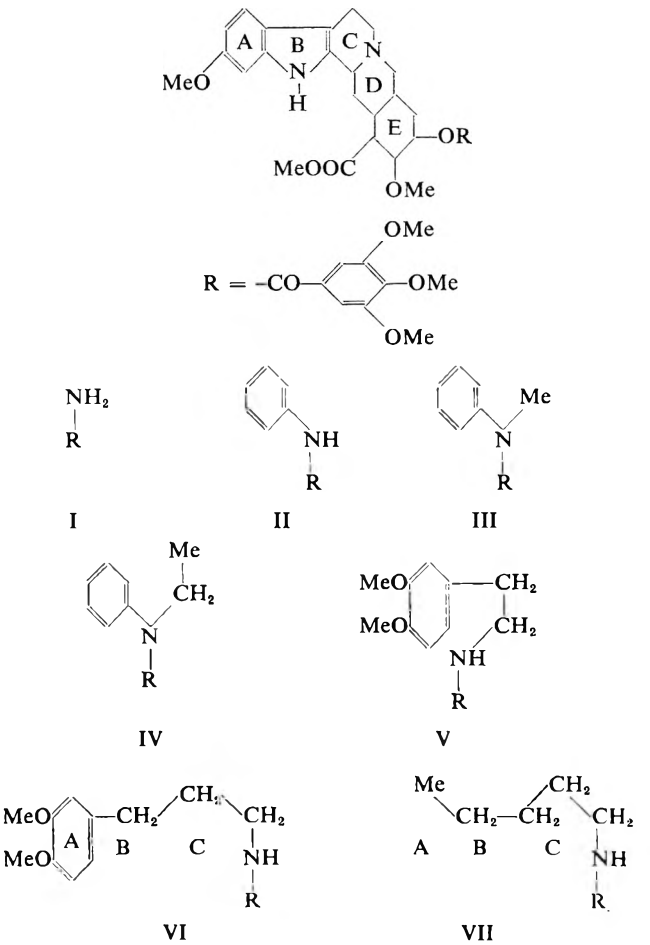


FIG. 1

EXPERIMENTAL

Expt. 1. 3-(3,4-*Dimethoxyphenyl*)*propylamine.* β-(3,4-Dimethoxyphenyl)propionamide (prepared according to the method of Buck and Perkin[7] (8 g.) in tetrahydrofuran (150 ml.) was added dropwise to a boiling suspension of $LiAlH_4$ (3 g.) in ether (150 ml.). The reaction mixture was refluxed for 72 hours and cooled. The excess $LiAlH_4$ was destroyed by the dropwise addition of aqueous tetrahydrofuran with vigorous shaking and solution filtered. The residue was washed

thoroughly with warm tetrahydrofuran and the mixed tetrahydrofuran extracts dried over anhydrous sodium sulphate; after removal of the solvent the residue was distilled under reduced pressure. Yield: 6·3 g. (85 per cent), b.p. 125 to 130°/1·5 mm., n_D^{22} 1·5338.

Hydrochloride. The above amine (2·1 g.) in ether (100 ml.) was treated with dry hydrogen chloride and the precipitated hydrochloride recrystallised from ethanol and ether. Yield: 1·8 g. (72 per cent), m.p. 162 to 163°. Found: C, 57·05; H, 7·86; N, 6·29; Cl, 14·93; per cent. $C_{11}H_{17}O_2N$,HCl requires C, 57·10; H, 7·79; N, 6·06; Cl, 15·15 per cent.

Expt. 2. 3,4,5-*Trimethoxybenzamide* (*I*). 3,4,5-Trimethoxybenzoyl chloride (10 g.) in ether (50 ml.) was cooled below 10° and excess ammonia (sp.gr. 0·88) (50 ml.) added dropwise with constant stirring. The reaction mixture was stirred well for about 30 minutes at the same temperature and then allowed to stand at room temperature for another 30 minutes with occasional shaking. The precipitated amide was recrystallised from benzene. Yield: 8·09 g. (88 per cent), m.p. 176 to 177° (lit. 176 to 177°).

Expt. 3. 3,4,5-*Trimethoxybenzanilide* (*II*). 3,4,5-Trimethoxybenzoyl chloride (4·6 g.) in tetrahydrofuran (15 ml.) was added dropwise to a cooled solution (below 10°) of aniline (3·72 g.) in ether (50 ml.). The mixture was stirred at room temperature for about an hour and then allowed to stand for a further 2 hours. The precipitated aniline hydrochloride was removed and the solvent removed by distillation under reduced pressure. The solid residue was recrystallised from methanol. Yield: 4·9 g. (85 per cent) m.p. 137 to 139°. Found: N, 4·90 per cent, requires N, 4·87 per cent.

Expt. 4. 3,4,5-*Trimethoxy*-N-*methylbenzanilide* (*III*). *N*-Methylaniline (2·3 g.) in ether (25 ml.) was added dropwise to a cooled solution (below 10°) of 3,4,5-trimethoxybenzoyl chloride (2 g.) in tetrahydrofuran (10 ml.). The mixture was stirred well at room temperature for 1 hour. The precipitated hydrochloride was removed and the solution was washed with 3N hydrochloric acid. The extract was dried over anhydrous sodium sulphate and the solvent removed. The residue was recrystallised from methanol. Yield: 1·65 g. (63 per cent), m.p. 80·5 to 82°, b.p. 178 to 180°/0·3 mm. Found: C, 66·94; H, 6·37; N, 4·62 per cent. $C_{17}H_{19}O_4N$ requires C, 67·74; H, 6·34; N, 4·62 per cent.

Expt. 5. N-*Ethyl*-3,4,5-*trimethoxybenzanilide* (*IV*). 3,4,5-Trimethoxybenzoyl chloride (2·3 g.) in dry ether (25 ml.) was added dropwise to a cooled solution (below 10°) of *N*-ethylaniline (2·45 g.) in ether (25 ml). with constant shaking. Immediate formation of *N*-ethylaniline hydrochloride was observed. The mixture was stirred at room temperature for 30 minutes and then allowed to stand for 40 hours. The *N*-ethylaniline hydrochloride was removed by filtration and the solvent by distillation under reduced pressure. The residue was distilled *in vacuo*. Yield: 2·8 g. (87 per cent), b.p. 165 to 170°/0·2 mm., m.p. 70·5 to 72° (aqueous methanol). Found: C, 68·50; H, 6·96; N, 4·37 per cent. $C_{18}H_{22}O_4N$ requires C, 68·40; H, 6·96; N, 4·43 per cent.

Expt. 6. N-(3,4-*Dimethoxyphenethyl*)-3,4,5-*trimethoxybenzamide* (*V*). 3,4,5-Trimethoxybenzoyl chloride (1·2 g.) in tetrahydrofuran (20 ml.) was added dropwise to a cooled and well stirred solution of homoveratrylamine (1·8 g.) in ether (25 ml.). The mixture was stirred at room temperature for 20 minutes and then allowed to stand overnight.

The precipitated homoveratrylamine hydrochloride was separated by filtration, the solution was dried over anhydrous sodium sulphate, and the solvent removed by distillation under reduced pressure. The solid residue was recrystallised from aqueous methanol. Yield: 1·5 g. (74 per cent), m.p. 134 to 135°. Found: C, 64·00; H, 6·65; N, 3·73 per cent. $C_{20}H_{25}O_6N$ requires C, 63·98; H, 6·69; N, 3·73 per cent.

Expt. 7. N-[3-(3,4-*Dimethoxyphenyl*)*propyl*]-3,4,5-*trimethoxybenzamide* (*VI*). 3,4,5-Trimethoxybenzoyl chloride (1·5 g.) in tetrahydrofuran (15 ml.) was added dropwise to a cooled (below 10°) solution of 3-(3,4-dimethoxyphenyl)propylamine (1·95 g.) in tetrahydrofuran (20 ml.) with constant stirring. The reaction mixture was allowed to stand overnight at room temperature and the precipitated hydrochloride of 3-(3,4-dimethoxyphenyl)propylamine removed by filtration and the solvent by distillation under reduced pressure. The solid residue was recrystallised from ether. Yield: 1·5 g. (75 per cent), m.p. 105 to 106°. Found: C, 64·66; H, 7·27; N, 3·61 per cent. $C_{21}H_{27}O_6N$ requires C, 64·80; N, 6·97; N, 3·60 per cent.

Expt. 8. 3,4,5-*Trimethoxy*-N-*pentylbenzamide* (*VII*). 3,4,5-Trimethoxybenzoyl chloride (2·3 g.).) in tetrahydrofuran (10 ml.) was added dropwise to a cooled (below 10°) stirred solution of pentylamine (1·74 g.) in ether (35 ml.). The reaction mixture was strongly acidified with hydrochloric acid and extracted with ether. The ether extract was dried over anhydrous sodium sulphate and the solvent removed. The solid residue was recrystallised from ether. Yield: 1·85 g. (66 per cent), m.p. 108 to 110°. Found: C, 64·31; H, 8·01; N, 4·88 per cent. $C_{15}H_{23}O_4N$ requires C, 64·15; H, 8·23; N, 4·98 per cent.

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ELECTROCARDIOGRAPHIC CHANGES PRODUCED IN RABBITS BY VASOPRESSIN (PITRESSIN) AND THEIR ALTERATION BY PROLONGED TREATMENT WITH A COMMERCIAL HEART EXTRACT

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Received November 9, 1959

The electrocardiographic changes produced in rabbits by large doses of vasopressin are interpreted to arise from an initial, unstable, state of apical accelerated repolarisation followed by a more enduring state of subendocardial injury. When rabbits were treated, daily, with a commercial heart extract for two weeks and then given vasopressin two days after treatment had stopped, signs of apical accelerated repolarisation were unaltered but the signs of subendocardial injury were substantially suppressed.

THERE are several reports that a protein-free extract of heart muscle from freshly-killed young animals contains an unidentified principle which produces coronary dilatation in perfused, isolated, mammalian hearts[1–3] and improves the efficiency of contractions in heart-lung preparations[4] and in fatigued, isolated, cat papillary muscles[5]. Increased coronary blood flow[6] and antagonism to vasopressin-induced coronary vasoconstriction[7] have been observed in intact dogs. In all these preparations, the pharmacological effects of the extract are brief. However, the reports of clinical trials with the heart extract, in patients with coronary artery disease, attribute relatively long periods of improvement to the actions of the drug[8,9]. As clinical trials with new drugs in coronary artery disease are notoriously difficult to assess, it seeemed important to find out if this extract could produce any enduring changes in the pharmacological behaviour of the hearts of experimental animals. Lindner, Loudon and Werner[7] have shown that after three daily injections of the extract had been given to dogs, the electrocardiographic changes produced by vasopressin were reduced in comparison with the control period. However, two days after stopping the injections the cardiac sensitivity to vasopressin had returned to control levels.

This paper describes similar experiments in rabbits but with a longer period of treatment with the heart extract before the animals were challenged with vasopressin. As the electrocardiographic responses to vasopressin in rabbits were found to differ from those described for dogs[10,11,14], the effects of vasopressin in the untreated rabbits are given in detail in this paper.

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METHODS

In all experiments, the animals were pretreated with either the heart extract or saline and then the cardiac responses to vasopressin were followed electrocardiographically. Rabbits were used for all experiments.

A water-soluble, protein-free, extract of heart muscle was supplied in ampoules. Each ampoule contained 1 ml. of extract equivalent to 0·97 g. of fresh heart, having between 100 to 115 mg. per cent total nitrogen content. A water-insoluble fraction was supplied in pills. Each pill contained 0·2 g. of dried material, equivalent to 0·82 g. of fresh heart, having between 11·3 to 12·3 g. per cent total nitrogen content. The treated groups of animals were given 1 ml. of water-soluble extract intramuscularly and one pill orally every day. The control groups of animals were given 1 ml. of sterile saline (0·9 per cent. w/v NaCl) intramuscularly every day but "dummy" pills were omitted. The animals were treated from Monday to Friday for two consecutive weeks. All control and treated animals were then given vasopressin (Parke-Davis, Pitressin, 20 i.u./ml.) on the Monday of the third week.

Electrocardiograms were taken with the animal, unrestrained, in the sitting position but movements were discouraged by surrounding the animal with a wooden barrier. A bipolar chest lead was used by inserting needle electrodes subcutaneously on both sides of the chest at the level of the 5th to 6th interspaces. The electrocardiograms were recorded with a New Electronic Products Multichannel Galvanometer Recorder. The overall frequency response of amplifier and galvanometer was flat to about 180 cycles/second. The amplifier time constant was 2 seconds and the sensitivity was adjusted to give 2 cm. deflection for 1 mV input.

Control electrocardiograms were taken over a period of 5 minutes and the vasopressin, in doses of 2 or 4 i.u./kg., was injected into an ear vein. Records were then taken at frequent intervals for at least 8 minutes.

Three experiments were carried out. In the *first experiment*, Himalayan doe rabbits about 9 months old were used. There were 6 control and 6 treated animals and at the end of the experiment all animals were given 2 i.u./kg. of vasopressin. The *second experiment* was a repetition of the first experiment but younger animals, about 3 to 4 months old, were used, and 4 i.u./kg. of vasopressin were given to each animal. To get satisfactory records from these animals they were sedated with 0·2 ml./kg. of 10 per cent w/v sodium thialbarbitone given intravenously. In the *third experiment*, 6 Dutch rabbits of similar age to those of group 1 (6 to 9 months old), were given 4 i.u./kg. of vasopressin and then the same animals were given the same dose of vasopressin after the standard period of treatment with the heart extract.

RESULTS

Effects of Vasopressin on the Electrocardiograms of Untreated Rabbits

The effects of vasopressin on heart rates, RS-T segments, and T waves are shown, for the three experiments, in Figures 1 and 2 and Table I.

Heart rates. After the injection of vasopressin, the rabbits went into

a shock-like state, usually with head-drop, and occasionally had to be prevented from falling over on their sides. The onset and recovery from this state of collapse seemed to be paralleled by cardiac slowing. The heart rate reached a minimum about 2 minutes after the injection and there was usually only moderate recovery after 8 minutes, when the recordings were stopped.

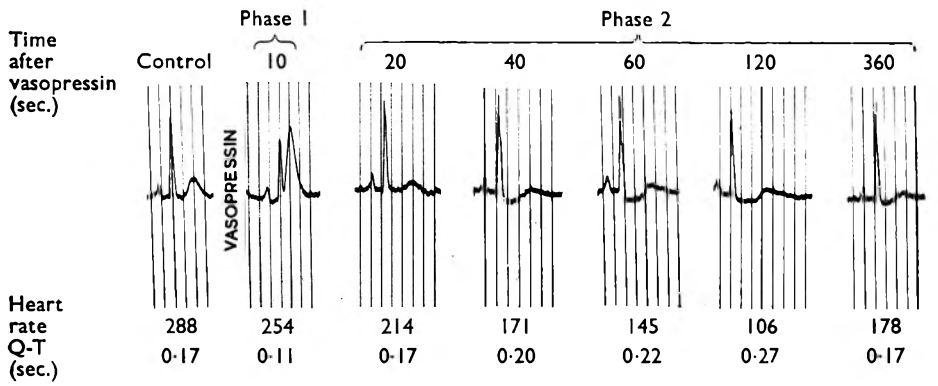


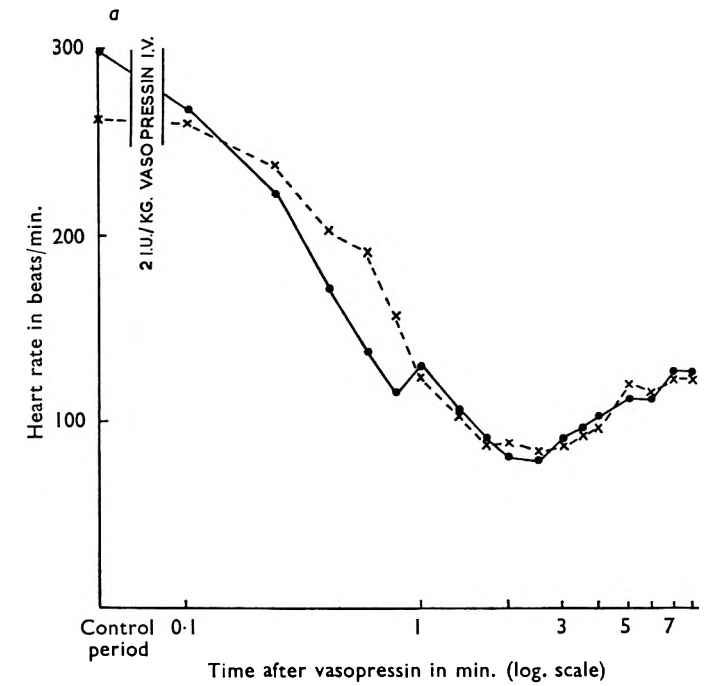
Fig. 1. Effects of vasopressin on the electrocardiogram of an untreated rabbit. Explanations of phases 1 and 2 are given in the text. Time marks = 0·04 sec.

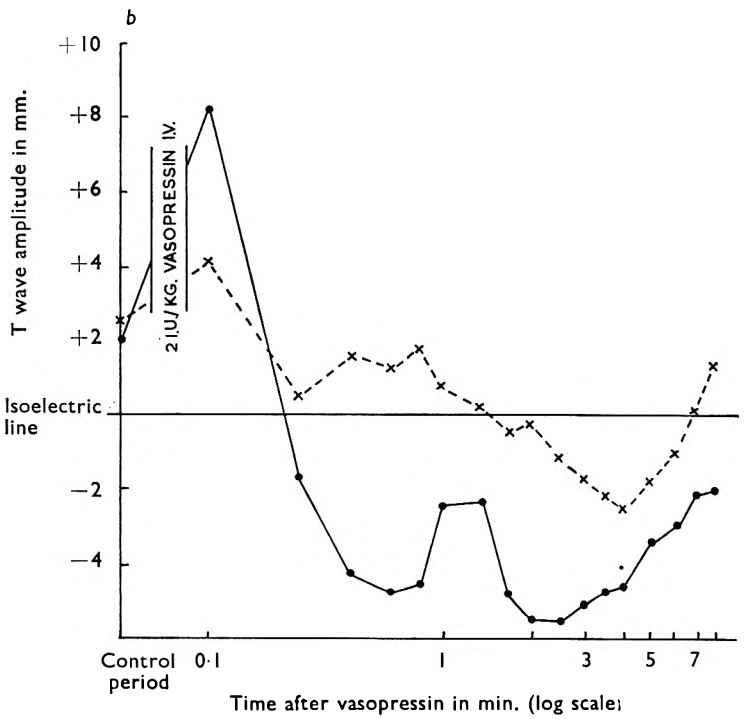
T waves. 30 control electrocardiograms were taken; 22 were found to have upright T waves, 3 had biphasic and 5 had inverted T waves.

After vasopressin, the first change was a marked elevation of T waves. The elevated T was accompanied by a shortened Q–T interval and hence high branching of the T wave from the downstroke of the R wave usually appeared (Fig. 1). These changes appeared within 5 to 10 seconds of the injection and had disappeared again within 25 to 30 seconds. After this phase, the T waves became depressed, and inverted T waves usually appeared within $1\frac{1}{2}$ to 2 minutes of the injection and were slowly returning towards the control appearance after 8 minutes. This phase of depressed or inverted T waves was associated with long Q–T intervals. As the prolonged Q–T intervals occurred during the period of marked bradycardia, Hegglin and Holzmann's[12] empirical correction for frequency was applied to some of the results and showed that there was probably no significant relative increase of Q–T compared with the control values.

RS–T segments. With one minor exception, all electrocardiograms were found to have isoelectric RS–T segments during the control period. After the vasopressin injection there was a brief phase of elevated RS–T segments corresponding to the phase of elevated T and shortened Q–T intervals.

The RS–T segment usually became isoelectric again within 20 to 30 seconds after the injection. Between 30 to 60 seconds the RS–T segments became depressed, concave or flat, in all control experiments. In most experiments some RS–T segment depression was still present after 8 minutes. This phase of RS–T segment depression corresponded with


a
300
200
100
Heart rate in beats/min.
2 I.U./KG. VASOPRESSIN I.V.
Control period
0·1
1
3
5
7
Time after vasopressin in min. (log. scale)


b
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T wave amplitude in mm.
2 I.U./KG. VASOPRESSIN I.V.
Control period
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Time after vasopressin in min. (log scale)

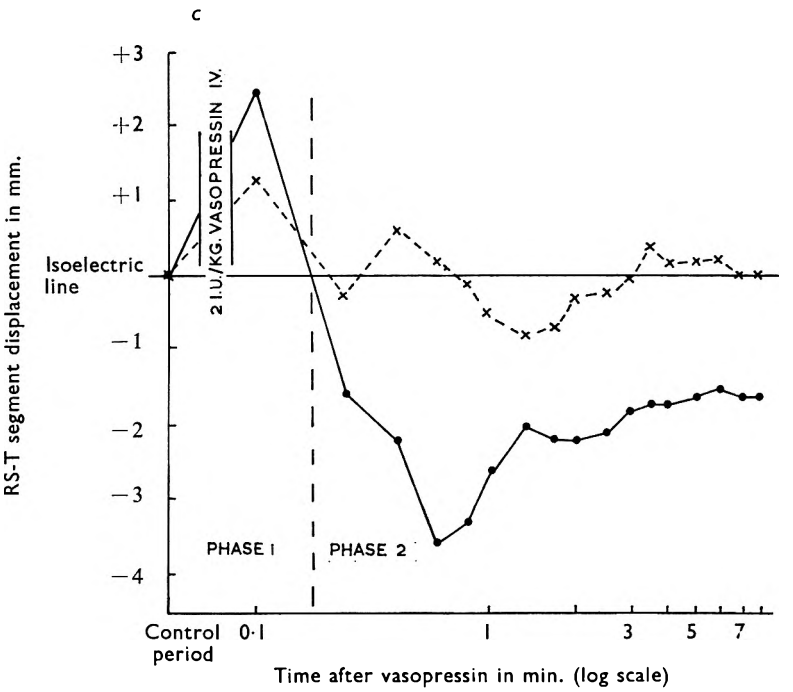

Fig. 2 *a,b,c.* Time-Course of electrocardiographic changes following vasopressin in untreated rabbits and in rabbits after treatment with heart extract.
The results are from Experiment 1 and each point plotted is the mean from each group of six animals. The standard errors of the means are included in Table I.

●——●, Untreated, ×- - -×, treated groups.

the phase of depressed or inverted T waves and normal or prolonged Q–T intervals.

On the basis of these changes in T, Q–T and RS–T the electrocardiograms of the control animals have been divided into two phases:

Phase 1 immediately follows the injection of vasopressin, lasts for 20 to 30 seconds, and is characterised by elevated T and RS–T and shortened Q–T intervals. *Phase* 2 succeeds phase 1, lasts for several minutes, and is characterised by depressed RS–T, depressed or inverted T, and relatively normal or prolonged Q–T intervals.

Effects of Pretreatment with Heart Extract on the Electrocardiograms after Vasopressin

Heart rates. In the three experiments there were no significant differences in heart rate responses to vasopressin between the treated and control groups of animals (Table I). By inference, it appears that pretreatment with the heart extract did not influence the generalised vasoconstriction produced by vasopressin.

T waves. There were no significant differences in T wave amplitude,

TABLE I

EFFECTS OF VASOPRESSIN ON HEART RATES, RS-T SEGMENT DISPLACEMENT AND T WAVE AMPLITUDE IN UNTREATED RABBITS AND RABBITS TREATED WITH HEART EXTRACT

Experiment No.	Time after vasopressin (sec.)	Heart rate: beats/min.			RS–T segment displacement: mm.			T wave amplitude: mm.		
		Untreated	Treated	P	Untreated	Treated	P	Untreated	Treated	P
1	Control	298 ± 8	272 ± 10	>0·1	0	0		+2·1 ± 0·92	+2·5 ± 1·25	>0·8
Phase 1	10	268 ± 17	260 ± 14	>0·7	+2·5 ± 1·70	+1·3 ± 0·80	>0·5	+8·3 ± 2·91	+4·1 ± 3·36	>0·3
Phase 2	40	171 ± 15	202 ± 21	>0·2	−3·6 ± 0·16	+0·2 ± 0·21	<0·01	−4·7 ± 1·06	+1·3 ± 1·27	<0·02
	120	81 ± 6	88 ± 15	>0·6	−2·2 ± 0·51	−0·3 ± 0·20	<0·05	−5·5 ± 1·12	−0·2 ± 1·15	>0·05
	360	127 ± 8	123 ± 7	>0·7	−1·6 ± 0·49	+0·2 ± 0·18	>0·02	−2·9 ± 1·08	+0·1 ± 1·21	<0·1
2	Control	291 ± 6	296 ± 5	>0·5	0	0		+1·6 ± 1·02	+2·5 ± 1·30	>0·6
Phase 1	10	261 ± 11	284 ± 7	>0·1	+2·3 ± 1·52	+1·6 ± 0·77	>0·6	+10·0 ± 2·58	+12·5 ± 2·86	>0·5
Phase 2	40	169 ± 17	207 ± 22	>0·2	−4·0 ± 0·73	+0·2 ± 0·85	<0·01	−2·9 ± 1·10	+2·8 ± 1·25	<0·02
	120	212 ± 9	147 ± 16	>0·2	−1·6 ± 0·48	−1·0 ± 0·36	>0·3	−2·7 ± 0·98	+0·6 ± 1·41	>0·1
	360	150 ± 11	160 ± 7	>0·4	−0·9 ± 0·28	−0·2 ± 0·19	<0·1	−2·0 ± 1·01	+2·4 ± 1·47	>0·05
3	Control	286 ± 11	285 ± 9	>0·9	0	0		+2·0 ± 0·57	+1·7 ± 0·85	>0·7
Phase 1	10	270 ± 12	288 ± 10	>0·3	+2·2 ± 0·61	+1·5 ± 0·73	>0·4	+12·6 ± 1·58	+7·2 ± 2·76	<0·2
Phase 2	40	177 ± 23	156 ± 21	>0·5	−1·4 ± 0·33	−0·1 ± 0·20	<0·02	+1·3 ± 0·25	+0·4 ± 0·60	>0·2
	120	134 ± 10	110 ± 7	>0·1	−3·6 ± 0·41	−0·9 ± 0·44	<0·01	−1·4 ± 0·47	+1·1 ± 0·69	<0·05
	360	146 ± 6	153 ± 5	>0·4	−0·4 ± 0·28	0		+0·6 ± 1·00	+0·3 ± 0·92	>0·8

Figures are the means and standard errors of the individual measurements.
P is the probability, estimated by *t* test, that the observed differences occurred by chance.

between treated and untreated animals, during phase 1 of the electrocardiograms. However, at the peak of phase 2, the T waves of the treated animals were significantly less depressed or inverted (Table I).

RS–T segments. There were no significant differences in RS–T segment elevation, between treated and untreated animals, during phase 1 of the electrocardiograms. At the peak of phase 2, however, the RS–T segments of the treated animals were significantly less depressed.

Discussion

Vasopressin produces coronary vasoconstriction and electrocardiographic signs of myocardial ischaemia[10]. Similar electrocardiographic signs do not appear when the cardiac muscle mass is uniformly ischaemic[13]. There is evidence, however, that the subendocardial region of the left ventricle is particularly susceptible to anoxaemia. Thus Lepeschkin[14] has emphasised that the subendocardial layers receive blood only during diastole while the subepicardial layers receive blood throughout the whole cardiac cycle. He believes that this explains why the subendocardial muscle layers are more sensitive than the rest of the heart to all types of coronary insufficiency. Dearing, Barnes and Essex[15] have shown that large doses of vasopressin produce cardiac necrosis which is most marked in the subendocardial region.

Using epicardial-facing electrodes, as in the present experiments, experimental subendocardial injury is known to produce RS–T segment depression, depressed or inverted T waves, and normal or prolonged Q–T intervals[14]. In the present experiments, similar changes were found during phase 2 of the electrocardiograms. This is the basis for believing that phase 2 is the result of subendocardial injury.

The significance of phase 1 of the electrocardiograms is more difficult to interpret. With epicardial-facing electrodes, elevation of RS–T and T imply subepicardial changes. The shortened Q–T intervals indicate that there has been a shortening of the recovery phase of the monophasic action potentials or accelerated repolarisation. Nahum and Hoff[16] have shown that warming the apex of the heart will produce similar electrocardiographic changes. Hence, phase 1 has been tentatively interpreted to arise from a brief period of accelerated repolarisation involving the whole apical muscle mass which appears before well marked injury has developed subendocardially.

In the present experiments, it was found that, in the groups of animals treated with heart extract, phase 2 of electrocardiographic changes after vasopressin was less marked than in the control animals, but that the phase 1 changes were not significantly different from the controls. If the interpretations of the two phases are substantially correct, then the heart extract treatment has apparently protected the hearts against subendocardial injury but has not influenced the initial phase of apical accelerated repolarisation.

This differential effect on the electrocardiogram and the persistence of the effect for at least two days after treatment had stopped, make it

difficult to explain the results in terms of coronary vasodilatation. However, Witzleb, Gollwitzer-Meier and Donat[4] have inferred from their experiments that the heart extract has a damping effect on the oxidative metabolism of the heart and increases the efficiency of myocardial contractions. This effect seems to be a possible explanation for the protection against subendocardial injury found in the present experiments. This would be an interesting explanation because Cossio[17] has recently suggested that iproniazid (marsilid) relieves ischaemic pain by depressing certain oxidative enzymes in the heart followed by an increased efficiency of oxygen utilisation.

It is usually difficult enough to assess the significance of experiments with tissue extracts said to contain unidentified active principles but more so in this case when the extract contains both water-soluble and water-insoluble components. Many experiments have been carried out with the water-soluble fraction but there appear to have been no pharmacological studies with the water-insoluble components. Both components were used, without prejudice, in the present experiments to mimic the clinical situation. Clearly, a separate pharmacological evaluation of each of these components is needed.

The results presented in this paper are consistent with previous reports about the activity of this heart extract, and taken together seem sufficiently interesting to warrant more experimental and clinical investigations on the nature of the active substances involved.

Acknowledgement. The heart muscle extract (Recosen) used in these experiments was made available by Dr. N. Levinson of Robapharm Ltd., Basle, Switzerland, to whom I am also indebted for supplying much useful information about this preparation.

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ESTERS OF *N*-METHYLPYRROLIDINYLALKANOLS AS LOCAL ANAESTHETICS

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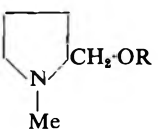
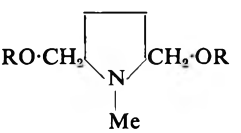
Received September 16, 1959

Three new pyrrolidylalkanol esters, 2-*p*-methoxybenzoyloxymethyl-l-methyl-, 2,5-di(*p*-methoxybenzoyloxymethyl)-1-methyl- and 2,5-di(2-*p*-methoxybenzoyloxypropyl)-1-methyl-pyrrolidine have been prepared and tested as local anaesthetics. The activity of the last compound has been shown to be almost identical with that of cocaine by the intracutaneous route, though it is without action on the cornea.

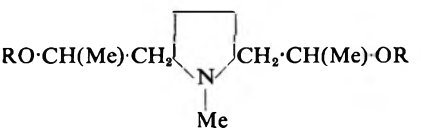
The preparation of a number of esters and phenylcarbonates of the 1-methylpyrrolidinylalkanols (R=H) I, II and III has been described[1].



(I)



(II)



(III)

R = C_6H_5·CO–, C_6H_5CH:CH·CO–, *p*-NH_2·C_6H_4·CO–, C_6H_5·NH·CO–

Preliminary pharmacological tests indicated that local anaesthetic activity was present in all three series of compounds and this paper reports the preparation and activity of the *p*-methoxybenzoates (I, II and III where R = *p*-MeO·C_6H_4·CO–).

2-Hydroxymethyl-1-methylpyrrolidine (1; R = H) was prepared by the method of Blicke and Chi Jung Lu[2], and this with anisoyl chloride in dry benzene gave the hydrochloride of 2-*p*-methoxybenzoyloxymethyl-1-methylpyrrolidine directly. The preparation of 2,5-di(hydroxymethyl)-1-methylpyrrolidine (II; R = H) and of 2,5-di(2-hydroxypropyl)-1-methylpyrrolidine (III; R = H) has been described[1]. These were treated in acetone solution with anisoyl chloride and excess sodium hydroxide solution to give the esters (II and III; R = *p*-MeOC_6H_4·CO–), which were converted to the hydrochlorides.

EXPERIMENTAL

All m.p.s. are uncorrected. Microanalyses are by Mr. G. S. Crouch, School of Pharmacy, University of London.

(I) 2-*p-Methoxybenzoyloxymethyl*-1-*methylpyrrolidine hy drochloride.* A Mixture of 0·86 g. of 2-hydroxymethyl-1-methylpyrrolidineand 1·54 g. of anisoyl chloride was refluxed for 1 hour in 20 ml. of dry benzene and left overnight. The deposited solid was recrystallised from isopropanol/light petroleum to give 1·47 g. of *product*, m.p. 204°. Found: C, 59·0; H, 7·0; N, 4·9. $C_{19}H_{20}O_3N$, Cl requires C, 58·83; H, 7·06; N, 4·90 per cent.

Picrate. Yellow needles from ethanol, m.p. 208–209° (decomp.).

(II) 2,5-*Di*(*p-methoxybenzoyloxymethyl*)-1-*methylpyrrolidine hydrochloride.* 2,5-di(hydroxymethyl)-1-methylpyrrolidine (II; R = H) (1·45 g.) was dissolved in acetone (20 ml.) and anisoyl chloride (6·8 g.) added. The solution was cooled, made strongly alkaline with 20 per cent NaOH solution (30 ml.) and shaken at intervals for 2 hours. After dilution with water the solution was extracted with ether, the ether dried (Na_2SO_4), the solvent removed, and the residue taken up in dry ether. Treatment with dry hydrogen chloride gave a colourless oil which solidified on scratching. Recrystallisation from isopropanol gave fine white needles (2·7 g.) of m.p. 193°. Found: C, 60·5; H, 6·1; N, 3·3. $C_{23}H_{28}O_6NCl$ requires C, 61·40; H, 6·24; N, 3·12 per cent.

Picrate. Yellow needles from ethanol, m.p. 140–141° (decomp.).

(III) 2,5-*Di*(2-*p-methoxybenzoyloxypropyl*)-1-*methyl-pyrrolidine hydro*)-*chloride.* This was prepared in a similar manner to the previous compound from 2,5-di(2-hydroxypropyl)-1-methylpyrrolidine (III; R = H) (1·57 g.) and anisoyl chloride (5·4 g.), as white needles from isopropanol, m.p. 140–142°. Yield, 1·8 g. Found: C, 64·6; H, 7·1; N, 2·7. $C_{27}H_{36}O_6$ requires C, 64·06; H, 7·17;
N, 2·77 per cent.

Picrate. Yellow needles from ethanol, m.p. 220° (decomp.).

TABLE I

EFFECTS ON MICE

Ester HCl	Dose in mg. by intraperitoneal route	Number of animals dead	Other effects
(I)	20	4/4	Convulsions, death in 2 to 3 min.
	10	0/4	Slight tremors
	4	0/4	Sedation
(II)	10	0/4	—
(III)	10	0/4	Marked sedation
Cocaine HCl	5	4/4	Convulsions, rapid death
	2	4/4	Convulsions, rapid death
	1	2/4	Tremors
	0·5	1/4	Tremors

PHARMACOLOGY

By courtesy of Professor G. A. H. Buttle, School of Pharmacy, University of London.

Effects in Mice

The compounds were injected intraperitoneally into groups of 4 animals all weighing between 20 to 22 g. The effects of 20, 10 and 4 mg. of compound I and of 10 mg. of compounds II and III are compared in Table I with the effects produced by cocaine hydrochloride, 5, 2, 1 and 0·5 mg. i.p.

Effects in the Eye

Solutions, in water, of the ester hydrochloride, 5 mg./ml., were prepared by warming (with III there was a tendency to recrystallise on cooling). One drop of each solution was placed in the eye of a guinea pig. In no case was any anaesthetic effect noted. Compound III caused some slight irritation. A solution of III in glycerol also produced no local anaesthetic effect.

Intracutaneous Method

The substances were compared by intracutaneous injection into guinea pigs, the backs of the animals being shaved the day before the test. Positive response was counted as the failure to elicit a squeak from the animal when a pin was applied to the weal produced by the injection. Six pricks were made to each weal at 5-minute intervals after injection. The number of pricks failing to cause a squeak (out of a total of 36) was taken as the degree of local anaesthesia present. The results are shown in Table II.

TABLE II

INTRACUTANEOUS METHOD WITH GUINEA PIGS

Time min.	Number of pinpricks (out of 6) failing to elicit squeak				
	Cocaine HCl 0·5 mg./ml.		1 mg./ml. (I)	1 mg./ml. (II)	1 m.g/ml. (III)
	(i)	(ii)			
5	6	6	6	6	6
10	6	5	6	6	6
15	5	4	6	6	6
20	6	4	6	6	5
25	5	0	5	6	6
30	5	0	5	6	6

From the results all three compounds appear to have a local anaesthetic activity of at least half that of cocaine.

A further assessment of the local anaesthetic activity of compound III was made by the guinea pig weal method. Preparation of the solution in saline was desirable as distilled water was found to have a marked anaesthetic effect, however, the presence of NaCl depressed the solution of the drug so dextrose, 5 per cent, was used, which did not itself have any degree of anaesthesia. Cocaine hydrochloride was also dissolved in 5 per cent dextrose. The results are shown in Table III.

TABLE III

ASSESSMENT OF COMPOUND III BY GUINEA PIG WEAL METHOD

Time min.	Number of pinpricks (out of 6) failing to elicit squeak)											
	Compound III						Cocaine HCl					
	mg./ml.						mg./ml.					
	0·5		0·25		0·125		0·5		0·25		0·125	
	(i)	(ii)	(i)	(ii)	(i)	(ii)	(i)	(ii)	(i)	(ii)	(i)	(ii)
5	6	6	6	6	6	6	6	6	6	6	6	6
10	6	6	6	6	4	6	6	6	6	5	5	6
15	6	6	6	5	2	5	6	6	6	4	2	5
20	6	6	5	4	0	4	6	6	5	4	1	5
25	6	6	5	1	—	1	6	6	5	3	0	4
30	6	6	4	1	—	1	6	6	5	2	—	0
Total	72		55		35		72		57		40	

CONCLUSIONS

Compound III, the hydrochloride of 2,5-di(2-*p*-methoxybenzoyloxypropyl)-1-methylpyrrolidine has local anaesthetic activity almost identical with that of cocaine when injected intracutaneously into guinea pigs. It differs from cocaine in having no effect in anaesthetising the cornea.

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OBSERVATIONS ON THE MECHANISM OF ACTION OF TRANQUILLISERS—A STUDY OF THEIR EFFECT ON MONOAMINE OXIDASE, D- AND L-AMINO ACID OXIDASES AND CATALASE

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Received September 8, 1959

The effect of eight tranquillisers, widely differing chemically, on monoamine and L-amino acid oxidases of liver, D-amino acid oxidase of kidney and the catalase of liver, have been investigated. Reserpine was found to stimulate D- and L-amino acid oxidases and to inhibit monoamine oxidase. Rescinnamine was much less effective in this respect. Phenothiazine derivatives showed significant inhibitory action on the catalase activity. Benactyzine and diphenhydramine had a mild inhibitory action on monoamine oxidase while meprobamate and mephenesin did not show any effect.

THE fascinating problem of the mode of action of tranquillisers has been made more complicated by the diverse chemical nature of these agents. As the nature of biochemical lesions in mental disturbances is still obscure, the mechanism of the beneficial action of this group of drugs is not yet understood. Brodie, Shore and Pletscher[1] observed similarity between the central effects of 5-hydroxytryptamine (5-HT) and reserpine, both containing an indole unit. The central effects of reserpine may be mediated through the continuous release in the brain of 5-HT[2] which is then largely metabolised by monoamine oxidase[3]. The action of chlorpromazine has been associated with 5-HT antagonism[4], amongst other mechanisms, while the effect of meprobamate[5] in anxiety states may be due to selective block of interneuronal circuits and that of benactyzine may be related to anti-acetylcholine activity[6].

In this investigation, the possible interference of these drugs with some of the enzyme systems concerned with the metabolism of amines and amino acids has been studied. The effect of the representative members of the rauwolfia alkaloids, phenothiazine derivatives, alkyl diols and diphenylmethane derivatives on monoamine and amino acid oxidases and catalase activity, has been investigated.

EXPERIMENTAL

Method and Material

Reserpine, rescinnamine, chlorpromazine, promethazine, meprobamate, mephenesin, benactyzine and diphenhydramine were selected for the present study.

The solutions were made in distilled water (2 mg./ml.). With reserpine and rescinnamine, the solutions were prepared in a concentration of 0·25 mg./ml. in 0·1N H_2SO_4 by gentle warming and adjusting the final pH to 5·5. Once dissolved, the solutions remained stable at room temperature.

Estimation of Amine and Amino Acid Oxidases

The activities were measured in a Warburg apparatus using fresh liver and kidney tissues of the rat as enzyme preparations. The tissues were homogenised in a Waring micro blender and the final concentration adjusted to 100 mg. liver tissue/ml. in phosphate buffer, pH 7·2 for monoamine oxidase; 100 mg. kidney tissue/ml. in distilled water for D-amino acid oxidase and 300 mg. liver tissue/ml. in sodium phosphate buffer, pH 8·3 for L-amino acid oxidase. In the side bulb of the Warburg flask, 0·2 ml. of tyramine or 5-HT creatinine sulphate (0·25M) for monoamine oxidase, 0·4 ml. of DL-alanine (5 per cent), for D-amino acid oxidase and 0·2 ml. L-leucine (0·1M) for L-amino acid oxidase were kept as substrates and tipped in after setting the assembled manometers at 37°. The main space of the flask contained the enzyme preparation, buffers and graded doses of drugs. The centre well contained 0·2 ml. of 2N NaOH for absorbing CO_2. The rate of oxygen consumption was measured for 1 hour in the monoamine oxidase and D-amino acid oxidase and for 3 hours in the L-amino acid oxidase estimations.

Catalase Activity

This was estimated by the method of Euler and Josephson[7], using 0·1N H_2O_2 as substrate. Diluted rat liver homogenate in water was used as enzyme preparation. The unreacted hydrogen peroxide was titrated by the iodine thiosulphate method.

RESULTS

The effect of reserpine and rescinnamine on the enzyme systems is shown in Tables I, II and III. The figures are the mean of 8 experiments in each case.

It may be observed from Table I that reserpine and rescinnamine, 2·5 μg./ml., inhibit monoamine oxidase, while at 100 μg./ml., reserpine produces a 70 per cent inhibition which is 2·5 to 3 times more than that produced by rescinnamine in the same concentration.

TABLE I

EFFECTS OF RESERPINE AND RESCINNAMINE ON MONOAMINE OXIDASE OF RAT LIVER

Concentration μg./ml.	Reserpine		Rescinnamine	
	μl. O_2/hr.	Inhibition per cent ± S.D.	μl. O_2/hr.	Inhibition per cent ± S.D.
Control	39·00	—	51·00	—
2·5	37·83	3·0 ± 0·32	49·92	2·1 ± 0·20
5·0	33·40	14·3 ± 0·70	46·81	8·2 ± 0·70
20·0	21·76	44·2 ± 2·60	43·91	13·9 ± 0·60
80·0	15·32	60·7 ± 4·10	37·74	26·0 ± 1·70
100·0	11·66	70·1 ± 3·50	37·75	26·0 ± 1·50

Chlorpromazine and promethazine were also found to inhibit monoamine oxidase but to a lesser extent than reserpine. In a lower concentration, 4 to 8 μg./ml., these drugs did not show any appreciable action and only 45 per cent depression was observed at 160 μg./ml.

Benactyzine and diphenhydramine were required in even higher doses for depressing the enzymatic activity. Diphenhydramine, at 320 μg./ml., could elicit only a 45 per cent depression. The relative potency of diphenhydramine to benactyzine was found to be 3 : 1. Meprobamate and mephenesin did not show any inhibitory action on monoamine oxidase.

TABLE II

EFFECTS OF RESERPINE AND RESCINNAMINE ON D-AMINO ACID OXIDASE OF RAT KIDNEY

Concentration μg/ml.	Reserpine		Rescinnamine	
	μl. O_2/hr.	Stimulation per cent ± S.D.	μl. O_2/hr.	Stimulation per cent ± S.D.
Control	75·00	—	69·00	—
2·0	80·25	7·0 ± 0·80	69·00	—
4·0	87·08	16·1 ± 0·90	69·80	1·1 ± 0·16
20·0	94·73	26·3 ± 1·60	77·63	12·5 ± 0·80
80·0	120·75	61·0 ± 3·50	90·05	30·5 ± 1·70
160·0	117·90	57·2 ± 4·20	86·60	25·5 ± 1·90

From Table II, it will be observed that reserpine, 2 μg./ml., stimulated D-amino acid oxidase, this reached 60 per cent with 80 μg./ml. Rescinnamine produced only 30 per cent stimulation at the same concentration.

Chlorpromazine, promethazine, diphenhydramine, benactyzine, meprobamate and mephenesin did not show any appreciable action on D-amino acid oxidase even at 400 μg./ml.

TABLE III

EFFECTS OF RESERPINE ON L-AMINO ACID OXIDASE OF RAT LIVER

Concentration μg./ml.	Period of incubation					
	1st hour		2nd hour		3rd hour	
	μl. O_2	Stimulation per cent ± S.D.	μl. O_2	Stimulation per cent ± S.D.	μl. O_2	Stimulation per cent ± S.D.
Control	5·0	—	9·0	—	11·0	—
5·0	6·6	32·5 ± 2·0	13·5	50·0 ± 2·6	18·0	63·3 ± 4·6
10·0	9·0	80·0 ± 3·8	22·5	150·0 ± 8·4	29·0	163·6 ± 9·2
25·0	13·0	160·0 ± 9·4	28·0	211·1 ± 10·2	36·0	227·2 ± 13·6
50·0	13·5	170·0 ± 8·6	29·1	223·3 ± 9·6	34·0	209·0 ± 12·1

From analysis of Table III, it will be seen that reserpine showed a marked stimulatory action on L-amino acid oxidase. At 5 μg./ml. 32, 50 and 63 per cent stimulation was observed during the first, second and third hour of incubation respectively. The stimulation reached 200 per cent at 25 and 50 μg./ml. With rescinnamine at the higher concentration, the stimulation did not exceed 27 per cent.

Inhibition of catalase activity was observed with chlorpromazine and promethazine where 10 μg./ml. produced a 10 to 14 per cent inhibition which was increased to 42 per cent at 100 μg./ml. The other drugs did not show any effect on L-amino acid oxidase and catalase enzyme systems.

DISCUSSION

Reserpine is known to deplete noradrenaline and 5-HT contents of brain[8]. Our observations indicate that reserpine can affect different

enzymes concerned with amino acid and amine metabolism. The diminished 5-HT level may be due to the reduced rate of synthesis but there is no evidence that the amount of metabolites of 5-HT in urine is reduced by reserpine. Stimulation of L-amino acid oxidase by reserpine might be responsible for the low level of 5-HT because of a quicker oxidation of tryptophan[9]. Rescinnamine, which is pharmacologically weaker than reserpine, shows less action on these enzyme systems.

Brain 5-HT levels are not affected by chlorpromazine[10]. This might be due to the drug's lack of action on amino acid oxidase.

The phenothiazine derivatives inhibit catalase activity, an observation which seems to be significant. The hydrogen peroxide formed during the oxidation of amino acids and amines is disposed of rapidly by the catalase enzyme system and the possibility of a narcobiotic action of chlorpromazine, due to accumulation of H_2O_2, may deserve consideration.

According to Berger[10], benactyzine does not increase the excretion of 5-hydroxyindole acetic acid in urine. In our investigation also it did not have any effect on amino acid oxidase.

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THE DEVELOPMENT OF TOLERANCE TO MORPHINE IN RATS CONCURRENTLY TREATED WITH CHLORPROMAZINE*

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Received November 2, 1959

Using a modified hot plate method for measuring analgesia in rats, the analgesic dose of morphine was estimated to be about 5 mg./kg. This dose increased by 50 per cent the time the animals withstood exposure before reacting to the thermal stimulation. When chlorpromazine 1·5 mg./kg. was given simultaneously with morphine, a similar degree of analgesia was obtained with about 2 mg./kg. of the narcotic. The sensitivity decreased markedly in rats receiving daily injections of analgesic doses of morphine. Chlorpromazine did not retard the development of tolerance to morphine in these rats. Some of the toxic effects of morphine, however, were less marked in the rats treated with both drugs than those treated with morphine alone.

AFTER repeated administration of morphine to an animal or to man its analgesic effect decreases. This reduction is a manifestation of tolerance. The rate at which tolerance develops is related to the dose For example, Schmidt and Livingston[1] reported that, when dogs were treated daily with 30 to 60 mg./kg. of morphine, tolerance to the narcotic effect developed within one month, but when the dose was 2 to 10 mg./kg. tolerance developed only after the dogs had been treated for 15 to 20 weeks.

Since chlorpromazine has been found to potentiate the analgesic action of morphine[2,3] it has been used clinically with the narcotic, less of which is then necessary to obtain an adequate analgesic effect.[4,5] Tolerance to this combination of drugs might be expected to develop more slowly than to morphine alone, and the present investigation was initiated to ascertain whether chlorpromazine affected the development of tolerance to morphine in rats.

EXPERIMENTAL

Morphine sulphate (Merck) and chlorpromazine hydrochloride (Poulenc) were used. The weights of the drugs refer to their salts.

The animals used were male Wistar rats initially weighing 120 to 160 g.

Method

The rate at which tolerance to morphine developed was assessed during a period of seven weeks, when the rats were given single daily injections of suitable doses of the narcotic or of the combination of the narcotic and chlorpromazine. The principle is similar to that of Galysh and others[6].

* Presented at the Annual Meeting of the Pharmacological Society of Canada, Kingston, Ont., Canada, June 9–11, 1958.

The analgesic effect was tested using a modification of Eddy and Leimbach's hot plate method[7]. The animals were placed one at a time in a large dry metal vessel fitted tightly on a water bath at 55·5°. This temperature was found to have no deleterious effect on the paws of the rats, even when they were repeatedly exposed to this stimulus either at 10-minute intervals on the same day or once daily for several weeks. The rats were found to respond to this thermal stimulation by licking the front paws or jumping out of the hot vessel. The time required to elicit either response was designated the exposure time (ET). In a preliminary test on 100 untreated rats 63 responded by licking their front paws. The mean ET for this group was $6{\cdot}63 \pm 0{\cdot}21$ seconds. The mean ET of the 37 rats which responded by jumping out of the vessel was $6{\cdot}54 \pm 0{\cdot}28$ seconds. Thus there was no significant difference in the mean ET between these two types of response. Both were therefore used as the end point in subsequent experiments.

In testing the analgesic effect of morphine, the ET of each rat in a group of 30 was first determined. Ten minutes later morphine was given by intraperitoneal injection, and the ET determined after a further 30 minutes. The per cent increases (Y) in the ET of these rats and the three logarithmic doses (X) were used in the analysis of variance and the computation of the regression equation ($Y = a + bX$). The AD50 value is the dose of morphine estimated to increase the ET by 50 per cent. It was calculated from the regression equation by substituting 50 for Y and solving for X. The variance of the AD50 was also estimated.

For comparison the changes in the ET in some experiments were converted to quantal data; an increase of 50 per cent or more was considered a positive analgesic effect. The variance of the AD50 calculated from these quantal data was considerably larger than that obtained from the graded responses, which were therefore used throughout these experiments.

The AD50 of morphine was then determined in another 30 rats, each of which was also injected intraperitoneally with chlorpromazine (1·5 mg./kg.). This dose of chlorpromazine had no analgesic action when given alone, but it potentiated the analgesic action of morphine.

Both groups of 30 rats were then given daily injections of morphine alone (Group A) at the determined AD50 of 5·09 mg./kg., or of morphine with chlorpromazine 1·5 mg./kg. (Group B) at the determined AD50 of 1·95 mg./kg. At the end of one week, the AD50 values were again determined as described above. They were found to have been increased, and so the new AD50 figures were used in the second week of dosing. This process was repeated at the end of the third and subsequent weeks for a total of seven weeks, the dose of chlorpromazine being kept constant throughout.

Since the experimental period for the rats was relatively long, it was considered desirable to ascertain the influence of age on the analgesic effect of morphine. The AD50 of morphine was therefore determined in two further groups of 30 animals at the beginning and at the end of the seven-week period, throughout which no drug was administered.

These rats were weighed weekly, and after determining the final AD50 values the animals were killed and some organs weighed.

Results

The initial AD50 of morphine in the first group (A) of rats which received this analgesic alone was 5·09 mg./kg., whereas in the second group (B) which in addition had received chlorpromazine (1·5 mg./kg.) the AD50 was 1·95 mg./kg. The AD50 values obtained with these two groups during the entire experimental period are listed in Table I, from which it is evident that there is a considerable increase in both groups.

TABLE I

THE AD50 VALUES OF MORPHINE (IN MG./KG.) DETERMINED WEEKLY IN RATS RECEIVING DAILY INJECTIONS OF (A) MORPHINE OR (B) MORPHINE AND CHLORPROMAZINE (1·5 MG./KG.) DURING A PERIOD OF 7 WEEKS

	Weeks							
Group	0	1	2	3	4	5	6	7
A	5·09	6·62	10·26	9·27	13·06	19·10	20·61	24·72
B	1·95	2·96	2·74	2·47	9·27	8·10	9·27	9·44

To estimate the rate of tolerance development in these two groups with more precision, regression lines were computed from the logarithmic AD50 values and the time in weeks at which they were determined. The slope (b) of the regression line and the variance (s_b) are respectively for Group A, 0·093 and 0·0016 and for Group B, 0·094 and 0·0070. Tolerance to morphine developed at a similar rate in these groups. In calculating the regression line the AD50 values were weighted with the reciprocals of their variance. This procedure theoretically improves the accuracy of the estimated slope. It also reduces the variance of this estimate as the s_b was 0·0016 when the AD50 values were weighted, whereas from the unweighted values it was 0·0129. The AD50 values determined in the third and fourth weeks in Group B were inaccurate as their variances were large. However, in spite of these marked deviations the regression of AD50 plotted against time was highly significant.

TABLE II

THE INITIAL AND FINAL AD50 VALUES ± S.E. (MG./KG.) OF MORPHINE IN RATS RECEIVING DAILY INJECTIONS OF (A) MORPHINE, (B) MORPHINE AND CHLORPROMAZINE, AND IN RATS GIVEN (C) MORPHINE AND (D) MORPHINE AND CHLORPROMAZINE AT THE BEGINNING AND END OF THE EXPERIMENTAL PERIOD ONLY

Group	Initial	Final
A	5·09 ± 0·92	24·72 ± 2·31
B	1·95 ± 0·19	9·44 ± 0·94
C	4·70 ± 1·12	6·30 ± 0·51
D	2·16 ± 0·22	2·97 ± 0·32

In Table II are listed the initial and final AD50 values of morphine in the two groups (C and D) of rats which received no daily treatment.

There was a slight but not significant increase in the AD50 values at the end of this period. This is in sharp contrast to the marked increases observed in the Groups A and B, the initial and final values of which are also listed in this Table for comparison.

Although the combination of chlorpromazine and morphine was not superior to morphine alone as far as development of tolerance is concerned, it appeared to be less toxic; for example, the growth was slightly but significantly more retarded in the rats treated with morphine (Group A) than those treated with the combination (Group B). This is shown in Table III. Alopecia areata was observed in all the animals during the

TABLE III

THE BODY WEIGHT IN G. (MEAN ± S.E.) OF THE FOUR GROUPS OF RATS (GROUP DESIGNATIONS AS IN TABLE II)

Group	Initial	Final
A	139 ± 2·9	231 ± 4·8
B	138 ± 4·1	249 ± 4·7
C	138 ± 1·6	258 ± 7·9
D	138 ± 2·9	268 ± 7·2

latter half of the experimental period, but the rats in Group A were affected to a greater extent than those in Group B. From the weights of some of the organs (see Table IV), it may be noted that the increase in

TABLE IV

THE WEIGHT (MEAN ± S.E.) OF ADRENALS, THYMUS AND THYROID IN MG./G. BODY WEIGHT OF RATS TREATED WITH (A) MORPHINE, (B) MORPHINE AND CHLORPROMAZINE AND (C) NEITHER

Group	Adrenals	Thymus	Thyroid
A	0·137 ± 0·003	1·17 ± 0·07	0·051 ± 0·001
B	0·133 ± 0·002	1·34 ± 0·06	0·046 ± 0·002
C	0·126 ± 0·002	1·70 ± 0·06	0·053 ± 0·002

the weight of the adrenals and the decrease in the weight of the thymus were more marked in Group A than in Group B. However, the weight of the thyroid was reduced in Group B but not in Group A.

DISCUSSION

A potentiation of the analgesic action of morphine by chlorpromazine has been observed in rats. The AD50 of morphine was reduced by three-fifths when a relatively small dose of chlorpromazine (1·5 mg./kg.) was given simultaneously. The magnitude of potentiation was similar to that reported by others; for example, Wirth[3] found that the analgesic dose of morphine was reduced by a half or two-thirds in the presence of chlorpromazine. According to the data presented by Courvoisier and

others[2], the AD50 of morphine was about 25 mg./kg. when it was given alone. However, when the rats had recieved chlorpromazine (5 mg./kg.), the AD50 of morphine was about 6 mg./kg. The greater potentiation was evidently related to the larger dose of chlorpromazine.

Although the rats in Groups C and D were less sensitive to morphine at the end of the 7 weeks than at the beginning, the increase in the AD50 over the period was very small (Table II). The increase in the AD50 values in the rats in Groups A and B was therefore a result of tolerance to the drug administered.

The rate at which tolerance to morphine developed in the rats was slightly slower than that observed by Galysh and colleagues[6], and the coefficient of variance was smaller. This is one of the advantages of using weighted AD50 values in calculating the regression line.

The present results show that tolerance to morphine developed at the same rate in rats treated with morphine and chlorpromazine (in spite of much smaller doses of the narcotic), as in those treated with morphine alone. The mechanism underlying this unexpected finding is not evident. It is not likely to be a result of a tolerance to chlorpromazine, since we found that the potentiating effect of this drug was identical in two other groups of rats: one group had received chlorpromazine daily for 7 weeks and the other had received saline.

There was a significant reduction in the increase in body weight in rats treated with morphine. Loss of hair, an increase in the weight of adrenals and a decrease in the weight of the thymus were also noted. These effects of morphine have been reported by others[8–10]. However, it is of interest to note that these changes were less marked in rats treated with morphine and chlorpromazine. Since the changes may be considered as signs of toxicity, the combination of both drugs was less toxic than morphine alone.

Another difference between the rats treated with morphine and those treated with the combination was the effects on spontaneous motor activity. The injection of morphine normally induced sedation. After 3 to 4 weeks' daily treatment this narcotic elicited excitation instead of sedation. This stimulation of the central nervous system after morphine injection in morphine-tolerant rats has been observed by others[9,11]. Excitation, however, was not seen in the rats treated with morphine and chlorpromazine.

The weight of the thyroid was less in rats treated with morphine and chlorpromazine than in those treated with morphine alone. This effect was therefore likely to be related to chlorpromazine, which has been reported to impede the uptake of ^{131}I by the thyroid in guinea pigs[12] and to prevent the histological changes in the thyroid of rats induced by exposure to cold[13].

Acknowledgement. The authors wish to express their gratitude to Dr. C. I. Bliss for his advice on some of the statistical problems. The sample of chlorpromazine was generously supplied by Poulenc Limited, Montreal.

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ON THE INTERFACIAL TENSION BETWEEN GELATIN AND SODIUM ALGINATE SOLUTIONS AND BENZENE

PART I. EFFECT OF TIME AND CONCENTRATION ON INTERFACIAL TENSION

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Received December 10, 1959

A cell is described for determining both surface and interfacial tensions by the sessile drop method. The interfacial tension between gelatin solution or sodium alginate solution and benzene has been determined. Both substances lowered the interfacial tension, the gelatin attained equilibrium quickly. Both tension-concentration curves were similar in shape. It is suggested that a condensed multilayer having the physical properties required to stabilise emulsions, is built up immediately below the surface.

SHOTTON[1] showed that acacia solutions are slow to attain equilibrium at the interface with benzene and that a static method for the determination of interfacial tension is necessary.

The slow change in surface tension or the phenomenon of surface ageing could be due to either a slow diffusion process between the bulk and the interface which continues until the equilibrium is attained[2], or a re-orientation of solute molecules at the interface. A linear molecule may re-orientate by the unfolding of the chain[3].

With protein solutions adsorption takes place at the surface and they may become denatured to give an insoluble film. Johlin[4] considered it would appear irrational to measure the surface tensions of such solutions.

EXPERIMENTAL AND RESULTS

Materials

Water. Distilled water containing a very small amount of potassium permanganate was redistilled in an all glass still.

Benzene. Analar benzene was recrystallised.

Gelatin. One sample of a pharmaceutical grade gelatin (B.P.C.) supplied by Young's Gelatin Co. Ltd. was used throughout this work. *Purification.* A de-ashed gelatin was prepared by using a method described by Holmes[5] and Janus, Kenchington and Ward[6] using a mixed bed ion exchange resin. The pH of a 1 per cent solution of the deionised gelatin was 6·4 at room temperature (20° approx.). *Density of solution.* The density of the gelatin solutions was proportional to the concentration. To calculate the interfacial tension of gelatin solutions the density was obtained from the graph.

Sodium alginate. A food grade sodium alginate supplied by Alginate Industries Ltd., under the trade name "Manucol SS/LB" of low viscosity

was used. It contained 0·4 per cent of calcium as impurity. *Purification.* The method of Black and Woodward[7] was used. Alginic acid was precipitated from a solution of sodium alginate with sulphuric acid and washed. This acid was then dissolved in sufficient sodium hydroxide solution to give a pH of approximately 6·7 which was the neutral point shown on the titration curve. The process was repeated and finally sodium alginate precipitated with ethanol and dried under vacuum. The equivalent weights of two batches of the purified sample were determined by electrometric titration and found to be 184 and 175.

Measurement of Interfacial Tension

The apparatus was similar to that used by Shotton[1]. Interfacial tensions were calculated by Porters'[8] method.

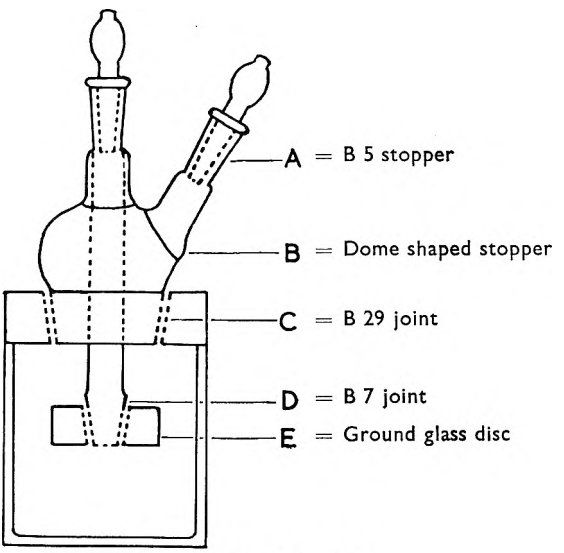

FIG. 1. The optical cell

The cell, Figure 1, was a cube of 4 cm. edge made from optically flat glass to which was cemented a glass block bored to receive a B.29 cone joint. The dome shaped stopper which fitted into the glass block had two openings, and to the central one was fixed a glass tube on which was fitted an accurately ground glass disc, 27 mm. in diameter, cut from a mirror blank with a radius of curvature of 1 metre.

The diameter of the disc was accurately measured and this was used to determine the magnification of the photographs.

Flasks containing water and benzene were mutually saturated and left at 35° in the cabinet overnight with the glass apparatus to attain temperature equilibrium. The sessile drop was formed by withdrawing a known volume of benzene from the flask and transferring it slowly through the opening in the dome of the cell, the cell having been previously assembled and filled with water. A photograph was taken after approximately 3

minutes and thereafter at intervals of 1, 2, 4, 8, 24, 48, and 72 hours. The diameter at the equator and the height of the vertex above the equator was measured.

The change of interfacial tension of benzene:water with time as determined by each of the two methods of drop formation is given in Table I. Harkins and Cheng[9] obtained the value of 34·09 dynes/cm.

TABLE I

THE CHANGE IN INTERFACIAL TENSION WITH TIME OF BENZENE:WATER AT 35° C. (DYNES/CM.)

Method	Time after formation							
	3 min.	1 hr.	2 hr.	4 hr.	8 hr.	24 hr.	48 hr.	72 hr.
Drop of benzene in water	34·2	33·9	33·6	33·6	33·60	33·6	33·6	33·6
Drop of water in benzene	34·2	34·0	33·8	33·7	33·7	33·6	33·6	33·6

Interfacial Tension of Solutions

Interfacial tension of gelatin solution against benzene. The gelatin solutions were the same as those prepared by the method of Davis, Salisbury and Harvey[10]. Two solutions of different concentrations were used for each experiment and saturated with benzene, care being taken to prevent emulsification. A drop of benzene was formed in the solution and photographed at the intervals indicated above. The results are summarised in Figure 2.

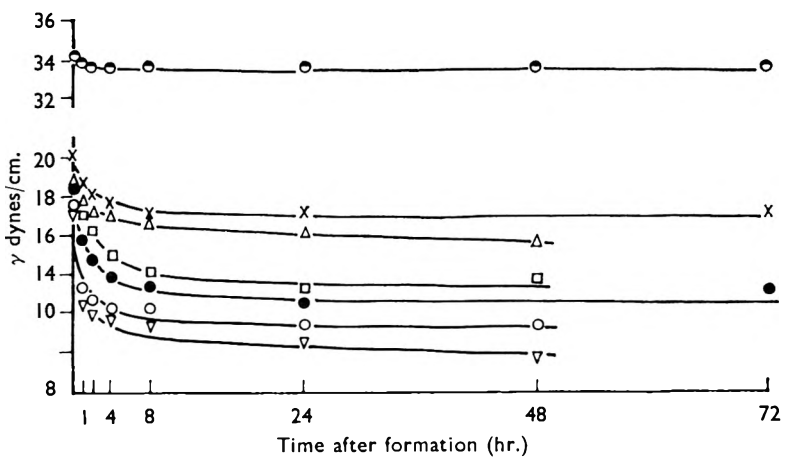

FIG. 2. The effect of time on the interfacial tension between gelatin solutions and benzene (temp. = 35°C). Per cent, w/v.

◓ water × 0·05 △ 0·17 □ 0·35 ● 0·755 ○ 0·988 ▽ 3·825

Effect of pH on interfacial tension. A 1 per cent gelatin solution was prepared containing 0·3 per cent analar sodium chloride to prevent

flocculation. The interfacial tension of this solution against benzene was very little different from that of a solution of gelatin alone. The amount of sodium hydroxide or hydrochloric acid to give the required

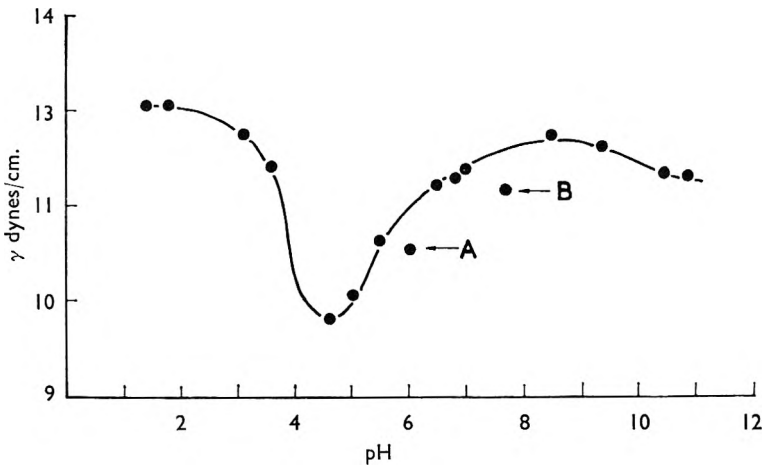

FIG. 3. The effect of pH on the interfacial tension between 1 per cent gelatin solutions and benzene (temp. = 35°C). Values taken after 24 hours.
(A and B see text, p. 114).

pH was obtained from the titration curve of a 1 per cent gelatin solution. The effect of pH on the interfacial tension of 1 per cent gelatin solutions measured after 24 hours, is shown in Figure 3.

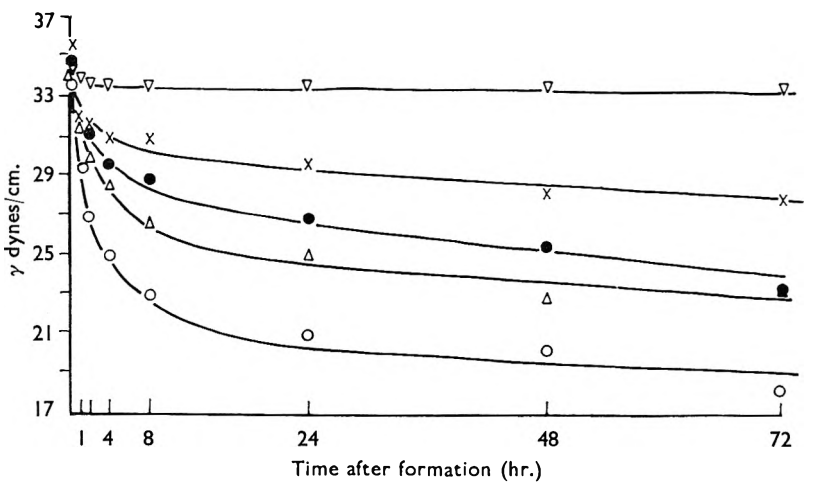

FIG. 4. The effect of time on interfacial tension between sodium alginate sslutions and benzene (temp. = 35°C.) Per cent, w/v.

▽ water × 0·1 drop = solution △ 0·1 drop = benzene
● 0·25 drop = solution ○ 0·25 drop = benzene

Interfacial tension of sodium alginate solutions. The required amount of purified sodium alginate was accurately weighed allowing for the moisture content of the purified material. Solutions were prepared by dissolving the materials in distilled water, heated to 75°. The interfacial tension of each solution was determined using both methods of forming the drop, i.e., a drop of benzene in solution or a drop of solution in benzene. The two systems were photographed side by side and the results summarised in Figure 4.

Discussion

The photographs of the drop were taken without altering the focus or the distance of the cell, but the value obtained for the magnification of the disc varied within $\pm$ 1 per cent. Hence an average for the magnification factor was taken to calculate the actual dimensions of the drop. According to Porter[10] the correction factor (Δ) is known with the greatest accuracy in the region of $h^2/r^2 = 0{\cdot}25$. The volume of the drop was adjusted so that h^2/r^2 reached a value of 0·20–0·30 after 24 to 72 hours. Taking into account all sources of variation, it is considered that the results obtained using this method have an experimental error of about 3 per cent initially, reducing to about 2 per cent for the later readings.

Change of Interfacial Tension with Time

The greatest rate of fall in the interfacial tension of gelatin and sodium alginate solutions against benzene took place in the first hour and was then less rapid up to 8 hours, after which period the change was very slow.

On forming a drop of benzene in a solution a fresh surface is created and diffusion into the interface takes place causing a rapid drop in interfacial tension. The interface becomes more concentrated with time and the subsequent slow change in interfacial tension may be due to a re-orientation of the molecules in the surface layer.

According to Cheesman and Davis[3] the protein molecule is normally rigid due to the forces of cohesion and the van der Waals force of attraction between the non-polar groups. At an oil-water interface the cohesive energy is reduced by the penetration of the oil between the cohesive side chain, and hence the molecule tends to unfold.

In the case of sodium alginate the molecule is probably held rigid due to the repulsion between the ionised carboxyl groups and may undergo re-orientation on reaching the interface by directing the polar groups towards the water.

Reynolds[12] observed that the interfacial tension of 0·5 per cent gelatin solution at 18° fell from 21·0 dyne/cm. after fifteen minutes to 19·9 dyne/cm. after 24 hours, whereas a 0·35 per cent solution at 35° fell from 18·5 dyne/cm. at 3 minutes to 13·9 dyne/cm. in 24 hours. Johlin[4,13,14] determined the surface tension of gelatin solution using different methods and he emphasised the need for a static method.

Matthews[2] attributed the flattening of the curves to either slow diffusion to the interface or the formation of a condensed monolayer, however the times he allowed to attain equilibrium were too short. Shotton using

acacia suggested that phenomena was due to "multilayers" being built up at the interface. Supporting evidence has been published recently[15].

Effect of Concentration on Interfacial Tension

The interfacial tension was lowered by an increase in concentration of the aqueous solution. The solutions of gelatin and sodium alginate when kept in contact with benzene became opalescent and considerable difficulty was experienced in photographing the drops and measuring them accurately. Hence the system of forming the drop was reversed, the drop being formed with the opalescent solution in benzene. The results obtained for this system were consistently and appreciably higher than those obtained by using a drop of benzene in the solution, the discussion on this apparent anomaly is given in Part II.

It is difficult to compare the above results with those of previous work as different methods and samples have been used. Holmes and Child[11] determined the interfacial tension by Donnan pipette and found an initial decrease in interfacial tension of gelatin solutions from 0 to 0·3 per cent, and remained constant on further increase in concentration. This could have been due to the non-attainment of surface equilibrium.

Matthews[2] stated that the type of $\Delta\gamma$/concentration curve depended upon the nature of the interfacial film which in turn depended upon the shape of the solute molecule that constitutes the film. He suggested that pectin would form a film of the intermediate type showing a tendency to form a condensed film but the lateral forces of attraction between the molecules would not be sufficient to bring this about. Alginic acid bears a structural resemblance to pectin. Pectins are regarded as poly-galacturonic acid in the form of the methyl esters and alginic acids are also poly-galacturonic derivatives. It is to be expected that these two substances will behave similarly at the interface.

The Effect of pH on Interfacial Tension of Benzene:Gelatin Solutions

The interfacial tension had the lowest value at pH 4·55, indicating that the isoelectric point of the gelatin used was in this region. At the isoelectric point the molecule is neutral and may readily form aggregates. Under these conditions adsorption at the interface is most probable and surface interfacial tension is at a minimum.

On either side of the isoelectric point the value for interfacial tension increased steadily on the addition of acid or alkali. On the acid side a maximum was reached at pH 2·5 and did not increase on further addition of acid, whereas with alkali it reached a maximum at pH 8·6 and decreased on further addition of alkali. This was probably due to the formation of sodium gelatinate which dissociated to form the electro-negative protein ions which were less readily adsorbed at the interface than undissociated molecules. The lowering of the interfacial tension above pH 8·6 could be due to suppression of the dissociation of sodium gelatinate by the presence of excess of sodium ion or, possibly, to the hydrolysis of gelatin. The results given at A and B in Figure 3 were not repeatable and it is possible that some contamination had occurred.

The Stabilisation of Emulsions with Hydrophilic Colloids

Emulsions were prepared using gelatin, sodium alginate and acacia solutions and 50 per cent of benzene. It was found that gelatin emulsions could be made with 0·3 and 0·4 per cent solutions by hand shaking, whereas using acacia or sodium alginate solutions, emulsions could be prepared only with a 10 per cent solution and a mechanical stirrer.

Sodium alginate emulsions were unstable, when compared with those made with acacia, the emulsification being difficult to achieve even when using a high concentration of alginate. The difference in the ease of emulsification can be probably attributed to the differences in the surface activity and the nature of interfacial film. It is suggested that emulsions formed by hydrophilic colloids are stabilised by an interfacial film having certain physical properties. Condensed multilayers which are charged by ionisation have the required properties to favour the formation of o/w emulsions.

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ON THE INTERFACIAL TENSION BETWEEN GELATIN AND SODIUM ALGINATE SOLUTIONS AND BENZENE

PART II. THE EFFECT OF THE RELATIVE POSITIONS OF THE TWO PHASES ON THE INTERFACIAL TENSION

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Received December 10, 1959

Using the systems of benzene and either gelatin solution or sodium alginate described, the results of the sessile drop method depended upon whether the drop was formed from benzene or from the solution. It is suggested that the difference in the results may be accounted for by some form of structure in the solution.

SOLUTIONS of gelatin and sodium alginate above 1 per cent when in contact with benzene became opalescent and considerable difficulty was encountered in photographing the drop of benzene through the solution. The opalescence developed with time and more readily in the stronger solutions. The solutions kept in stoppered tubes with benzene became opalescent after 24 hours, but cleared if the benzene layer was removed. On visual examination of a drop of solution from the bulk or from the interface, particles of irregular size and opaque appearance resembling solid were observed and this suggested the existence of a gel like interfacial film. It is improbable that spontaneous emulsification could have taken place as the interfacial tension values were comparatively high.

Experiments were repeated using a sessile drop of the solution in benzene to enable the drop to be photographed through the clear benzene. Although the experiments were made using the same solutions and with the two systems side by side the results were consistently and appreciably different; those obtained using a sessile drop of solution in benzene being much higher than when a drop of benzene was used as shown in Figures 1 and 2.

DISCUSSION

In Figures 1 and 2 the curves are the average of the replicate experiments. The gelatin solutions showed only a little change in interfacial tension with time after 24 hours and the values at 24 hours were taken as representing equilibrium figure; whereas for sodium alginate 72 hours was found to be suitable. The variation of the results obtained using the two systems is outside the experimental limits.

The two methods are based on the same principle in that the shape of the drop is dependent on the interfacial tension according to Bashforth and Adam's treatment of revolution and thus the ratio of h^2/r^2 is dependent on the interfacial tension. If the solutions possess some form of structure a certain amount of force would be necessary to break down this structure and induce the liquid to flow.

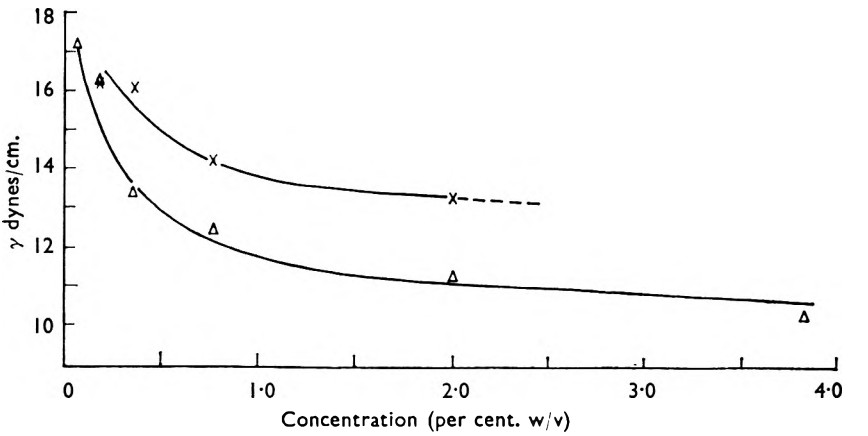

FIG. 1. The effect of the relative positions of the two phases on interfacial tension (temp. = 35°). Values after 24 hours. Gelatin solutions.

× Drop of solution in benzene △ Drop of benzene in solution

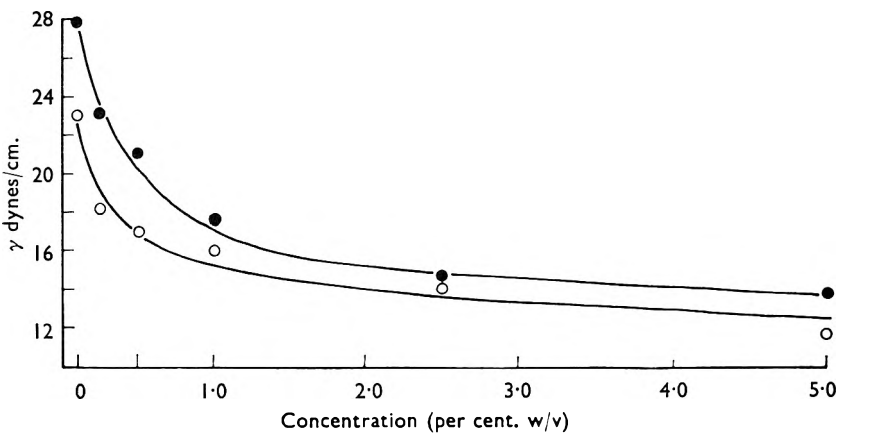

FIG. 2. The effect of the relative positions of the two phases on interfacial tension (temp. = 35°). Values after 72 hours. Sodium alginate solutions.

● Drop of solution in benzene ○ Drop of benzene in solution

The total force at the interface should be the same in both instances since the interfacial tension and the surface area of the drop, whether of benzene or solution, nominally are the same.

Since the drop is altering continuously towards a shape corresponding to the true interfacial tension, a strain will be induced in the structure in the solution. For a given amount of movement of the interface, as shown by the change in 'h', the strain produced in the drop of solution will be very much greater than that induced in the much larger bulk of solution surrounding the drop of benzene. The drop of benzene will therefore flatten to a greater extent than the drop of solution in order to produce the

same amount of strain in the structure so giving a lower result for the interfacial tension which should be nearer to the true value.

If it is possible to assume that the sodium alginate and gelatin solutions possess some form of rigidity or structure within them, then the anomaly of the results could be explained by the above hypothesis.

The strain may arise by the deformation of the structure present in the bulk solution or in the region of the interface. However, it would be expected that the interface would be similar in structure and magnitude in both instances so that it seems reasonable to suppose that the difference in the results is mainly due to a very weak gel-like structure arising in the undisturbed liquid through association between the polar groups in the molecule.

Sheppard[1] and Bogue[2] have shown that gelatin solutions exhibit both plastic and viscous flow. Using concentrations from 1 to 8 per cent of gelatin, Bogue observed that below 34° gelatin solution showed a plastic flow and had a yield value, whereas above 34° only viscous flow was exhibited. In cases where plastic flow was noticed the solution had a permanent and fixed resistance to deformation which would occur only when a minimum pressure was applied. The transition from gel to sol form exists over a range of temperature and depends upon the concentration of the solution. The experiments were made at 35° where a residual structure may be expected in gelatin solutions. If this structure increased with the age of the solution, then it could account for the results obtained.

It was observed by Belton[3] while determining the surface tension of gelatin solution using a bubble pressure method that the pressure required to blow the first bubble was greatest and fell successively until 5 or 6 bubbles were blown. He suggested that the unstirred solution possessed a structure which was broken down in the neighbourhood of the jet, until the surface tension of the stirred solution was observed.

In the case of solutions which possess some form of structure a similar behaviour may be expected. Under these conditions it is difficult to ascertain whether the surface has attained an equilibrium corresponding to the actual interfacial tension or not, and the values obtained should be regarded as relative ones.

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A STUDY OF BACTERIOLOGICAL MEDIA
THE EXAMINATION OF PROTEOSE-PEPTONE*

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Received December 3, 1959

The free and total amino acid content of three batches of Protecse-Peptone is presented. The presence of hydroxyproline and the high content of glycine and alanine suggest that a part of the protein used in the manufacture belongs to collagen. The three batches have shown similar strepogenin activity.

IN previous papers "Oxoid" peptone[1,2], Bacto-Casitone[3,4] and Casamin E[5] were examined for their constituent amino acids and peptides. This communication describes the quantitative estimation of the free and total amino acids in three batches of Proteose-Peptone (B120)[6], which is particularly adapted for use for the production of various bacterial toxins[6-8].

EXPERIMENTAL AND RESULTS

Three batches of Proteose-Peptone designated A, B and C were examined for the free and total amino acids and for their strepogenin activity using methods previously described[3,5]. The results are given in Tables I and II.

TABLE I

THE QUANTITATIVE ESTIMATION OF THE FREE AND TOTAL AMINO ACIDS OF PROTEOSE-PEPTONE

Amino acid	Free amino acid g./100 g.			Total amino acids g./100 g.		
	A	B	C	A	B	C
Gly	0·36	0·29	0·32	10·6	11·1	11·1
Ala	0·64	0·51	0·57	6·4	6·5	6·1
Val	0·48	0·38	0·48	4·3	4·1	4·2
Leu's	1·99	1·67	1·93	9·4	8·8	8·8
Ser	0·63	0·50	0·56	3·4	3·4	3·4
Thr	0·59	0·44	0·53	4·3	4·3	4·2
Tyr	0·64	0·47	0·7	1·9	1·8	1·9
Phe	1·41	1·22	1·46	3·6	3·2	3·4
Met	0·56	0·77	0·93	1·6	1·6	1·5
Arg	0·34	0·33	0·43	6·7	6·8	6·7
His	0·30	0·17	0·14	2·0	2·0	2·0
Cys	—	—	—	0·45	0·5	0·54
Lys	0·36	0·30	0·33	5·3	5·0	5·4
Pro	0·37	0·25	0·29	5·4	5·4	5·3
Asp, Glu	2·34	2·05	2·15	18·2	18·0	18·5
Try	0·23	0·15	0·23	—	—	—
Hypro	—	—	—	3·9	4·3	3·8
Total	11·24	9·50	11·06			

* This is communication No. 1544 from the Sterling Chemistry Laboratory, Yale University, New Haven, Conn., U.S.A.

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TABLE II

THE EFFECT OF PROTEOSE-PEPTONE ON THE GROWTH OF *L. CASEI*

Time hours	Control	Batch A mg.			Batch B mg.			Batch C mg.			Wilson Liver L. mg.		
		0·1	0·5	1·0	0·1	0·5	1·0	0·1	0·5	1·0	0·1	0·5	1·0
					A– Medium of Steele and others*								
24	0	16	23	63	10	22	63	12	23	52	10	15	20
48	5	83	182	232	68	167	239	82	191	232	33	78	143
72	204	129	238	269	122	221	279	132	241	266	124	132	205
					Acid Production**								
48	9	0·95	3·05	4·75	0·95	3·25	4·70	1·05	3·55	4·85	0·6	1·35	2·8
72	4·15	3·35	7·15	9·35	3·05	6·40	8·75	3·30	7·55	8·25	1·95	3·3	5·35
					B– Medium of Kodicek and Mistry*								
24	54	90	169	210	81	164	191	102	159	210	63	105	143
48	272	278	303	325	280	302	320	280	297	318	266	285	295

* Scale reading of Klett-Summerson colorimeter.
** ml. of 0·077 N sodium hydroxide.

The chromatograms of DNP-amino acids and peptides were similar to those obtained with Bacto-Casitone[3] and Casamin E[5] with the exception of a spot that appeared on standing of the ether extract and seemed to be an artifact. The peptide spots were found to consist of a mixture of peptides as evidenced by *N*-terminal amino acid analyses.

DISCUSSION

Proteose-Peptone is recommended as an ingredient of bacteriological media for the production of toxins. It has been examined to see whether it has any characteristic features to distinguish it from the bacteriological media previously examined[1–5]. From Table I it is seen that there exists some variation in the individual free amino acids among the three batches examined. The total free amino acids varies from 9·5 to 11·2 per cent, this is lower than that for Bacto-Casitone 15·9 to 17·5 per cent and Casamin E 24·7 to 41·8 per cent. Proteose-peptone thus contains a slightly higher per cent of peptides than the other media reported. The total amino acids were similar in the three batches showing that the proteins used for their manufacture are identical. The high glycine, alanine and arginine and the presence of hydroxyproline may reflect the features of the protein used for the preparation of Proteose-Peptone. As the collagen exhibits the characteristic feature of containing hydroxyproline and having a high proportion of glycine[9], it seems likely that a part of the protein used in the manufacture belongs to this group. It is seen also from Table I that the pattern in which the free amino acids exists in Proteose-Peptone is different from Bacto-Casitone[3] and Casamin E[5]. In the latter two, pancreatic digestion was used for their preparation. Bovine pancreatic juice[10] is composed of trypsin, chymotrypsin and two carboxypeptidases. From the specificity of trypsin and chymotrypsin it is anticipated that peptides containing arginine and lysine in the *C*-terminal position are liberated by the action of the former while peptides with aromatic amino acids in the *C*-terminal position are produced by the action of the latter. The resulting peptides are further acted upon by carboxypeptidases resulting in the liberation of the basic and aromatic amino acids. As a result of the action of the pancreatic enzymes there is a high proportion of the free

aromatic and basic amino acids and a low content of proline. This was found to be the case in Bacto-Casitone and Casamin E. In the case of Proteose-Peptone the liberation of arginine and lysine is about one-tenth that in Bacto-Casitone and Casamin E. This suggests that a different procedure of enzymatic digestion has been used for its preparation.

The spots examined were found to be a mixture of peptides and there appears to be some variation in the peptide constituents in corresponding spots.

The stimulatory effect on the growth and lactic acid production of *L. casei* was similar in magnitude in the three batches as seen in Table II. The pattern of stimulation in the two basal media was similar to results obtained previously with Bacto-Casitone and Casamin E.

There was no significant difference between the strepogenin activity of Proteose-Peptone and that of Bacto-Casitone and Casamin E. The strepogenin effect can be evoked by peptides which differ considerably in their sequence structures as well as their amino acid content[1]. This may explain the similarity of the strepogenin effect of Bacto-Casitone, Casamin E and Proteose-Peptone which differed markedly in the amino acid content of their peptides. Various peptones[7,8] including a casein hydrolysate, were examined as constituents of a medium for toxin production of *Corynebacterium diphtheriae* and it was found that Proteose-Peptone was the most satisfactory. It seems most likely that the toxin factor is more specific than strepogenin, and that the peptides responsible for the toxin production may differ from those with strepogenin effect. Peptides with strepogenin activity were found not to enhance the multiplication of cultured appendix cells[12].

Acknowledgements. I wish to thank Professor Harold G. Cassidy for his encouragement and advice throughout the work. This work was supported by research grants RG 3207 (C3, C3S1, C4, C5) from the Division of Research Grants, National Institutes of Health, Public Health Service.

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A NOTE ON THE COLORIMETRIC ASSAY OF CORTISONE AND HYDROCORTISONE

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Received November 19, 1959

A colorimetric method of estimation of cortisone and hydrocortisone in pharmaceutical preparations using 2,6-di-t-butyl-*p*-cresol has been simplified in its details of procedure and compared with the tetrazolium method. Preparations of cortisone and hydrocortisone containing penicillin, streptomycin and neomycin have also been examined.

Schulz and Neuss[1] have proposed a method for the estimation of corticosteroids which is based on the interaction of cyclic ketones with phenols[2] to form coloured complexes. In this method the two steroids, cortisone and hydrocortisone react with an alkaline solution of 2,6-di-t-butyl-*p*-cresol to form yellow-brown and blue colours respectively. A comparison of the absorbance of the sample is made with the colour obtained by an approximate concentration of the standard. We have endeavoured to simplify and standardise the procedure so that a standard curve for definite concentrations can be obtained. The modified method has been compared with the tetrazolium reagent method[3] of the United States Pharmacopeia[4].

The pharmaceutical preparations studied were cortisone and hydrocortisone skin and eye ointments, lotions, injections and tablets and also combined preparations of the steroids with antibiotics.

Experimental

Apparatus. Unicam Spectrophotometer, SP600; reflux condenser, ground-glass joints; round bottomed flasks 100 ml. with ground glass joints; steam bath, capable of being maintained at 100°.

Reagents. Ethanol 90 per cent w/v; DTBPC reagent: 2,6-di-t-butyl-*p*-cresol 0·5 per cent, solution in 90 per cent ethanol; N sodium hydroxide freshly prepared and standardized; strong solution of the standard corticosteroid in ethanol (0·5 mg./ml.).

Procedure

Standard curve. Dilute 0·25, 0·5, 0·75, 1·0, 1·25 and 1·5 ml. of the strong standard solution of the steroid to 25 ml. with ethanol separately in volumetric flasks. Pipette 5·0 ml. of each solution into a 100 ml. round bottomed flask, add 5·0 ml. of the DTBPC reagent followed by 10 ml. of the sodium hydroxide solution. Reflux for 30 minutes in a steam bath maintained at 100°. (A boiling water bath with a stirrer to maintain the temperature at 100° can also be used). Without removing the condenser, immerse the flask for two minutes in cold water at 10 to 12°, rotating it gently. Remove the flask from the water and allow it to stand at room

temperature for 10 minutes. Filter rapidly through a filter paper (Whatman No. 41) avoiding loss of ethanol due to evaporation. Determine the absorbance of the filtrate at 625 mμ for hydrocortisone and 471 mμ for cortisone, in a 1 cm. cell with distilled water as blank. The time interval between the reading and removing the flask from the steam bath should be between 20 and 22 minutes. Carry out a blank determination on 5·0 ml. of ethanol and the reagents in the same manner and subtract the blank from the above readings. The curve is plotted with concentration and absorbance as abscissa and ordinate respectively. Standard curves for cortisone and hydrocortisone are shown in Figures 1 and 2.

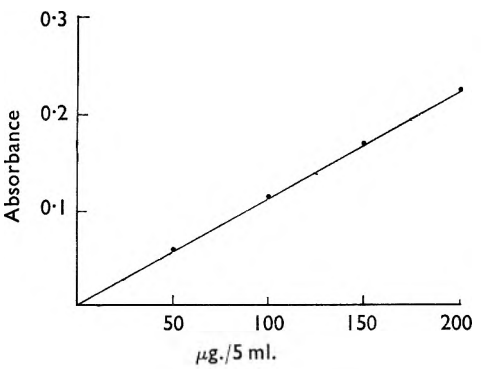

FIG. 1. Standard curve for hydrocortisone.

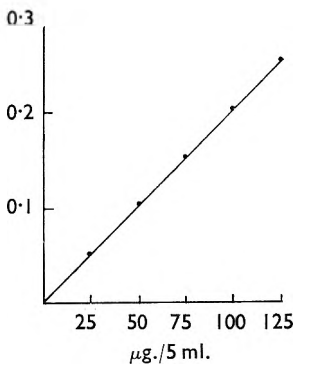

FIG. 2. Standard curve for cortisone acetate.

Ointments, lotions and injections. Weigh or pipette a sufficient quantity of the sample to contain about 10 mg. of the steroid in a 100 ml. volumetric flask. Extract the steroid with ethanol by warming on a water bath. Cool and make up to volume. Filter through sintered glass (porosity, 15 to 40 μ). Pipette 10 ml. of the filtrate and make up to 50 ml. in a volumetric flask. Pipette 5 ml. of the final dilution (equivalent to 100 μg.) into a 100 ml. round bottomed stoppered flask and proceed as described under the method for the standard curve. Correct the absorbance by treating the reagents in the same way and using 5 ml. of ethanol in place of the sample. (With ointments having a greasy base, make a blank determination on the paraffins used in the preparation of the ointment).

Tablets. Weigh sufficient quantity of powdered tablets to contain 10 mg. of the steroid into a volumetric flask. Extract the steroid as above with 100 ml. of chloroform. Filter, taking care to avoid loss of chloroform by evaporation. Prepare suitable sub-dilutions as described above, so that 5·0 ml. contains about 100 μg. of the steroid. Evaporate 5·0 ml. of the chloroform extract to dryness in a 100 ml. round-bottomed stoppered flask and dissolve the residue in 5·0 ml. of ethanol. Develop and read the colour as in the procedure described above.

Recovery. Weigh a definite quantity of the standard steroid and mix thoroughly with the pharmaceutical preparation (in the case of the tablets,

in the powdered granules). Proceed as described and calculate the quantity of the steroid found in the sample from the total quantity determined by the procedure and report as per cent recovery of the standard steroid.

Comparison with Tetrazolium Method. Proceed as described in the United States Pharmacopeia[4] for the determination of the steroid in the sample. The results are summarised in Table I.

TABLE I

Steroid	Sample	Age	Claimed per cent	Tetrazolium method	DTBPC method	
				Found per cent	Found per cent	Per cent recovery of added steroid
Cortisone (acetate)	Cortisone eye ointment (greasy base)	6 months	1·0	0·98	1·0	100·1
,,	Cortisone tablets	3 months	8·3	7·7	7·9	97·3
Hydrocortisone (alcohol)	Hydrocortisone skin ointment	5 months	1·0	0·98	1·04	101·8
,,	Hydrocortisone skin lotion	12 months	0·50	0·51	0·55	101·5
Hydrocortisone (hemisuccinate sodium)	Intravenous hydrocortisone	17 months	74·6		65·3	101·8
Hydrocortisone (acetate)	Hydrocortisone with neomycin skin ointment	30 months	1·0		0·93	100·5
,,	Hydrocortisone with penicillin and dihydrostreptomycin ointment (greasy base)	2 months	0·50		0·49	100·5

Discussion

We found that magnetic stirring was not necessary and we preferred a constant boiling steam bath. The cooling period of ten minutes in the original method[1] was found to be unnecessarily long and rapid cooling for two minutes followed by standing for 10 minutes at room temperature stabilised the colour for another 15 minutes and the reading was made after a total time of 20 minutes from the removal of the flask from the bath.

The use of 5 per cent aqueous sodium hydroxide solution as suggested by Schulz and Neuss gave readings which were not reproducible and did not show a linear relationship. To eliminate the error due to the quantitative difference in the alkali strength in the determinations, it was decided to use freshly standardised N sodium hydroxide solution, as a result of which reproducible readings for a calibration curve were obtained.

The strength of DTBPC reagent was reduced to 0·5 per cent and this did not effect the accuracy of results. The ethanol was standardised at 90 per cent as 95 per cent strength gave turbidity to the reaction mixture on dilution. Also further dilution to 25 ml. after filtration or addition of 5·0 ml. of ethanol to make up the volume as directed in the original method was found to introduce errors. With these modifications, we found that

with cortisone, Beer's Law was obeyed up to 125 μg./5 ml., and with hydrocortisone, the highest concentration was 200 μg./5 ml.

Reagent blanks in different determinations ranged from 0·003 to 0·006 at 625 mμ and from 0·028 to 0·032 at 471 mμ.

The DTBPC method of estimation of cortisone and hydrocortisone offers an advantage over the official tetrazolium method because of simplicity of operation as well as the development of different colours for the two steroids. We found that the tetrazolium method gave low results in our estimations, while the results by the DTBPC method were slightly high. Antibiotics like penicillin, streptomycin and neomycin did not interfere in the determination of the steroids in the preparations examined by us. The calibration curve holds good to $\pm$ 2 per cent for routine determinations and needs to be checked frequently. For very accurate determinations, we found that there was no alternative to taking comparative readings of samples and standards of near concentrations and determining the steroid content by ratio.

Acknowledgements. We acknowledge the helpful suggestions made by Mr. D. C. M. Adamson, Chief Analyst, Glaxo Laboratories Ltd., Greenford, during the preparation of this paper.

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PHARMACOPOEIAS AND FORMULARIES

INTERNATIONAL PHARMACOPOEIA FIRST EDITION. SUPPLEMENT*

REVIEWED BY K. R. CAPPER

This Supplement completes the first edition of the International Pharmacopoeia. The completion is marked by the inclusion of a cumulative index to Volumes I and II and the Supplement, and of lists of the monographs in the three books. The 94 monographs in the Supplement include a number for long-established drugs, such as Salicylic Acid, Fluorescein Sodium and Zinc Oxide, as well as many which have achieved official recognition here only in recent editions of the British Pharmacopoeia and its Addenda. Some indeed are not yet in the British Pharmacopoeia, for example, Acetrizoic Acid, Methandriol and Methoxamine Hydrochloride, and, as the B.P. is less than two years old, those who compiled the International Pharmacopoeia have obviously intended to produce a pharmacopoeia which is as up-to-date as practicable. The completed first edition of the International Pharmacopoeia contains well over 500 monographs of which about one-third are for preparation and the full list of monographs is more or less what would be expected in any national pharmacopoeia published towards the end of 1959.

The specifications in the International Pharmacopoeia are not intended to have legal status as such in any country but to serve as a guide to national specifications and in this way will undoubtedly help those countries which have not the potential to set up the complex organisations of expert committees which serve the pharmacopoeia commissions here, in the U.S.A. and elsewhere. It would be equally advantageous if the International Pharmacopoeia could help to achieve standardisation of standards in the national pharmacopoeias and reduce the analytical costs of pharmaceutical manufacturers. With the first edition completed, it is timely to ask how far the first edition of the International Pharmacopoeia has fulfilled either of these functions? The first it has done; any country which insists that is drugs should conform to the specifications of the International Pharmacopoeia will ensure that its medicines will be of high quality. The specifications in the tolerances laid down, and in the details of the limit tests and assays, bear comparison with those in any national pharmacopoeia. If the pharmaceutical industries in any country are not efficient enough to reach the standards of the more advanced countries, they receive no encouragement or compromise in the International Pharmacopoeia. It could, however, be contended that this function could be equally well served, as it has been in the past, by the United States and British Pharmacopoeias on which the Ph.I. monographs are so largely based. There is little to suggest, however, that it will achieve the second aim of an international pharmacopoeia —that of reducing the heterogeneity of national standards. To do so it would need to show some advance on the specifications in these. The lengthy list of experts given in the preface is impressive but it is not clearly stated which of these actually contributed towards the preparation of the Supplement monographs. There is some reason to believe that the work has been principally

* Pp. xx + 224 (including Index). World Health Organization, Geneva, 1959. Published in English and French. English version available from The Pharmaceutical Press, 17, Bloomsbury Square, London, W.C.1. 25s. (Postage: U.K., 1s. 6d., overseas, 2s.)

done by the secretaries of pharmacopoeia commissions and other experts on pharmacopoeia-making. That this should have been so is reasonable enough. The result, however, has been that most of the 500 or so monographs reproduce, with minor differences, those of one or other of the major national pharmacopoeias distributed in a manner which admirably couples impartiality with a nice recognition of national prestige. There is, however, little evidence of an original and authoritative approach in the individual methods for tests and assays. A minor example of a major weakness is shown in the inclusion of a limit test of lead (as in the B.P.) in some monographs and for heavy metals (as in the U.S.P.) in others—8 drugs must comply with the first and 9 with the latter, 2 with both. The Expert Advisory Panel must rely heavily on the key persons in the principal pharmacopoeia commissions as these persons have experience in producing this sort of book and they can best give a consensus of national opinion. Nevertheless, unless their efforts are supplemented by those with experience in applying the specifications in the laboratory, the International Pharmacopoeia will never be anything more than an admirable example of national compromise with an international label. There are listed in the preface those with such experience but there are no pharmaceutical analysts from Great Britain although there are several of international reputation who might have been asked to serve. Now that the huge task of preparing the first edition is complete, special attention might be given to this aspect and especially to standardising methods.

The section of the Supplement which is of greatest general interest is that which contains the Appendices. Two of these are especially useful. Appendix 16 contains a full list of International Biological Standards and reference samples, a list which is remarkably comprehensive and includes Standards for veterinary as well as human preparations. References to papers relevant to the standards are contained in the recently published 12th Report of the Expert Committee on Biological Standardisation (W.H.O. Technical Report Series, No. 172). It is regrettable that so many of these references are to unpublished working documents of the W.H.O. Expert Committee on Biological Standardisation. Reference substances for assays depending on spectrophotometric analysis and other physico-chemical methods are badly needed to make this procedure a reliable method of assay; Appendix 17 contains a modest, but promising, list of 8 authentic chemical substances for this purpose. Appendix 7 gives graphs for preparing isotonic solutions similar to those in the Danish Pharmacopoeia and equally useful. The table of doses for children given in other pharmacopoeias but not in the B.P. will also be welcomed. The Supplement completes an International Pharmacopoeia which, if no better than the best national pharmacopoeias, is little, if at all, worse. That this should be so is remarkable in the circumstances and reflects great credit on those concerned.

BOOK REVIEW

INDUSTRIAL GUMS. Polysaccharides and Their Derivatives. Edited by Roy L. Whistler. Pp. xi + 766 (including Index). Academic Press, 1959. New York ($25.00); London (173s. 6d.).

This book is a reference work on gums with especial emphasis on production, properties, economics and industrial application, but also contains useful information on their chemistry. Obviously some definition of "gum" is necessary in view of the indiscriminate use of this term in commerce. Resins, rubber products, and chicle, are excluded by the definition used, namely, "plant polysaccharides or their derivatives which are dispersible in either cold or hot water to produce viscous mixtures or solutions". A surprising number of products is included in this definition and equally surprising are the large amounts used; it is estimated that the annual consumption in the U.S. alone is about 500,000 tons.

The introductory chapter gives an interesting general account of the economic factors affecting costs, and the physico-chemical factors affecting the industrial application of gums. This is followed by informative chapters on seaweed products, agar, alginates, carrageenan, fucoidan and laminaran; well known gums such as acacia, ghatti, karaya and tragacanth; the seed gums, carob, quince, psyllium, linseed and tamarind. Four chapters are devoted to cellulose derivatives, and one each to pectins, dextrins and dextrans. Among the lesser known products which may have important uses in the future are, chitin and its derivatives, amylose, amylopectin, wheat hemicelluloses, guar gum and Ti (derived from an Hawaiian plant).

Since 32 authors have contributed different chapters there is naturally some uneveness in treatment. Thus acacia is given 86 pages, whereas the more costly product tragacanth which "is still widely used today in foods and drugs" is dismissed in 4½ pages, less than half the space devoted to laminaran. The account of the formation and preparation of tragacanth is very brief, although such information is not without importance to the chemist and would explain the fact that electron microscope studies "show that gum tragacanth solutions contain cellulose microfibrils". Traces of cellulose walls occur because the gum is produced from the whole tissue of the pith and rays; they are normally visible under an ordinary microscope. On p. 215 the surprising statement is made that catechu and cutch are varieties of gum arabic as they come from *Acacia* species. However, this is a small slip in an otherwise full and informative chapter.

It would be impossible to deal adequately in a review with the wealth of information contained in this book, which is indeed a well-documented reference work on the industrial aspects of gums.

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