RESEARCH PAPERS

THE LOCAL ANAESTHETIC PROPERTIES OF A SERIES OF *N*-SUBSTITUTED *p*-AMINOBENZOIC ACID ESTERS OF TROPINE

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Received June 28, 1960

The local anaesthetic properties and toxicities of a series of *N*-substituted *p*-aminobenzoic acid esters of α- and β-tropine were investigated. No consistent differences in local anaesthetic activity were observed between the corresponding α-tropine and β-tropine esters. Substitution at the *p*-amino group by straight chain alkyl radicals having up to four carbon atoms increased both local anaesthetic activity and toxicity. The propyl derivative was as potent as amethocaine but its therapeutic index was lower.

THE effect of replacing one amino hydrogen atom by an alkyl radical on the local anaesthetic properties of diethylaminoethyl *p*-aminobenzoate was reported by Eisleb[1]. Surface anaesthetic activity was found to be little affected when the substituent was methyl but it increased rapidly from ethyl to propyl and then remained fairly constant with straight chains to octyl when it fell rapidly so that the *p*-dodecylaminobenzoic acid ester was almost ineffective.

No systematic examination of the effects of similar substitution in *p*-aminobenzoic acid esters of tropine appears to have been made. The *p*-aminobenzoic acid esters of α-tropine and β-tropine (*pseudo*tropine) are known[2,3] but the only *N*-substituted derivatives for which pharmacological data is available are those with a *p*-*N*-butyl group which are analogous in structure to amethocaine (2-dimethylaminoethyl *p*-butylaminobenzoate). Since both of these tropine esters showed promise as local anaesthetics[3], it seemed worth while examining the properties of other substituted *p*-aminobenzoyl tropeïnes.

METHODS

Surface anaesthesia. The potency of each compound was determined by comparison with amethocaine hydrochloride using a modification of the method described by Chance and Lobstein[4]. A solution of the local anaesthetic was applied to the cornea of a guinea pig for 15 seconds and tests for anaesthesia were carried out at minute intervals for 10 minutes. These tests consisted of touching the cornea with the rounded tip of a fine flexible glass rod and observing whether the corneal blink reflex was elicited. Each compound was evaluated on three guinea pigs which were tested daily for 6 days. Solutions of standard and test compounds dissolved in 0·9 per cent (w/v) NaCl solution at concentrations x, 2x and 4x were applied to the eyes in random order so that at the end of 6 days each solution had been tested on each eye. The mean percentage response for each concentration of drug was plotted against concentration on

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logarithmic probability paper and relative potency estimated from the concentrations giving 50 per cent effects.

Infiltration anaesthesia. The intradermal weal test of Bülbring and Wajda[5] was used. Each compound was usually evaluated on six guinea pigs against procaine hydrochloride. Solutions of standard and test compounds in 0·9 per cent (w/v) NaCl solution at concentrations of x, 2x and 4x were injected intradermally in random order at six sites marked on the back of the animal. Depilation was done on the day before the test with barium sulphide cream after clipping the hair short. The mean response for each concentration of drug was plotted against the logarithm of the concentration and relative potencies were estimated by comparing the concentrations needed to give 50 per cent effects.

Conduction anaesthesia. This was determined in mice weighing between 20 and 30 g. which were tested by pinching the tail with rubber covered

TABLE I

N-SUBSTITUTED *p*-AMINOBENZOIC ACID ESTERS OF TROPINE AND *pseudo*TROPINE

Cpd. No.	m.p. of salt	m.p. or b.p. of ester°C	Found*				Theory			
			C	H	N	Cl	C	H	N	Cl
5	272	200–2/2 mm.	62·1	7·3	9·1	11·3	61·9	7·1	9·1	11·5
6	291–2	205–7/2 mm.	63·4	7·8	8·4	10·9	63·0	7·7	8·6	11·0
7	106–7	—	70·9	7·7	6·8	—	70·8	7·5	6·6	—
8	225–6	206–8/2 mm.	64·0	8·0	8·4	10·4	63·8	8·0	8·3	10·5
9	248–9	112–4	63·7	8·1	8·5	10·3	63·8	8·0	8·3	10·5
10	232–4	190–2/0·5 mm.	63·8	8·2	8·2	10·4	63·8	8·0	8·3	10·5
14	242–3	—	67·6	6·7	7·0	9·1	68·4	7·0	7·2	9·2

* Analyses by Messrs. Weiler and Strauss, Oxford.
Compound 3 contained 10·6 per cent ionisable chlorine (theory 10·7) m.p. 281–2°.

artery forceps. Only those which squeaked on the first or second application of this stimulus were employed in the test. Compounds were dissolved in 0·9 per cent (w/v) NaCl solution and 0·05 ml. of solution was injected subcutaneously bilaterally at the root of the tail. The animals were retested at intervals and the proportion failing to respond to two stimuli recorded.

Duration of local anaesthesia was determined on the rabbit cornea after flooding the eye with drug solution for 30 seconds. Drugs were dissolved in citrate buffer pH 6·0 (Na_2HPO_4 0·2M, citric acid 0·1M). Amethocaine hydrochloride which was used as the standard was applied to one eye and the compound under test to the other. The cornea was then touched every 4 minutes with a fine glass rod and the time for return of the blink reflex noted.

Toxicity tests were carried out in albino mice weighing between 18 and 22 g. Drugs were dissolved in 0·9 per cent (w/v) NaCl solution and 0·5 ml. of solution injected intraperitoneally for each 20 g. weight. The LD50 values with 95 per cent fiducial limits were calculated by the method of Litchfield and Wilcoxon[6].

The mydriatic properties of compounds were estimated by the method of Pulewka modified by Ing, Dawes and Wajda[7]. Atropine sulphate was used as standard and pupil diameters were measured 30 minutes after intraperitoneal injection of a compound.

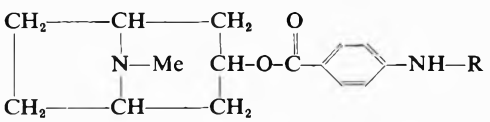
Chemical

p-*Alkylaminobenzoic acids.* These were most conveniently prepared by the action of alkyl bromides on potassium *p*-aminobenzoate[3], with the exception of the *p*-methylamino acid which was obtained in 92 per cent yield by the method of Cosulich and Smith[8].

p-*Alkylaminobenzoyl chlorides.* The hydrochlorides of these were made in 70–90 per cent yields by addition of the acid (60–90 minutes) to thionyl

TABLE II

THE RELATIVE LOCAL ANAESTHETIC POTENCIES AND TOXICITIES OF *N*-SUBSTITUTED *p*-AMINCBENZOIC ACID ESTERS OF TROPINE AND *pseudo*TROPINE



Cpd. No.	C	R	Salt used	Relative potency			LD50 mg./kg. with P, 0·95 limits (mice intraperitoneal)
				Infiltration anaesthesia (guinea pigs)	Surface anaesthesia (guinea pig cornea)	Mydriasis (mice intraperitoneal)	
1	α	H	Hydrochloride	0·2	0·03	<0·01	33 (30–37)
2	β		Hydrochloride	0·2	—	<0·01	88 (75–104)
3	α	H*	Hydrochloride	0·4	—	—	—
4	α		Acetate	0·2	0·2	<0·01	21·5 (18·7–25·0)
5	α	Me	Hydrochloride	0·3	0·2	<0·01	28 (24–33)
6	α	Et	Hydrochloride	0·7	0·5	—	12·2 (11·4–13·2)
7	α		Phenylacetate	0·8	0·4	0·03	12·1 (11·1–13·2)
8	α	Prn	Hydrochloride	1·2	1·0	0·02	11·3 (8·4–15·1)
9	β		Hydrochloride	0·8	0·8	<0·02	12·7 (10·8–15·1)
10	α	Pri	Hydrochloride	0·6	0·2	0·01	21 (19–23)
11	α	Bun	Hydrochloride	1·0	0·8	—	15·2 (14·0–16·5)
12	β		Hydrochloride	1·0	0·3	<0·01	20 (18–23)
13	α		Acetate	0·6	1·1	<0·01	20 (17–25)
14	α		Hydrochloride	0·2	0·3	<0·01	19 (16–24)
Cinchocaine			Hydrochloride	—	1·3	—	29·5 (25·7–33·9)
Amethocaine			Hydrochloride	1·0	1·0	—	50 (39–65)
Procaine			Hydrochloride	0·1	—	—	220 (188–255)
Atropine			Sulphate	—	—	1·0	—

All values are given in terms of base.

* = Derivative of 2-chloro-4-amino benzoyltropeïne.

C = Conformation of acyloxy group.

chloride (2 ml./g.) stirred at 3–6° (cf. Graf and Langer[9]). After a further 3 hours or after complete solution was achieved the product was precipitated by addition of excess dry ether and was in general sufficiently pure for use.

Esters of the above acids. The amino acid chloride hydrochloride was added to a tropanol hydrochloride (10 per cent excess) in dry chloroform (3 ml./g.). After gentle reflux until evolution of hydrogen chloride had ceased, the salts were extracted with water and the solution basified with ammonium hydroxide. Solvent was removed from the dry ether extract and the residue was either distilled or used directly for preparation of a salt. Yields were 60–80 per cent. Analyses are shown in Table I.

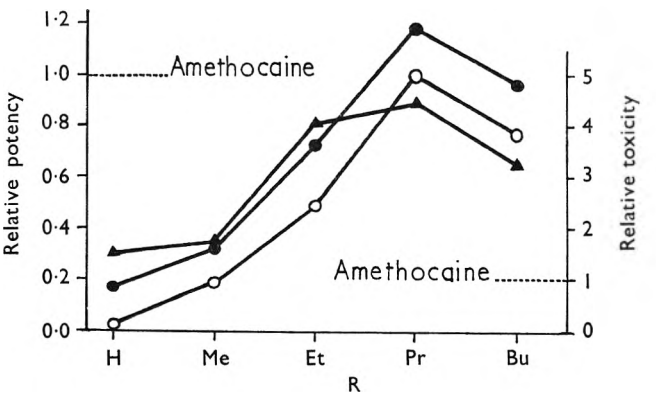


FIG. 1. Local anaesthetic potency and toxicity of α-tropine esters of substituted *p*-aminobenzoic acids *p*-$RHN{\cdot}C_6H_4COOH$. All compounds were tested as the hydrochlorides.

●—● Infiltration anaesthesia in guinea pigs.
○—○ Surface anaesthesia in guinea pigs.
▲—▲ Toxicity by I.P. injection in mice.

RESULTS

The results obtained with all compounds in tests for infiltration and surface anaesthetic properties are summarised in Table II. *p*-Aminobenzoyl α-tropeïne was approximately twice as potent as procaine by the intradermal weal test and about one-thirtieth as potent as amethocaine on the guinea pig cornea. Substitution of one hydrogen of its primary amino group by straight chain alkyl groups containing from one to four carbon atoms gave compounds which were both more effective as local anaesthetics and more toxic. These effects are shown in Figure 1. A methyl group augmented activity only slightly but ethyl and propyl groups each gave an increase in potency. Butyl was no more effective than propyl and the results suggest that a peak effect is obtained with the propyl derivative. In this homologous series results obtained for surface anaesthesia paralleled those obtained by infiltration. There was a rough parallelism too between toxicity and local anaesthetic activity.

By intraperitoneal injection in mice the least toxic member of this series was *p*-aminobenzoyl α-tropeïne which had an LD50 of 33 mg./kg.

The corresponding values for the butyl and propyl derivatives were 15·2 mg./kg. and 11·3 mg./kg. respectively. These last two compounds were as effective as amethocaine and cinchocaine in producing local anaesthesia but both were considerably more toxic, the LD50 for cinchocaine being 29·5 mg./kg. and that of amethocaine 50·0 mg./kg.

TABLE III

TOXICITY OF LOCAL ANAESTHETICS BY INTRAVENOUS INJECTION IN MICE

Compound employed	LD50 mg./kg. of base (P 0·95 limits)
Cinchocaine hydrochloride	6·2 (5·5–7·0)
Amethocaine hydrochloride	8·8 (7·9–9·8)
p-Propylaminobenzoyl α-tropeïne hydrochloride	4·6 (4·0–5·2)

TABLE IV

THE RELATIVE DURATION OF ANAESTHESIA PRODUCED BY *p*-PROPYLAMINOBENZOYL α-TROPEÏNE HYDROCHLORIDE AND AMETHOCAINE HYDROCHLORIDE ON THE RABBIT CORNEA

Compound	Concentration mg./ml.	Mean duration of anaesthesia in min. with SD	Proportion of animals anaesthetised
Amethocaine HCl	0·5	22	4/5
	1·0	31 ± 12	5/5
	2·0	44 ± 13	5/5
	4·0	80*	5/5
p-Propylaminobenzoyl α-tropeïne HCl	0·5	6	1/5
	1·0	48 ± 12	5/5
	2·0	77 ± 11	5/5
	4·0	109**	5/5

* Local anaesthesia lasted for over 2 hours in 1/5 rabbits.
** Local anaesthesia lasted for over 2 hours in 4/5 rabbits.

On intravenous injection into albino mice *p*-propylaminobenzoyl α-tropeïne was found to be more toxic than amethocaine and cinchocaine (Table III).

Direct comparison of the duration of anaesthesia on the rabbit cornea after amethocaine hydrochloride and *p*-propylaminobenzoyl α-tropeïne hydrochloride showed that although the threshold concentration was lower for amethocaine, at concentrations giving complete anaesthesia the action of *p*-propylaminobenzoyl α-tropeïne hydrochloride was more prolonged (Table IV).

p-Propylaminobenzoyl α-tropeïne hydrochloride was much less effective than lignocaine hydrochloride in producing anaesthesia in mice (Fig. 2). The peak effect with lignocaine hydrochloride 0·5 per cent was obtained

within 10 minutes of injection and the response to a painful stimulus was abolished in 90 per cent of mice at that time. *p*-Propylaminobenzoyl α-tropeïne hydrochloride produced its maximum effect between 20 and 30 minutes after injection, 0·2 and 0·4 per cent solutions being effective in only 53 and 72 per cent of animals respectively. Both concentrations produced toxic signs (tremors and ataxia) and at the higher dose 2 out of 15 animals died within 20 minutes of injection.

The esters examined showed negligible mydriatic properties in mice after intraperitoneal injection (Table II).

The isopropyl derivative of *p*-aminobenzoyl α-tropeïne was less toxic than the propyl analogue but its local anaesthetic properties were correspondingly reduced. Neither substitution of benzyl at the primary amino

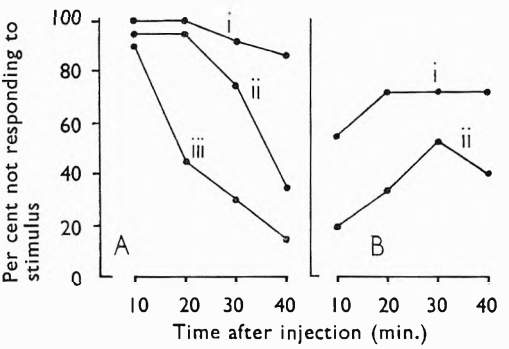


FIG. 2. Conduction anaesthesia in mice. A. Lignocaine hydrochloride. (i) 2 per cent, 15 animals; (ii) 1 per cent, 20 animals; (iii) 0·5 per cent, 20 animals. B. *p*-Propylaminobenzoyltropeïne hydrochloride. (i) 0·4 per cent, 15 animals; (ii) 0·2 per cent, 15 animals. With 0·4 per cent solution two mice died within 20 minutes of injection.

group or chlorine at the *ortho* position in the benzene ring of *p*-aminobenzoyl α-tropeïne was of significance in increasing local anaesthetic activity or reducing toxicity.

In the hope of obtaining less toxic compounds and yet retaining local anaesthetic potency the *p*-aminobenzoic acid ester or β-tropine and its *p*-propyl and *p*-butyl derivatives were prepared since esters of the β isomer have been reported to be less toxic than those of α-tropine[3]. A marked difference in toxicity was observed only in the case of the β-tropine ester of *p*-aminobenzoic acid which was much less toxic than the α form. By the intradermal weal test there were no consistent differences between isomers but in the test for surface anaesthesia the butyl β-tropine ester was considerably less effective than the corresponding α form.

In view of the claim by Rabinovitch and others[3] that acetic or phenylacetic acid esters of tropine were more effective as local anaesthetics than the hydrochlorides, the acetates or phenylacetates of three compounds were tested but were found to have no striking advantages over their hydrochlorides. The local anaesthetic properties of *p*-ethylaminobenzoyl α-tropeïne as the phenylacetate differed only slightly from those of the

hydrochloride. With *p*-butylaminobenzoyl α-tropeïne the potency of the acetate by intradermal injection was less than that of the hydrochloride but on application to the cornea the situation was reversed, the acetate being slightly more effective.

Discussion

Our results confirmed the high activity of *p*-butylaminobenzoyl α-tropeïne reported by Rabinovitch and others[3] and showed that in potency, the local anaesthetic properties of both it and *p*-propylaminobenzoyl α-tropeïne compared favourably with those of amethocaine or cinchocaine when tested by infiltration or application to a mucous surface. However, none of the tropine or *pseudo*tropine esters tested were superior to currently available local anaesthetics. Enhanced local anaesthetic activity was accompanied by increased toxicity and the therapeutic index of the most active compound tested was considerably less than that of amethocaine.

Because the tropine esters were so toxic and it was probable that peak local anaesthetic activity was obtained with the propyl derivative the series was not extended to include compounds in which the length of substituent on the primary amino group was increased beyond butyl.

The results obtained in intradermal weal tests with *p*-aminobenzoyl tropeïne and its *N*-substituted propyl and butyl derivatives did not give any indication that stereoisomerism at the 3 position is of importance in determining the local anaesthetic properties of tropine esters[10,11] and a significant reduction in toxicity by replacing tropine by *pseudo*tropine was only seen in the case of the *p*-aminobenzoic acid ester[3].

Acknowledgements. The authors are indebted to Miss P. Cameron, Miss C. Mackay, Miss I. J. Palmer and the late P. D. Graham for assistance with the experimental work.

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STUDIES IN THE FIELD OF DIURETICS

Part IV. The Condensation of Some Halogeno-2,4-Disulphamylbenzene Derivatives with Basic Reagents

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Received May 13, 1960

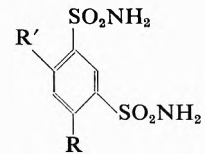
The condensation of some halogeno-2,4-disulphamylbenzenes with basic reagents has been examined. Some of the resulting products have been converted into 1,2,4-benzothiadiazine-1,1-dioxide derivatives. By using 2-aminopyridine in the reaction the novel azaphenthiazines (VII) were obtained.

After the discovery of the diuretic properties of 5-chloro-2,4-disulphamyltoluene[1] (disulphamide) (I; R = Me, R′ = Cl), the stability of this product towards various reagents was examined as a matter of routine. Observations made in the course of this work upon the reactivity of the halogen atom towards basic reagents (cf.[2]) are described herein, together with collateral studies on the ring closure of some basic derivatives to novel thiadiazine types.

5-Chloro-2,4-disulphamyltoluene surprisingly proved stable to heating with strongly alkaline reagents such as aqueous potassium hydroxide, ethanolic sodium ethoxide, or sodium formate in ethanediol (cf.[3]). In striking contrast, its fully methylated derivative, 5-chloro-2,4-bisdimethylsulphamyltoluene (II; R = Cl, R′ = R″ = Me), reacted readily with ethanolic sodium ethoxide or with aqueous ethanolic potassium hydroxide to yield the 5-ethoxy substitution product (II; R = OEt, R′ = R″ = Me). It was apparent from this behaviour that the chlorine atom of disulphamide was markedly deactivated under strongly alkaline conditions, presumably through ionisation of the sulphamyl groups with formation of sulphamyl anions SO_2NH^-. If this were indeed the case, it followed that replacement of the halogen atom by a nucleophilic group would be facilitated under experimental conditions unfavourable to ionisation of the sulphamyl residues. The reaction of 5-chloro-2,4-disulphamyltoluene with ethanolic methylamine was consequently examined and, as anticipated, smooth reaction was effected (at 100° under pressure) with formation of 5-methylamino-2,4-disulphamyltoluene (I; R = NHMe, R′ = Me) in excellent yield. Extension of this reaction to ethanolamine gave the 5-(2-hydroxylethylamino)-derivative (I; R = $NH.CH_2CH_2OH$, R′ = Me), which approached the parent compound in diuretic potency, in tests made by Dr. A. David and his colleagues. This observation led to the preparation of the basic derivatives listed in Table I. These compounds, however, proved to be inferior to disulphamide in animal assays.

Reaction of 5-(2-hydroxyethylamino)-2,4-disulphamyltoluene with formamide and with formic acid (cf.[4]) yielded 4-(2-hydroxyethyl)-6-methyl-7-sulphamyl-1,2,4-benzothiadiazine-1,1-dioxide (III; R = OH) and the corresponding formate (III; R = O.CHO), respectively. These cyclic derivatives were degraded to the parent base by short heating with

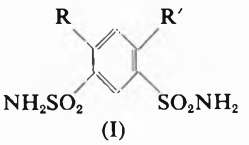
TABLE I



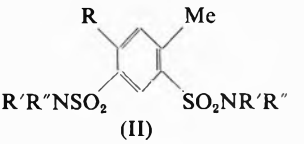
	R	R′	Formula	m.p. °C.	Found				Required			
	Substituent at position 4	6			C	H	N	S	C	H	N	S
1	NH.CH_2.CH_2.OH	—	$C_8H_{12}O_5N_2S_2$	190–191	32·4	4·6	13·9	—	32·5	4·4	14·2	—
2	NH.CH_2.CHOH.Me	—	$C_9H_{14}O_5N_2S_2$	206–208	35·4	5·0	13·3	20·5	34·9	4·9	13·6	20·7
3	NH.$(CH_2)_3$.OH	—	$C_9H_{14}O_5N_2S_2$	177–179	34·9	4·8	14·0	—	34·9	4·9	13·6	20·7
4	N(CH_2.CH_2.OH)$_2$	—	$C_{10}H_{17}O_6N_2S_2$	160–162	35·0	5·3	12·4	18·8	35·4	5·1	12·4	18·9
5	NH.Ph	—	$C_{12}H_{12}O_4N_2S_2$	226–227	44·0	3·9	13·1	19·4	44·0	4·0	12·8	19·6
6	N<$(CH_2)_4$>NH	—	$C_{10}H_{16}O_4N_2S_2$	217 (d.)†	37·7	4·9	17·1	20·1	37·5	5·0	17·5	20·0
7	$NHNMe_2$	—	$C_8H_{14}O_4N_4S_2$	198–199	32·7	4·7	19·3	21·7	32·7	4·8	19·0	21·8
8	NH.CH_2.CHOH.CH_2.OH	—	$C_9H_{14}O_6N_2S_2$	190–192	32·9	4·4	12·3	—	33·2	4·6	12·9	—
9	Me	NH.Me	$C_8H_{12}O_4N_2S_2$	243	34·4	4·4	15·3	—	34·4	4·7	15·0	—
10	Me	NH.CH_2.CH_2.OH	$C_9H_{14}O_5N_2S_2$	216	35·0	4·6	13·3	—	35·0	4·9	13·6	—
11	Me	NH.CH_2.CHOH.Me	$C_{10}H_{16}O_5N_2S_2$	187–189	37·5	5·2	13·2	—	37·1	5·3	13·0	19·8
12	Me	NMe.CH_2.CH_2.OH	$C_{10}H_{16}O_5N_2S_2$	224–225 (d.)	37·5	5·1	12·8	19·7	37·1	5·3	13·0	19·8
13	Me	NH.$(CH_2)_3$.OH	$C_{10}H_{16}O_5N_2S_2$	171–173	37·6	5·5	12·8	19·3	37·1	5·3	13·0	19·8
14	Me	NH.CH_2.C(Me)OH.C_2H_5	$C_{13}H_{22}O_5N_2S_2$	174–176	42·9	6·1	—	—	42·7	6·4	—	—
15	Me	NH.CH_2.CHOH.CH_2OH	$C_{11}H_{18}O_6N_2S_2$	164–168	35·7	5·2	12·5	19·0	35·4	5·1	12·4	18·9
16	Me	$NH.NH_2$	$C_7H_{12}O_4N_4S_2$	227 (d.)	30·2	4·4	19·9	22·8	30·0	4·3	20·0	22·8
17	Me	NH.$NH.CO.NH_2$	$C_8H_{13}O_5N_5S_2$	237 (d.)	30·0	4·1	21·6	19·7	29·7	4·1	21·7	19·8
18	Me	NH.CH_2.CH_2.NH_2.HCl	$C_9H_{17}O_4N_4S_2Cl$	267 (d.)	31·7	5·0	16·3	10·4*	31·3	5·0	16·3	10·3*
19	Me	NH.CH_2.CH_2.NH.$CO.NH_2$	$C_{10}H_{17}O_5N_5S_2$	214 (d.)	34·1	5·0	19·8	—	34·2	4·9	19·9	—
20	Me	N<$(CH_2)_5$	$C_{12}H_{19}O_4N_2S_2$	211–212	43·4	5·9	12·6	—	43·2	5·8	12·6	—
21	Me	N<$(CH_2)_4$>NH	$C_{11}H_{18}O_4N_3S_2$	246 (d.)	40·0	5·4	17·0	19·1	39·5	5·4	16·8	19·2
22	Et	NH.CH_2.CH_2.OH	$C_{10}H_{17}O_5N_2S_2$	202–203	37·0	5·2	13·2	20·0	37·1	5·3	13·0	19·8
23	Pr^n	NH.CH_2.CH_2.OH	$C_{11}H_{19}O_5N_2S_2$	179–180	39·1	5·6	12·2	—	39·2	5·7	12·5	—
24	Cl	$NH.NH_2$	$C_8H_9O_4N_4S_2Cl$	243 (d.)	24·2	3·1	18·9	12·1*	24·0	3·0	18·6	11·8*
25	Cl	NH.CH_2.CH_2.OH	$C_9H_{12}O_5N_2S_2Cl$	200–202	29·6	4·0	12·9	10·8*	29·1	3·7	12·8	10·8*
26	$NH.NH_2$	$NH.NH_2$	$C_6H_{10}O_4N_6S_2$	228 (d.)	24·2	4·0	29·0	—	24·3	4·1	28·4	—
27	NH.CH_2.CH_2.OH	NH.CH_2.CH_2.OH	$C_{10}H_{18}O_6N_4S_2$	200–202	34·0	5·4	15·5	—	33·9	5·1	15·8	—

* = Cl. † d. = decomposition.

ethanolic hydrochloric acid. When the hydroxyethylamino-derivative (I; R = $NH.CH_2CH_2OH$, R′ = Me) was condensed with one equivalent of formaldehyde in 2-ethoxyethanol containing a trace of hydrogen chloride as catalyst, 4-(2-hydroxyethyl)-6-methyl-7-sulphamyl-3,4-dihydro-1,2,4-benzothiadiazine-1,1-dioxide (IV; R = Me, R′ = H) was obtained. This



(I)



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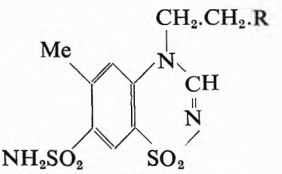

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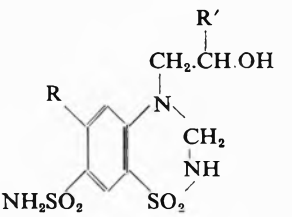

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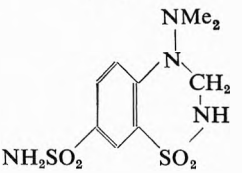

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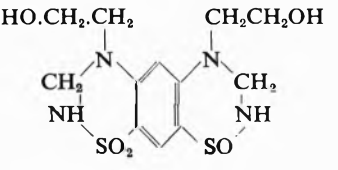

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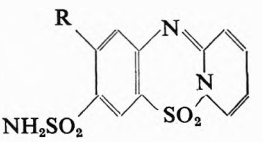

(VII)

analogue of hydrochlorothiazide[5] proved stable to short heating with ethanolic hydrochloric acid. The 4-(2-hydroxypropyl)-derivative (IV; R = R′ = Me) was prepared in a similar way.

Condensation of 5-chloro-2,4-disulphamyltoluene with hydrazine hydrate on the steam bath gave a high yield of 5-methyl-2,4-disulphamylphenylhydrazine (I; R = $NHNH_2$, R′ = Me), which formed normal condensation products with acetone and with benzaldehyde. Attempts to obtain cyclic structures from these condensation products, or from the original phenylhydrazine, by reaction with formaldehyde under a variety of experimental conditions proved unsuccessful. *Inter alia*, the phenylhydrazine was converted into the semicarbazide (I; R = $NHNHCONH_2$, R′ = Me) and into the azide (I; R = $N\langle^{N}_{N}$, R′ = Me) by reaction with potassium cyanate and nitrous acid, respectively. In addition, disulphamide was condensed with ethylenediamine to give 5(2-aminoethylamino)-2,4-disulphamyltoluene (I; R = $NH_2CH_2CH_2NH$, R′ = Me), which was

characterised as the urea (I; R = $NH_2CONHCH_2CH_2NH$, R′ = Me) and with piperazine to give 5-piperazino-2,4-disulphamyltoluene. Reaction with 1,1-dimethylhydrazine under reflux, or with aniline at 160°, could not be achieved.

2,4-Disulphamylfluorobenzene (I; R = F, R′ = H) proved to be more reactive than the chlorotoluene (I; R = Cl, R′ = Me) in reactions with bases. Thus it condensed readily with 1,1-dimethylhydrazine to give the dimethylphenylhydrazine (I; R = $NHNMe_2$, R′ = H), smoothly converted into 4-dimethylamino-7-sulphamyl-3,4-dihydro-1,2,4-benzothiadiazine-1,1-dioxide (V) by reaction with formaldehyde. Aniline similarly gave the anilino-derivative (I; R = NHPh, R′ = H). The behaviour of 2,4-disulphamylchlorobenzene (I; R = Cl, R′ = H), however, resembled that of the 5-chlorotoluene derivative.

1,5-Dichloro-2,4-disulphamylbenzene (I; R = R′ = Cl) was converted by ethanolic ammonia in an autoclave at 150° into 5-chloro-2,4-disulphamylaniline, the immediate precursor of the chlorothiazide and hydrochlorothiazide group of diuretic agents. With 2-hydroxyethylamine, it was possible to obtain mono- (I; R = $NHCH_2CH_2OH$, R′ = H) and bis- (I; R = R′ = $NHCH_2CH_2OH$) condensation products, converted by formaldehyde into 6-chloro-4-(2-hydroxyethyl)-7-sulphamyl-3,4-dihydro-1,2,4-benzothiadiazine-1,1-dioxide (IV; R = Cl, R′ = H) and the novel tricyclic structure (VI), respectively. Mono- and bis- condensation products were also obtained from hydrazine.

Both 5-chloro-2,4-disulphamyltoluene and 1,5-dichloro-2,4-disulphamylbenzene failed to react with 2-aminopyridine at temperatures up to 180°. 2,4-Disulphamylfluorobenzene and 5-chloro-2,4-disulphamylfluorobenzene, in contrast, reacted to give condensation products formulated as 8-sulphamyl-11-azaphenthiazine derivatives (VII). These novel structures, however, were without diuretic activity in the saline-loaded rat.

Experimental

Attempted alkaline hydrolysis of 5-chloro-2,4-disulphamyltoluene. A solution of the disulphamide (4·0 g.) in water (40 ml.) containing potassium hydroxide (10 g. = 12·5 equiv.) was heated under reflux for 4 hours. It was then cooled and acidified with dilute nitric acid. Unchanged material (3·5 g.) was collected which had m.p. 260–261° after crystallisation from aqueous ethanol. The acid-aqueous liquid gave no precipitate with silver nitrate solution.

Reaction of 5-chloro-2,4-dimethylsulphamyltoluene with sodium ethoxide. A solution of the compound (8·5 g.) in ethanol (90 ml.) containing sodium ethoxide [prepared from sodium (1·2 g.)] was heated under reflux for 6 hours. The solution was concentrated to half-bulk, diluted with a small amount of water and neutralised with hydrochloric acid. *5-Ethoxy-2,4-dimethylsulphamyltoluene* (8 g.) separated on cooling and had m.p. 126–128° after crystallisation from aqueous methanol. Found: C, 44·5; H, 6·2; N, 8·1; S, 18·5. $C_{13}H_{22}O_5N_2S_2$ requires C, 44·6; H, 6·3; N, 8·0; S, 18·3 per cent.

5-*Methylamino*-2,4-*disulphamyltoluene*. (*a*) A solution of 5-chloro-2,4-disulphamyltoluene (20 g.) in 30 per cent ethanolic methylamine (200 ml.) was heated on the steam bath for 14 hours. *Unchanged* material (19·5 g.), m.p. 256–258°, was recovered.

(*b*) The disulphonamide (47·1 g.) was dissolved in 30 per cent ethanolic methylamine (300 ml.) and the solution heated in the autoclave at 100° for 5 hours. After removal of excess amine the residual solid was crystallised from aqueous methanol to yield the *product* (40 g.), m.p. 243°.

5-*Methylaminotoluene*-2,4-*disulphonmethylamide*. 5-Chlorotoluene-2,4-disulphonchloride (12·94 g.) was added in portions with cooling to 33 per cent ethanolic methylamine (100 ml.) and the solution heated at 60° for 16 hours. After evaporation to dryness the residual solid was crystallised from aqueous ethanol to yield the product (12 g.), m.p. 206–207°. Found: C, 39·0; H, 5·7; N, 13·9; S, 21·0. $C_{10}H_{17}O_4N_3S_2$ requires C, 39·1; H, 5·5; N, 13·7; S, 20·8 per cent.

N-(*β-Hydroxyethyl*)-5-*methyl*-2,4-*disulphamylaniline*. A solution of 5-chloro-2,4-disulphamyltoluene (50 g.) in 2-hydroxyethylamine (50 ml.) was heated on the steam bath for 10 hours, when the hot mixture was poured into water (200 ml.) with stirring. The *product* (53 g.) which separated, had m.p. 216° after crystallisation from water.

Reaction of the foregoing compound (6·2 g.) with 98 per cent formic acid (30 ml.) under reflux for 3 hours, followed by removal of the excess formic acid at reduced pressure and crystallisation from aqueous ethanol yielded 4-(*β-formyloxyethyl*)-6-*methyl*-7-*sulphamyl*-1,2,4-*benzothiadiazine*-1,1-*dioxide*, m.p. 280° (decomp.). Found: C, 38·4; H, 3·6; N, 12·1. $C_{11}H_{13}O_6N_3S_2$ requires C, 38·0; H, 3·7; N, 12·1 per cent.

With formamide (30 ml.) at 130° for 3 hours, the compound (6·2 g.) yielded 4-(*β-hydroxyethyl*)-6-*methyl*-7-*sulphamyl*-1,2,4-*benzothiadiazine*-1,1-*dioxide*, m.p. 275–277° after crystallisation from aqueous ethanol. Found: C, 37·3; H, 4·2; N, 13·3. $C_{10}H_{13}O_5N_3S_2$ requires C, 37·6; H, 4·1; N, 13·2 per cent. The m.p. of this compound was strongly depressed on admixture with the foregoing formyl derivative.

4-(*β-Acetoxyethyl*)-3,6-*dimethyl*-7-*acetylsulphamyl*-1,2,4-*benzothiadiazine*-1,1-*dioxide*. A solution of *N*-(*β*-hydroxyethyl)-5-methyl-2,4-disulphamylaniline (6·2 g.) in acetic anhydride (30 ml.) was heated under reflux for 3·5 hours. The *product* (7·0 g.) separated (from 50 per cent ethanol) in minute shining plates, m.p. 237° (decomp.). Found: C, 43·4; H, 4·7; N, 9·8; S, 14·9. $C_{15}H_{19}O_7N_3S_2$ requires C, 43·2; H, 4·6; N, 10·1; S, 15·3 per cent.

4-(*β-Hydroxyethyl*)-6-*methyl*-7-*sulphamyl*-3,4-*dihydro*-1,2,4-*benzothiadiazine*-1,1-*dioxide*. A suspension of *N*-(*β*-hydroxyethyl)-5-methyl-2,4-disulphamylaniline (6·2 g.) in ethoxyethanol (50 ml.) was treated with 40 per cent formaldehyde solution (2 ml.) and ethoxyethanol (1 ml.) saturated with hydrogen chloride added. The suspended solid dissolved rapidly on warming and the solution was heated at 140° for 2 hours. Excess of solvent was removed under reduced pressure and the residue crystallised from water. The *product* (5·4 g.) had m.p. 214°. Found: C, 37·8; H, 5·0; N, 13·1; S, 19·8. $C_{10}H_{15}O_5N_3S_2$ requires C, 37·4; H, 4·7;

N, 13·1; S, 19·9 per cent. Infra-red spectra in Nujol confirmed that the compound had a free hydroxyl group. The compound (0·7 g.) was recovered unchanged after heating under reflux with 3N hydrochloric acid (20 ml.) for 3 hours.

4-(*β-Hydroxypropyl*)-6-*methyl*-7-*sulphamyl*-3,4-*dihydro*-1,2,4-*benzothiadiazine*-1,1-*dioxide*, was obtained by condensation of *N*-(β-hydroxypropyl) 5-methyl-2,4-disulphamylaniline (12·9 g.) in ethoxyethanol (100 ml.) with 40 per cent formaldehyde solution (4 ml.) using a trace of hydrogen chloride as catalyst as described in the preceding preparation. The *product* had m.p. 221–222° after crystallisation from water. Found: C, 39·5; H, 5·2; N, 12·3; S, 19·1. $C_{11}H_{17}O_5N_3S_2$ requires C, 39·4; H, 5·1; N, 12·5; S, 19·1 per cent.

5-*Methyl*-2,4-*disulphamylphenylhydrazine*. A solution of 5-chloro-2,4-disulphamyltoluene (56·8 g.) in 100 per cent hydrazine hydrate (100 ml.) was heated on the steam bath for 5 hours. Excess of hydrazine was removed under reduced pressure and the residue crystallised from water to yield the *product* (52·3 g.), m.p. 227° (decomp.). Found: C, 30·3; H, 4·4; N, 19·9; S, 22·8. $C_7H_{12}O_4N_4S_2$ requires C, 30·0; H, 4·3; N, 20·0; S, 22·8 per cent. The *hydrochloride* had m.p. 206° (decomp.) after crystallisation from methanol-ether. Found: C, 26·7; H, 4·3; N, 17·3; Cl, 11·1; S, 19·9. $C_7H_{13}O_4N_4S_2Cl$ requires C, 26·5; H, 4·1; N, 17·7; Cl, 11·2; S, 20·2 per cent. The phenylhydrazine formed an iso*propylidene* derivative with acetone, which had m.p. 247–248° after crystallisation from ethanol. Found: C, 37·3; H, 5·1; N, 17·5; S, 19·9. $C_{10}H_{16}O_4N_4S_2$ requires C, 37·5; H, 5·0; N, 17·5; S, 20·0 per cent. The *benzylidene* derivative had m.p. 279–281° (decomp.) after crystallisation from aqueous methanol. Found: C, 45·9; H, 4·6; N, 14·8. $C_{14}H_{16}O_4N_4S_2$ requires C, 45·6; H, 4·4; N, 15·2 per cent.

5-*Methyl*-2,4-*disulphamylphenyl azide*. A solution of the phenylhydrazine (5·6 g.) in 2N hydrochloric acid (35 ml.) was treated at 0–5° with sodium nitrite (1·4 g.). The *product* (4·4 g.), separated in pale-yellow prisms of m.p. 194° (decomp.) after crystallisation from 50 per cent ethanol. Found: C, 29·2; H, 3·3; N, 23·8; S, 21·7. $C_7H_9O_4N_5S_2$ requires C, 28·9; H, 3·1; N, 24·1; S, 22·0 per cent. The *azide* was recovered unchanged after heating with 6N hydrochloric acid or 10 per cent potassium hydroxide at 100° for 2 hours.

NN-*Dimethyl*-2,4-*disulphamylphenylhydrazine*. A mixture of 2,4-disulphamylfluorobenzene (5·1 g.) and 1,1-dimethylhydrazine (4·8 g.) was heated under reflux for 4 hours when excess of the hydrazine was boiled off. The residual solid was crystallised from 20 per cent ethanol to yield the *product* (4·55 g.), m.p. 198–199°.

4-*Dimethylamino*-7-*sulphamyl*-3,4-*dihydro*-1,2,4-*benzothiadiazine*-1,1-*dioxide*. The foregoing dimethylphenylhydrazine (5·9 g.) was dissolved in ethoxyethanol (40 ml.) containing paraformaldehyde (0·6 g.) and a trace of hydrogen chloride as catalyst. The mixture was heated on the steam bath for 6 hours when excess of solvent was removed under reduced pressure. The viscous residue was stirred with aqueous sodium acetate to yield the *product* which formed pale-yellow crystals, m.p. 211–213°

after crystallisation from water. Found: C, 35·3; H, 4·3; N, 18·3; S, 20·8. $C_9H_{14}O_4N_4S_2$ requires C, 35·3; H, 4·6; N, 18·3; S, 20·9 per cent.

1-(5-*Methyl*-2,4-*disulphamylphenyl*)-1,2-*diaminoethane hydrochloride.* A solution of 5-chloro-2,4-disulphamyltoluene (28·4 g.) in 1,2-diaminoethane (30 g.) was heated at 140° for 3 hours when excess of amine was removed under reduced pressure. The residual gum was dissolved in water (2 volumes) and the solution brought to pH 6 by the addition of concentrated hydrochloric acid. The *product* (31 g.) had m.p. 267° (decomp.) after crystallisation from ethanol-ethyl acetate or from 95 per cent ethanol.

5-*Chloro*-2,4-*disulphamyl aniline.* A mixture of 2,4-disulphamyl-1,5-dichlorobenzene (30 g.), ethanol (700 ml.) and liquid ammonia (100 ml.) was heated in an autoclave at 150° for 7 hours. The solution was concentrated to about 300 ml., when the *product* (22 g.) crystallised on cooling. It had m.p. 256–258° after crystallisation from water. Found: C, 25·3; H, 3·1; N, 14·6. Calc. for $C_6H_8O_4N_3S_2Cl$: C, 25·2; H, 2·8; N, 14·7 per cent.

5-*Chloro*-2,4-*disulphamyl*-N-(*β-hydroxyethyl*)-*aniline.* 2-Hydroxyethylamine (6·5 ml., 2 mole equiv.) was added to a hot solution of 2,4-disulphamyl-1,5-dichlorobenzene (15·2 g.) in ethane diol (25 ml.) and the mixture heated at 130° for 1·5 hours. Excess of diol was removed at 100°/0·2 mm. and the viscous residue stirred with water (125 ml.). The solid (13·6 g.) which separated had m.p. 200–202° after crystallisation from water.

1,5-*Di*-(*β-hydroxyethyl*)-*amino*-2,4-*disulphamylbenzene.* A solution of 2,4-disulphamyl-1,5-dichlorobenzene (15·2 g.) in 2-hydroxyethylamine (24 ml., 8 mole. equiv.) was heated on the steam bath for 15 hours when excess of base was distilled off at 0·1 mm. Trituration of the residue with water (100 ml.) yielded solids (12 g.) which had m.p. 200–202° after crystallisation from water. The melting point was strongly depressed on admixture with the foregoing mono-substituted product.

6-*Chloro*-4-(*β-hydroxyethyl*)-7-*sulphamyl*-3,4-*dihydro*-1,2,4-*benzothiadiazine*-1,1-*dioxide.* A suspension of 5-chloro-2,4-disulphamyl-*N*-(*β*-hydroxyethyl)-aniline (16·5 g.) and paraformaldehyde (1·65 g.) in 2-ethoxyethanol (100 ml.) was warmed on the steam bath and treated with a saturated solution of hydrogen chloride in 2-ethoxyethanol (2 ml.), when all solids dissolved immediately. The solution was heated for 2 hours when the solvent was distilled off under reduced pressure. The solid residue (17 g.) crystallised from water to yield the *product* (11·2 g.), m.p. 204–206°. Found: C, 32·1; H, 3·4; N, 11·9; Cl, 10·4. $C_9H_{12}O_5N_3S_2Cl$ requires C, 31·6; H, 3·6; N, 12·3; Cl, 10·4 per cent. The melting point was strongly depressed on admixture with the starting material.

4,5-Di-(*β-Hydroxyethyl*)-1,8-*dithia*-2,4,5,7-*tetraza*-1,2,3,4,5,6,7,8-*octahydroanthracene*-1,1,8,8-*tetroxide.* A suspension of 1,5-di-(*β*-hydroxyethyl)-amino-2,4-disulphamylbenzene (14·2 g.) and paraformaldehyde (2·64 g.) in 2-ethoxyethanol (50 ml.) was heated on the steam bath and treated with a saturated solution of hydrogen chloride in 2-ethoxyethanol (2 ml.), when all solids dissolved. The solution was heated for 15

minutes when the *product* (10·5 g.) separated. It had m.p. 238°, not raised by a further crystallisation from water. Found: C, 38·1; H, 4·6; N, 15·2; S, 16·7. $C_{12}H_{18}O_6N_4S_2$ requires C, 38·1; H, 4·8; N, 14·8; S, 16·9 per cent.

2,4-*Disulphamyl*-N-(*β,γ-dihydroxypropyl*)-*aniline*. A solution of 2,4-disulphamylchlorobenzene (27 g.) in 2,3-dihydroxypropylamine (50 ml.) was heated on the steam bath for 3 hours. Excess of amine was removed at 0·1 mm. when the residual gum solidified on trituration with water. The *product* had m.p. 190–192° after crystallisation from water.

2,4-*Disulphamyl*-N-*bis*-(*β-hydroxyethyl*)-*aniline*. A solution of 2,4-disulphamylfluorobenzene (15 g.), and di-β-hydroxyethylamine in 2-ethoxyethanol (50 ml.) was heated under reflux for 2 hours and excess of volatile material then removed at 100°/0·1 mm. The viscous residue was extracted with hot chloroform to remove excess of di-β-hydroxyethylamine and its hydrochloride. The viscous material solidified overnight and the *product*, obtained by recrystallisation from water, had m.p. 160–162°.

5-*Chloro*-2,4-*disulphamylphenylhydrazine*. A suspension of 2,4-disulphamyl-1,5-dichlorobenzene (31 g.), in 20 per cent aqueous hydrazine hydrate (100 ml.) was heated under reflux. All solid dissolved after about 10 minutes then the *product* (26 g.) crystallised rapidly. It had m.p. 243° (decomp.) after crystallisation from water.

2,4-*Disulphamyl*-1,5-*phenylenedihydrazine*. A mixture of 2,4-disulphamyl-1,5-dichlorobenzene (6·2 g.), and 90 per cent hydrazine hydrate (10 ml.) was heated under reflux when the solid dissolved rapidly and crystals began to separate after about 15 minutes. Heating was continued for 1 hour when the mixture was cooled and the *product* (4·9 g.) collected. It had m.p. 228° (decomp.) after crystallisation from water.

3-*Sulphamyl*-5a-aza-*isophenthiazine*-5,5-*dioxide*. A mixture of 2,4-disulphamylfluorobenzene (5·1 g.) and 2-aminopyridine (1·9 g.) was heated with occasional stirring at 150° for 12 hours. Trituration of the hot residue with methanol furnished solids (2·47 g.), m.p. 338° (decomp.). Crystallisation from ethane diol yielded the *product* in yellow leaflets, m.p. 343° (decomp.). Found: C, 42·2; H, 3·3; N, 13·4; S, 20·4. $C_{11}H_9O_4N_3S_2$ requires C, 42·4; H, 2·9; N, 13·5; S, 20·6 per cent.

2-*Chloro*-3-*sulphamyl*-5a-aza-*isophenthiazine*-5,5-*dioxide*. An intimate mixture of 5-chloro-2,4-disulphamylfluorobenzene (5·8 g.) and 2-aminopyridine (3·8 g.) was heated at 140–160° for 4 hours with occasional stirring. Trituration of the hot residue with methanol yielded the *product* (1·4 g.), m.p. 375° (decomp.). The m.p. was not raised by crystallisation from ethane diol. Found: C, 38·4; H, 2·1; N, 12·0; Cl, 10·4; S, 18·8. $C_{11}H_8O_4N_3S_2Cl$ requires C, 38·2; H, 2·3; N, 12·1; Cl, 10·3; S, 18·6 per cent.

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THE EFFECT OF PYROGEN FROM *E. COLI* ON THE ACTIVITY OF SUCCINIC ACID DEHYDROGENASE IN LIVER MITOCHONDRIA

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Received July 7, 1960

To investigate more accurately the effect of pyrogen from *E. coli* on liver metabolism, its influence on mitochondrial fractions and on the activity of succinic acid dehydrogenase in this fraction has been studied. It appears that the pyrogen acts by directly activating the enzyme system chosen. Antagonism by malonate confirms the enzymatic and specific character of the phenomenon. No effect of pyrogen was found on the activity of lactic acid dehydrogenase. The results further the conception of a peripheral action of pyrogen.

In our previous studies[1–3] it was possible, amongst other things, to demonstrate increase of oxygen consumption in the sections of liver by the pyrogen from *Escherichia coli*. This action takes place both after the administration of pyrogen incubated with serum, directly into the section of liver *in vitro*, and also in rabbits killed at the peak of post-pyrogenic fever. This suggests the possibility that the increase of temperature here is of a peripheral character and the increase of metabolism is a primary phenomenon.

To investigate more accurately the effect of our pyrogen on liver metabolism we have studied its influence on mitochondrial fractions and on the activity of succinic acid dehydrogenase in this fraction.

Material and Methods

Rabbits of determined sensitivity to pyrogen were used. After killing the animals and bleeding, the tissue for examination was immediately taken and placed on ice in a small vessel.

Pyrogen from *E. coli* was obtained by the method of Palmer and Gerlough[4] as modified by Westphal, Luderitz, Eichenberger, and Keiderling[5]. The pyrogen was added to Warburg vessels and incubated for 1 hour at 37° with rabbit serum diluted with an equal amount of fluid of the following composition: NaCl, 0·154M 48 ml., KCl, 0·154M 1 ml., $CaCl_2$ 0·11M 1 ml., phosphate buffer 1/15M pH 7·2 5 ml., glucose 0·1 g.

After homogenising in 0·25M sucrose the liver homogenate was fractionated by centrifugation[6,7]. Nuclei and large residues were separated by centrifugation at 700 *g* and mitochondria were obtained at 4,500 *g*. After resuspension and rinsing they were again separated at 12,500 *g*. All the procedures were carried out below 4°.

The activity of succinic acid dehydrogenase was determined in the Warburg apparatus employing Slater and Bonner's[8] manometric method in which oxygen consumption is determined during the oxidation of sodium succinate in the presence of potassium cyanide as inhibitor of cytochrome and methylene blue as acceptor of hydrogen. For this purpose

there was added to the Warburg vessels: 0·8 ml. phosphate buffer, 0·2M; 0·2 ml. pyrogen in the amount of 2 ng. or 0·002 ng.; 0·2 ml. sodium succinate, 0·4M; 0·3 ml. methylene blue, 0·01M; 0·3 ml. KCN, 0·1M; 0·5 ml. suspension of mitochondria from 50 mg. of tissue and into the middle part 0·2 ml. of 10 per cent NaOH.

Succinic acid dehydrogenase was inhibited by adding 0·2 ml. of sodium malonate, 1·6M.

The activity of lactic acid dehydrogenase was estimated by means of Green and Brosteaux's[9] method which consists of the measurement of oxygen consumption during the oxidation of sodium lactate in the presence of methylene blue. The following were added into the Warburg vessels: 0·2 ml. sodium lactate, 2M; 0·2 ml. of pyrogen, 2 ng.; 0·1 ml. methylene blue, 0·0017M; 1·8 ml. mitochondria suspension from 180 mg. of tissue in phosphate buffer 7·2, and into the middle part 0·2 ml. of 10 per cent NaOH.

RESULTS

Succinic acid dehydrogenase is one of the most abundant enzymes concerned with oxygen consumption. Thus, it was the first enzyme which attracted our attention after we observed the increase of respiration

TABLE I

THE EFFECT OF PYROGEN ON THE ACTIVITY OF SUCCINIC ACID DEHYDROGENASE

Substrate	Number of experiments	O_2 consumption in microlitres by 1 mg. of dry mass of mitochondria during 2 hours	P
Mitochondria	35	88·2 ± 2·96	—
Mitochondria with incubated serum	31	114·8 ± 3·06	$P_1 < 0{\cdot}001$
Mitochondria with 2 ng. incubated pyrogen	26	167·2 ± 3·20	$P_1 < 0{\cdot}001$ $P_2 < 0{\cdot}001$
Mitochondria with 0·002 ng. incubated pyrogen	24	230·4 ± 11·29	$P_1 < 0{\cdot}001$ $P_2 < 0{\cdot}001$
Mitochondria from a rabbit killed at the peak temperature	37	120·4 ± 3·22	$P_1 < 0{\cdot}001$
Mitochondria with 0·002 ng. incubated pyrogen and sodium malonate	20	14·7 ± 1·95	$P_4 < 0{\cdot}000001$

of liver sections by pyrogens. Numerous authors[10–12] have demonstrated the main site of succinic acid dehydrogenase to be the mitochondria, which we, in turn, have used as an enzyme source. Pyrogen incubated in the serum was added to the vessel in amounts of 2 ng. and 0·002 ng. for 2·5 ml. of liquid. The latter quantity corresponds approximately to the amount of pyrogen which would reach 100 mg. of liver with a pyrogen injection of 0·02 ng./kg. In one group of the experiments the activity was examined in mitochondria obtained from rabbits killed at the peak of fever after intravenous injection of pyrogen in the dose of 0·2 ng./kg.

To confirm the specificity of the observed mechanism in one group of experiments we made measurements in the environment containing 0·2 ml. of 1·6M sodium malonate in addition to the proper substrate.

Double control was possible by estimating the activity of mitrochondria in normal environment and in the environment enriched by serum. The results obtained are presented in Table I.

Another enzyme which we considered in our study was lactic acid dehydrogenase. Because of its less abundant appearance as a source of mitochrondria we have used 180 mg. of liver for each vessel. To pyrogen incubated with serum we have added the concentration of 2 ng./2·5 ml. of liquid. The results obtained are presented in Table II.

TABLE II

THE EFFECT OF PYROGEN ON THE ACTIVITY OF LACTIC ACID DEHYDROGENASE

Substrate	Number of experiments	O_2 consumption in microlitres by 1 mg. of dry mass of mitochondria during 2 hours	P
Mitochondria	27	16·35 ± 0·65	—
Mitochondria with incubated serum	18	12·10 ± 0·68	$P_1 < 0·001$
Mitochondria with 2 ng. incubated pyrogen	20	13·90 ± 0·65	$P_1 = 0·02$ $P_2 = 0·07$

DISCUSSION

The system of succinic acid dehydrogenase chosen by us is one of the more important oxidising systems and the basic link of the Krebs cycle. From our studies it appears that pyrogen from *E. coli* acts by directly activating this system. This activity is reflected by the effect of minute amounts of pyrogen which confirms its observed action on the metabolism of liver cells. Antagonism of the described phenomenon by malonate confirms its enzymatic and specific character. No effect of pyrogen was found on the activity of lactic acid dehydrogenase. Our results are a further step in favour of the conception of a peripheral action of pyrogen which does not negate and does not exclude the existence of a central action suggested by numerous authors. The problem whether the system of succinic acid dehydrogenase is the only one which becomes activated under the influence of pyrogen remains an open question.

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THE ANTI-INFLAMMATORY ACTION OF GRISEOFULVIN IN EXPERIMENTAL ANIMALS

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Received July 19, 1960

When tested by the cotton-pellet method in rats or the tuberculin-hypersensitivity test in guinea pigs, griseofulvin had a marked anti-inflammatory action. The activity was, weight for weight, less than that of the corticosteroids tested concurrently. The anti-inflammatory action of griseofulvin appears to be independent of any effect upon the pituitary-adrenal axis.

Griseofulvin was first isolated in 1939 from *Penicillium griseofulvum* Dierckx[1], and its chemical structure was established some years later[2]. It was then shown to have antifungal activity *in vitro*, especially against dermatophytes. It is effective orally in the treatment of ringworm in guinea pigs[3], cattle[4] and man[5], producing no untoward effects. It seems that this *in vivo* antifungal action is greater than might have been predicted from *in vitro* tests. In experiments with guinea pigs infected with *Microsporum canis* and treated with griseofulvin, Gentles[3] observed that the "highly inflammatory reaction which developed in all the control animals was prevented". Cochrane and Tullett[6] investigated the griseofulvin treatment of acute inflammatory cattle ringworm in man and reported that the antibiotic caused a rapid disappearance of general malaise, pain and discomfort; they also observed "a rapid disappearance of all inflammatory signs". The work reported here was initiated to enquire whether griseofulvin has an anti-inflammatory action in addition to known antifungal properties.

Experimental

Drugs Tested

In all the experiments on rats and mice, griseofulvin was administered orally as a suspension in 5 per cent gum acacia, each dose in a volume of 0·5 ml./ 100 g. weight. In guinea pigs the antibiotic was administered orally as a suspension in physiological saline with 1:4000 w/v of the wetting agent Triton WR1339, each dose in a volume of 0·5 ml./540 g. weight.

For comparison cortisone acetate was administered orally to rats and mice, except when otherwise stated in the text, in a similar volume per dose; a stock suspension containing 25 mg./ml. was diluted in saline or distilled water to the desired concentration. The guinea pigs received hydrocortisone acetate by subcutaneous injection, each dose of 25 mg./kg. in a volume of 0·5 ml./500 g. weight. Hydrocortisone was used instead of cortisone in guinea pigs, being better tolerated on subcutaneous

injection. In some experiments, rats were each injected daily with a subcutaneous dose of a long-acting corticotrophin preparation (Cortrophin ZN, Organon) of five international units.

Methods

The cotton pellet test in rats[7]. Male albino rats 100–120 g. weight were used. Sterilised cotton wool pellets of known weight (7–10 mg.) were implanted subcutaneously in groups of 5 rats under ether anaesthesia; 4 pellets were placed in each animal, one in each groin and one in each axilla. Griseofulvin was administered orally at doses of 62·5–500 mg./kg. weight on 4 consecutive days, the first dose being given immediately after implantation of the pellets. On the fifth day the rats were killed with chloroform and the pellets dissected; all fat and extraneous tissue was removed, and the pellets dried overnight in an hot air oven at 60°. Groups of control rats received oral doses of tap water in place of the griseofulvin suspension. The weight of tissue in each pellet was calculated, and the mean value for each group was compared with that of the controls. Some additional experiments were done on adrenalectomised rats.

The tuberculin skin sensitivity test. A modification of the methods of Long and Miles[8] was used. Albino guinea pigs of both sexes and weighing 500–600 g. were maintained on a pelleted diet and tap water. They were made sensitive to tuberculin by intramuscular injection of 0·1 mg. (moist weight) of *Mycobacterium BCG* 4–6 weeks before the test and were randomised into groups of 8–10. One group was left untreated and the others were treated with either griseofulvin at doses from 10–250 mg./kg. weight, or with hydrocortisone acetate, 25 mg./kg. The animals were tested by injecting old tuberculin intradermally at two doses, 10 T.U. and 100 T.U. in 0·1 ml. volume, into the depilated flank. Three treatment doses of griseofulvin or hydrocortisone were given at 18 and 2 hours before and 6 hours after the tuberculin test.

The diameters of the zones of oedema caused by the injections were measured with calipers. By plotting the average diameters against the log dose of tuberculin in the control group a standard curve was obtained, from which the apparent potencies of the tuberculin in the treated groups were measured. Thus, for example, if the standard tuberculin when measured on the treated guinea pigs appeared to have 40 per cent of its true potency, this was recorded as a "60 per cent reversal" of the local tuberculin sensitivity in the treated group.

The formalin foot test. The method of Selye[9], as modified by Buttle, D'Arcy, Howard and Kellett[10], was used.

Male albino rats, weighing 100–200 g. were maintained on a pellet diet and tap water. Griseofulvin was administered orally to groups of 8 animals at doses ranging from 62·5–2,500 mg./kg. weight. One and a half hours afterwards, the volume of the left hind-foot of each rat was measured by means of the apparatus described by Buttle and his colleagues[10], and 0·1 ml. of a 3 per cent solution of formaldehyde was injected into the plantar aponeurosis of the foot. A second dose of griseofulvin was administered 3 hours after the formalin injection.

Control groups of rats were also injected with formalin solution and received oral doses of 5 per cent gum acacia alone. The changes in volume of the injected feet were noted in each treated and control rat at periods of $4\frac{1}{2}$ and 6 hours after the formalin injection. For some experiments, the rats were adrenalectomised before being used in the tests.

The "cold stress" test. Normal and adrenalectomised mice were tested for protection against cold stress by a method previously described[11]. The animals were maintained on a pelleted diet and tap water.

Protection of histamine-sensitised mice. Parfentyev and Goodline[12] showed that mice were sensitised to the lethal effect of injected histamine by the intravenous injection of *H. pertussis* vaccine and that the sensitivity was reversed by the administration of cortisone. This method was used

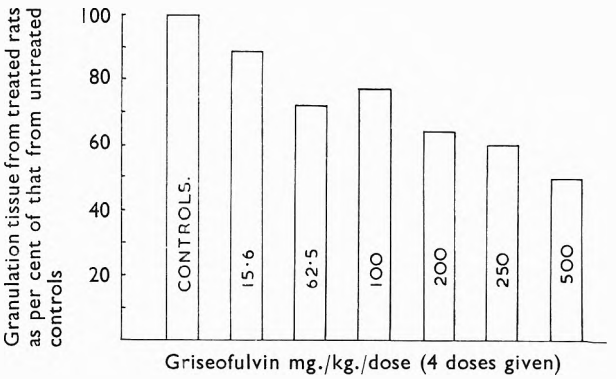

FIG. 1. The inhibitory effect of griseofulvin on the formation of granulation tissue around subcutaneously implanted cotton pellets. Griseofulvin administered orally daily for 4 consecutive days. Each column represents amount of granulation tissue, expressed as a percentage of that from untreated control animals. Groups of 5 or 10 rats used; four cotton pellets implanted in each rat.

to compare the effect of griseofulvin in oral doses up to 200 mg./kg., with that of cortisone by subcutaneous doses up to 100 mg./kg. Female albino mice of the A2G strain[13] (15–16 g.) were used and maintained on a pelleted diet and tap water.

The liver glycogen-deposition test. The actions of griseofulvin and cortisone on the deposition of glycogen in the livers of fasting adrenalectomised mice were compared by the method of Venning, Kazmin and Bell[14].

Effects on the adrenal cortex. The alterations in weight and histological appearance of the adrenal cortex after prolonged administration of griseofulvin and cortisone in rats were examined by a method previously described[15]. In similar experiments the effect of prolonged daily administration of griseofulvin was compared with that of Cortrophin ZN.

RESULTS

In the cotton pellet test on rats, griseofulvin inhibited the formation of granulation tissue around subcutaneously implanted cotton pellets. The combined results of several experiments are shown graphically in Figure 1,

from which it can be seen that griseofulvin produces a graded reduction in formation of granulation tissue over the oral dose range 15·6–500 mg./kg. daily for 4 consecutive days. Griseofulvin is less active than cortisone in the cotton pellet test. Figure 2 shows the results of three experiments in

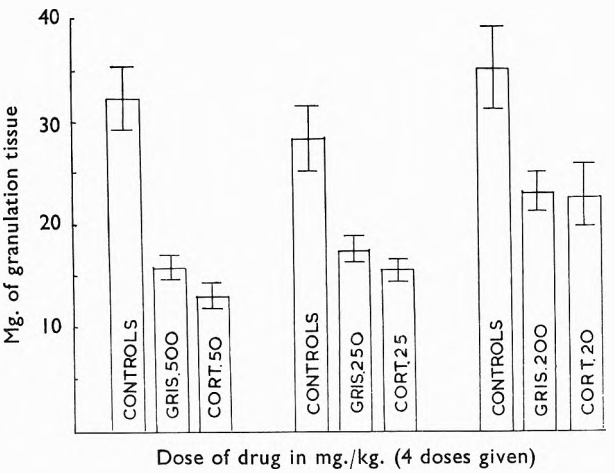

FIG. 2. Comparison between effects of griseofulvin (Gris.) and cortisone acetate (Cort.) on formation of granulation tissue around subcutaneously implanted cotton pellets. Both drugs orally administered daily for 4 consecutive days. Each column represents mean weight of granulation tissue in a group of five rats; four cotton pellets implanted in each rat; the vertical lines represent the standard errors.

TABLE I

THE EFFECT OF GRISEOFULVIN ON SKIN SENSITIVITY TO TUBERCULIN IN TUBERCULIN-POSITIVE GUINEA PIGS

Substance	Dose level (mg./kg./dose*)	Route of administration	No. of observations	Mean percentage reduction in tuberculin sensitivity and range
Griseofulvin	250	Oral	1	85
Griseofulvin	100	Oral	2	75 (62–87)
Griseofulvin	50	Oral	6	66 (58–82)
Griseofulvin	25	Oral	2	47 (36–57)
Griseofulvin	10	Oral	2	22 (20–23)
Controls—no treatment	0	—	8	0
Hydrocortisone acetate ..	25	Subcutaneous	5	68 (54–78)

* Three doses of griseofulvin were given orally, 18 and 2 hours before and 6 hours after intradermal injections of tuberculin.

which an attempt was made to compare directly the two compounds at equivalent response levels. The results indicate that griseofulvin has about one-tenth the activity of cortisone acetate in this test, since 200 mg./kg. griseofulvin produced a response similar to that of 20 mg./kg. of cortisone acetate; 250 mg./kg. griseofulvin was equivalent to 25 mg./kg. cortisone acetate and 500 mg./kg. griseofulvin was similar in its effect to 50 mg./kg. cortisone acetate.

In tuberculin-positive guinea pigs, the oral administration of griseofulvin caused a reduction in skin sensitivity to tuberculin, the percentage reduction being graded to the amount of griseofulvin (Table I). In this test

oral griseofulvin appears to have about one-third the activity of subcutaneous hydrocortisone, since a 25 mg./kg. dose of the latter caused 68 per cent reversal and 50–100 mg./kg. of griseofulvin gave the same effect. In other experiments, not recorded in detail here, it was found that

TABLE II

COMPARISON BETWEEN EFFECTS OF GRISEOFULVIN AND CORTISONE ACETATE ON INDICES OF ADRENOCORTICAL ACTIVITY IN THE RAT

Test	Griseofulvin		Cortisone acetate	
	Dose	Result	Dose	Result
(1) Protection of adrenalectomised mice against cold stress	5 g./kg. orally in 2 doses	No protection	62·5 mg./kg. orally in 2 doses	Protection
(2) Protection of mice sensitised to histamine (by *B. pertussis* vaccine) against intraperitoneal challenge with histamine	200 mg./kg. orally in 3 doses	No protection	100 mg./kg. subcutaneously in 2 doses	Protection
(3) Deposition of glycogen in livers of fasting adrenalectomised mice	2·5 g./mouse orally in divided doses	No glycogen deposition	5 mg./kg. orally	Deposition of glycogen
(4) Effect of prolonged administration on:				
(i) Weight of adrenal gland	2 g./kg. orally daily for 6 weeks	(i) No effect	200 mg./kg. orally daily for 6 weeks	(i) Decrease in adrenal weight
(ii) Adrenal tissue (histological examination)		(ii) No effect		(ii) Atrophy of zona fasciculata; depletion of sudanophilic lipoid

TABLE III

EXPERIMENTS TO DETERMINE WHETHER THE ANTI-INFLAMMATORY ACTIVITY OF GRISEOFULVIN ON THE RAT IS INDEPENDENT OF THE PITUITARY-ADRENAL AXIS

Test	Griseofulvin+	Cortisone acetate+	Cortrophin-ZN*
(1) Cotton pellet test in adrenalectomised rats Cotton pellet test in normal rats	Inhibition of formation of granulation tissue to an equal extent	Inhibition of formation of granulation tissue to an equal extent	—
(2) Formalin foot tests in adrenalectomised rats Formalin foot test in normal rats	Inhibition of formalin-induced swelling to an equal extent	Inhibition of formalin-induced swelling to an equal extent	—
(3) Protection of normal mice against cold stress	No protection	Protection	—
(4) Effect of prolonged administration on adrenal weight in normal rats	No effect	Adrenal atrophy	Adrenal hypertrophy

+ Dose levels as in Table II.

* Five I.U./rat subcutaneously daily for 6 weeks.

subcutaneous griseofulvin had a much smaller effect, presumably owing to the slow absorption of the insoluble antibiotic from the site of injection.

The results by the formalin foot technique were inconsistent. Griseofulvin given orally to rats at the high dose levels of 2 × 5,000 mg./kg. and 2 × 2,500 mg./kg. inhibited the formalin-induced swelling of the rats'

feet but at lower doses, down to 125 mg./kg., the results were variable. It appears that the formalin foot technique is not sufficiently sensitive to assist in evaluating this action of griseofulvin, and the results are not therefore reported here in detail.

The results of the "cold stress" tests in adrenalectomised mice, the histamine sensitisation tests in mice, the liver glycogen-deposition tests and the tests on the weight and histological appearance of the adrenal cortex are summarised in Table II. They show that, although cortisone acetate gives a positive result in all of these tests, griseofulvin at much higher dose levels has no activity.

Griseofulvin and cortisone acetate were further compared by cotton pellet tests and formalin foot tests in *adrenalectomised* and normal rats and in *normal* mice for protection against cold stress. Griseofulvin was also compared with Cortrophin ZN for any possible effect in producing hypertrophy of the adrenal cortex on prolonged administration to normal rats. The results are summarised in Table III.

Discussion

Griseofulvin was shown by the cotton pellet method in rats and the tuberculin skin sensitivity method in guinea pigs to have "anti-inflammatory" activity. This evidence was supported by some of the results of the formalin foot tests. The activity was about one-third to one-tenth that of cortisone acetate, depending on the method.

Our further experiments questioned whether the action of griseofulvin was due to a cortisone-like activity or to an entirely different mechanism. From the results of the cold-stress tests in adrenalectomised mice, the histamine sensitisation tests in mice and the liver glycogen-deposition tests in adrenalectomised mice and from the studies on the weight and morphology of the adrenal cortices of rats (Table II), it was evident that the anti-inflammatory effect of griseofulvin could not be due to any "cortisone-like" action.

Another possible explanation for the action of griseofulvin was that the substance might owe its activity in the anti-inflammatory tests to an activation of the pituitary-adrenal axis of the experimental animals. Tests by the cotton pellet and formalin foot methods in normal and adrenalectomised rats, by cold stress tests in normal mice and for a possible effect in producing hypertrophy of the adrenal cortex on prolonged administration in normal rats (Table III) showed that this could not be occurring. Whereas in the cotton pellet and formalin foot tests griseofulvin and cortisone acetate were active whether the animals were adrenalectomised or not, normal mice (with their adrenals intact) were not protected against cold stress by griseofulvin but were by cortisone. Moreover, in normal rats prolonged administration of cortisone produced atrophy of the adrenals and Cortrophin-ZN caused hypertrophy, but griseofulvin had no effect.

It is therefore clear that the anti-inflammatory action of griseofulvin does not depend on stimulation of the adrenal gland or on potentiation of its normal steroid secretion. In the cold stress test, if griseofulvin had

any action in increasing either output or utilisation of adrenocortical secretion, this would be reflected by protection of the animals. Moreover, since griseofulvin on prolonged administration to rats does not cause hypertrophy of the adrenals as judged by weight and tissue structure, it appears that it can neither cause an increased secretion of pituitary corticotrophin nor exert a "corticotrophin-like" action of its own.

It thus appears likely that griseofulvin owes its anti-inflammatory activity to some direct action of unknown nature at the site of inflammation.

Acknowledgements. We express our thanks to Mr. J. J. Grimshaw for invaluable co-operation in the statistical analyses and gratefully acknowledge the technical assistance of Miss C. Spearing.

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THE IDENTIFICATION AND DETERMINATION OF NITROGENOUS ORGANIC BASES WITH AMMONIUM REINECKATE

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Received July 13, 1960

The identification and determination of several important bases as their reineckates is described. The ultra-violet and visible spectra of reineckates were studied and used for the determination of molecular weights and solubilities and for the determination of bases. This paper also records as yet unreported constants of the characteristic mono- and di-reineckate derivatives of several clinically important compounds. A method for the regeneration of the conjugate bases from the reineckates using ion exchange resins is also given.

Many organic bases react with ammonium reineckate to form derivatives which are useful for their identification and determination. Microscopic examination and melting point determinations of the isolated complexes serve as useful means for the characterisation of many basic reineckates[1–6]. Reineckates can be quantitatively determined either gravimetrically, colorimetrically or by titration[7–16].

Although microscopic examination and melting point determinations are useful they are often insufficient to permit unequivocal identification of reineckates which possess similar micro-crystals or overlapping decomposition temperatures. The ultra-violet and visible absorption spectra are now shown to offer additional parameters for distinguishing these reineckates. The regeneration of the conjugate bases from their reineckates using ion exchange resins has also been investigated.

EXPERIMENTAL

Apparatus, reagents and solutions. Beckman Model DU Spectrophotometer; 1 cm. quartz cells; A.R. acetone; ammonium reineckate solution, approximately 2 per cent solution prepared by dissolving 2 g. of ammonium reineckate in 100 ml. cold water and filtering through a Whatman No. 42 paper; ion exchange resin Permutit De-Acidite FF. The bases used as listed in the tables were commercial products and were not further recrystallised.

Preparation of Reineckates of Mono-basic Compounds

Excess ammonium reineckate solution was added slowly with constant stirring to a solution of the base in 0·1N hydrochloric acid. After cooling to 0° the precipitate was filtered and washed with water to remove excess ammonium reineckate solution. The products were purified by recrystallisation in 60 per cent ethanol at 60° (higher temperatures cause decomposition of some reineckates). Usually two recrystallisations gave compounds sufficiently pure for physico-chemical characterisation.

Preparation of Di-reineckates of Dibasic Compounds

About 100 mg. of the dibasic compound or its salt was dissolved in 20 ml. of 0·1N hydrochloric acid. Excess ammonium reineckate solution

was added and the precipitate formed was filtered and dried over phosphorus pentoxide. The di-reineckates thus obtained were sufficiently pure for chromium determination. Repeated recrystallisation from 60 per cent ethanol gave the mono-derivatives of most of the substances studied with the exception of quinine, doxylamine and mepyramine di-reineckates.

TABLE I

PHYSICO-CHEMICAL CHARACTERISATION OF AMINE-REINECKATES

Base	Mol. composition of reineckate	Decomposition temperature °C.	Chromium analyses		Molecular weight		E (1 g.mol./l., 1 cm.)	Solubility in water g./100 ml.	
			Calc. per cent	Found per cent	Calc.	Found		27° C.	5° C.
Opium alkaloids									
Morphine	$C_{21}H_{26}CrN_7O_3S_4$	204–208	8·61	8·65	604	599	105·8	0·008	0·008
Codeine	$C_{22}H_{28}CrN_7O_3S_4$	189–193	8·41	8·47	618	618	106·4	0·021	0·011
Thebaine	$C_{23}H_{28}CrN_7O_3S_4$	196–200	8·25	8·27	630	631	106·6	0·002	0·003
Narcotine	$C_{26}H_{30}N_7O_7S_4$	150–153	7·10	7·18	732	745	104·9	0·010	0·010
Papavarine	$C_{24}H_{28}N_7O_4S_4$	207–211	7·91	8·05	—	—	110·0	0·0	0·0
Synthetic narcotics									
Ketobemidone	$C_{19}H_{28}CrN_7O_3S_4$	173–176	9·18	9·12	566	564	106·1	0·007	0·005
Pethidine	$C_{19}H_{28}CrN_7O_3S_4$	136–138	9·18	9·26	566	565	106·3	0·019	0·011
Alphaprodine	$C_{20}H_{28}CrN_7O_3S_4$	172–174	8·96	9·01	578	583	107·4	0·008	0·006
Pipidone	$C_{28}H_{36}CrN_7OS_4$	164–166	7·78	7·82	668	665	106·2	0·021	0·006
Methadone	$C_{25}H_{34}CrN_7OS_4$	160–164	8·28	8·36	628	630	106·7	0·019	0·011
Phenadoxone	$C_{27}H_{36}CrN_7O_2S_4$	162–165	7·76	7·79	670	675	107·1	0·009	0·010
Racemorphan	$C_{21}H_{30}CrN_7OS_4$	165–167	9·02	8·97	576	581	107·3	0·013	0·007
Metopon	$C_{22}H_{28}CrN_7O_4S_4$	246–253	8·42	8·32	618	628	106·9	0·035	0·012
Diacetylmorphine	$C_{25}H_{30}CrN_7S_4O_5$	180–185	7·55	7·58	688	682	105·7	0·020	0·015
Sulphonamides									
Sulphacetamide	$C_{12}H_{17}CrN_8O_3S_5$	134–137	9·76	9·67	—	—	—	0·380	0·360
Sulphathiazole	$C_{13}H_{16}CrN_9O_2S_6$	172–175	9·08	9·22	574	573	106·4	0·076	0·028
Sulphadiazine	$C_{14}H_{17}CrN_{10}O_2S_5$	192–194	9·15	9·06	569	567	106·1	0·045	0·022
Sulphapyridine	$C_{15}H_{16}CrN_9O_2S_5$	177–180	9·16	9·27	568	568	106·5	0·019	0·019
Sulphamerazine	$C_{15}H_{19}CrN_{10}O_2S_5$	189–191	8·94	8·81	583	580	106·1	0·053	0·028
Sulphamethazine	$C_{16}H_{21}CrN_{10}O_2S_5$	144–147	8·71	8·52	597	595	106·1	0·032	0·022
Antihistamines									
Diphenhydramine	$C_{21}H_{28}CrN_7OS_4$	178–180	9·07	9·12	574	575	106·8	0·016	0·04
Promethazine	$C_{21}H_{27}CrN_8S_5$	155–157	8·62	8·51	603	604	106·8	0·001	0·0
Pecazine	$C_{23}H_{29}CrN_8S_5$	188–190	8·29	8·27	629	626	106·2	0·0	0·0
Antazoline	$C_{21}H_{24}CrN_8S_4$	159	8·91	8·85	584	581	106·1	0·032	0·0
Phenindamine	$C_{23}H_{28}CrN_7S_4$	148–150	8·96	8·80	580	574	105·4	0·008	0·005
Doxylamine	$C_{21}H_{29}CrN_8S_4O$	145	8·84	8·84	589	587	106·3	0·004	0·0
Doxylamine	$C_{24}H_{36}Cr_2N_{16}OS_8$*	153–155	11·45	11·45	—	—	—	0·008	0·0
Mepyramine	$C_{21}H_{29}CrN_9OS_4$	142–143	8·62	8·59	603	604	106·6	0·003	0·0
Mepyramine	$C_{35}H_{47}Cr_2N_{15}OS_8$*	135–138	11·05	11·08	—	—	—	0·004	0·0
Methapyrilene	$C_{18}H_{26}CrN_9S_5$	152–155	8·96	8·78	580	581	106·7	0·027	0·0
Methapyrilene	$C_{22}H_{32}Cr_2N_{16}S_9$*	144–147	11·55	11·55	—	—	—	0·013	0·007
Chlorothan	$C_{18}H_{25}ClCrN_8S_5$	162–165	8·46	8·44	615	614	106·3	0·009	0·008
Chlorothan	$C_{22}H_{32}ClCr_2N_{16}S_9$*	134–138	11·15	11·15	—	—	—	0·0	0·0
Chlorpheniramine	$C_{20}H_{26}ClCrN_8S_4$	130–135	8·78	8·80	593	593	106·5	0·0	0·0
Chlorpheniramine	$C_{24}H_{32}ClCr_2N_{14}S_8$*	103–107	11·4	11·30	—	—	—	0·001	0·0
Chlorcyclizine	$C_{22}H_{28}ClCrN_8S_4$	190–193	8·42	8·42	619	616	106·1	0·024	0·016
Chlorcyclizine	$C_{26}H_{36}ClCr_2N_{14}S_8$*	162–164	11·09	11·02	—	—	—	0·0	0·0
Thonzylamine	$C_{20}H_{29}CrN_{10}OS_4$	154–157	8·60	8·43	605	604	106·3	0·0	0·0
Thonzylamine	$C_{24}H_{38}Cr_2N_{16}OS_8$*	136–138	11·24	11·29	—	—	—	0·028	0·015
Antergan	$C_{21}H_{29}CrN_8S_4$	150–153	9·08	9·11	573	574	106·7	0·004	0·0
Antergan	$C_{25}H_{36}Cr_2N_{14}S_8$*	105–107	11·65	11·71	—	—	—	0·0	0·0
Others									
Quinine	$C_{24}H_{31}CrN_8O_2S_4$	216–218	8·10	8·00	643	640	106·2	0·007	0·003
Quinine	$C_{28}H_{38}Cr_2N_{16}O_2S_8$*	145–149	10·80	10·92	—	—	—	0·004	0·00
Cocaine	$C_{21}H_{28}CrN_7O_3S_4$	159–162	8·35	8·39	622	624	106·8	0·009	0·005
Phenazone	$C_{15}H_{18}CrN_8OS_4$	155–157	10·25	10·17	—	—	—	0·034	0·007
Amphetamine	$C_{13}H_{20}CrN_7S_4$	132	11·46	11·43	454	452	106·2	0·120	0·117
Caffeine	$C_{12}H_{17}CrN_{10}O_2S_4$	137–138	10·10	9·84	—	—	—	0·625	0·312
Theobromine	$C_{11}H_{15}CrN_{10}O_2S_4$	165–167	10·45	10·23	—	—	—	0·990	0·445
Aniline	$C_{10}H_{14}CrN_7S_4$	195–197	12·65	12·68	412	411	106·2	—	—
Pyridine	$C_9H_{12}CrN_7S_4$	198–202	13·05	13·17	398	395	105·5	0·037	0·018

* Di-reineckates.

Preparation of Mono-reineckates of Dibasic Compounds

Procedure 1. Repeated recrystallisation of di-reineckates in 60 per cent aqueous ethanol. The di-reineckates were recrystallised thrice from 60 per cent aqueous ethanol at a temperature not exceeding 60°. Under these conditions most of the di-reineckates gave the pure mono-derivatives with the exceptions already stated.

Procedure 2. Formation of the reineckates at 70°. An aqueous solution containing a salt of a dibasic compound was heated to 70°. Ammonium reineckate solution was added with stirring. The solution was cooled and the precipitate filtered. Chromium analyses on the products indicated that all the dibasic substances gave the mono-reineckates with the exception of quinine, doxylamine and mepyramine.

Procedure 3. Formation in alkaline media. An aqueous solution of a salt of a dibasic compound was added to a slightly ammoniacal solution of ammonium reineckate. The mono-reineckate was filtered and dried over phosphorus pentoxide. Chromium analyses showed that compounds prepared by this procedure were always the mono-reineckates. This procedure is a general one for the direct preparation of the mono-derivatives of dibasic compounds. Prolonged standing of the ammoniacal solution is to be avoided as occasionally a purplish contaminating precipitate was also formed. However, the pure mono-reineckates can still be obtained by recrystallisation of the contaminated reineckates from 60 per cent ethanol.

Determination of Molecular Weights

About 10 to 15 mg. of the recrystallised reineckate complex was dissolved in 5 ml. acetone in a 5 ml. volumetric flask. The optical density was measured at 525 mμ and the molecular weight of the reineckate calculated from the following formula:

$$M = w/A \times \epsilon/v \times 1{,}000 \quad .. \quad .. \quad .. \quad .. \quad .. \quad (1)$$

where M = molecular weight of the reineckate in g., w = mg. of the reineckate used, A = observed optical density, ϵ = gram-molecular extinction coefficient of the reineckate ($\epsilon_{525 m\mu} = 106{\cdot}5$), and v = volume of acetone used. The molecular weights obtained by this procedure for certain compounds are shown in Table I, column 5.

Determination of the Solubilities of the Reineckates in Water

The reineckate complex was added to water in a 25 ml. volumetric flask until no more went into solution. At this stage more reineckate was added to ensure that the solution was saturated. This solution was allowed to stand in a bath of the required temperature for one hour with constant shaking. The solution was filtered through a Whatman No. 42 filter paper and the optical density of the filtrate measured at 525 mμ. The solubilities of the reineckates were calculated from the following formulae:

$$w = A/\epsilon \times v/1{,}000 \times M \quad .. \quad .. \quad .. \quad .. \quad .. \quad (2)$$

$$w = A/2\epsilon \times v/1{,}000 \times M \quad .. \quad .. \quad .. \quad .. \quad .. \quad (3)$$

where w = mg. of reineckate dissolved in 25 ml. of water, A = observed optical density, v = volume of water used (25 ml.) and M = molecular weight of the reineckate, and ϵ = gram-molecular extinction coefficient of ammonium reineckate in water ($\epsilon_{525m\mu} = 108{\cdot}5$). Equation (2) is used for the calculation of the solubilities of mono-reineckates and equation (3) for di-reineckates.

The solubilities of the various reineckates studied are shown in Table I, column 7.

Quantitative Determination of Organic Bases

In a 50 ml. beaker about 10 mg. of the base or its salts was dissolved in 5 ml. of 0·1N hydrochloric acid. The beaker was placed in an ice bath

TABLE II

ASSAY OF DRUGS* BY AMMONIUM REINECKATE

Compounds	Mol. composition of compounds	Mol. wt.	Amount used mg.	Optical density A at 525 mμ	Amount calc. mg.
Synthetic Narcotics					
Levomethorphan hydrobromide	$C_{16}H_{25}NO{\cdot}HBr$	352·32	13·8	0·416	13·75
Dextromethorphan hydrobromide	$C_{18}H_{26}NO{\cdot}HBr$	352·32	9·25	0·280	9·25
Levomethadone hydrochloride	$C_{21}H_{27}NO{\cdot}HCl$	345·90	24·0	0·741	24·15
Dextromethadone hydrochloride	$C_{21}H_{27}NO{\cdot}HCl$	345·90	11·4	0·345	11·2
Pipadone	$C_{24}H_{31}NO{\cdot}HCl$	386·00	13·2	0·363	13·2
Ketobemidone	$C_{15}H_{21}NO_2{\cdot}HCl$	283·79	13·8	0·518	13·8
Acetoxyketobemidone	$C_{17}H_{23}NO_3{\cdot}HCl$	325·84	19·9	0·650	19·8
DL-Methadone hydrochloride	$C_{21}H_{27}NO{\cdot}HCl$	349·90	22·4	0·700	22·9
Antihistamines					
Promethazine hydrochloride	$C_{17}H_{20}N_2S{\cdot}HCl$	320·89	15·8	0·531	16·0
Diphenhydramine hydrochloride	$C_{17}H_{21}NO{\cdot}HCl$	291·83	15·0	0·556	15·2
Antazoline hydrochloride	$C_{17}H_{19}N_3{\cdot}HCl$	301·83	17·05	0·607	17·15
**Chlorothan citrate	$C_{14}H_{18}ClN_3S{\cdot}C_6H_8O_7$	487·98	17·7	0·786	17·9
**Chloropheniramine maleate	$C_{16}H_{19}ClN_2{\cdot}C_6H_4O_4$	390·88	10·0	0·557	10·2
**Chlorcyclizine hydrochloride	$C_{18}H_{21}ClN_2{\cdot}HCl$	337·30	16·1	1·030	16·3
**Doxylamine succinate	$C_{17}H_{22}N_2O{\cdot}C_4H_6O_4$	388·47	12·65	0·727	12·9
**Methapyrilene hydrochloride	$C_{14}H_{19}N_3S{\cdot}HCl$	297·86	14·0	1·020	14·2
**Thonzylamine hydrochloride	$C_{16}H_{22}N_4O{\cdot}HCl$	322·85	12·1	0·804	12·2
**Antergan	$C_{17}H_{22}N_2{\cdot}HCl$	290·81	12·3	0·925	12·6
**Mepyramine maleate	$C_{17}H_{23}N_3O{\cdot}C_4H_4O_4$	401·47	9·2	0·492	9·3
Sulphonamides					
Sulphamerazine	$C_{11}H_{12}N_4O_2S$	264·32	10·0	0·413	10·25
Sulphathiazole	$C_9H_9N_3O_2S$	255·33	10·0	0·430	10·25
Sulphapyridine	$C_{11}H_{11}N_3O_2S$	249·30	15·0	0·650	15·2
Sulphadiazine	$C_{10}H_{10}N_4O_2S$	250·29	15·0	0·645	15·1
Others					
Antipyrine	$C_{11}H_{12}N_2O$	188·22	15·9	0·863	15·25
2-Azobicyclo (3,3,1) nonane hydrochloride	$C_8H_{15}N{\cdot}HCl$	161·67	12·55	0·810	12·3

* Drugs used are commercial products, not recrystallised.

** Substances which form di-reineckates.

and 10 ml. of ammonium reineckate solution added. The contents of the beaker was cooled to 0°. The precipitate was filtered through a sintered glass filtering funnel and washed with 1 ml. portions of ice cold water until the wash liquid was colourless. To remove excess water from the precipitate suction was continued. The precipitate was dissolved in acetone and transferred to a 10 ml. volumetric flask and diluted with acetone to exactly 10 ml. volume. The absorption was measured at 525 mμ. The amounts of bases or their salts present can be calculated

by using equation (4) for substances which form mono-reineckates, or equation (5) for compounds which form di-reineckates.

$$w = A/106{\cdot}5 \times v/1{,}000 \times M \quad .. \quad .. \quad .. \quad .. \quad (4)$$

$$w = A/213{\cdot}0 \times v/1{,}000 \times M \quad .. \quad .. \quad .. \quad .. \quad (5)$$

w = weight of base or salt in mg., A = observed optical density, v = volume of acetone used, M = molecular weight of base or salt. Tables II and III show some recovery experiments using this procedure.

TABLE III

ASSAY OF PURE ALKALOIDS* AND MANUFACTURED OPIATES* USING AMMONIUM REINECKATE

Alkaloid	Mol. composition of alkaloid	Mol. wt.	Amount used mg.	Optical density A at 525 mμ	Amount calc. mg.
Morphine	$C_{17}H_{19}NO_3{\cdot}H_2O$	303·35	5	0·180	5·1
			10	0·356	10·1
Morphine hydrochloride	$C_{17}H_{19}NO_3{\cdot}HCl{\cdot}3H_2O$	375·84	10	0·286	10·1
Codeine	$C_{18}H_{21}NO_3$	299·36	5	0·176	4·95
			10	0·346	9·8
Codeine phosphate	$C_{18}H_{21}NO_3{\cdot}H_3PO_4{\cdot}1\frac{1}{2}H_2O$	424·38	17·9	0·455	18·1
Thebaine	$C_{19}H_{21}NO_3$	311·37	5	0·170	5·0
			10	0·342	10·0
Thebaine hydrochloride	$C_{19}H_{21}NO_3{\cdot}HCl{\cdot}H_2O$	365·85	10	0·287	9·9
Narcotine	$C_{22}H_{23}NO_7$	413·41	5	0·132	5·1
			10	0·258	10·0
Narcotine hydrochloride	$C_{22}H_{23}NO_7{\cdot}HCl{\cdot}\frac{1}{2}H_2O$	458·88	10	0·230	9·9
Dihydromorphine	$C_{17}H_{21}NO_3{\cdot}H_2O$	305·37	17·7	0·630	17·9
Dihydromorphine hydrochloride	$C_{17}H_{21}NO_3{\cdot}HCl$	323·80	21·8	0·707	21·5
Dihydrocodeinone hydrochloride	$C_{18}H_{21}NO_3{\cdot}HCl$	335·82	21·0	0·650	20·5
Benzylmorphine hydrochloride ..	$C_{24}H_{25}NO_3{\cdot}HCl$	411·91	22·2	0·562	21·8
Dihydrocodeinone	$C_{18}H_{21}NO_3$	299·37	23·0	0·827	23·5
Morphine-N-oxide	$C_{17}H_{19}NO_4$	301·33	15·9	0·563	15·9

* Drugs used are commercial products not recrystallised.

Ultra-violet Absorption Spectra

About 10 mg. of the reineckate salt was dissolved in 100 ml. of 95 per cent ethanol and 10 ml. of this solution was further diluted to 50 ml. with 95 per cent ethanol to give a solution containing about 2 mg. of reineckate per 100 ml. of ethanol. The spectra obtained are shown in Figures 3 and 4, a–d.

Regeneration of the Conjugate Bases from their Reineckates

The anion exchange column (1 cm. diameter) was filled with Permutit De-Acidite FF resins to a drain height of about 10 cm. The resin was converted to the OH form by treatment with 50 ml. 0·5N sodium hydroxide. The column was then washed with water until the effluent has a pH of 7.

About 10 to 20 mg. of the reineckate in 50 ml. of acetone was passed through the column until the eluate gave a negative test with Mayer's reagent. This acetone eluate was evaporated to dryness on a steam bath and the residue which is the conjugate base can be subjected to further confirmatory tests if required.

The column was re-activated by washing first with distilled water followed with 50 ml. of 0·5N sodium hydroxide solution. Results obtained with this procedure were very satisfactory.

Results and Discussions

The Formation and Recrystallisation of Reineckates

The formation of the reineckates depends on the pKb values of the bases and the pH of the reaction media[17]. This view was later shared by Poethke and others[4]. It is believed that the reaction proceeds via the protonation of the base B to the conjugate acid BH^+ which then reacts with the reineckate ion to form the complex thus:

$$BH^+ + [Cr(NH_3)_2(SCN)_4]^- \rightarrow BH\,[Cr(NH_3)_2(SCN)_4] \quad .. \quad (6)$$

The equilibrium between the conjugate acid BH^+ and water is

$$BH^+ + H_2O \rightleftharpoons H_3O^+ + B \quad .. \quad .. \quad .. \quad .. \quad .. \quad (7)$$

This equilibrium is governed by the strengths of the bases and the pH values of the reaction media. The relation between these variables may be expressed by the following equation:

$$\log[B]/[BH^+] = pH + pKb - 14 \quad .. \quad .. \quad .. \quad .. \quad (8)$$

Since dibasic substances have two pKb values equation (8) above can thus be used to explain the formation of mono- and di-reineckates in different pH media. The mechanism of the reaction between ammonium reineckate and organic bases has been studied in detail and will form the subject of another paper.

The formation of both the mono- and di-reineckates in acid media presented no difficulties. Recrystallisation of the mono-reineckates from 60 per cent ethanol gave the pure products. However, recrystallisation of all the di-reineckates studied with the exception of quinine, doxylamine and mepyramine yielded the mono-derivatives under these conditions. Many of the di-reineckates are unstable to heat. When ammonium reineckate is added to acidic aqueous solution containing dibasic compounds at 70° the mono-reineckates are usually obtained with the exception of the three compounds mentioned earlier.

Spectral Characteristics of the Absorption Spectra of the Reineckates, their Uses and Limitations

The spectral curves of several reineckates studied in acetone solution between 350–600 mμ were found to be similar to that of ammonium reineckate itself (Fig. 1) with the exception of papaverine[12] and cotarnine reineckates. This phenomena has been observed by other workers[3,13,16]. The absorption of the reineckates is attributed exclusively to the reineckate moiety of the molecule and is independent, with the exceptions stated, of the conjugate base. Examination of the ammonium reineckate curve shows two maxima at 395 mμ and 525 mμ. The average values of $\epsilon_{525 m\mu}$ for reineckates listed in column 6 of Table I are 106·5 and these are in excellent agreement with the observed values for ammonium reineckate itself.

From the spectral relationships stated, the following formula can be found when a cell of 1 cm. path length is used:—

$$w/M = A/\epsilon \times v/1{,}000 \quad .. \quad .. \quad .. \quad .. \quad .. \quad (9)$$

This equation can be used for the determination of the molecular weights and solubilities of the reineckates and also for the quantitative determination of certain organic bases without the use of calibration curves.

The accuracy of the spectrophotometric method of molecular weight determination depends mainly on the accuracy of the weighing process,

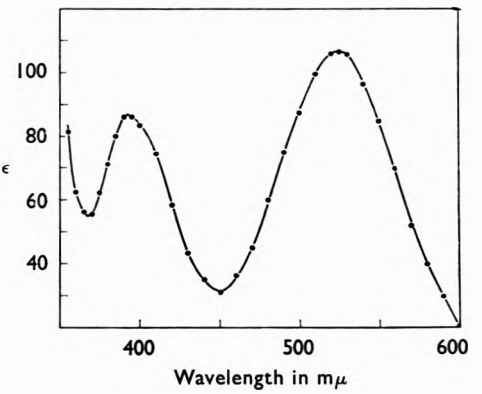

FIG. 1. Ultra-violet absorption curve of ammonium reineckate monohydrate in acetone.

the exact determination of the optical density of the solution at the chosen wavelength and the purity of the reineckates studied. Normally about 15 mg. of the reineckate, representing about 7 mg. of the conjugate base is used and a small error in weighing or the reading of the absorbance may lead to an appreciable error in the value of the molecular weight. If

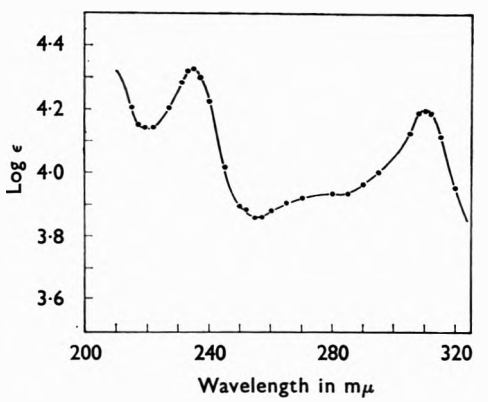

FIG. 2. Ultra-violet absorption curve of ammonium reineckate monohydrate in ethanol.

larger quantities are used better results are obtained. Table I, column 5, lists the molecular weights of the reineckates obtained by this method. The molecular weights of the free bases can be obtained by subtracting 319, which is the molecular weight of the reinecke acid, from these values.

The solubilities of the various reineckates are shown in Table I, column 7. This method of determination of the solubilities of the reineckates is superior to existing methods in that it is simple and measures directly

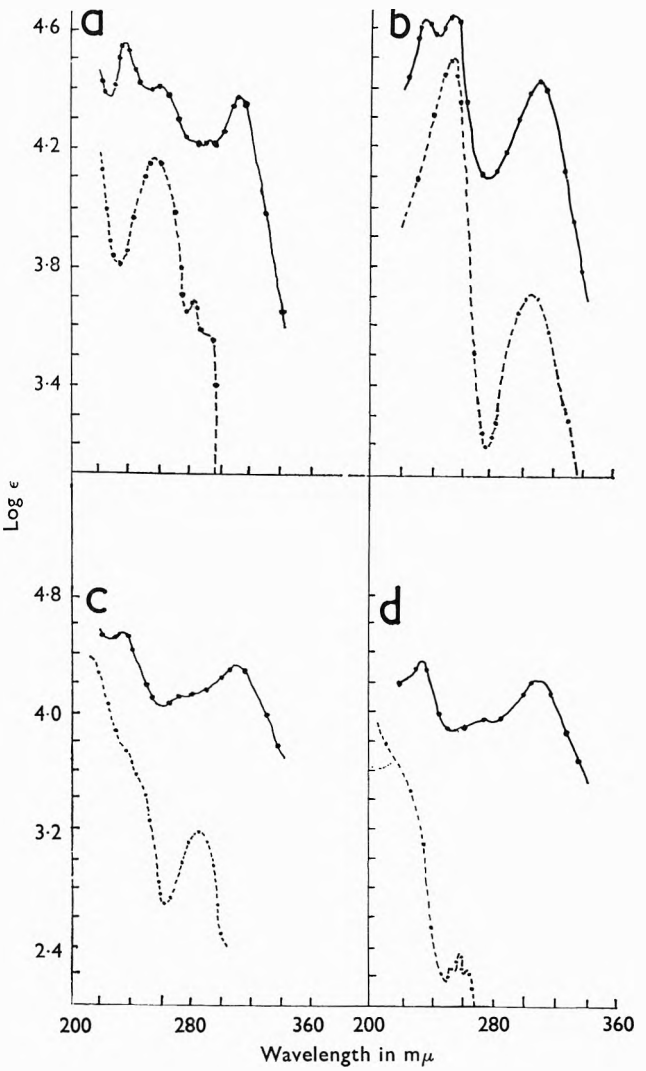

FIG. 3. Ultra-violet absorption curves for: a, strychnine reineckate (·——·) and base (· - - - ·); b, pecazine reineckate (·——·) and hydrochloride (· - - - ·); c, morphine reineckate (·——·) and base (· - - - ·); d, pethidine reineckate (·——·) and base (· - - - ·). Solvent 95 per cent ethanol.

the amounts that have gone into solution. An examination of these data reveals that in general the solubilities of the reineckates decrease with temperature. The solubilities of the reineckates appear to be a function of the pKb values of the conjugate bases and are not dependent

on whether they are derived from primary, secondary or tertiary amines as reported[19].

The data in Tables II and III illustrate that the formulae (4) and (5) can be used for the quantitative determination of many of the bases without the use of standard calibration curves. The recoveries are generally good as shown in the tables. These formulae are only applicable to substances whose reineckates are not too soluble in water and are obviously not applicable for the determination of weak bases such as caffeine, theobromine and sulphacetamide.

The ultra-violet absorption spectra of ammonium reineckate is shown in Figure 2. This curve has a maximum at 235 mμ and another at 310 mμ together with an almost flat portion of the curve between its minima at 255 mμ and 300 mμ. All the spectra in Figures 3 and 4, a–d, shown, with the exception of morphine and pethidine, are characteristic of the bases they represent in that they all possess maxima at the wavelengths

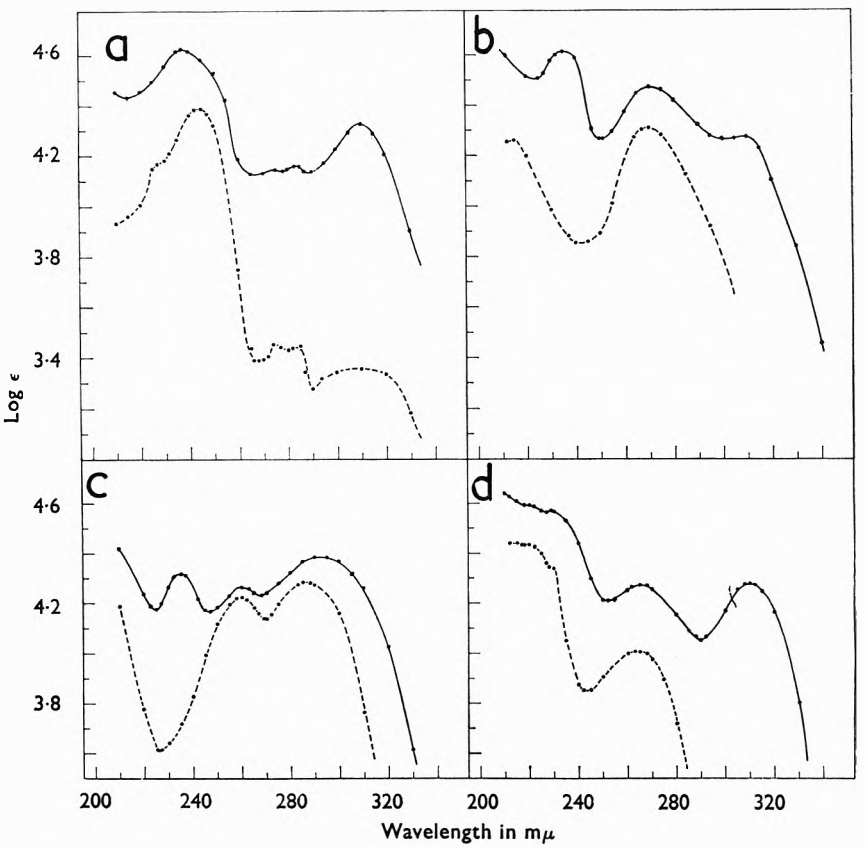

FIG. 4. Ultra-violet absorption curves for: a, thonzylamine monoreineckate (·——·), and hydrochloride (· - - - ·); b, sulphamerazine reineckate (·——·) and sulphamerazine (· - - - ·); c, sulphathiazole reineckate (·——·) and sulphathiazole (· - - - ·); d, phenindamine reineckate (·——·) and tartrate (· - - - ·). Solvent 95 per cent ethanol.

corresponding to the maxima of the spectra of the conjugate bases. Besides morphine and pethidine, the spectra of codeine, ketobemidone and methadone reineckates were also found not to have any maxima at the wavelengths corresponding to the maxima of the spectra of these substances.

Recovery of the Amines from their Reineckates

The regeneration of the conjugate bases from their reineckate derivatives using Permutit De-Acidite FF ion exchange resins presented no difficulties. The reaction between the resin and the reineckates can be represented by the following equation:

$$[RCH{\cdot}N(R^1)_3{}^+ OH^-] + BH\,[Cr(NH_3)_2(SCN)_4] \rightarrow \{RCH{\cdot}N(R^1)_3{}^+\,[Cr(NH_3)_2(SCN)_4]^-\} + B + H_2O \quad .. \quad (10)$$

It was found that the free base B set free in accordance with equation (10) remained in the acetone solution and that very little acetone was required to elute from the resin bed any material which had precipitated during the ion exchange reaction. Evaporation of the acetone yielded substances pure enough for further confirmatory tests.

This method of regeneration of the bases is superior in simplicity of operation to the Kapfhammer method[18], that is by treating an acetone solution of the reineckate with silver sulphate and then with barium chloride. The only limitation to the use of the present procedure for the liberation of the conjugate bases is that the De-Acidite FF resin is a strong anion exchanger and holds back amphoteric bases such as morphine and certain sulphonamides on the column. However, these substances can be eluted from the column by using 10 per cent acetic acid solution. The use of this procedure for the isolation of alkaloids from plant materials has already been reported[20].

Acknowledgements. The author is greatly indebted to Mr. L. G. Chatten and Dr. C. G. Farmilo (Food and Drugs Directorate, Ottawa, Canada) for providing the antihistamines and narcotic samples for this study. He is also deeply appreciative of the valuable discussion and criticisms given by Dr. Loke Kwong Hung (Biochemistry Department, University of Malaya, Singapore). Above all the author wishes to express his profound gratitude to Mr. Chia Chwee Leong (Chief Chemist, Department of Chemistry) whose encouragement and assistance brought this study to a successful completion.

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SOME OBSERVATIONS ON THE PHARMACOLOGY OF 10-METHOXYDESERPIDINE

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Received May 16, 1960

10-Methoxydeserpidine caused a slow sustained fall in the arterial blood pressure of the anaesthetised cat when doses of 2 to 4 mg./kg. were given, an effect seen only when the initial blood pressure was high. Unlike reserpine and deserpidine, 10-methoxydeserpidine did not cause ptosis or loose stools in mice or rats when used at doses of from 10 to 80 mg./kg. and although pentobarbitone sleeping time was not increased, at higher doses of 40 to 80 mg./kg. there was reduction of motor activity, respiratory depression and some animals died. Unlike deserpidine the 10-methoxy derivative had no effect on the pressor responses to compression of the abdominal aorta or stimulation of the splanchnic nerve, but depressed the response to occlusion of the common carotid arteries. The LD50 of 10-methoxydeserpidine was found to be 82 $\pm$ 2·6 mg./kg. in mice. Methyl reserpate and reserpic acid showed reserpine-like activity in intact animals and isolated tissues only at very high doses.

Extracts of *Rauwolfia serpentina* and the alkaloid reserpine are employed in the treatment of hypertension and mental illness. Reserpine and extracts which contain it may cause side effects, among the more important of which is mental depression. This has led to a search for compounds with reserpine-like antihypertensive properties, but which do not cause mental depression. Velluz[1], Velluz, Peterfalvi and Jequier[2], Gros, Peterfalvi and Jequier[3] and Peterfalvi and Jequier[4] have shown that 10-methoxydeserpidine meets this requirement. In addition it was less toxic than reserpine in mice and as actively hypotensive as reserpine in anaesthetised cats and rabbits. In the rat, made hypertensive with sodium chloride and desoxycorticosterone acetate it exerted a marked hypotensive action, and abolished the response to carotid artery occlusion[4]. In clinical trials[3,5] 10-methoxydeserpidine reduced both systolic and diastolic blood pressures in hypertensive patients.

Deserpidine itself has been studied by a number of workers who have reported its effects upon intact animals[6–13]. It has reserpine-like hypotensive and sedative activity[6–9,11,13] and in all other respects appears to have typical reserpine-like pharmacological properties, indicating that the removal of the 11-methoxy group causes no qualitatively important effects. We have compared the properties of deserpidine with those of 10-methoxydeserpidine on isolated tissues and organs and in intact anaesthetised animals and have also tested reserpic acid and methyl reserpate; compounds which lack the trimethoxybenzoic acid moiety of the reserpine molecule. Plummer and his associates[13] and Bein[14] point out that, in experimental animals, reserpic acid lacks the sedative and hypotensive effects of reserpine. Methyl reserpate has about one-third

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TABLE I

COMPARISON OF THE PHARMACOLOGICAL EFFECTS OF 10-METHOXYDESERPIDINE WITH DESERPIDINE, RESERPIC ACID HYDROCHLORIDE AND METHYL RESERPATE

Preparation	10-Methoxydeserpidine	Deserpidine	Reserpic acid hydrochloride	Methyl reserpate
B.P. Anaesthetised cat. (See also refs. 4, 7 and 8)	2–6 mg./kg. No immediate effect. In cats with low initial blood pressure level no fall even up to 6 hr. after first dose of drug. 9 mg. caused slight fall after 3 hr. When initial blood pressure level high, slow sustained fall in blood pressure with 2–4 mg./kg.	2–4 mg./kg. Immediate, short-lived fall, gradual secondary fall (40–60 mm.) at maximum in 1–1½ hr. Bradycardia	2–4 mg./kg. Immediate, short-lived fall, slight secondary fall after 2 hr.	2–4 mg./kg. Little or no immediate or delayed fall unless initial blood pressure high when 4 mg. caused 20–30 mm. fall and 5–10 mg. 30–40 mm. fall, after 1–1½ hr.
	2–6 mg./kg. No antagonism but slight increase in pressor response to Ad. or NA. (2–5 μg./kg.)	1–2 mg./kg. Increase in pressor responses to Ad. or NA. (1–2 μg./kg.)	2–7 mg./kg. No effect on pressor responses to Ad. or NA. (1–4 μg./kg.)	5–7 mg./kg. Little or no modification of pressor responses to Ad. or NA. (1–2 μg./kg.)
	5 mg./kg. No modification of depressor responses to 5 μg./kg. Hm. or Ach.	1–2 mg./kg. No modification of depressor responses to 1–5 μg./kg. Hm. or Ach.	2–5 mg./kg. No modification of depressor responses to 1–5 μg./kg. Hm. or Ach.	2–5 mg./kg. No modification of depressor responses to 1–5 μg./kg. Hm. or Ach.
Vasopressor reflexes: (i) Bilateral occlusion of common carotid arteries. (See also refs. 4, 8)	2–4 mg./kg. No immediate or delayed effect when initial blood pressure low. Reversible depression when initial level high (>150 mm. Hg)	2 mg./kg. Delayed reduction in response (40 min.)	2–5 mg./kg. No effect	4–5 mg./kg. No effect
(ii) Compression of abdominal aorta	2–4 mg./kg. No immediate or delayed effect	1–2 mg./kg. Marked reversible inhibition of responses	2–5 mg./kg. No effect	4–5 mg./kg. No effect
(iii) Electrical stimulation of left greater splanchnic nerve	2–6 mg./kg. Slight increase in magnitude of response	1–2 mg./kg. Delayed inhibition of pressor response (40–60 min.). Recovery in 2–3 hr.	2–5 mg./kg. No effect	4–5 mg./kg. No effect
(iv) Electrical stimulation central end of cut vagus. (See also ref. 7)	2–6 mg./kg. Abolition of pressor response followed by reversal (Fig. 1)	1–2 mg./kg. Abolition and reversal of responses (delayed 20–40 min.). Recovery incomplete after 3 hr.	5 mg./kg. No effect	5 mg./kg. No effect
Nictitating membrane. (See also refs. 4, 7)	5 mg./kg. No effect	1–2 mg./kg. No effect	4–5 mg./kg. No effect	4–5 mg./kg. No effect

TABLE I *—continued*

Preparation	10-Methoxydeserpidine	Deserpidine	Reserpic acid hydrochloride	Methyl reserpate
Respiration (cat). See also refs. 4, 8)	2–5 mg./kg. Slight respiratory depression in most cases but in some marked depression of respiration (Fig. 2)	5 mg./kg. Slight respiratory depression	5 mg./kg. No effect	1–2 mg./kg. No effect
B.P. Anaesthetised rat	300 μg.–1 mg. Fall in blood pressure after about 20 min. Maximum in 1 hr. Slight enhancement of pressor responses to Ad. or NA. (1 μg.). No effect on depressor response to 5-HT (5 μg.)	100–200 μg. Immediate, sharp fall of small magnitude, prolonged secondary fall (40–50 mm. after 40–60 min.). Enhancement of pressor responses to Ad. and NA. (1–2 μg.) 100–200 μg. No effect on depressor response to 5 μg. 5-HT	1–2 mg. Immediate sharp fall. No secondary fall. Delayed (20–40 min.) enhancement of pressor responses to Ad. and NA. (1–2 μg.) 1–2 mg. As deserpidine	1–2 mg. Immediate sharp fall. No secondary fall. No effect or slight inhibition of responses to Ad. and NA. (1–2 μg.) 1–2 mg. As deserpidine
Cardiac muscle: (i) Rabbit heart	20–120 μg. Decrease in rate, tone and amplitude and increase in outflow (Fig. 3)	40–100 μg. Decrease in rate, tone and amplitude (irreversible). Increase in outflow	125–500 μg. Slight fall in tone and increase in outflow	0·5–1·0 mg. No effect on tone or amplitude. Small increase in outflow 20–50 μg/ml. Perfused for 15 min. caused reversible depression in rate, tone and amplitude.
(ii) Guinea pig or rabbit auricles	18–30 μg. Slight reversible reduction in responses to NA. and Ad. (1–2 μg.) 18–60 μg./ml. No effect on response to Ach. (0·5 μg./ml.)	4–14 μg./ml. Reversible reduction in rate and amplitude 4–30 μg. ml. Slight reversible reduction in responses to NA. and Ad. (1–2 μg.). No influence on response to Ach. (0·1–0·5 μg./ml.)	200–500 μg./ml. No effect on responses to NA. and Ad. (1–2 μg.) 10–20 μg./ml. No effect on responses to Ach. (0·1–0·5 μg./ml.)	20–50 μg./ml. No effect on responses to NA. and Ad. (1–2 μg.) 8–20 μg./ml. No effect on responses to Ach. (0·1–0·5 μg./ml.)
Vascular smooth muscle: (i) Horse carotid arteries	20–200 μg./ml. Slight reversible inhibition of Ach. (0·01–0·1 μg./ml.) Ad. (0·1–0·2 μg./ml.) NA. (0·1–0·2 μg./ml.) and 5-HT (0·5 μg./ml.) - induced contractions	5–10 μg./ml. Marked, incompletely reversible inhibition of Ach.-induced contractions. (Ach. 0·01–0·04 μg./ml.). Reversible antagonism to Ad. or NA.-induced contractions. (Ad. or NA. 0·01–0·02 μg./ml.)	100–200 μg./ml. Reversible inhibition of Ach.-induced contractions (Ach. 0·01–0·04 μg./ml.). No effect on tone	100–200 μg./ml. Reversible inhibition of Ach.-induced contractions (Ach. 0·01–0·04 μg./ml.). No effect on tone

TABLE I—*continued*

Preparation	10-Methoxydeserpidine	Deserpidine	Reserpic acid hydrochloride	Methyl reserpate
(ii) Rat hindquarters	500 μg.–2 mg. No direct vasodilatation. No effect on Ad., NA. or 5-HT (0·5–1 μg.) vasoconstriction	50–300 μg. No direct vasodilatation. Marked reduction in constrictor responses to Ad. and NA. (1–2 μg.). No effect on responses to $BaCl_2$ (0·25–1 mg.) or 5-HT (1 μg.)	1–2 mg. No direct vasodilatation. Reversible reduction in constrictor responses to Ad. or NA. (1–2 μg.) and as deserpidine	0·5–1 mg. No direct vasodilatation. Slight reduction in constrictor responses to Ad. and NA. (1–2 μg.) and as deserpidine
Intestinal smooth muscle: (i) Guinea pig ileum	20–75 μg./ml. No direct action or slight relaxation. Inhibition of stimulant effects of Ach. (0·1–1·0 μg./ml.) Hm (0·01–0·1 μg./ml.) $BaCl_2$ (0·25–1 mg./ml.) and 5-HT (1·0–5·0 μg./ml.). Recovery delayed but complete	5–10 μg./ml. No direct action. Marked inhibition of stimulant effect of Ach. (0·01–0·1 μg./ml.) Hm (0·01–0·1 μg./ml.) $BaCl_2$ (0·25–1 mg./ml.) and 5-HT (1–5 μg./ml.). Recovery delayed but complete	50–100 μg./ml. No direct action. Marked inhibition of stimulant effects of Ach. (0·01–0·1 μg./ml.) Hm (0·01–0·1 μg./ml.) $BaCl_2$ (0·25–1 mg./ml.) and 5-HT (1–5 μg./ml.). With lower doses effects rapidly reversible, higher doses as deserpidine	150–500 μg./ml. As reserpic acid hydrochloride
(ii) Rabbit duodenum	20–75 μg./ml. Relaxation of gut with inhibition of peristalsis. Recovery 20 min.	5–10 μg./ml. Relaxation of gut with inhibition of peristalsis. Recovery 20–40 min. 10–20 μg./ml. Marked antagonism to Ach. (0·01 μg./ml.)-induced contractions. Recovery 1–1½ hr. Antagonism to Ad. (0·1 μg./ml.) - induced relaxation. Recovery 20 min.	50–100 μg./ml. Brief reversible relaxation, reduction of tone and inhibition of peristalsis 18–50 μg./ml. Reversible antagonism to Ach. (0·01 μg./ml.)-induced contractions. No effect on Ad. (0·1 μg./ml.)-induced relaxation of gut	100–200 μg./ml. Brief reversible relaxation, reduction of tone and inhibition of peristalsis. Reversible antagonism to Ach. (0·01 μg./ml.)-induced contractions.
Skeletal muscle. Frog rectus abdominis	50–100 μg./ml. Contraction of muscle (winter frogs). Irreversible antagonism to Ach. (1·0–3·0 μg./ml.)	5–10 μg./ml. No direct action. Slight irreversible inhibition of effects of 0·1–1·0 μg./ml. Ach.	50–100 μg./ml. No direct action. Slight reversible inhibition of effects of 0·1–1·0 μg./ml. Ach.	100–200 μg./ml. As reserpic acid hydrochloride

Note: The following abbreviations are used in this Table. Ach. for acetylcholine chloride: Ad. for adrenaline bitartrate: NA. for noradrenaline bitartrate: 5-HT for 5-hydroxytryptamine creatinine sulphate: Hm for histamine acid phosphate: and $BaCl_2$ for barium chloride.

of the potency of reserpine, as shown by the mouse ptosis assay[15], but has no reserpine-like activity in the dog[14].

Experimental and Results

The methods used were similar to those reported previously[16]. The results are set out in Table I. In some instances we have repeated the experiments of others and where this has been done it is indicated in the table. Rat blood pressure was recorded by the method of Dekanski[17] using Condon's manometer[18]. Experiments on isolated spirals of horse carotid arteries were as described by Kirpekar and Lewis[19]. Ptosis in mice and rats was measured by the method of Rubin and Burke[15]. Effects upon barbiturate sleeping time were measured by

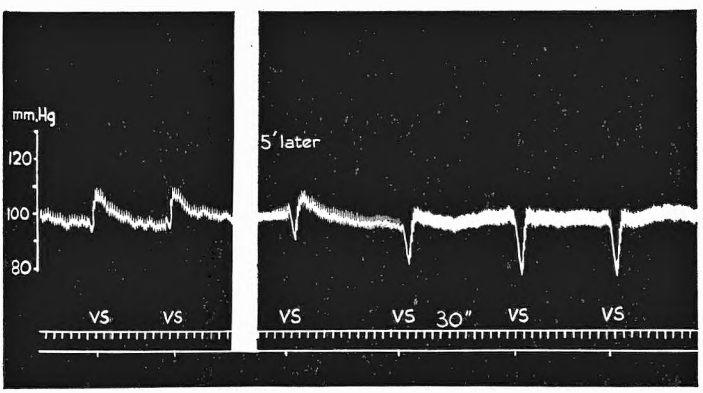

Fig. 1. Influence of 6 mg./kg. 10-methoxydeserpidine on the response to stimulation of the central end of the cut vagus of the cat. The drug was given 5 min. before the subsequent stimulus. Stimulation (VS) was with 10 sec. bursts of square wave impulses at 10 volts, 2 msec. duration and at a frequency of 1,000/min.

the method of Cronheim and his colleagues[20] but using 60 mg./kg. of sodium pentobarbitone. The intraperitoneal LD50 was estimated in groups of 20 mice, 15 to 16 g., by the method of Miller and Tainter[21].

Solutions of methyl reserpate, reserpic acid hydrochloride, deserpidine and 10-methoxydeserpidine were prepared by dissolving the solid in the minimal amount of glacial acetic acid and adjusting to volume with distilled water. The pH of the final solution was from 3·4 to 3·6. Control solutions prepared in the same way and at the same pH were used throughout for purposes of comparison.

Discussion

10-Methoxydeserpidine was found to cause a delayed fall in the arterial blood pressure of the anaesthetised cat when the initial blood pressure level was high. The effects of deserpidine on the arterial blood pressure were more marked and we have confirmed the observations of earlier workers[4,7,8]. 10-Methoxydeserpidine was less potent than deserpidine

in lowering the rat's arterial blood pressure but otherwise had similar effects. It caused bradycardia in the cat and both deserpidine and 10-methoxydeserpidine caused an increase in the pressor responses to adrenaline and noradrenaline. 10-Methoxydeserpidine reduced the pressor response to carotid artery compression, an effect shown by deserpidine. Unlike deserpidine the 10-methoxy compound did not inhibit the pressor response to compression of the abdominal aorta, had very little effect on the response to splanchnic nerve stimulation and did not

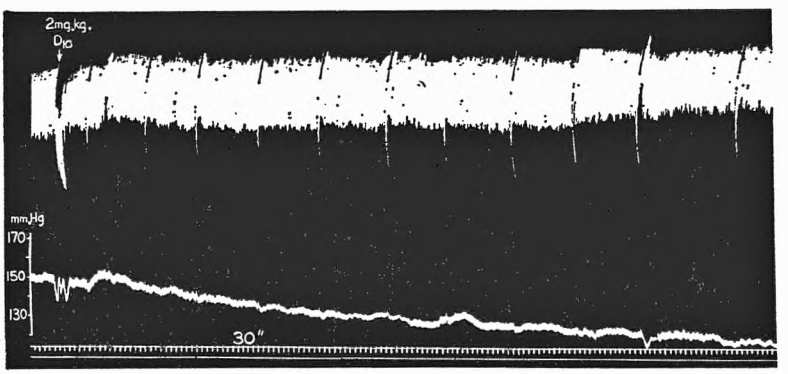

Fig. 2. Effect of 10-methoxydeserpidine (D_{10}) on respiration (upper record) and blood pressure (lower record) of the pentobarbitone-anaesthetised cat.

cause ptosis, diarrhoea or sedation in rats at doses of up to 20 mg./kg. In mice 40 to 80 mg./kg. of 10-methoxydeserpidine caused drowsiness and a reduction in motor activity but it was much less effective even at these dose levels than deserpidine or reserpine.

Sleeping time experiments indicated that at higher dose levels of 10-methoxydeserpidine there is a significant ($P = 0{\cdot}1$) increase in pentobarbitone sleeping time which may be related to respiratory depression and not therefore a typical reserpine-like effect.

The LD50 was found to be $82 \pm 2{\cdot}6$ mg./kg. in mice, a much lower figure than that obtained elsewhere[4]. Death was due to respiratory failure, the heart continuing to beat some time after respiration had ceased.

This change in pharmacological properties must presumably be attributed to properties conferred on the molecule by the change in position of the methoxy group from the 11- to the 10- position. More marked central activity is found in reserpine and in deserpidine. In the former there is a 10-methoxy group which is absent from the latter which may mean that the 10-methoxy group in 10-methoxydeserpidine either hinders the fit of the molecule on to the central receptor sites or perhaps prevents it from reaching them.

In the isolated perfused rat hindquarters 10-methoxydeserpidine did not inhibit vasoconstriction due to adrenaline, noradrenaline or 5-hydroxytryptamine. Otherwise its properties, as far as we have ascertained them (Table I), are similar to those of deserpidine.

Methyl reserpate and reserpic acid had little effect on the blood pressure of the cat and rat, even when doses about ten times greater than those of deserpidine or 10-methoxydeserpidine were used and, as pointed out by earlier workers, removal of the ester group caused almost complete

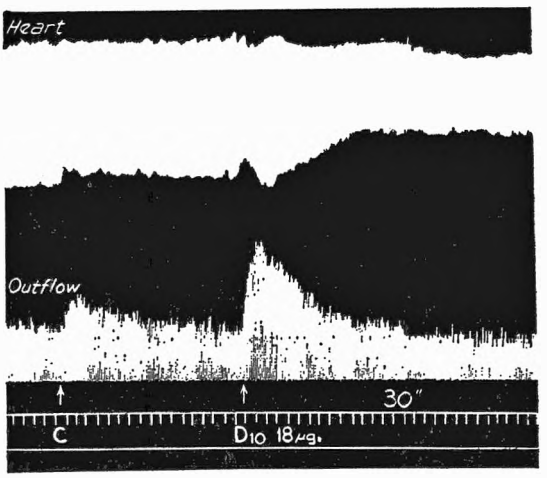

FIG. 3. Rabbit heart. Influence of 10-methoxydeserpidine on heart (upper record) and outflow from heart (lower record). At D_{10}, 18 µg. 10-methoxydeserpidine and at C control solution injected into the cannula.

loss of typical reserpine-like activity[13–15]. On isolated organs typical reserpine-like effects were shown but very large doses were needed. This seems to indicate that removal of the ester group causes a sharp fall in potency but does not completely eliminate all reserpine-like activity.

Acknowledgements. We thank Dr. J. J. Segall of Roussel Laboratories Ltd., for supplies of 10-methoxydeserpidine, Dr. G. T. Bassill of Ciba Ltd., for supplies of reserpic acid hydrochloride, methyl reserpate and deserpidine, and Miss Sheena McPhee for technical assistance.

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MICELLAR SIZE AND SURFACE ACTIVITY OF SOME C_{18} α-MONOGLYCERIDES IN BENZENE

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Received July 15, 1960

Some C_{18} α-monoglycerides in benzene have been studied by the light-scattering and surface tension methods. The glycerol esters of stearic, oleic and linoleic acids had micellar weights of less than 7,000; glycerol monoricinoleate had a micellar weight of over 18,000. All the monoglycerides showed some surface activity in benzene, glycerol monoricinoleate having the greatest, the other unsaturated monoglycerides the least.

A characteristic of surface-active substances is the presence of both hydrophilic and lipophilic regions in the molecule. The hydrophilic region will be polar or ionic and the lipophilic region a relatively non-polar hydrocarbon chain or ring. The balance between the hydrophilic and lipophilic regions can be varied in several ways.

In biological systems substances possessing surface activity are numerous. Among the more important are the lecithins and monoglycerides—the latter can be regarded as non-ionic *in vivo* whilst the former possess a zwitter-ion structure and are non-ionic only at their isoelectric points. Both these types may possess saturated and unsaturated fatty acid components which will in turn vary the extent of solubility in aqueous and non-aqueous systems. The double bonds will modify the lipophilic region of a molecule and consequently its surface activity. This is important in biological systems where these substances play an active role in solubilisation and transportation of lipids.

The effect of double bonds on the micellar size and surface activity of some C_{18} α-monoglycerides has been investigated. The influence of an hydroxyl group, in the hydrocarbon chain of ricinoleic acid, on the surface activity of glycerol monoricinoleate was also examined.

Experimental

Preparation of the α-Monoglycerides

The stearic, oleic, linoleic and ricinoleic monoesters of glycerol were prepared by a method similar to that of Averill, Roche and King[1]. The fatty acid chlorides were first prepared by slowly adding 0·06 mole of fatty acid to 0·18 mole of oxalyl chloride and refluxing for 4 hours in an all-glass still. Excess oxalyl chloride was removed at 2–3 mm. pressure.

Acetone-glycerol was prepared by reacting 100 g. freshly distilled anhydrous glycerol with 200 ml. acetone saturated with dry hydrogen chloride over fused anhydrous sodium sulphate. The acetone-glycerol was purified by shaking with excess lead carbonate to remove hydrogen chloride, filtered and dried over anhydrous sodium sulphate.

The acetone-glycerol α-ester compounds were prepared by mixing equimolecular proportions of acetone-glycerol and quinoline in a 25 ml. round-bottomed flask immersed in an ice-cold bath. An equimolecular quantity of cold fatty acid chloride was added and the mixture allowed to stand for 2 days. 25 ml. of ether and 1·0 ml. ice-cold 0·5N sulphuric acid were added to the mixture, shaken in a separating funnel and the acid layer drawn off. The ether layer was shaken several times with 5 ml. portions of sodium bicarbonate solution and washed well with water. The solution was then dried over anhydrous sodium sulphate and filtered.

The protecting acetone group was removed from the α-ester by cooling an etheral solution of the ester in an ice-cold bath and slowly adding 5 ml. 10 N sulphuric acid. After 30 minutes 15 ml. water was added and the

TABLE I

REFRACTIVE INDEX INCREMENTS FOR α-MONOGLYCERIDES

Monoglyceride	M.p.	I.no.	Refractive index	dn/dc	Monomer weight	Micellar weight
Monostearate	81·8°	—	—	0·0634	358·5	4,730
Monooleate	35·8°	70·3	1·4625 (40°)	0·0658	356·5	6,900
Monolinoleate	12·5–14°	142·0	1·4767 (20°)	0·0520	354·5	5,400
Monoricinoleate	5°–6°	67·7	1·4717 (20°)	0·0654	372·5	18,600

contents placed in a freezing mixture when the monoglycerides separated out. The stearic ester was washed, dried over anhydrous sodium sulphate and crystallised from a mixture of 1 : 1 ether and light petroleum. The unsaturated esters were treated in a similar way but crystallised over a longer period of time and at a much lower temperature.

Preliminary light-scattering experiments with the α-monostearate showed that traces of free fatty acid considerably increased the scattered light. Adequate washing of an etheral solution of the monoglycerides with sodium bicarbonate soution and water was carried out to ensure the complete absence of hydrolysis products.

Apparatus

The light-scattering instrument constructed in our laboratory and based on that designed by Hughes, Johnson and Ottewill[2] has been described[3]. Subsequent modifications to the apparatus have also been reported[4]. All measurements were made with the mercury green line, $\lambda = 5{,}461$ Å. Ludox was used as a calibrating liquid.

The specific refractive index increment was determined by means of a Rayleigh interference refractometer modified for monochromatic light as described by Bauer[5]. All measurements were taken at 20°.

Surface tensions were measured by the ring method using the chainomatic balance assembly previously described[6].

All measurements were taken at 20°.

RESULTS AND DISCUSSION

Light-Scattering

The refractive index increments for the α-monoglycerides are given in Table I.

The scattering intensity at 90° was corrected for solvent effects and plotted in terms of Kc/R_{90} against concentration for comparison with Zimm's basic scattering equation[7],

$$\frac{Kc}{R_\theta} = \frac{1}{MP(\theta)} + 2Bc$$

where K contains optical constants of the scattering system;

c = concentration of monoglyceride g.ml.$^{-1}$;
R_θ = reduced intensity of the scattered light at angle θ;
B = interaction constant;
M = molecular weight; and
$P(\theta)$ = particle scattering factor to correct for internal interference.

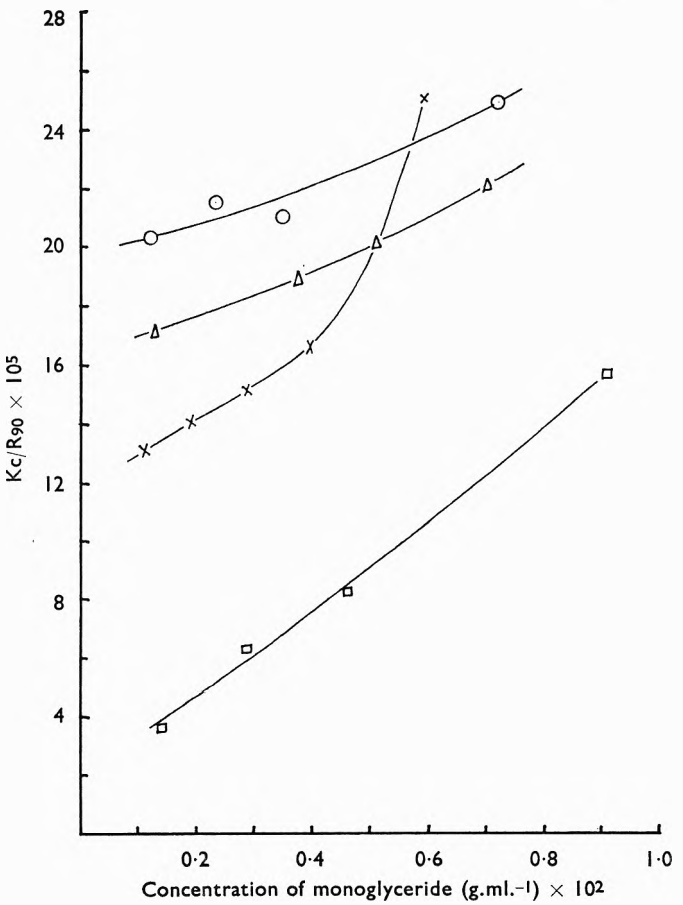

FIG. 1. 90° scattering curves for the α-monoglycerides in benzene at 20° C.

⊙ Monostearin × Monoolein △ Monolinolein
□ Monoricinolein

Since micelles and not single molecules contribute to the scattering of light, Debye[8] has suggested that the concentration term should be $c - c_o$ where c_o is the concentration of monomers (critical micelle concentration). The light scattered by the particles was, however, so small that a critical micelle concentration was not measurable. For a similar reason values for the molecular weight were not obtained by extrapolation but by averages of the values of Kc/R_{90} in the more dilute region (below 0·5 per cent w/v).

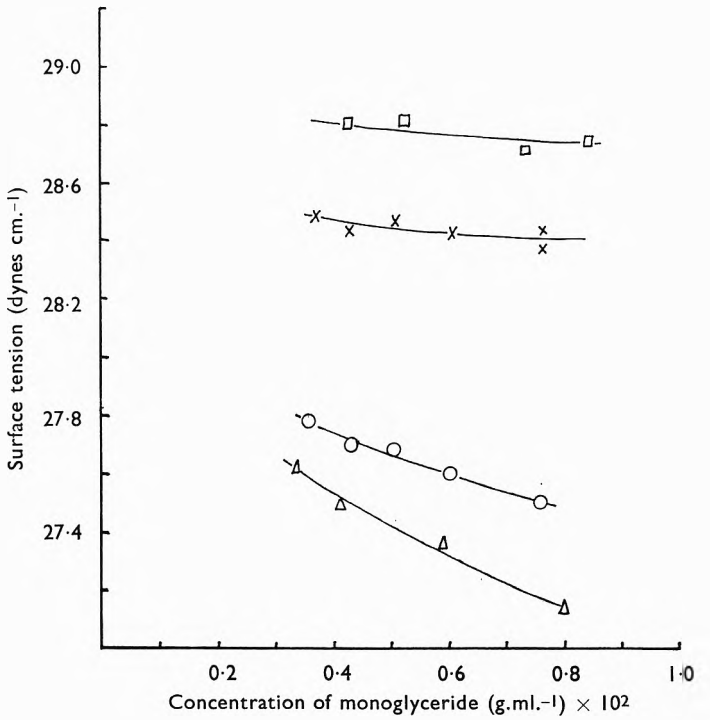

FIG. 2. Surface tension effects of some α-monoglycerides on benzene at 20° C.

○ Monostearin × Monoolein □ Monolinolein
△ Monoricinolein

The depolarisation of scattered light after correcting for solvent effects was small (less than 0·04) for all the monoglycerides.

The dissymmetry of scatter at 30° either side of 90° to the incident beam was never greater than 1·05 and on account of the low level of scatter the contribution of dust particles towards this value may have been significant.

The monoglycerides form dispersions in water but are soluble in benzene. From the dual character of the monoglyceride molecules they would be expected to form micelles in a non-aqueous solvent having a long-chain hydrocarbon periphery surrounding the hydrophilic glycerol nucleus, that is, "inverted" forms of the classical soap micelle.

The monostearate particles were the smallest of the four glycerides examined and contained approximately 13 monomers per micelle (Fig. 1). Introduction of a double bond into the hydrocarbon chain resulted in a larger particle size formation shown by glycerol monooleate. It is possible that monooleate is slightly less soluble than monostearate which would have resulted in the formation of larger aggregates; the polar effect of the double bond in the hydrocarbon chain may also have influenced the orientation of the long chains in micelle formation.

Results obtained from the light scattered by monolinoleate particles differed from those expected from a further increase in polar character of the long-chain fraction (two double bonds) of the glyceride molecule. The dissymmetry of scattering was very low and the possibility of increased scatter arising from impurities was negligible. It was first suspected that hydrolysis may have occurred producing some free fatty acid which contributed to the increased scatter; furthermore, some solubilisation of the free glycerol within the micelles could also have increased the scatter. Repetition of the sodium bicarbonate purification treatment did not produce any substantial change in scattering. The smaller particle size was then attributed to a closer but probably more complex packing of the hydrocarbon chains.

The presence of an hydroxyl group in the long chain made a significant increase in size of the micelles shown by the scattering from glycerol monoricinoleate. Due to this hydroxyl group the ester would have a lower solubility in benzene than the other monoglycerides and the formation of larger aggregates could be expected. The hydroxyl group was also likely to hinder the orientation of hydrocarbon chains to a close packing in the micelles.

All the monoglycerides showed some surface activity in benzene (Fig. 2). The lowering of the surface tension of benzene by monooleate and monolinoleate was very small and less than that shown by monostearate. They are all soluble in benzene but the presence of a polar region at the double bonds of the unsaturated monoglycerides appeared to decrease their surface activity. The large micellar size of monoricinoleate calculated from the light-scattering measurements was partly attributed to it being less soluble in benzene. The hydroxyl group present in monoricinoleate showed a greater tendency to orient itself in the surface layer, again due to the solubility relationship, away from the hydrocarbon solvent, so bringing about a greater lowering of the surface tension.

Acknowledgement. I thank Dr. L. Saunders for his continued interest.

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THE ABSORPTION AND ELIMINATION OF METABISULPHITE AND THIOSULPHATE BY RATS

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Received May 30, 1960

Rats eliminated 55 per cent of the sulphur of metabisulphite and 23 per cent of that of thiosulphate into the urine in the 4 hours after the oral administration of these compounds. This sulphur was excreted almost entirely as inorganic sulphate.

A search of the literature yielded much information about the formation of sulphite and its detoxification in the animal body, but little evidence about the absorption of sulphite from the gastrointestinal tract and the manner of its excretion. Strong indication exists that orally administered metabisulphite solution is absorbed by rats, for Lockett and Natoff[1] found that the ingestion of 11·1 to 25·5 mg. of sodium metabisulphite per 100 g. weight per day did not increase the wet weight of the faeces excreted. Such metabisulphite would have required 0·57 to 1·3 ml. of water for its isotonic solution. Less than a third of this volume of water would have caused a significant increase in the wet weight of the faeces if added to them, for the average wet faeces excreted was 2·31 $\pm$ 0·01 g. per 100 g. weight per day. The object of the present work has been to make some estimate of the speed and degree of this absorption and discover in what form ingested metabisulphite is excreted by this species.

Experimental

Female Wistar rats were fed diet 41 b of Stein and were housed in the laboratory for at least 1 week before use.

Experiments of Group I

Eight rats weighing 147·8 $\pm$ 1·8 g. were divided into pairs matching in weight. Two pairs drank only sodium metabisulphite (2,000 p.p.m. as SO_2) and two pairs water, for 3 days: all were deprived of food for the last 24 hours of this period. Then each animal received a volume of its drinking fluid equivalent to 5 per cent of weight by stomach tube. Immediately afterwards the bladders were emptied by gentle suprapubic pressure, each pair was put into a separate metabolism cage, and the urine was collected for 4 hours. A cross-over test was made 4 days later in which those pairs which had previously drunk the metabisulphite solution drank the water, and *vice versa*. The whole procedure was repeated.

Experiments of Group II

Twelve rats, weighing 260·5 $\pm$ 1·6 g., were assigned to four groups for tests in which oral treatments were allocated to groups according to

a 4 × 4 Latin Square design and were administered by stomach tube at intervals of 3 days. On each occasion an hydrating volume of water equivalent to 2·5 per cent body weight was given after a fast of 18 hours and was followed, 1 hour later, by a volume of test solution equal to 5 per cent of body weight. Urine was collected from each group separately throughout the next 4 hours (see Experiments Group I). The test solutions used were NaCl, 0·74 per cent; $Na_2S_2O_3$, 5 H_2O, 4·51 per cent; Na_2SO_4,10 H_2O, 5·86 per cent; $Na_2 S_2 O_5$, 3·46 per cent; all w/v in water.

Experiments of Group III

These differed from those of Group II in that the rats used weighed 267·5 ± 2·4 g., and were assigned to six groups. Treatments were given by intraperitoneal injection, in volume equivalent to 3 per cent of body weight, and consisted of 0·9 per cent NaCl w/v; 13·4 ml. 1·46 per cent anhydrous Na_2SO_4, 8·0 ml. 1·95 per cent $Na_2S_2O_5$ and 8·0 ml. 2·54 per

TABLE I

THE EFFECT OF THE INGESTION OF 5 PER CENT WEIGHT OF A SOLUTION OF SODIUM METABISULPHITE ON THE URINARY EXCRETION OF SULPHUR BY RATS IN THE SUCCEEDING 4-HOUR PERIOD

	Water load	Metabisulphite load	*t* calc. n = 14
Urine volume, ml.	8·1 ± 0·68 (16)	9·3 ± 0·64 (16)	1·36
Inorganic sulphur as SO_4" in mg. ..	10·7 ± 2·13 (16)	33·2 ± 3·35 (16)	5·56
Reducing power as SO_3" in mg. ..	1·3 ± 0·27 (16)	2·0 ± 0·29 (16)	2·53
Organic sulphur as SO_4"	0·3 ± 0·03 (16)	0·4 ± 0·12 (16)	0·70

The values shown are means for pairs of rats ± S.E. of the mean (number of observations).

cent $Na_2S_2O_3$,5 H_2O all w/v in water, each diluted to 100 ml. with 0·9 per cent NaCl w/v, administered to each group in turn in a four-part cross over test according to a random block design.

Chemical Methods

The total and inorganic sulphur of urine was estimated as sulphate by the method of Bray, Humphris, Thorpe, White and Wood[2], modified only by substitution of a Gallenkamp photoelectric colorimeter N.3615 for a Spekker photoelectric absorptiometer, and replacement of the Chance Neutral Filter H 508 by one with a neutral density of 1·15. Since sulphite, thiosulphate and thiocyanate reduce iodine, they were collectively estimated in urine as an increase in the reduction of iodine above control levels. 1 ml. urine was slowly added to 5 ml. of 0·1N aqueous iodine. After 2 minutes 0·2 ml. HCl (B.P.) was added and the excess iodine was titrated with 0·01N thiosulphate using mucilage of starch as indicator. Blank tests were made throughout.

RESULTS

Changes Induced in the Urinary Excretion of Sulphur by the Oral Administration of Sodium Metabisulphite in Rats

Comparison was made of the sulphur-containing compounds excreted in the urine of rats in the 4-hour period immediately following the

administration by stomach tube of either water or a solution of sodium metabisulphite in Experiments of Group I. The results of the eight tests made are summarised in Table I. The sulphate equivalent of the mean oral dose of metabisulphite administered per rat was 44·4 mg. Of this, 53 per cent was excreted in the urine in the first 4 hours after its administration. Much the larger part of this sulphur was in inorganic form, and was sulphate. There was also a small but significant increase in the reducing power of the urine attributable to the presence of sulphite, thiosulphate or thiocyanate.

Comparison of the Changes Induced in the Urinary Excretion of Sulphur by the Oral Administration of Sulphate, Thiosulphate and Metabisulphate to Rats

Experiments of Group II were used to make comparison of the sulphur containing compounds excreted in the urine in the 4 hours immediately after the administration by stomach tube of solutions of sodium chloride,

TABLE II

THE URINARY EXCRETION OF WATER AND SULPHUR BY GROUPS OF THREE RATS IN THE 4 HOURS AFTER THE ORAL ADMINISTRATION OF AQUEOUS SOLUTIONS OF SODIUM CHLORIDE, SULPHATE, METABISULPHITE AND THIOSULPHATE IN A VOLUME EQUIVALENT TO 5 PER CENT WEIGHT

Salt in loading fluid	NaCl	Na_2SO_4	$Na_2S_2O_5$	$Na_2S_2O_3$
Water load excreted per cent	46·5 ± 9·32 (4)	6·9 ± 0·82 (4)	65·5 ± 4·40 (4)	45·2 ± 11·61 (4)
Inorganic sulphur as SO_4'' in mg.	8·9 ± 0·62 (4)	22·2 ± 3·94 (4)	197·4 ± 6·47 (4)	79·2 ± 11·11 (4)
Organic sulphur as SO_4'' in mg.	1·5 ± 0·21 (4)	3·4 ± 0·37 (4)	2·1 ± 1·61 (4)	4·0 ± 1·42 (4)
Sulphur load excreted per cent	—	7·1 ± 1·15 (4)	55·1 ± 6·24 (4)	23·1 ± 3·11 (4)

The values shown are means + S.E. (number of groups).

metabisulphite, thiosulphate and sulphate in a volume equivalent to 5 per cent of body weight. Equivalent amounts of sodium were present in all these solutions and equal amounts of sulphur in the last three. The results of these experiments are summarised in Table II. The differences between the proportions of the sulphur load excreted in the 4 hours immediately after the ingestion of the three sulphur compounds were highly significant ($P = 0·01$ by t test). Approximately 55 per cent of the sulphur load of metabisulphite, 23 per cent that of thiosulphate and 7 per cent that of sulphate were eliminated. The sulphur load was excreted solely in inorganic form in each case.

Comparison of the Changes Induced in the Urinary Excretion of Sulphur by the Intraperitoneal Injection of Isotonic Solutions of metabisulphite, thiosulphate, and sulphate

Intraperitoneal injections of isotonic aqueous sodium metabisulphite in volume equivalent to 3 per cent of body weight caused an immediate restlessness rapidly followed, in each of 12 rats, by cyanosis, prostration and cardiovascular collapse. Half the animals died at this stage, within 35 minutes of the injection. The remainder had begun to recover at 40 minutes and appeared normal half an hour later. No such toxic

effects resulted from similar intraperitoneal injections of sodium thiosulphate or sulphate. The toxic symptoms of intraperitoneal metabisulphite were not seen when the dose was reduced to one-quarter by dilution of the original isotonic solution 1 part in 4 with isotonic sodium chloride. After trial, one-twelfth the LD50 concentration of metabisulphite was selected for use in comparison of the effect of intraperitoneal injections of isotonic solutions of metabisulphite, thiosulphate and sulphate in volume equivalent to 3 per cent of weight on the urinary excretion of sulphur. The results of one such experiment, typical of three, are shown in Table III. In these Group III experiments the total sulphur injected, expressed as mg. of sulphate per 100 g. weight, amounted to 4·81 when metabisulphite or thiosulphate, and 3·95 when sulphate solutions were used. Neither the form of excretion nor the extent of the excretion of this sulphur differed whether metabisulphite, thiosulphate or sulphate had been given by the intraperitoneal route. In the first 4 hours after injection

TABLE III

COMPARISON OF THE URINARY SULPHUR OUTPUTS IN THE 4 HOURS FOLLOWING THE INTRAPERITONEAL INJECTION OF SODIUM METABISULPHITE, THIOSULPHATE, SULPHATE AND CHLORIDE IN ISOTONIC SOLUTIONS, IN VOLUME EQUIVALENT TO 3 PER CENT WEIGHT

Loading solution	Sodium metabisulphite	Sodium thiosulphate	Sodium sulphate	Sodium chloride
Water load per cent	36·7 ± 5·23 (6)	44·3 ± 9·38 (6)	39·6 ± 4.42 (6)	37·2 ± 5·8 (6)
S.G.	1030 ± 3·43 (6)	1020 ± 3·59 (6)	1022 ± 2·94 (6)	—
Inorganic sulphur as SO_4'' in mg.	28·4 ± 1·56 (6)	27·6 ± 2·88 (6)	21·3 ± 6·73 (6)	6·50 ± 3·11 (6)
Total sulphur as SO_4'' in mg.	30·0 ± 1·35 (6)	29·2 ± 2·95 (6)	23·2 ± 1·84 (6)	7·70 ± 1·58 (6)
Reducing power as SO_3 in mg.	2·0 ± 0·90 (6)	2·4 ± 0·42 (6)	2·4 ± 0·33 (6)	—
Sulphur load excreted per cent	88·6 ± 5·29 (6)	84·9 ± 11·7 (6)	87·7 ± 14·16(6)	—

The values shown are means + S.E. (numbers of groups).

80 to 90 per cent of the administered sulphur was eliminated as inorganic sulphate; this was accompanied by very small amounts of iodine reducing substances. The diureses evoked by these sulphur containing solutions matched that caused by nearly equivalent sodium loading (Table III).

DISCUSSION

The rates of the urinary elimination of sulphur, provided in the forms of metabisulphite, thiosulphate or sulphate, were indistinguishable when these compounds were given by intraperitoneal injection (Table III) but differed when the oral route was substituted (Table II). The form in which the sulphur appeared in the urine was the same whether metabisulphite, thiosulphate or sulphate was administered. Therefore it may be inferred that the rates of absorption of these compounds from the gastrointestinal tract differ. Rats absorb metabisulphite more rapidly than thiosulphate, and sulphate is, as expected, absorbed only to a very limited extent.

Administered thiosulphate and metabisulphite are not excreted as such by rats because the ingestion or injection of these two iodine reducing substances caused no greater increase in the iodine reducing power of the urine during their renal excretion than did that of sulphate (Tables I and

III) and that increase was very small (Table I). All three compounds were excreted in the urine almost solely in the form of sulphate. It must therefore be concluded that the only important detoxification system for metabisulphite and thiosulphate in the rat is an oxidative one (Table III). Fridovich and Handler[3,4], have shown that the oxidation of sulphite by rat livers does take place. An intermediate thiosulphonate is formed and hydrolysed to yield a suphydryl compound and a sulphate. These reactions were shown to involve the reversible participation of a flavo-protein and hypoxanthine proved an obligatory co-factor at pH values of and in excess of 7·6.

The small amount of iodine reducing substance in normal rat urine may be, by analogy, thiosulphate (Table I) for small amounts of thio-sulphate are usually present in the urine of man[5] and in that of cats and dogs[6]. This thiosulphate of normal urine probably arises from the sulphur containing amino acids of the diet[7]. Since rat liver converts mercaptopyruvate rapidly and quantitatively to thiosulphate in the presence of sulphite[8] the slight increase in the iodine reducing power of rat urine during the elimination of the metabisulphite should probably be attributed to the excretion of slightly more than the usual trace of thiosulphate.

Acknowledgements.—The expenses of this work were defrayed from a grant made by Unilever & Co. Ltd., which also provided a personal grant to B. Bhaghet for assistance during training in research.

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A NOTE ON SODIUM SALICYLATE AND TISSUE 5-HYDROXYTRYPTAMINE IN THE RAT

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Received June 7, 1960

The treatment of rats with large doses of sodium salicylate induces a marked decrease in the 5-hydroxytryptamine level of the skin without depleting the stores in the ileum.
After the addition of sodium salicylate to rat incubated skin, an increased release of 5-hydroxytryptamine into the surrounding fluid was noted in only 3 instances out of 10 using Vane's preparation of the rat fundus for the assay.

The subcutaneous injection of very small doses of 5-hydroxytryptamine (5-HT) causes a local inflammatory response in the rat[1]. The generalised[2], as well as the local[3] egg white anaphylactoid inflammatory reaction in rats is intimately connected with the release of 5-HT. The pretreatment of the rats by substances which release 5-HT, such as 48/80 or reserpine can mitigate or prevent the generalised[2] and local egg white reaction[3].

Sodium salicylate antagonises the inflammatory action of 5-HT[4] and has been shown to inhibit *in vitro* the anaphylactic release of histamine

TABLE I

The influence of sodium salicylate (1·5 g./kg. weight for 4 days, i.p.) upon the content of 5-HT in the rat skin and ileum

	5-HT μg./g. tissue ± S.E. of the mean	
	Controls	Sodium salicylate treated animals
Skin	1·087 ± 0·161	0·504 ± 0·112 P <0·01
Ileum	1·020 ± 0·210	1·100 ± 0·190

from the guinea pig lung[5,6] and from the rabbit blood[7]. As body 5-HT often has a fate similar to that of body histamine, we have studied the influence of sodium salicylate treatment upon the 5-HT stored in the rat skin and intestine.

Methods

White rats of both sexes were treated with daily sodium salicyclate by intraperitoneal injection (1·5 g./kg.) for four days. On the fifth day the animals were killed. The abdominal skin was shaved, cut into small pieces and extracted for 5-HT by the method of Coreale[8]. A piece of ileum was washed and also extracted. Control animals were injected with saline for four days. The samples of tissue extracts were tested for their 5-HT activity on Vane's[9] rat fundus preparation.

The *in vitro* experiments were done with the rat skin. A part of the abdominal skin was shaved, cut into small pieces and put into Krebs'

solution (1 g. of the skin in 10 ml. at 37°). This solution was then tested for its 5-HT activity before and after the addition a given dose of sodium salicylate. A small volume (0·1–0·2 ml.) of this fluid was added to a bath (10 ml.) with the isolated rat fundus preparation. For this preparation Krebs solution (at 37°) containing 10^{-7} atropine and 10^{-6} antazoline was used.

Results

As shown in Table I, the treatment with sodium salicylate lowered the 5-HT content of the rat skin. The quantity found in the skin after four days of treatment with 1·5 g./kg. salicylate daily amounted to only one half of the normal value. The difference was significant at the 1 per cent level of probability ($P<0·01$). In contrast to the skin, the quantity of 5-HT in the rat ileum was not lowered by sodium salicylate treatment but seemed to be slightly increased. However, the difference from the control value was not significant.

The *in vitro* experiments showed that the addition of sodium salicylate caused an increased in the activity of the incubation fluid in only three out of ten experiments. The addition of sodium salicylate to the bath with the Vane's preparation did not change its responsiveness when the drug was added in concentrations up to 10^{-5} g./ml. The same concentrations did not contract the test preparation. Hence, it seems feasible to assume that the increase of the activity of the incubation fluid, which was obtained after the addition of sodium salicylate, was caused by an increase in the concentration of the active substance in this fluid. As the bath fluid of the test preparation contained atropine and antazoline and as the effect of the incubation fluid was abolished by bromolysergic acid diethylamide it is thought that the active substance was 5-HT. This result would mean that the addition of sodium salicylate caused an increased release of 5-HT from the rat skin, which is in accordance with the *in vivo* experiments. The fact that the increase of 5-HT activity of the incubation fluid occurred in only 3 out of 10 experiments may be explained by assuming that minute quantities of 5-HT were released initially by the skin, so that the test preparation could not register the differences of the activity induced by sodium salicylate.

It should be mentioned that sodium salicylate when present in the bath with the Vane's rat fundus preparation in concentrations higher than 10^{-5}, reduced or inhibited both 5-HT and incubation fluid effects.

Discussion

The present experiments have shown that the treatment with sodium salicylate increased the release of 5-HT from the stores in the rat skin. It is interesting that only the skin 5-HT level was so lowered; the 5-HT content of the intestine not being significantly changed. The cause of this difference in the susceptibility of 5-HT stores in these two preparations to the action of sodium salicylate is not known. However, similar findings were described for reserpine[2] and cortisone[10]. Both these drugs more readily influenced the 5-HT stores in the skin than in the intestine.

It has been shown that sodium salicylate lowers the permeability of the subcutaneous tissue in the rat[4] and in the rabbit[12], acting unspecifically through the adrenal hormones. As variations in tissue 5-HT store might be expected to influence the tissue permeability, the present experiments suggest that the influence of sodium salicylate upon the tissue permeability may be related with its action upon the level of 5-HT in store. Therefore, the anti-inflammatory action of sodium salicylate may be related to its action on tissue 5-HT or other inflammatory substance(s) stored in various tissues or both. It might be expected that the tissue, depleted of humoral inflammatory agent by treatment with sodium salicylate, would react less readily to various factors which are thought to produce the inflammation by releasing such tissue agents from their stores.

It is interesting to note that 5-HT, itself injected in very high doses, had an anti-inflammatory action in the rat[3]. However, this action of 5-HT was probably mediated through a modification of the hormonal secretion of the adrenals[14].

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STABILITY OF ATROPINE SOLUTIONS: BIOLOGICAL AND CHEMICAL ASSAYS

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Received August 15, 1960

Atropine solutions stored at room temperature for 2 to 6 years were assayed biologically using the anti-acetylcholine action as end point. The activity of the 6-year old solutions was about 25 per cent below the labelled strength. The loss of activity was proportional to the duration of storage. Colorimetric assays on some of these samples confirmed these results. The spasmolytic and lethal activities of two other samples of partially hydrolysed atropine solutions were also determined. The presence of the hydrolysis products did not alter the ratio of these two activities of atropine. But, the biological activities of the sample, in which the hydrolysis amounted to 44 per cent, were greater than those attributable to the unhydrolysed atropine.

There is a growing practice to store atropine solutions for use in accidental poisonings with certain pesticides. It was therefore desirable to ascertain the stability of these solutions. Using a chemical method, Kondritzer and Zvirblis[1] found that atropine solution was relatively stable in acidic media. They estimated that at a temperature of 30° and pH 4·5, the hydrolysis of atropine would amount only to 25 per cent in about 100 years. On the other hand, Huycke[2] noted that one-third of the pharmacological activity of the atropine solutions tested was lost after the solutions had been stored for 4 to 5 years.

Schriftman and Kondritzer have reported that tropine and tropic acid, the hydrolysis products, did not interfere with the chemical analysis of atropine[3]. The effects of these substances on the biological activities of atropine have not been ascertained.

Experimental

Material. Samples of five batches of atropine tartrate solution and two batches of atropine sulphate solution of commercial origin were used. The atropine tartrate samples contained 2 mg./ml. in terms of atropine sulphate and in addition 2·4 per cent (w/v) glycerol and 0·4 per cent (w/v) phenol. The atropine sulphate samples contained 0·3 mg./ml. and in addition, sodium acetate 2·177 mg./ml. and acetic acid 0·48 mg./ml. The pH of all samples was about 4·5.

Two large samples of partially hydrolysed atropine sulphate solution were prepared by adjusting the pH to 8·8 with NaOH and heating at 70°. Heating was discontinued when approximately the desired degree of hydrolysis, as shown by chemical analysis, was achieved. These samples were subsequently acidified with hydrochloric acid, and untreated atropine sulphate solution was then added to adjust to the final concentration and

degree of hydrolysis to the preselected values. The mixtures were assayed colorimetrically. The provisional standard used was Merck's atropine sulphate powder.

Biological assay. This was made on the isolated guinea pig ileum. The ileum was suspended in the tissue bath of an automatic biological assay apparatus (Casella Electronics). The tissue bath of about 6 ml. in capacity was filled with Tyrode's solution which was kept at 37° and aerated with a mixture of 95 per cent of oxygen and 5 per cent CO_2. The control unit of the apparatus was set so that the following events took place in each cycle: (1) Wash out and refill of the tissue bath with Tyrode's solution, (2) Rest I, 30 seconds, (3) Wash out and refill, (4) Rest II, 30 seconds, (5) Wash out and refill with Tyrode's solution containing acetylcholine (10^{-8}) and (6) Contact, 40 seconds. The duration of each cycle was about 2 minutes.

The intestinal contraction induced by acetylcholine during Event 5 and 6 of each cycle was recorded by means of an electrical stylus on a kymograph. The power supply to the stylus and the kymograph were automatically turned on before Rest II had turned off at the end of Contact. After the response of the intestine to acetylcholine had become stabilised, different concentrations ($2–5 \times 10^{-9}$) of atropine were added during Event 3. The depressant effect of atropine on the response to the subsequent dose of acetylcholine was used as the criterion for the assay. A dose of atropine was given only after every five acetylcholine doses to ensure nearly complete recovery of the intestine from the effect of atropine. The sample to be tested and the standard solution of atropine were given alternately and 1×2-dose design[4] was adopted in these assays. Each batch was assayed twice and the weighted mean potency and its confidence limits were estimated.

In addition to the spasmolytic activity, the lethal activity of the two large samples of partially hydrolysed atropine was compared to that of the standard. This was done on adult male rats of the Wistar strain. Their weight was between 150 and 200 g. The rats were randomly distributed in nine groups of 15 each. Three graded doses of each sample were given to these rats. The mortality rates were converted to probits. The LD50 and the relative potencies were computed by probit analysis[5,6].

Chemical assay. The chemical assay was made by the Vitali-Morin method[7], except that dimethyl formamide was used instead of acetone. The sample, neutralised if alkaline, was placed in a 20 ml. beaker and evaporated to dryness over a steam bath. After the addition of 0·3 ml. of fuming nitric acid (Merck reagent grade, sp.gr. 1·5, 90 per cent HNO_3) the solution was evaporated as before. After cooling the beaker, 5·00 ml. of dimethyl formamide (Anachemia) was added to dissolve the nitrated product and 3·00 ml. of the resulting solution was transferred to a 1 cm. cuvette. Colour was developed by the addition of 164·8 μl. of 3 per cent KOH dissolved in methanol. The solutions were mixed rapidly and the absorbance determined at 555 mμ exactly 60 seconds after mixing. Absorbance decreased approximately 2 per cent per minute. A blank was run using water instead of atropine solution. Beer's law was followed

up to 50 μg./3·16 μl. of final coloured solution. The blank was renewed when it began to show turbidity.

Results and Discussion

A section of the kymographic tracing of a typical biological assay is reproduced in Figure 1. The intestinal contractions were induced by acetylcholine. After the first dose of atropine, the standard preparation

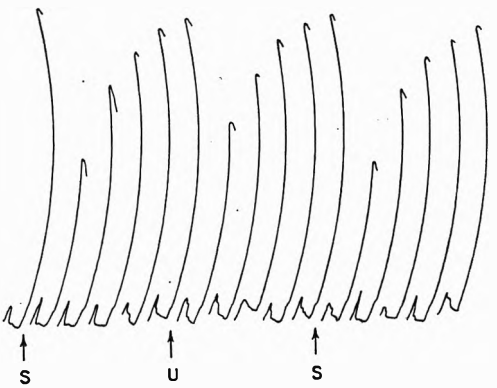

Fig. 1. A portion of the tracing of a typical biological assay. The intestinal contractions were induced by acetylcholine (10^{-8}). At the two S marks, Tyrode's solution containing the atropine standard (3×10^{-9}), instead of plain Tyrode's solution, was introduced into the tissue bath, before Rest II. This was washed out before adding the subsequent doses of acetylcholine. At U, Tyrode's solution containing a sample of aged atropine (4×10^{-9}) was added.

(S), the contraction was reduced by 47·6 per cent. The intestine gradually recovered after repeated doses of acetylcholine. The second dose of atropine was one of the aged samples (U) and it depressed the contraction by 34·2 per cent. Another dose of the standard (S) induced a 47·3 per cent depression of the intestinal contraction. The experiment was continued in this manner with a single concentration of the atropine standard, while two concentrations of the aged atropine sample were given alternately. The two concentrations of the aged sample were so selected that one would produce a greater and the other a smaller spasmolytic effect than the standard.

In Table I are listed the results obtained in the biological as well as the chemical assays. It may be noted that there was no significant difference between these two methods. These results also indicate that there was a definite inverse relation between the strength of the sample and its duration of storage. The regression equations calculated from the biological and chemical assays, according to the least square principle[8] are respectively:

$$E = 100{\cdot}8 - 0{\cdot}3376\ X \text{ and}$$
$$E = 92{\cdot}4 - 0{\cdot}2086\ X,$$

E being the estimated strength and X the duration of storage in months. From these equations, the spasmolytic activity of a 5-year old atropine

solution is estimated as 80·5 per cent of the original potency and the chemical strength 82·0 per cent. These two estimates are not significantly different.

Since the clinical dose of atropine may vary considerably, a 20 per cent decrease in potency was considered insignificant; it would only necessitate the administration of a slightly larger dose to obtain the same effect when

TABLE I

THE STRENGTHS OF AGED ATROPINE SOLUTIONS AS DETERMINED BY BIOLOGICAL AND CHEMICAL ASSAYS. THE VALUES (WEIGHTED MEAN AND CONFIDENCE LIMITS) ARE PERCENTAGES IN TERMS OF THE STANDARD

Sample*	Age**	Biological	Chemical
1	73	73·4 (68·4–78·7)	—
2	70	73·8 (66·8–81·5)	—
3	67	77·4 (72·9–82·2)	74·6 (69·7–79·9)
4	66	79·9 (73·4–86·9)	80·6 (74·9–86·8)
5	53	94·3 (85·8–103·6)	84·0 (77·9–90·6)
6	30	92·8 (85·2–101·2)	84·5 (81·8–87·2)
7	25	86·7 (81·6–92·2)	87·5 (85·0–90·0)

* Samples 1 and 2 are atropine sulphate solutions, and the others are atropine tartrate solutions.
** Age in months from date of packaging to date of assay.

5-year old atropine solution is used. However, old atropine solutions would be more undesirable if the hydrolysis products would augment the toxic but not the therapeutic action of atropine. According to the results summarised in Table II, these two biological activities of atropine were increased slightly and almost equally by the hydrolysis products when they were present in higher proportions.

The LD50 of the atropine sulphate standard in these rats was 255 mg./kg. with confidence limits (P = 0·05) at 232–281 mg./kg. This is similar to the LD50 of 280 (225–350) mg./kg. reported by Cahen and Tvede[9].

TABLE II

THE EFFECT OF THE HYDROLYSIS PRODUCTS ON THE SPASMOLYTIC (ISOLATED GUINEA PIG ILEUM) AND LETHAL (LD50 IN RATS) ACTIVITIES OF ATROPINE. THE VALUES (WEIGHTED MEAN AND CONFIDENCE LIMITS) ARE PERCENTAGES IN TERMS OF THE STANDARD (SAMPLE 1)

Sample	Unhydrolysed atropine per cent	Spasmolytic activity	Lethal activity
1	100	100	100
2	70·8	74·4 (68·4–80·8)	71·2 (62·8–80·6)
3	56·2	63·8 (58·4–69·8)	69·4 (64·8–74·4)

Five of the seven samples assayed on the ileum (Samples 3–7, Table I) had been assayed by Huycke[2] on the mouse pupil (Sample 4–8 in his paper). It is evident from these results that there was a smaller loss in the spasmolytic activity than the mydriatic. The difference is especially notable since the spasmolytic tests were carried out about 15 months after mydriatic tests. The reason for this discrepancy is unknown. However, it is possible that the hydrolysis products may affect these two biological actions of atropine in different ways.

The rate of deterioration of atropine as indicated by the biological and chemical assays, although slower than that reported by Huycke, is faster than that estimated by Kondritzer and Zvirblis[1]. Whether the excipients in the commercial atropine solutions have any effect on the rate of hydrolysis is unknown.

Acknowledgement. The authors wish to thank Dr. L. I. Pugsley for his interest in this study.

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BOOK REVIEW

RADIOACTIVE TRACERS IN CHEMISTRY AND INDUSTRY. By Pascaline Daudel. Translated into English by U. Eisner. Pp. x + 210 (including index). Charles Griffin and Company Ltd., London, 1960. 36s.

There is a wealth of information to be found in Mme. Daubel's book and it should be read by all who are interested in radiochemical techniques.

There are five chapters, the first of which deals with General Principles. It describes in detail the preparation of radioelements by the Szilard-Chalmers effect. It goes on to describe methods by which a labelled molecule can be prepared by total synthesis, irradiation, or by exchange reactions. The chapter closes with a brief account of the methods used to detect and measure radiation. In the opinion of the reviewer this should be augmented or left out altogether, in future editions.

The application of tracer techniques to the study of reaction mechanisms is given in chapter two. Numerous examples are described and these will be of value to the organic and inorganic chemist alike. Typical of these is the use of ^{128}I to study the reaction between potassium periodate and labelled potassium iodide. The potassium iodate formed in this reaction was found to be completely inactive and must have been derived from the periodate. Only by the use of tracers could this mechanism be shown. Many organic examples are described and typical of these is the work of Dauben and others on a study of the mechanism of the Willgerodt reaction. The chapter is concluded by nine pages of references to original work.

A typical exchange reaction—the Walden inversion is described in the next chapter. This is concerned with the application of tracers to a study of exchange reactions; but it is marred by an excessive number of references, there are 23 pages of them compared with 22 pages of text.

The description of the use of tracer techniques in chemical analysis is extremely valuable. But as with all chapters in this book the maximum benefit will be derived if the reader has an elementary knowledge of radiochemistry since in one or two places terms are used without explanation, for example the reference to the Compton compensating device, on page 113.

Chapter 5, the last chapter, has been written by Dr. N. Robinson. This describes the application of tracers to industrial problems.

There are very few misprints; the type and diagrams are clear but the book is rather expensive for its size (36s.).

R. FLEMING

LETTER TO THE EDITOR

Claudogens—A New Term for Antifertility Steroids

SIR,—It is becoming increasingly difficult to define the precise mechanism of action of antifertility steroids. Such highly progestational agents as 17α-acetoxy-6α-methyl-16-methylenepregn-4-ene-3,20-dione,[1] 17α-acetoxy-6-methylpregna-4,6-diene-3,20-dione[2] and 17α-acetoxy-6α-methylprogesterone[3], for example, can undoubtedly exert their antifertility effect by preventing ovulation. At the same time it has been suggested[4] that compounds of this type may effectively bar conception at doses lower than those required to inhibit ovulation by congealing the cervical mucous and so rendering it inimical to the sperm. Certain 19-norsteroids can additionally interfere with the implantation and postimplantation stages under selected experimental conditions. Other instances of multiplicity of biological actions at differing dose levels will be known to workers in this field.

In these circumstances it seems desirable to introduce a descriptive term applicable to steroidal products with antifertility action. It is suggested that the word "claudogenic", incorporating the prefix "claudo" derived from *claudere* (to halt) would be an appropriate adjective for this purpose. The active agent could then be termed a "claudogen".

V. PETROW.

Research Department,
The British Drug Houses Ltd.,
London, N.1.
September 7, 1960.

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