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Research Papers

Toxicity and tissue distribution studies on the hydrochloride, bismuth iodide complex and a resinate of emetine

K. J. CHILD, B. DAVIS, M. G. DODDS AND E. G. TOMICH

The hydrochloride, the bismuth iodide complex and a resinate of emetine have been compared on mice, cats and dogs for toxicity, on cats for emetic activity and on rats for distribution of emetine in the tissues. Emetine hydrochloride, injected subcutaneously, was as toxic as emetine hydrochloride or emetine bismuth iodide administered orally, but only one-sixth as emetic. Given orally emetine resinate was considerably less toxic and emetic than the other preparations. The patterns of emetine distribution in the tissues of rats were similar for all the preparations, but the tissue concentrations were much lower with emetine resinate. In infected rats the resinate was as amoebicidal as the hydrochloride or the bismuth iodide complex. It is suggested that emetine resinate might be better than emetine bismuth iodide for the treatment of intestinal amoebiasis.

EMETINE is potentially very toxic (Klatskin & Friedman, 1948), yet it has retained its pre-eminence in the treatment of amoebic infection despite the challenge of several new amoebicides (Woodruff, Bell & Schofield, 1956; Woodruff, 1959; Adams, 1960; Bell & Woodruff, 1960; Carneri, Coppi, Almirante & Logemann, 1960; Dooner, 1960; Wilmot, Powell, McLeod & Elsdon-Dew, 1962).

Professor W. H. Linnell suggested to us that a study of emetine resinates might be rewarding. Several resinates were made, and one was compared with emetine hydrochloride and the bismuth iodide complex.

Materials and methods

Emetine hydrochloride B.P. (71% emetine base*). An aqueous solution of the hydrochloride was used for subcutaneous administration to mice, rats, cats and dogs, and a solution in 0·5% sodium carboxymethylcellulose for oral administration to mice and rats. For oral administration to cats and dogs, the hydrochloride as powder was presented in hard gelatin capsules.

Emetine bismuth iodide B.P. (28% emetine base*).

Emetine resinate. Emetine adsorbed from aqueous solution on a polystyrene sulphonic acid ion exchange resin (Permutit Zeo Karb 225, SRC 16) gave a resinate containing 23% emetine base† after drying.

Both emetine bismuth iodide and resinate were presented as suspensions in 0·5% sodium carboxymethylcellulose for oral administration to mice and rats and as powders in hard gelatin capsules for oral dosing to cats and dogs. The bismuth iodide was administered to dogs also as enteric coated tablets.

From Glaxo Research Ltd., Greenford, Middlesex

* Determined by Kjeldahl nitrogen and B.P. assay for emetine.
† Determined by Kjeldahl nitrogen.

TOXICITY

Acute systemic toxicity in mice. Groups of 10 female mice (A2G strain, body weights 18–22 g) were dosed subcutaneously with the hydrochloride or orally with all three preparations. Group mortalities were recorded 14 days later and the LD50 values were calculated by the method of de Beer (1945).

Subacute toxicity in dogs. Mongrel dogs of either sex were dosed according to the schedules in Table 2. When possible, dosing was continued for 56 days, but some of the animals died earlier or became so ill that they had to be destroyed.

ACUTE EMETIC ACTIVITY IN CATS

Adult cats of either sex which had been fasted overnight received single subcutaneous doses of the hydrochloride or single oral doses of all three preparations, and a positive score was recorded for each animal that vomited within 24 hr. The dose (mg base/kg) that should produce emesis in 50% of the cats receiving it (ED50) was calculated for each preparation. No animal was dosed more than once weekly.

TISSUE EMETINE LEVELS IN RATS

Each of 96 female rats of the WAG strain (initial body weight approximately 100 g) received the equivalent of 100 μg emetine base (approximately 1 mg/kg) once daily for up to 10 days. The hydrochloride was administered subcutaneously and all three preparations orally; 24 animals received each treatment.

Three rats from each group were killed with coal gas 24 hr after receiving their first, second, third, sixth or tenth doses; the remaining rats were killed 5, 12 or 19 days after their last doses. The emetine concentrations in the heart, kidneys, liver, lungs and spleen were determined on the bulked organs from each group of 3 animals by the spectrophotofluorometric method of Davis, Dodds & Tomich (1962).

Results

TOXICITY

Acute systemic toxicity in mice. The LD50 values are given in Table 1. Emetine hydrochloride and bismuth iodide were equally toxic by the oral

TABLE 1. ACUTE TOXICITY OF EMETINE PREPARATIONS TO MICE

Preparation	Emetine hydrochloride		Emetine bismuth iodide	Emetine resinate
Route	subcutaneous	oral	oral	oral
Doses (mg base/kg)	Mortalities per group of 10 mice			
20	0	0	0	–
25	0	1	0	–
30	2	5	2	–
35	9	9	7	–
40	10	10	9	–
145	–	–	–	–
218	–	–	–	–
290	–	–	–	–
1370	–	–	–	1
2280	–	–	–	4
LD50 (mg base/kg)	32	30	33	>2280

TABLE 2. SUBACUTE TOXICITY OF EMETINE PREPARATIONS TO DOGS

Sex of dog	Compound	Route	Treatment mg base/kg/day	Initial weight	Final weight	Fate	Cumulative dose mg base/kg
Female	Emetine hydrochloride (solution)	Subcutaneous	1·4	7·5	5·3	Died on day 9	7·0
Female	Emetine hydrochloride (solution)	Subcutaneous	1·4	6·0	4·2	Died on day 8	7·0
Female	Emetine hydrochloride (solution)	Subcutaneous	1·0	3·4	3·4	Died on day 7	5·0
Male	Emetine hydrochloride (solution)	Subcutaneous	1·0	3·7	3·6	Died on day 13	10·0
Female	Emetine hydrochloride (in gelatin capsules)	Oral	1·0	5·0	4·1	Died on day 16	11·0
Female	Emetine hydrochloride (in gelatin capsules)	Oral	1·0	3·3	3·2	Died on day 13	10·0
Female	Emetine bismuth iodide (in enteric coated tablets)	Oral	1·7	8·0	7·7	Very ill on day 15: Killed	17·0*
Male	Emetine bismuth iodide (in enteric coated tablets)	Oral	1·7	8·5	8·3	Very ill on day 15: Killed	17·0*
Female	Emetine bismuth iodide (in gelatin capsules)	Oral	1·0	4·3	3·0	Died on day 16	11·0
Female	Emetine bismuth iodide (in gelatin capsules)	Oral	1·0	3·4	3·2	Died on day 10	7·0
Male	Emetine resinate (in gelatin capsules)	Oral	1 for 2 weeks 1 for 2 weeks 4 for 2 weeks 8 for 2 weeks	2·7	8·0	Appeared normal on day 56	148·0
Female	Emetine resinate (in gelatin capsules)	Oral	2 for 2 weeks 4 for 2 weeks 8 for 2 weeks 16 for 2 weeks	2·6	9·1	Appeared normal on day 56	296·0

‡ Some tablets appeared unchanged in the faeces.

route and as toxic as the hydrochloride by the subcutaneous route. Orally the resinate exhibited one-eightieth the toxicity of the hydrochloride.

Subacute toxicity in dogs. The results are given in Table 2. Dogs were highly sensitive to the toxicity of emetine administered as hydrochloride or bismuth iodide. All four dogs injected subcutaneously with hydrochloride died after cumulative doses of 5 to 10 mg emetine base/kg, and two receiving it orally died after 10 or 11 mg/kg. Each of the two dogs dosed with enteric coated tablets of the bismuth iodide had to be killed after receiving a total dose of 17 mg emetine base/kg. Several enteric coated tablets were found in the faeces of these animals.

In contrast, two dogs dosed with resinate showed no ill effects: they grew well and appeared perfectly healthy after 56 days, by which time one had received a total of 148 and the other 296 mg emetine base/kg.

ACUTE EMETIC ACTIVITY IN CATS

From Table 3 it may be seen that emetine hydrochloride and bismuth iodide given orally were equally emetic and both were approximately 120 times more emetic than the resinate. The emetic activity of the hydrochloride by the oral route was 6 times that by the subcutaneous route.

TABLE 3. EMETIC ACTIVITY OF EMETINE PREPARATIONS IN CATS

Compound	Emetine hydrochloride						Emetine bismuth iodide		Emetine resinate			
Route	oral		subcutaneous				oral		oral			
Dose mg emetine base/kg	0·35	0·7	1·4	2·8	4·2	5·6	0·55	1·1	10	20	40	80
No. vomited / No. dosed	2/9	6/8	0/5	2/9	5/5	4/5	2/5	4/5	0/1	0/2	0/4	2/4
ED50*	0·6		3·5				0·7		ca. 80			

* ED50. Dose in mg emetine base/kg that causes emesis in 50% of cats.

TISSUE EMETINE DISTRIBUTION IN RATS

From Table 4 it may be seen that the emetine distribution pattern in the organs examined was similar for all three preparations and that of the hydrochloride was unaffected by the route of administration. The spleen had the highest concentrations of emetine, with the lungs, liver, kidneys and heart in decreasing order. The total amounts of emetine found in these organs during and after each treatment are given in Fig. 1. Cumulation occurred with all treatments and, except in the group which received emetine resinate, emetine was detectable in some organs 19 days after the last dose.

Emetine concentrations of similar order were found in the tissues after the oral administration of emetine bismuth iodide or hydrochloride, or after subcutaneous administration of the hydrochloride. Emetine resinate, however, produced emetine concentrations less than one-third of those produced by the hydrochloride.

TABLE 4. TISSUE EMETINE LEVELS IN RATS DURING AND AFTER 10 CONSECUTIVE DAILY DOSES OF DIFFERENT EMETINE PREPARATIONS

Treatment† and route	Days after beginning treatment	Cumulative dose to group (mg emetine base)	Emetine concentration (µg base per g wet tissue)*					Total emetine in tissues examined	
			Heart	Kidney	Liver	Lung	Spleen	(µg base)*	% of dose)*
Emetine hydrochloride Subcutaneous	1	0·3	1·0	2·6	2·9	4·9	6·8	68·4	22·8
	2	0·6	1·0	3·8	4·5	7·4	9·8	107·0	17·8
	3	0·9	2·1	5·1	5·5	10·5	12·4	148·0	16·4
	6	1·8	2·3	8·8	10·8	16·7	22·8	265·2	14·8
	10	3·0	3·6	11·7	11·7	17·7	21·2	327·2	10·9
	14	3·0	3·2	9·5	8·4	15·1	17·7	251·6	8·4
	21	3·0	1·5	4·0	3·2	7·6	9·8	105·6	3·5
	28	3·0	0·6	1·5	1·5	3·2	5·5	54·7	1·8
Emetine hydrochloride Oral	1	0·3	1·3	3·4	3·9	5·5	6·5	83·7	27·9
	2	0·6	1·6	4·9	6·5	17·9	10·7	156·5	26·1
	3	0·9	1·2	4·9	5·5	9·2	9·2	143·7	16·0
	6	1·8	2·4	8·0	10·7	16·5	19·2	251·5	13·9
	10	3·0	3·3	8·6	9·0	16·7	17·4	245·4	8·2
	14	3·0	1·8	5·7	5·2	10·4	16·1	164·7	5·5
	21	3·0	1·3	2·9	2·5	6·7	8·9	86·6	2·9
	28	3·0	0·4	0·4	0·6	1·5	3·1	21·6	0·7
Emetine bismuth iodide Oral	1	0·3	1·1	3·0	3·3	5·0	4·3	75·2	25·1
	2	0·6	1·5	5·2	6·1	8·8	13·7	126·9	21·2
	3	0·9	1·8	5·8	5·2	11·1	17·1	135·8	15·1
	6	1·8	2·4	8·3	10·1	13·6	20·3	241·0	13·4
	10	3·0	3·1	9·6	10·2	18·2	18·5	292·2	9·7
	14	3·0	1·3	4·9	6·0	17·4	15·3	175·1	5·8
	21	3·0	2·5	2·2	2·2	6·3	7·9	79·7	2·7
	28	3·0	0·0	0·7	0·0	2·2	3·1	15·3	0·5
Emetine resinate Oral	1	0·3	0·3	0·3	0·5	1·1	0·5	12·5	4·2
	2	0·6	0·8	1·3	1·9	2·4	2·4	33·1	5·5
	3	0·9	0·6	1·5	1·8	2·5	3·1	40·2	4·5
	6	1·8	0·6	1·5	2·4	5·0	5·3	63·9	3·6
	10	3·0	1·3	2·9	3·3	5·7	5·4	89·7	3·0
	14	3·0	0·5	0·5	1·3	2·5	2·8	35·3	1·2
	21	3·0	0·0	0·3	0·0	0·5	1·0	4·2	0·1
	28	3·0	0·0	0·2	0·2	0·3	0·6	5·5	0·2

* Group values (3 rats per group).
† Each rat received the equivalent of 100 µg emetine base once daily for 10 days.

Discussion

An ideal amoebicide should eliminate the parasites, wherever they may be located, without harming the host. Since emetine is toxic, any derivative that liberates the free base will fall short of this ideal.

Studies of distribution in the tissues of rats show that orally administered emetine hydrochloride and bismuth iodide are both well absorbed and that the levels obtained are similar to that after subcutaneous hydrochloride. Further, the bismuth iodide given orally was as toxic to mice as the hydrochloride given orally or subcutaneously, and orally the two compounds were equally emetic to cats. It would therefore seem that neither derivative is ideal for the treatment of intestinal amoebiasis. It should be emphasised, however, that in our experiments on the bismuth iodide the mice and rats were dosed with aqueous suspensions and the cats with gelatin capsules, whereas patients generally receive either gelatin capsules or enteric coated tablets. In dogs emetine bismuth iodide was less toxic than the hydrochloride; this was not surprising, because some of the enteric coated tablets were found in the faeces. If patients likewise eliminate some of their enteric coated tablets of emetine bismuth iodide

it explains why this preparation given in this form is not generally considered as effective as the hydrochloride in the treatment of hepatic amoebiasis, even though Woodruff (1959) has suggested that with a standard course of emetine bismuth iodide sufficient emetine is absorbed from the intestine for the purpose. Where, therefore, apparent paradoxes exist in the correlation of laboratory animal and clinical observations, these may be related to the differences in degree of absorption of emetine from the preparation administered and to species differences, such as that due to absence of a vomiting centre in the rat.

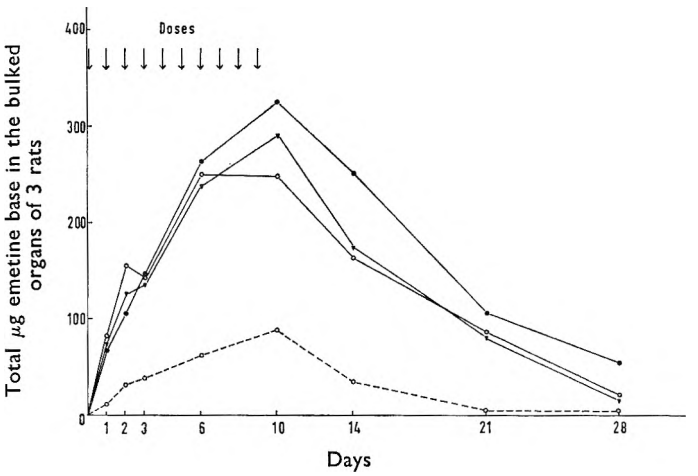

FIG. 1. Total amounts of emetine in the bulked organs of female rats (3 per group) during and after dosing once daily with various preparations of emetine equivalent to 100 μg emetine base per rat per day for up to 10 days. —●— Emetine hydrochloride—subcutaneous. —○— Emetine hydrochloride—oral. —▼—Emetine bismuth iodide—oral. ----○---- Emetine resinate—oral.

Combination of emetine with Zeo Karb 225, SRC 16, reduces its acute toxicity in mice, its subacute toxicity in dogs and its emetic action in cats. The reduced toxicity presumably reflects a lower degree of intestinal absorption, since tissue levels of emetine in rats after the resinate were lower than after the two other preparations.

The low toxicity of emetine resinate in animals is of interest because Muggleton, Heath & Johnson (personal communication) have shown that it is as effective as oral hydrochloride against intestinal *E. histolytica* infections in rats.

Preliminary results of clinical trials (Woodruff, personal communication) appear to confirm that the resinate is both effective and well tolerated by patients with intestinal amoebiasis.

Acknowledgements. We are grateful to Professor W. H. Linnell who prompted these studies and supplied us with the first resinate. Members of the Physical Chemistry Unit of Glaxo Research Ltd. prepared the

remaining resinates, including the one described, and Mr. P. G. Box and Miss O. Uvarov administered the drugs in the dog experiments. We thank Professor A. W. Woodruff for permitting us to report briefly on his findings before their publication.

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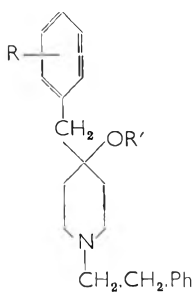
Halogen compounds related to the reversed esters of pethidine

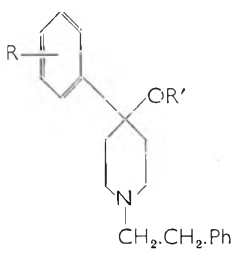
N. J. HARPER AND ALMA B. SIMMONDS

Some 1-phenethyl-4-piperidinols and their acyl esters have been prepared and investigated in a preliminary screening procedure as potential morphine-like analgesics or CNS depressants. 4-*p*-Fluorophenyl-1-phenethyl-4-propionoxypiperidine on oral administration to mice was found to be a potent morphine-like analgesic.

HARPER and Simmonds (1959) reported on a series of fluoro-compounds, namely *N*-substituted 4-fluorobenzyl-4-piperidinols and their acyl esters, which were structurally related to the prodine-type analgesics. Two of these compounds, 4-*o*-fluorobenzyl-1-phenethyl-4-piperidinol (I; R = *o*-F: R′ = H) and 4-*p*-fluorobenzyl-1-phenethyl-4-piperidinol (I; R = *p*-F: R′ = H), although devoid of morphine-like activity, appeared to possess some central nervous system depressant effect. It was therefore of interest to prepare the corresponding chloro-compounds (I; R = *o*- or *p*-Cl; R′ = H).



(I)



(II)

Since prodine-type compounds having a halogen substituent in the 4-phenyl group had not been prepared previously, it was also considered of interest to synthesise and assess pharmacologically, compounds of the type II (R = F, Cl, or CF_3, and R′ = H, CO·Me or CO·Et). Compounds with a 1-phenethyl group were chosen since prodine analgesics of this type have been shown to be potent morphine-like analgesics (Janssen & Eddy, 1960).

Chemistry

The piperidinols (I; R = *o*-Cl, or *p*-Cl and R′ = H; II: R = *p*-F, or *p*-CF_3 and R′ = H) were prepared by the addition of the appropriate Grignard reagent to 1-phenethyl-4-piperidone. The compound (II; R = *p*-Cl, R′ = H) was prepared by the aralkylation of 4-*p*-chlorophenyl-4-piperidinol, which was obtained from 4-*p*-chlorophenyl-1,2,3,6-tetrahydropyridine. The latter was prepared from *p*-chloro-α-methyl-

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styrene by the method of Schmidle & Mansfield (1956). The piperidinol (II; R = *m*-CF_3, R′ = H) was obtained by hydrolysis of the propionic ester.

The piperidinols were converted into their esters by refluxing with the appropriate acid anhydride in the presence of pyridine, or by treating the piperidinol-metallic complex with an acid anhydride.

4-*p*-Fluorophenyl-1-phenethyl-4-piperidinol and 4-*p*-chlorophenyl-1-phenethyl-4-piperidinol on refluxing with hydrobromic acid in aqueous methanol gave the corresponding tetrahydropyridine derivatives. 4-*p*-Chlorophenyl-1,2,3,6-tetrahydro-1-phenethylpyridine was also obtained by aralkylation of the corresponding secondary base.

Pharmacology

The piperidinols and their esters of type I (R = *o*- or *p*-Cl, R′ = H, CO·Me or CO·Et) (in the form of salts) were tested for analgesic activity by subcutaneous injection in mice using an adaptation of the hot-plate test as described by Janssen & Jageneau (1957). Significant activity was found only in one case, namely 4-*p*-chlorobenzyl-1-phenethyl-4-piperidinol (I; R = *p*-Cl, R′ = H), which had an ED50 of 27 mg/kg, comparable to that of the *p*-fluoro-analogue (I; R = *p*-F; R′ = H, ED50 22·5 mg/kg), previously prepared by Harper & Simmonds (1959). It was, however, less active than the *o*-fluoro-compound (I; R = *o*-F, R′ = H, ED50 16 mg/kg). Since the hot-plate test does not distinguish between morphine-type analgesics and other compounds which may increase the reaction time, mydriatic activity in mice was also determined as described by Jageneau & Janssen (1956). Janssen & Jageneau (1956, 1957) have shown that in many morphine-like analgesics there is a correlation between analgesic and mydriatic activities in mice. None of the above compounds had significant mydriatic activity.

In the case of the piperidinols of type II, only the compounds (II; R = *p*-F, R′ = H) (ED50 = 26 mg/kg) and (II; R = *p*-CF_3, R′ = H, ED50 = 30 mg/kg) had significant activity in the hot-plate test. In neither case did they appear to be morphine-like in character, since the compounds were devoid of mydriatic activity.

In the case of the esters of the type (II; R = *p*-F, *p*-Cl, *p*-CF_3 and *m*-CF_3 and R′ = CO·Me and CO·Et) which were tested by a hot-plate method as described by Beckett, Casy, Hall & Vallance (1961) only one (II; R = *p*-F, R′ = CO·Et) had morphine-like analgesic activity. (The Straub tail effect was noted at 32 mg/kg on oral administration.) This compound on subcutaneous administration to mice had an ED50 of 12 mg/kg (approximately four times the activity of pethidine), and on oral administration to mice had an ED50 of 41 mg/kg (three times the activity of codeine). This oral analgesic activity appeared to be associated with the *p*-fluoro-atom, since similar activity was not found in the *m*- or *p*-trifluoromethyl or *p*-chloro-analogues. A comparison with the unsubstituted analogues (II; R = H, R′ = CO·Et) is obviously of interest. Some preliminary investigations on the available acetoxy and

butyroxy compounds (II; R = H, R′ = CO·Me or CO·Pr^n) were carried out. In the hot-plate test these on oral administration to mice had ED50's of 71 and 62 mg/kg respectively, being 1·5 and 1·7 times, respectively, the activity of codeine and were thus less active than the *p*-fluoro-compound. Introduction of the *p*-fluoro-substituent also reduces toxicity, the LD50's in mice of the compounds (II; R = *p*-F, R′ = CO·Et) and (II; R = H, R′ = CO·Me) on oral administration being 500 and 125 mg/kg respectively.

The tetrahydropyridine derivatives prepared during this investigation did not have significant activity in the hot-plate test or mydriatic activity.

Experimental

4-p-*Fluorophenyl*-1-*phenethyl*-4-*piperidinol.* 1-Phenethyl-4-piperidone (6·8 g) in ether was added to a cooled, stirred, ethereal solution of *p*-fluorophenylmagnesium bromide prepared from *p*-bromofluorobenzene (11·7 g) and magnesium (1·75 g). The mixture was stirred for 2 hr, allowed to stand overnight and then decomposed by pouring on to crushed ice and acidifying with hydrochloric acid. The precipitated hydrochloride (9·5 g) was collected, dissolved in water and made alkaline with ammonia solution. Extraction with ether gave an oil which on treatment with ethanolic hydrogen chloride gave 4-p-*fluorophenyl*-1-*phenethyl*-4-*piperidinol hydrochloride*, m.p. 198° (from ethanol). Found: C, 67·2; H, 6·9; N, 4·3; Equiv. wt 344. $C_{19}H_{23}ClFNO$ requires: C, 67·9; H, 6·9; N, 4·2% Equiv. wt 336.

The ethereal layer from the decomposed reaction mixture was extracted with dilute hydrochloric acid and the combined acidic extracts made alkaline with ammonia solution. Extraction with ether gave an oil (3·1 g) which on treatment with ethanolic hydrogen bromide gave 4-p-*fluorophenyl*-1-*phenethyl*-4-*piperidinol hydrobromide*, m.p. 168° (from ethanol). Found: C, 59·4; H, 6·3; N, 3·6; Br, 21·1; Equiv. wt 383. $C_{19}H_{23}BrFNO$ requires: C, 60·0; H, 6·1; N, 3·7; Br, 21·0%. Equiv. wt 380.

In a similar manner, *p*-bromo-ααα-trifluorotoluene (18 g), magnesium (2·0 g) and 1-phenethyl-4-piperidone (10 g) gave 1-*phenethyl*-4-p-ααα-*trifluorotolyl*-4-*piperidinol hydrochloride* m.p. 178° (from ethanol). Found: C, 62·4; H, 6·3; N, 3·6; Equiv. wt 375. $C_{20}H_{23}ClF_3NO$ requires: C, 62·2; H, 6·0; N, 3·6%. Equiv. wt 386.

p-Chlorobenzyl chloride (10·7 g), magnesium (1·6 g) and 1-phenethyl-4-piperidone (6·8 g) gave 4-p-*chlorobenzyl*-1-*phenethyl*-4-*piperidinol hydrochloride* (10·4 g), m.p. 249° (from ethanol). Found: C, 65·6; H, 7·1; N, 3·9; Equiv. wt 366. 366. $C_{20}H_{25}Cl_2NO$ requires: C, 65·6; H, 6·9; N, 3·8%. Equiv. wt 366.

o-Chlorobenzyl chloride (36·0 g), magnesium (5·9 g) and 1-phenethyl-4-piperidone (15·5 g) gave 4-o-*chlorobenzyl*-1-*phenethyl*-4-*piperidinol hydrochloride* (20·7 g), m.p. 202° (from ethanol). Found: C, 65·6; H, 6·9; N, 3·9; Cl, 19·8; Equiv. wt 363. $C_{20}H_{25}Cl_2NO$ requires: C, 65·6; H, 6·9; N, 3·8; Cl, 19·4%. Equiv. wt 366.

THE HYDROLYSIS OF PROPYL BENZOATE IN AQUEOUS SOLUTIONS OF CETOMACROGOL. By A. G. Mitchell. *J. Pharm. Pharmacol.*, 1963, **15**, 761–765.

Corrigenda:

Page 762, under "Results" for "0·195" read "0·0195".

Page 764, Table 1, Column 2, for "c_s" read "c".

Page 764, Replace existing Fig. 3 with Fig. below.

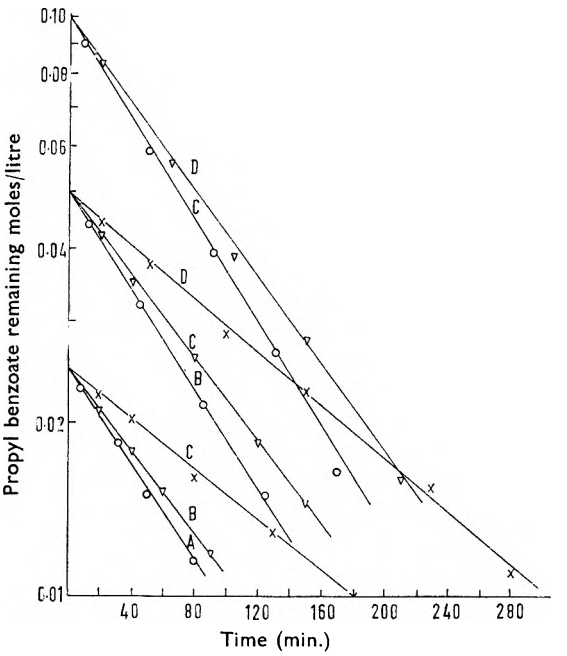

FIG. 3. First order rate plots for the alkaline hydrolysis of n-propyl benzoate in cetomacrogol at various saturation ratios. Temperature 35°. Saturation ratio × 0·25; ▽ 0·5; ○ 1·0. Cetomacrogol concentration (moles/litre) A, 0·0140; B, 0·0285; C, 0·0585; D, 0·128.

1-*Phenethyl*-4-m-ααα-*trifluorotolyl*-4-*piperidinol.* 1-Phenethyl-4-piperidone (20·3 g) in ether (60 ml) was added dropwise to a stirred cooled solution of the lithium aryl prepared by radical exchange from *m*-bromo-ααα-trifluorotoluene (36 g) and butyl-lithium in ether (100 ml). The butyl-lithium was prepared by the addition of butyl bromide (23·3 g) in ether (25 ml) to lithium (1·8 g) in ether (50 ml), cooled to −30° in an acetone/carbon dioxide bath. To the clear solution, propionic anhydride (14·3 g) in dry ether was added dropwise with stirring. The mixture became turbid and was stirred for a further 6 hr and then added to crushed ice and an excess of acetic acid. Some crystalline solid (the ester hydrobromide) was precipitated at this stage, and was collected and crystallised from ethanol to give 1-*phenethyl*-4-*propionyloxy*-4-m-ααα-*trifluorotolylpiperidine hydrobromide* (1 g), m.p. 224°. Found: C, 57·4; H, 5·5; N, 3·0; Equiv. wt 486. $C_{23}H_{27}BrF_3NO_2$ requires: C, 56·8; H, 5·6; N, 2·9%. Equiv. wt 486. The ether solution was washed with dilute acid, the combined acidic solutions were made alkaline with ammonia solution and extracted with ether. On evaporation an oil was obtained which on treatment with ethanolic hydrogen chloride gave 1-*phenethyl*-4-*propionyloxy*-4-m-ααα-*trifluorotolylpiperidine hydrochloride*, m.p. 229° (from ethanol). Found: C, 62·3; H, 6·4; N, 3·1; Equiv. wt 442. $C_{23}H_{27}ClF_3NO_2$ requires: C, 62·5; H, 6·2; N, 3·2%. Equiv. wt 422. 1-Phenethyl-4-propionyloxy-4-*m*-ααα-trifluorotolylpiperidine hydrochloride (2·8 g) was refluxed with a solution of potassium hydroxide (1·6 g) in 96% ethanol (50 ml) for 15 hr. The residue obtained by ether extraction of the diluted mixture gave an oil which on treatment with ethanolic hydrogen chloride gave 1-*phenethyl*-4-m-ααα-*trifluorotolyl*-4-*piperidinol hydrochloride*, m.p. 235° (from ethanol). Found: C, 62·2; H, 6·4; N, 3·5; Equiv. wt 389. $C_{20}H_{23}ClF_3NO$ requires: C, 62·2; H, 6·0; N, 3·6%. Equiv. wt 386.

4-p-*Chlorophenyl*-1-*phenethyl*-4-*piperidinol.* *p*-Chloro-α-methylstyrene (305 g) was stirred and heated at 40–60° with a solution of ammonium chloride (214 g) in formaldehyde solution (40%, 730 ml) for 4 hr. The mixture was then stirred with methanol for 2 days, evaporated under reduced pressure (15 mm), and stirred and heated with hydrochloric acid (600 ml), on a steam-bath for 4 hr. The mixture was diluted with water (250 ml), cooled in ice water, and made alkaline with concentrated potassium hydroxide solution. Toluene extraction gave an oil which on distillation gave 4-*p*-chlorophenyl-1,2,3,6-tetrahydropyridine, b.p. 164°/6 mm. This base on treatment with hydrochloric acid, followed by evaporation with benzene and ethanol, gave 4-p-*chlorophenyl*-1,2,3,6-*tetrahydropyridine hydrochloride*, m.p. 206° (from ethanol). Found: C, 57·9; H, 5·7; N, 5·8; Equiv. wt 231. $C_{11}H_{13}Cl_2N$ requires: C, 57·4; H, 5·7; N, 6·1%. Equiv. wt 230.

4-*p*-Chlorophenyl-1,2,3,6-tetrahydropyridine hydrochloride (10 g), suspended in acetic acid (30 ml), was shaken with 25% hydrogen bromide in acetic acid (30 ml) and on standing overnight gave 4-bromo-4-*p*-chlorophenylpiperidine hydrobromide (11·5 g), m.p. 216° (Janssen, 1959, 213–215°). This solid on crystallisation from ethanol-ether gave

4-p-*chlorophenyl*-1,2,3,6-*tetrahydropyridine hydrobromide*, m.p. 211°. Found: C, 48·3; H, 4·9; N, 4·8; Equiv. wt 271; E_{max} 16,530 at 249 mμ. $C_{11}H_{13}BrClN$ requires: C, 48·1; H, 4·8; N, 5·1%. Equiv. wt 275.

The addition of an excess of sodium hydroxide solution to a solution in water of 4-bromo-4-*p*-chlorophenylpiperidine hydrobromide gave 4-*p*-chlorophenyl-4-piperidinol, m.p. 136° (from toluene) (Janssen, 1959).

A mixture of 4-*p*-chlorophenyl-4-piperidinol (4·5 g), phenethyl bromide (5 g), sodium bicarbonate (3·5 g) and toluene was refluxed for 3 days. The mixture was filtered, and the filtrate on concentration gave the free base 4-p-*chlorophenyl*-1-*phenethyl*-4-*piperidinol* (1·5 g), m.p. 128° (from ethanol). Found: C, 72·4; H, 7·0; N, 4·2; Equiv. wt 318. $C_{19}H_{22}ClNO$ requires: C, 72·3; H, 7·0; N, 4·4%. Equiv. wt 316. Further concentration gave the salt, 4-p-*chlorophenyl*-1-*phenethyl*-4-*piperidinol hydrobromide* (4·3 g), m.p. 199° (from ethanol). Found: C, 57·3; H, 5·8; N, 3·5; Equiv. wt 401. $C_{19}H_{22}BrClNO$ requires: C, 57·5; H, 5·8; N, 3·5%. Equiv. wt 397.

PREPARATION OF ACYL ESTERS

4-p-*Chlorophenyl*-1-*phenethyl*-4-*propionyloxypiperidine*. A mixture of the piperidinol (2 g), propionic anhydride (3 ml) and piperidine (3 ml) were refluxed together for 3 hr. Evaporation of the solvent gave an oil which on treatment with ethanolic hydrogen chloride gave 4-p-*chlorophenyl*-1-*phenethyl*-4-*propionyloxypiperidine hydrochloride*, m.p. 208° (from ethanol). Found: C, 64·3; H, 6·8; N, 3·5; Equiv. wt 413. $C_{22}H_{27}Cl_2NO_2$ requires: C, 64·7; H, 6·7; N, 3·4%. Equiv. wt 408.

In a similar manner the following were prepared:

1-*Phenethyl*-4-*propionyloxy*-4-p-ααα-*trifluorotolylpiperidine hydrochloride*, m.p. 230° (from ethanol). Found: C, 62·4; H, 6·3; N, 3·3; Equiv. wt 449. $C_{23}H_{27}ClF_3NO_2$ requires: C, 62·5; H, 6·2; N, 3·2%. Equiv. wt 442.

4-*Acetoxy*-1-*phenethyl*-4-m-ααα-*trifluorotolylpiperidine hydrochloride*, m.p. 231° (from ethanol). Found: C, 61·3; H, 6·1; N, 3·3; Equiv. wt 427. $C_{22}H_{25}ClF_3NO_2$ requires: C, 61·8; H, 5·9; N, 3·3%. Equiv. wt 428.

4-*Acetoxy*-4-o-*chlorobenzyl*-1-*phenethylpiperidine hydrochloride*, m.p. 221° (from ethanol). Found: C, 63·9; H, 6·8; N, 3·3; Equiv. wt 407. $C_{22}H_{27}Cl_2NO$ requires: C, 64·7; H, 6·7; N, 3·4%. Equiv. wt 408.

4-o-*Chlorobenzyl*-1-*phenethyl*-4-*propionyloxypiperidine hydrochloride monohydrate*, m.p. 129° (from ethanol). Found: C, 62·8; H, 7·4; N, 3·2; Equiv. wt 435. $C_{23}H_{31}Cl_2NO$ requires: C, 62·7; H, 7·1; N, 3·2%. Equiv. wt 440.

4-p-*Chlorobenzyl*-1-*phenethyl*-4-*propionyloxypiperidine hydrochloride monohydrate*, m.p. 200° (from ethanol). Found: C, 63·1; H, 7·3; N, 3·3; Equiv. wt 420. $C_{23}H_{31}Cl_2NO$ requires: C, 62·7; H, 7·1; N, 3·3%. Equiv. wt 440.

4-p-*Fluorophenyl*-1-*phenethyl*-4-*propionyloxypiperidine*. The Grignard complex obtained from *p*-bromofluorobenzene (16 g), magnesium (2·4 g) and 1-phenethyl-4-piperidone (10·2 g) was treated with propionic anhydride (9·1 g.) in ether, and gave p-*fluorophenyl*-1-*phenethyl*-4-*propionyl*-

oxypiperidine hydrochloride, m.p. 210° (from isopropanol). Found: C, 67·6; H, 6·9; N, 3·4; Equiv. wt 391. $C_{22}H_{27}ClFNO_2$ requires: C, 67·4; H, 6·9; N, 3·6. Equiv. wt 391.

4-p-*Fluorophenyl*-1-*phenethyl*-4-*propionyloxypiperidine hydrobromide*, m.p. 185° (from ethanol). Found: C, 60·7; H, 6·3; N, 3·4; Equiv. wt 440. $C_{22}H_{27}BrFNO_2$ requires: C, 60·6; H, 6·2; N, 3·2%. Equiv. wt 436.

4-*Acetoxy*-1-*phenethyl*-4-p-ααα-*trifluorotolylpiperidine*. This compound was prepared in a manner similar to that described for 1-phenethyl-4-propionyloxy-4-*m*-ααα-trifluorotolylpiperidine. Butyl-lithium was prepared from butyl bromide (16·4 g) and lithium (1·25 g). The butyl-lithium was treated with *p*-bromo-ααα-trifluorotoluene (25 g), 1-phenethyl-4-piperidone (14·2 g) and acetic anhydride (20 g). The addition of ice water gave a precipitate (35 g) which on recrystallisation from ethanol gave 4-*acetoxy*-1-*phenethyl*-4-p-ααα-*trifluorotolylpiperidine hydrobromide*, m.p. 259°. Found: C, 55·9; H, 5·5; N, 2·7; Equiv. wt 463. $C_{22}H_{25}BrF_3NO_2$ requires: C, 55·9; H, 5·3; N, 2·9%. Equiv. wt 472.

4-p-*Chlorophenyl*-1-*phenethyl*-1,2,3,6-*tetrahydropyridine*. (*a*) 4-*p*-Chlorophenyl-1,2,3,6-tetrahydropyridine (5·8 g), phenethyl bromide (7·6 g), sodium bicarbonate (3·5 g) and toluene (200 ml) were refluxed together for 3 days. The filtered solution on evaporation gave 4-p-*chlorophenyl*-1-*phenethyl*-1,2,3,6-*tetrahydropyridine*, m.p. 132° (from ethanol) (1·9 g). Found: C, 76·1; H, 6·7; N, 4·8; Equiv. wt 295; E_{max} = 18,500 at 253 mμ. $C_{19}H_{20}ClN$ requires: C, 76·6; H, 6·8; N, 4·7%. Equiv. wt 298.

(*b*) 4-*p*-Chlorophenyl-1-phenethyl-4-piperidinol hydrobromide (1 g) refluxed in a mixture of methanol (200 ml) and hydrobromic acid (60%, 50 ml) for 3 hr, gave on cooling 4-p-*chlorophenyl*-1-*phenethyl*-1,2,3,6-*tetrahydropyridine hydrobromide*, m.p. 231° (from methanol). Found: C, 59·8; H, 5·7; N, 3·5; Equiv. wt 389; E_{max} = 19,400 at 251 mμ. $C_{19}H_{21}BrClN$ requires: C, 60·2; H, 5·6; N, 3·7%. Equiv. wt 379.

Similarly, dehydration of the piperidinol gave 4-p-*fluorophenyl*-1-*phenethyl*-1,2,3,6-*tetrahydropyridine hydrobromide*, m.p. 201° (from ethanol). Found: C, 62·1; H, 5·9; N, 4·0; Equiv. wt 353; E_{max} = 17,500 at 241 mμ. $C_{19}H_{21}BrFN$ requires: C, 63·0; H, 5·9; N, 3·9%. Equiv. wt 362.

Equivalent weights of the bases were determined by titration with 0·02N perchloric acid in glacial acetic acid using Oracet Blue B as indicator. Titration of salts was carried out in the same solvent in the presence of mercuric acetate by the method of Pifer & Wollish (1951).

The ultra-violet absorption measurements were made on solutions in ethanol.

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Capillary permeability responses to snake venoms

H. J. FEARN, CAROLINE SMITH AND G. B. WEST

Russell's viper venom is many times more toxic to mice on intravenous administration than is saw-scaled viper venom, and yet on local intradermal injection the two venoms have equal capillary permeability effects in mice, rats, guinea-pigs and rabbits. Their effects are completely prevented by drugs possessing both anti-histamine and anti-5-HT properties. It is concluded that the increased capillary permeability induced by the venoms is largely mediated through the release of histamine and 5-HT.

THE clinical symptoms after snake bites often resemble the acute effects of histamine. There is, for example, widespread capillary endothelial damage and this often leads to acute thrombosis and death. Some viper venoms have also been shown to degranulate mast cells thereby releasing heparin and histamine (Higginbotham, 1959). We have now tested the toxicity to mice of two snake venoms and then studied their effects on capillary permeability in four mammalian species.

Methods

TOXICITY OF SNAKE VENOMS

The acute intravenous toxicities of Russell's viper venom (*Vipera russellii*) and of saw-scaled viper venom (*Echis carinatus*) were determined in groups of 10 adult albino mice (weight 18–22 g) using the method of Litchfield & Wilcoxon (1949). Mortality rates were measured 24 hr after dosage.

TESTS ON CAPILLARY PERMEABILITY

The abdominal skin of adult albino rats, guinea-pigs and rabbits was shaved with an electric razor and 24 hr later the animals were anaesthetised and injected intravenously with azovan blue dye (7 mg/kg). Injections of the agents stated below were then made intradermally on both sides of the midline of the shaved area; 20 min later, the animals were killed, the shaved skin was removed and firmly pinned to a cork board, and the extent of the colloid dye accumulation was estimated on the inner side of the skin by measuring the average diameter of the extent of dye spread. The mean response to neutralised saline (NaCl, 0·9% w/v) was $10 \pm 1{\cdot}5$ mm diameter (60 determinations).

In the experiments with mice, the skin was similarly shaved and 24 hr later the animals were injected intravenously with congo red (50 mg/kg). One intradermal injection was made into each animal and later the shaved skin was extracted with acetone (6 ml/g). The extent of the colloid dye accumulation was then estimated absorptiometrically (Hilger absorpticmeter: green filter, OG 1). In each experiment, groups of at least 3 animals were used and the results were averaged.

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AGENTS USED

The agents were histamine, 5-hydroxytryptamine (5-HT), compound 48/80, ovomucoid, dextran (Intradex), bradykinin, Russell's viper venom (*Vipera russelli*), saw-scaled viper venom (*Echis carinatus*) and saline. A few experiments were made with hooded cobra venom (*Naja naja*) and scorpion venom (*Leinrus quinquestriatus*). Each was used in a volume of 0·1 ml neutralised saline.

ANTAGONISTS

These were given either intravenously 30 min before the dye and active agent or intradermally together with the active agent but after the colloid dye.

DEPLETION OF AMINES

Compound 48/80 was injected intraperitoneally to deplete rats of their skin histamine and part of their skin 5-HT before the test; the twice daily doses were 1 mg/kg on the first day, 2 mg/kg on the second and 3 mg/kg on the third. Animals were then tested on the fourth and eighth days, that is, 1 and 5 days after the last dose of compound 48/80.

Results

TOXICITY OF VENOMS

The acute intravenous LD50 of Russell's viper venom in mice was 35·0 μg/kg (limits of error at P = 0·95, 30·2–40·6) whereas that of the saw-scaled viper venom was 620 μg/kg (limits, 470–818). Thus Russell's venom is more than 17 times more toxic than that of the saw-scaled viper. The complete results from which these LD50 values were calculated are shown diagrammatically in Fig. 1. With Russell's venom, deaths usually occurred within the first 30 min, the cause being pulmonary oedema; with the saw-scaled viper venom, deaths were much delayed, many dying at about 18 hr after injection and showing gross haemorrhage in the lungs and blood in the urine and faeces.

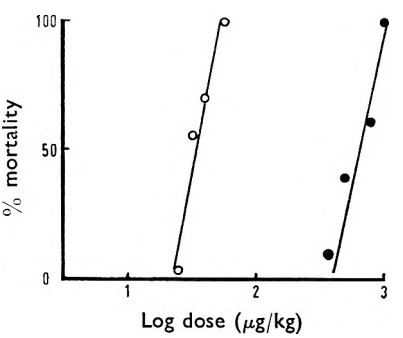

FIG. 1. The acute intravenous toxicities of Russell's viper venom (○—○) and of saw-scaled viper venom (●—●) using groups of 10 mice. Note that Russell's viper venom is many times more toxic.

THE RELATION BETWEEN INTRADERMAL DOSE AND RESPONSE

This is shown in Fig. 2 for most of the agents studied. By contrast with their relative activities on intravenous administration into mice, Russell's viper venom and saw-scaled viper venom were equally effective intradermally in the three species, doses of 0·1 to 4 μg being sufficient to give dose-response relationships. The local inflammatory reaction of the saw-scaled viper venom was always accompanied by minute petechial haemorrhages. Similar intradermal doses of cobra venom and of scorpion

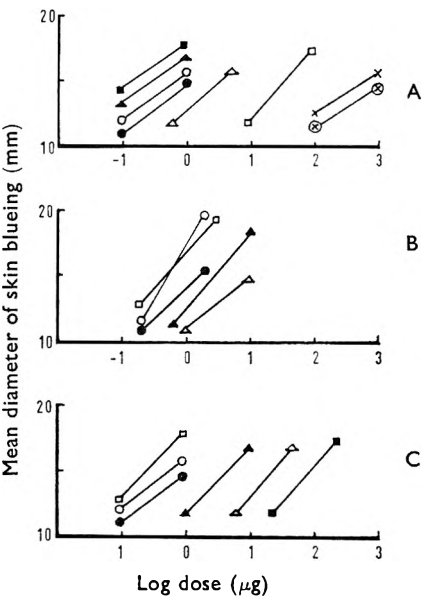

FIG. 2. Dye accumulation in the skin when Russell's viper venom (○), saw-scaled viper venom (●), histamine (□), 5-HT (■), bradykinin (△), compound 48/80 (▲), ovomucoid (×), and dextran (⊗) are injected intradermally into rats (A), rabbits (B) and guinea-pigs (C). Note that Russell's viper venom and saw-scaled viper venom are equi-active in all three species.

venom were also effective in rats. Of the other agents, histamine, ovomucoid and dextran were weakly active in rats, and so were 5-HT and bradykinin in guinea-pigs; furthermore, 5-HT was inactive in rabbits and ovomucoid and dextran were inactive in rabbits and guinea-pigs. These results are also shown in Table 1.

TABLE 1. THE RELATIVE EFFECTIVENESS OF DIFFERENT AGENTS ON INTRADERMAL INJECTIONS IN THE FOUR SPECIES. RESPONSES MEASURED ON A RELATIVE SCALE FROM 0 TO +++, EACH + REPRESENTING A 10-FOLD DECREASE IN EFFECTIVE DOSE

Agent	Rats	Mice	Rabbits	Guinea-pigs
Venoms	+++	++	+++	+++
Bradykinin	++	++	++	++
Compound 48/80 ..	+++	+	+−+	++
Histamine	+	++	+−+	+++
5-HT	+++	+++	0	+
Ovomucoid, dextran ..	+	0	0	0

EFFECT OF THE AGENTS IN MICE

With compound 48/80, histamine and 5-HT, the response decreased as the dose was increased, but the increase in capillary permeability produced by the venoms intensified as higher doses were used. This is shown in Fig. 3. The two venoms were equally effective intradermally in mice and were about 10 times less active than in the other three species.

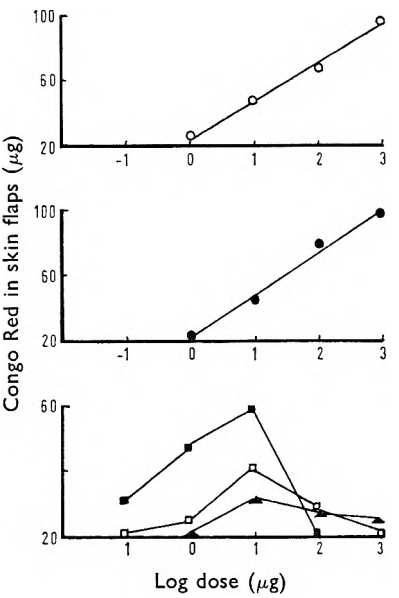

FIG. 3. Dye accumulation in the skin when Russell's viper venom (○), saw-scaled viper venom (●), histamine (□), 5-HT (■) and compound 48/80 (▲) are injected intradermally into mice. Note the different scales. Whereas the venom effects increase with higher doses, those of the standard substances decrease.

EFFECT OF AMINE DEPLETION

Chronic treatment of rats with compound 48/80 prevented the responses to compound 48/80, ovomucoid, dextran, histamine and 5-HT and reduced the venom effects. When tested 4 days later, the responses to all the agents had fully recovered although the skin histamine was still low at 20% of the control value (Bonaccorsi & West, 1963).

EFFECT OF ANTAGONISTS

Doses of 10 mg/kg of mepyramine, an antihistamine drug, completely inhibited the responses of histamine in rats and rabbits and markedly reduced those of the venoms and of compound 48/80. Doses of 2 mg/kg of 1-methyl-lysergic acid butanolamide (UML 491), an anti-5-HT drug, were effective in preventing the responses to 5-HT in rats and mice but were very weak against the venoms. Doses of 8 mg/kg of promethazine, an antihistamine and anti-5-HT drug, effectively prevented the responses

of the venoms, compound 48/80, histamine and 5-HT in guinea-pigs, rats and mice, although they were only weakly active against bradykinin. These results are shown in Table 2.

TABLE 2. THE RELATIVE EFFECTIVENESS OF DIFFERENT ANTAGONISTS ON INTRAVENOUS INJECTION IN THE FOUR SPECIES. REDUCTION OF THE LOCAL RESPONSE OF EACH AGENT IS MEASURED ON A RELATIVE SCALE FROM 0 TO +++

Agent	Mepyramine		UML 491		Promethazine		
	Rat	Rabbit	Rat	Mouse	Guinea-pig	Rat	Mouse
Venoms	++	++	+	+	+++	+++	+++
Bradykinin	+	+	+	+	+	+	+
Compound 48/80	++	++	+	0	+++	+++	+++
Histamine	+++	+++	0	0	+++	+++	+++
5-HT	0	—	+++	+++	+++	+++	+++
Ovomucoid, dextran	+	—	+++	—	—	+++	—

When the antagonists were given together with the active agents in rats, promethazine in doses of 2 μg was again the most effective compound. The venom responses were also completely prevented by similar doses of cyproheptadine, an antagonist with both antihistamine and anti-5-HT activities. The results of these local effects are shown in Table 3. Note that mepyramine exerted a slight inhibitory effect on the 5-HT response when given by this route, and UML 491 slightly reduced the histamine effect.

TABLE 3. THE RELATIVE EFFECTIVENESS OF ANTAGONISTS (2 μg) ON INTRADERMAL INJECTION IN RATS. REDUCTION OF RESPONSES IS MEASURED ON A RELATIVE SCALE FROM 0 TO +++

Agent	Dose (μg)	Mepyramine	UML 491	Promethazine	Cyproheptadine
Venoms	1	+	++	+++	+++
Bradykinin	10	+	+	+	+
Compound 48/80	1	+	++	++	++
Histamine	100	+++	+	+++	+++
5-HT	1	+	+++	+++	+++
Ovomucoid, dextran	100	+	+++	+++	+++

Discussion

The results show that Russell's viper venom, a thromboplastic agent, is many times more toxic to mice than is saw-scaled viper venom, a haemolytic agent, when given intravenously, and yet it is only equally active when given intradermally to mice, rats, guinea-pigs and rabbits and when the changes in capillary permeability are measured. The treatment of viper snake poisoning continues to be a therapeutic problem in many parts of the world in spite of available antivenene and it is of fundamental importance to elucidate the exact mechanism producing the increased capillary permeability as the extent of the capillary damage determines to a large extent the ultimate outcome of the snake bite.

The local responses of the venoms are effectively antagonised by promethazine or cyproheptadine and anti-5-HT compounds, and it seems

that the snake venoms exert their effects through the release of at least both 5-HT and histamine. This is, however, not the complete mechanism as the venoms, for example, are effective local inflammatory agents even when the skin histamine content is much reduced by chronic treatment with compound 48/80. They are also effective in mice at high doses when histamine and 5-HT are exhibiting tachyphylaxis. Bradykinin is also an effective initiator of the local inflammatory response but its actions in the four species studied are only feebly reduced by the more specific antagonists.

Sparrow & Wilhelm (1957) reported the species differences in susceptibility to capillary permeability factors such as histamine, 5-HT and compound 48/80, and the present results confirm that histamine is most active and 5-HT is least active in guinea-pigs and rabbits, whilst compound 48/80 is the least active in mice. Ovomucoid and dextran have again been shown to be ineffective by this test in mice, rabbits and guinea-pigs.

The administration of an antihistamine and anti-5-HT drug, together with the antivenene, is suggested in the management of viperine snake poisoning.

Acknowledgements. We wish to acknowledge the gifts of compound 48/80 from Burroughs, Wellcome and Co., London; bradykinin and UML 491 from Sandoz Products Ltd., London; and scorpion venom from Dr. K. R. Adam, Khartoum.

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Analysis of August rat liver for calcium, copper, iron, magnesium, manganese, molybdenum, potassium, sodium and zinc*

J. L. EVERETT, C. L. DAY AND F. BERGEL

The concentrations of Ca, Cu, Fe, Mg, Mn, Mo, K, Na and Zn in "August" pure line rat livers has been determined by emission spectroscopy, polarography and flame photometry. Differences were shown to exist between normal, regenerating and neoplastic livers, especially in their calcium, iron and manganese contents.

BERGEL, Everett, Martin & Webb (1957) described a semi-quantitative method of analysis of Wistar rat livers by emission spectrography. The present communication deals with the extension of these comparative analyses to include major elements and more refined methods and techniques, i.e., flame photometry with ion-exchange chromatography, polarography and emission spectrography; this time the livers of "August" rats (a genetically pure line strain) were used.

Animal material

The preparation and pre-treatment of tissue samples subjected to analysis were similar to those reported by Bergel & others (1957) except that after drying, the tissues were not submitted to a defatting process.

The following pooled normal livers from rats ("August" strain, fed on MILL HILL rat cake diet No. 44) represented our starting material for analytical procedures: foetal, new born, 5, 7, 10, 14, 17, 21, 24, 28, 35, 42 and 56 days old. In addition we had at our disposal, as pooled material: maternal livers from those mother animals which provided the foetal samples; regenerating livers, produced as described previously (hepatectomies by Miss E. Leuchars); the abnormal livers were produced by feeding 12 rats (6 weeks old) a diet of 10% protein with *p*-dimethylamino-azobenzene (600 mg/kg dry weight) for 4 months. Of 12 rat livers, 5 proved unsuitable, and the remaining 7 were submitted to histological examination. All the specimens consisted for the main part of tumour tissue, some of the tumours being of parenchymal cell origin and some of bile duct origin. Where surrounding liver tissue was also present, fatty degeneration or bile duct hyperplasia were evident. Details are as follows: Rat I. Multiple parenchymal-cell hepatomas and multiple cholangiomas. Rat II. Malignant cholangio-carcinoma. Rat III. Extensive bile duct hyperplasia and multiple cholangiomas. Rat IV. Malignant cholangiocarcinoma. Rat V. Multiple liver tumours, some of parenchymal cell origin and some of bile duct origin, also focal fatty infiltration. Rat VI. Large malignant parenchymal cell hepatoma. Rat VII. Areas of bile duct hyperplasia and two parenchymal cell hepatomas.

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* The second paper on major and minor metals in normal and abnormal tissues. The first paper was by Bergel, Everett & Webb (1957).

TABLE 1. ANALYTICAL RESULTS

Liver (age in days)	Dry wt (g)	Ash wt (g)	Ratio dry wt/ ash wt	Na (ppm)	K (ppm)	Ca (ppm)	Fe (ppm)	Cu (ppm)	Mg (ppm)	Mn (ppm)	Mo (ppm)	Zn (ppm)
Foetal	0·4019	0·0251	16·0	5,630	13,750	1,250	290	19·65	415	3·37	0·272	463
5	7·4358	0·4431	16·9	3,580	11,540	886	201	30·2	349	2·53	0·475	430
7	8·7587	0·4439	19·8	3,920	9,080	832	263	83·2	450	1·31	0·69	414
10	9·3366	0·4808	19·4	4,050	8,750	824	247	64·3	480	1·09	0·34	202
14	10·2940	0·6638	15·5	3,100	12,280	1,225	165	100·0	670	1·99	0·61	284
17	13·6223	0·8976	15·2	3,230	7,880	1,052	303	99·7	885	1·73	0·92	257
21	13·7257	0·7140	17·1	2,980	6,720	672	213	97·8	360	1·17	0·76	216
24	12·9083	0·5187	24·8	2,950	5,640	625	131	26·3	254	1·15	0·50	210
28	11·3644	0·5190	21·8	2,910	10,120	714	253	27·1	335	1·91	1·23	202
35	12·9491	0·5427	24·0	2,590	8,120	624	195	18·6	338	1·17	1·18	163
42	15·9080	0·8680	18·3	2,330	10,130	900	202	38·0	509	0·88	1·16	328
49	7·8700	0·3301	23·8	2,590	6,300	566	160	55·0	404	1·89	0·86	113
56	17·8309	0·6998	25·5	2,270	8,230	725	187	22·1	432	1·57	1·22	137
Maternal* ..	10·4855	0·7718	15·4	1,420	9,070	741	249	24·7	442	1·24	1·62	175
Regenerating†	7·3778	0·3806	19·4	6,620	5,930	592	173	21·9	788	1·15	1·42	248
Tumour‡ (6 month)												
Rat No. I ..	10·9227	0·6571	16·6	3,650	9,920	2,010	361	20·8	320	4·34	0·94	127
Rat No. II ..	11·6438	0·6047	19·3	1,960	6,730	713	456	33·2	394	1·24	1·06	155
Rat No. III ..	7·4280	0·3942	18·85	3,050	5,830	1,340	392	19·65	403	4·68	1·83	101
Rat No. IV ..	10·2544	0·5968	17·2	3,920	5,800	1,745	407	54·4	459	3·14	1·34	134
Rat No. V ..	13·4394	0·6755	19·8	3,570	8,320	1,350	404	54·5	223	4·66	0·91	138
Rat No. VI ..	8·2642	0·4563	18·2	3,240	9,320	1,375	357	57·0	710	4·40	1·42	149
Rat No. VII ..	12·6496	0·7482	16·9	3,080	5,910	2,170	438	39·7	398	2·84	0·73	160

* Maternal livers from pregnant rats.
† Regenerating liver. Removal of one lobe and then allowed to regenerate (5 days).
‡ Tumours arising from rats fed on a diet containing a carcinogenic Azo dye.

All results are quoted in ppm of dry tissue.

Analytical methods

Sodium, potassium and calcium were determined using the Evans Electroselenium Flamephotometer; iron, copper, zinc, manganese and molybdenum using the Southern Instruments K1000 Polarograph and magnesium using the Hilger Medium Quartz Spectrograph.

FLAME PHOTOMETRY

(*a*) Sodium and potassium. The ash (*ca.* 10 mg) was dissolved in 0·5 ml of concentrated hydrochloric acid (Analar) and the solution made up to 10 ml (1 mg/ml) with water. This solution was diluted × 100 for potassium and × 1,000 for the sodium and then aspirated into the flame of the photometer. Standard working curves were established, using a series of prepared standard solutions containing known quantities of both elements. It was found previously that by using the dilutions mentioned, interference from other elements in the flame was negligible.

(*b*) Calcium. The method described by Hemingway (1956) was used. 1 ml of the solution containing 1 mg/ml (solution as used previously for Na and K) was passed through a column containing a cation-exchange resin (Amberlite IR-120 (H) ground to the recommended size), to retain the calcium, free of phosphate. The column was washed with water and the calcium eluted with 5N nitric acid (Analar), until 10 ml of eluate was obtained which was then aspirated into the flame. Standard working curves were obtained by passing through the columns solutions, containing known amounts of calcium; they were eluted with 5N nitric acid.

POLAROGRAPHY

(*a*) Iron. The method as described by Hetman (1959) was applied. A known amount of ash was dissolved in concentrated hydrochloric acid, and the solution diluted with water. 5 ml of this solution was introduced into a 10 ml graduated flask, then the base electrolyte, consisting of 2 ml 10M sodium hydroxide, 1 ml M sucrose and 1 ml of a saturated solution of EDTA was added and the total brought up to the mark with water. The polarogram was recorded on a 5 ml aliquot, after de-aerating with N_2 for 3 min, applying cathodic reduction and using a Hg pool as reference electrode. Standards were determined, using the above procedure, and working curves established.

(*b*) Copper. For this metal the method of Carruthers & Suntzeff (1945) was used: a known amount of ash was dissolved in 1 ml of 0·1N hydrochloric acid and 1–2 drops of concentrated nitric acid was added to oxidise the iron to the ferric state. The solution was evaporated to dryness on a water-bath, the residue dissolved in 2 ml of 0·1N potassium thiocyanate and the solution polarographed after removal of the oxygen.

(*c*) Manganese. Following the method, described by Hamamoto (1934), a known amount of ash was dissolved in concentrated hydrochloric acid. The solution was gently heated, allowed to cool and sodium carbonate was added to give a weakly acidic reaction. The addition of saturated sodium acetate and heating gave a basic iron acetate as precipitate which was filtered off and discarded. To the filtrate was added a

small crystal of potassium chlorate which was allowed to dissolve; then 20 ml of 3N sodium hydroxide were added. On heating, the manganese was separated as $Mn(OH)_2$ and MnO(OH), the precipitate was filtered and ashed in a crucible. This ash was converted to the chloride, dissolved in 2 ml of 0·001N lithium chloride and polarographed after the removal of the oxygen. Standard working curves were established, using the same procedure.

(*d*) Molybdenum. Following the method described by Jones (1954) a known amount of ash was dissolved in concentrated hydrochloric acid and 0·1 ml sulphuric acid, and the solution diluted with water to 10 ml. 2 ml of a 2% ethanolic benzoin oxime solution was added and the total extracted with chloroform; the extracts were combined and the chloroform removed by evaporation. The residue was decomposed with acids, the solution cooled, and to it 4·8 ml of 1M sodium perchlorate were added. The final volume was polarographed after removal of oxygen.

(*e*) Zinc. The method of Cholak, Hubbard & Burkey (1943) was applied. A known amount of ash was dissolved in 0·5 ml concentrated hydrochloric acid and to the solution 30 ml of 20% ammonium citrate added with 4 drops of 0·1% thymol blue in water. The total was adjusted to pH 9·5 with ammonia; 4 ml of 1·25% aqueous solution of sodium diethyldithiocarbamate was added and a final volume of 100 ml reached by dilution with water. The solution was shaken with 5 ml portions of a chloroform solution of di-β-naphthylthiocarbazone (200 mg in 990 ml of chloroform and 10 ml of ethanol), until the original blue-green colour remained unchanged. The chloroform extract was washed with 50 ml of water and extracted by shaking it with 50 ml of 0·2N hydrochloric acid. The aqueous solution was evaporated to a volume of 1–2 ml, cooled and its pH adjusted to the change in methyl red with ammonia. The volume was brought to 5 ml with water, de-aerated with N_2 and polarographed. Standard solutions were made, using this procedure, and calibration curves established.

EMISSION SPECTROGRAPHY

Magnesium. A known amount of ash was dissolved in hydrochloric acid (conc.) and the solution diluted with water. An aliquot (0·1 ml) was evaporated on flat top 6·5 mm diameter graphite electrodes (National Carbon Co., U.S.A.) which had previously been dipped into a light petroleum solution of 1% apiezon grease to render them non-porous. The electrodes were arced for 45 sec at 9 A and the spectrograms recorded on Kodak Photoscript B10 plates. The density of the spectral line, Mg 2783, was measured using a Hilger microphotometer and correction was made for "background" in the usual manner. Seidl density values (Black, 1952) were used and the concentration read from a working curve. Standard working curves had been established, using known amounts of magnesium added to a synthetic base prepared by Messrs. Johnson Matthey, London, described by Bergel & others (1957).

Water for all determinations had been passed through an Elgastat Deioniser (type B102) before use.

Results and discussion

Sodium. Inside the normal liver group the sodium content decreased from 5,630 ppm in foetal livers to an average of 2,400 ppm in adult livers. The maternal livers showed a further reduction to 1,400 ppm while the first sample of regeneration livers apparently had a high level of 6,620 ppm. Amongst the seven hepatomas the content averaged 3,400 ppm.

As the high sodium content of the first sample of regenerating livers was rather surprising as compared with the average content (over the whole age range), a further six control livers with corresponding regenerating livers were analysed. When matched pairs from individual rats, i.e., normal and regenerating livers from the same animals were analysed, the regenerating group averaged 3,150 ppm, whereas the control group averaged 3,015 ppm. It was therefore concluded that comparisons of values must be between the same age groups.

Calcium. Normal livers averaged 650 ppm, whereas calcium in the hepatomas increased in content to 1,600 ppm. This is in direct contrast to the values found by Kishi, Fuitwara & Nakahara (1937) who by using a gravimetric method found that in transplantable rat hepatomas the calcium values decreased. Delong, Coman & Ziedman (1950) also found that the calcium values were low in human intestinal cancers.

In both findings the reference tissue did not bear so close a relationship to the tumour tissue as did our tissues. For instance, in the case of the human intestinal cancers the adjacent normal mucosa was used as the reference tissue; whilst in the other case the tissue was a "transplantable" hepatoma (which after serial transplantation no longer bears much relationship to the original source from which it derived). The present work on the determination of calcium will be extended to include a variety of primary and transplantable experimental tumours including cellular fractions together with pertinent reference tissue.

Potassium. Normal liver decreased in content from 13,700 ppm in foetal livers to an adult level of 7,000 ppm. There was no significant difference in the content of the hepatomas in comparison with those of the adult rat liver, but with regards the foetal livers the values were lower.

Copper. During the suckling period in the life of the rats the copper content of the livers was increased, as reported (Lorenzen & Smith, 1947), and declined to a normal level to 25 ppm after weaning. In five out of the seven hepatomas studied the values were above the normal average, and in three of these the levels were of the order of 55 ppm. These figures are similar to those given by Bergel & others (1957) which were obtained by emission spectrography whereas the present figures were obtained by polarography.

Iron. In the "normal" liver groups the iron values were 200 ppm over the whole age range. This figure is very similar to that given by Bergel & others (1957). The content of the tumorous tissue was 400 ppm. This rise in value was also reported in that communication where attention was drawn to the possible fluctuation of iron due to the presence or

absence of residual blood. It should be pointed out, however, that there appears to be a genuine rise in the Fe content of hepatomas.

Magnesium. The 788 ppm of magnesium found in the regenerating liver appears to be high but is within the values of "normal" livers, the highest figure being 885 ppm. No significant differences were found, when the "normal" values were compared with those of the hepatomas.

Zinc. Zn levels decreased from 460 ppm to 210 ppm from foetal to adult livers. On the whole these figures agree with the overall pattern given by Bergel & others (1957), although the plateau is on a higher level. This could be accounted for by the change of the analytical method to a polarographic one. There were no significant differences between the Zn values of the neoplastic tissues and those of the adult "normal" livers.

Manganese. The increased manganese content found in the hepatomas as compared with the content of normal livers is one of the outstanding features of this study. In the previous communication where livers from the Wistar hybrid rat were used the values for the liver tumours were similar to those of the later age groups.

Molybdenum. The previously found continual increase in Mo content from foetal to adult rat livers (Bergel & others, 1957) was confirmed in this present study, 0·272 ppm to 1·24 ppm. The Mo content of the regenerating liver of 1·42 ppm was not significantly different from that of the adult livers. Previously no Mo could be detected by the use of emission spectra in the regenerating livers.

Histograms for all these elements were prepared and are kept for inspection at our address.

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Uncaria gambier Roxb., the structure of its leaves and young shoots*

MUN SUM LEONG AND BETTY P. JACKSON

The morphological and histological characters of the leaves and young shoots of *Uncaria gambier*, which are used in the preparation of catechu, are fully described and illustrated.

SEVERAL attempts have been made in the past to establish the origin of the vegetable material found in catechu. Gilson (1893) has suggested that the hairs found in the drug are the same as those occurring on the surface of the sepals and petals of *Uncaria gambier*, the plant from which the extract is prepared. Brumwell (1911) has examined microscopically the leafy fragments found in catechu and after comparing them with the surface characters of the leaves of *Uncaria gambier* and of some allied plants, concluded that they were derived exclusively from *Uncaria gambier*. In the same paper Brumwell also reported the presence of large numbers of hairs in the water-insoluble residue of catechu but did not comment upon their origin. In addition, later authors (Trease, 1960; Wallis, 1960) have reported that the vegetable fragments found in catechu are part of *Uncaria gambier*, and this fact has also been stated in the official monographs of the more recent editions of the British Pharmaceutical Codex (1954, 1959, 1963).

The plant *Uncaria gambier*, family Rubiaceæ, was first described by Roxburgh (1832), and an account of its gross morphology is included in some of the standard botanical works (Baillon, 1891 (under *Ourouparia*); Bentley & Trimen, 1880). There is, however, practically no information available concerning the histological structure of the plant. Consequently, although earlier work has indicated that the vegetable fragments commonly found in catechu are derived exclusively from *Uncaria gambier*, it has not been possible to identify them and to relate their structure to that of the plant itself. In view of this, it was decided to carry out a full anatomical investigation of the leaves and young shoots of *Uncaria gambier* in order that the origin of the vegetable fragments occurring in catechu could be elucidated; at the same time, it was hoped that this would provide a standard for the material used in the preparation of the drug.

Materials

Two specimens of *Uncaria gambier* were used in this investigation, namely:

(1) Dried specimens of leaves and immature fruits, labelled "*Uncaria gambier*, Paris Exhibition, 1878", and obtained in 1960 from the Herbarium of the Pharmaceutical Society of Great Britain.

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* The subject-matter of this communication forms part of a thesis by one of us (M.S.L.) accepted by the University of London for the degree of Master of Pharmacy.

(2) Aerial parts, including flowers and fruits, preserved in ethanol containing 5% formalin, also fresh specimens of leaves, stems and hooks, all collected in 1962 from plants growing in a Gambier plantation in Johore. The fresh material had been collected and sent by air without any preliminary treatment. On arrival it was in a slightly moist condition, and it was allowed to dry at room temperature.

The two samples of material were authenticated by comparison with Herbarium specimens of *Uncaria gambier* at the Royal Botanic Gardens, Kew.

Macroscopical and Histological Characters

(i) Leaves

MACROSCOPICAL CHARACTERS

Fully grown leaves are lanceolate to ovate, averaging about 6 to 11 cm in length and about 4·5 to 6 cm in greatest breadth, somewhat thick and coriaceous; margin entire, apex acuminate and bluntly pointed; base asymmetric; midrib and lateral veins prominent on the lower surface; lateral veins, 4 to 6 on each side, each forming an angle of about 50° with the midrib and anastomosing about 1 to 2 mm from the margin. In the angles between the midrib and the lateral veins, on the lower surface only, there are small, dense patches of long, brownish hairs; other, smaller, hairs occur scattered over the midrib on both surfaces and over the lateral veins on the lower surface (see Fig. 1, A and B). Petiole, 0·6 to 2 cm long and often longitudinally furrowed.

HISTOLOGICAL CHARACTERS

Lamina. The upper epidermis consists of tetragonal to hexagonal tabular cells measuring about H 22 to 25 μ and Lev L and B 18 to 32 μ*; the outer periclinal walls thickened and covered by a cuticle having marked striations; stomata and covering trichomes absent (Fig. 1, D). The palisade consists of a single layer of cylindrical cells measuring about H 34 to 70 μ and Lev L and B 14 to 18 μ and containing numerous chloroplasts. Beneath the palisade there is a layer of subrectangular or sometimes triangular collecting cells measuring about H 21 to 36 μ and Lev L and B 10 to 18 μ; the remaining spongy mesophyll consists of 2 to 4 layers of round or elliptical, branched, loosely-packed cells with numerous, large air spaces, individual cells measuring about H 14 to 43 μ and Lev L and B 14 to 54 μ. Present throughout the mesophyll, and particularly abundant in regions near the veins, are rounded idioblasts each containing a well-defined cluster crystal of calcium oxalate measuring about 16 μ in diameter (Fig. 2, C).

* Cell measurements are recorded by symbols suggested by Moll and Janssonius (1923). For describing organs showing bilateral symmetry, i.e., leaf, stipule, sepal and petal, the symbols H, Lev L and Lev B are used. The symbol H = height, in a direction perpendicular to the surface of the organ; Lev = in the direction of the surface of the organ; Lev L and Lev B = parallel to the surface and at the same time in a longitudinal direction or transverse direction respectively. For describing the stem and hook the symbols R, T and L indicate the measurements made in the radial, transverse and longitudinal directions respectively.

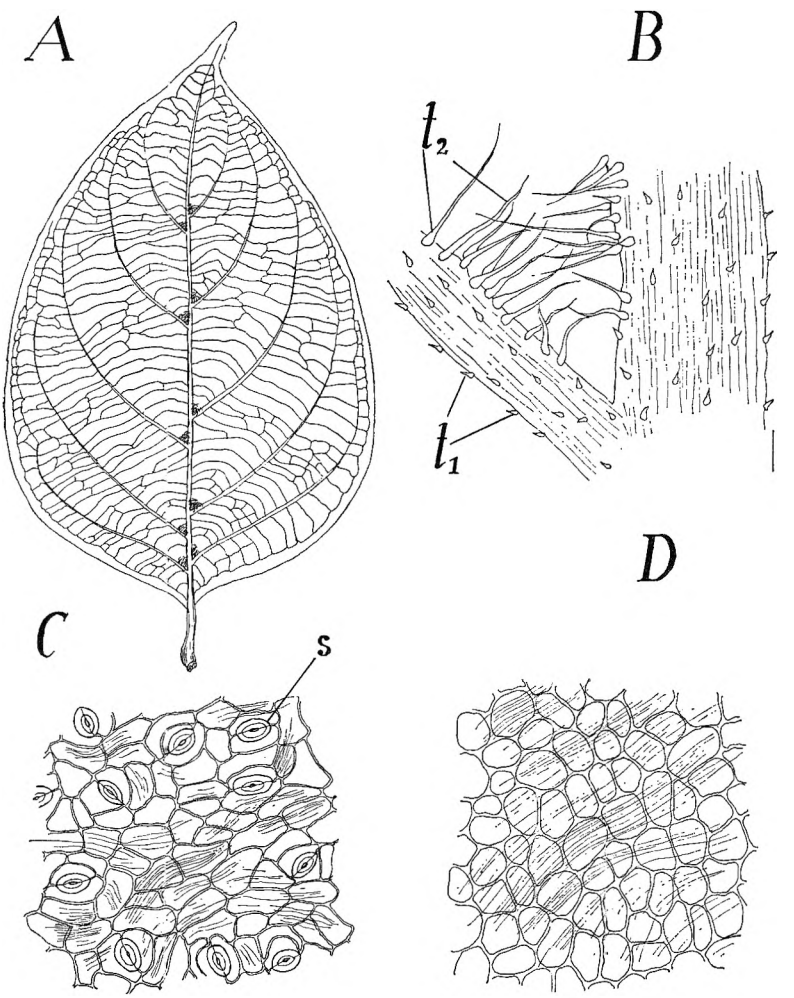

FIG. 1. Leaf of *Uncaria gambier* Roxb. *A*, entire leaf, $\times\frac{1}{3}$; *B*, diagram of part of the lower surface showing the distribution of the covering trichomes on the midrib and lateral veins, $\times 30$; t_1, short, conical trichomes; t_2, long covering trichomes; *C*, lower epidermis; *D*, upper epidermis; *s*, stoma; *C* and *D*, $\times 220$.

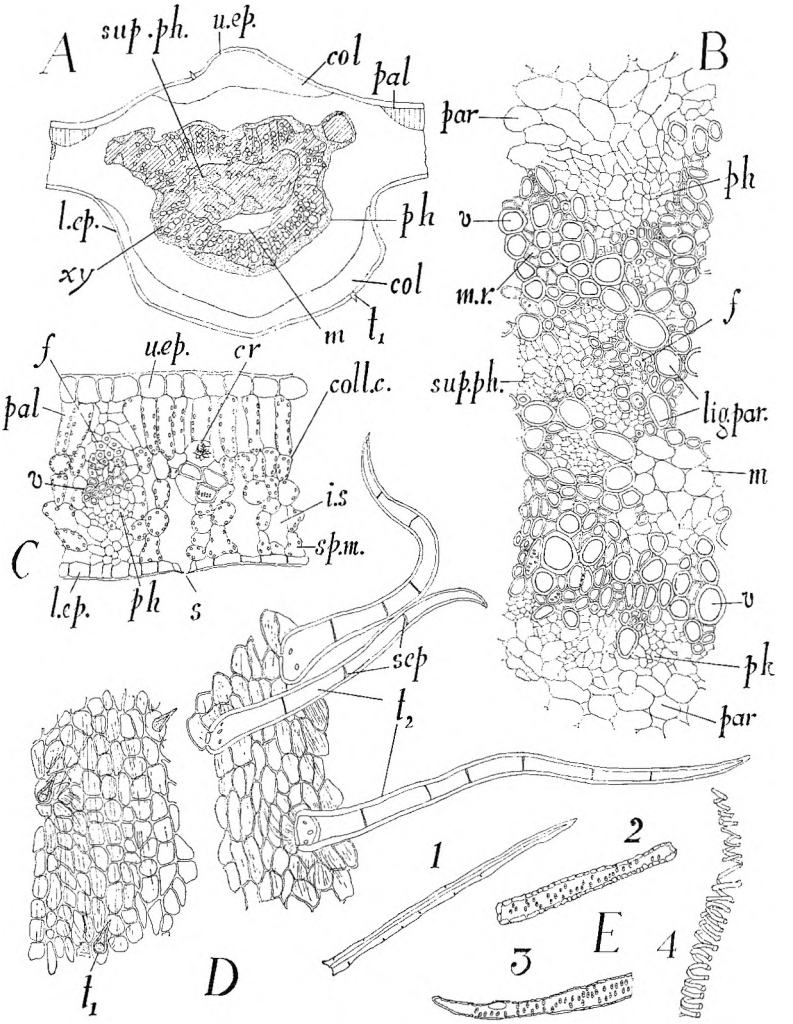

FIG. 2. Leaf of *Uncaria gambier* Roxb. *A*, diagram of a transverse section of the midrib, × 30; *B*, details of a transverse section of the meristele; *C*, transverse section of the lamina; *D*, two regions of the lower epidermis of the midrib in surface view showing two different types of trichomes; *E*, isolated elements from the midrib; *B*, *C*, *D* and *E*, × 160; *col*, collenchyma; *coll.c*, collecting cells; *cr*, crystals of calcium oxalate; *f*, fibres; *i.s.*, intercellular space; *l.ep.*, lower epidermis; *lig.par.*, lignified parenchyma; *m*, medulla; *m.r.*, medullary rays; *pal*, palisade; *par*, parenchyma; *ph*, phloem; *s*, stoma; *sep*, septa; *sp.m.*, spongy mesophyll; *sup.ph*, supernumerary phloem; t_1, short conical covering trichome; t_2, long covering trichomes; *u.ep*, upper epidermis; *v*, vessels; *xy*, xylem; *1*, fibre; *2*, lignified parenchyma; *3*, tracheidal-vessel; *4*, spiral vessel fragment.

The lower epidermis has a finely striated cuticle and consists of tabular cells measuring H 11 to 14 μ and Lev L and B 11 to 32 μ with straight or slightly sinuous anticlinal walls. Paracytic stomata are very numerous except over the lateral veins, where they are absent; they are slightly raised above the level of the epidermis, elliptical, measuring from 18 to 25 μ in length and about 10 μ in breadth (Fig. 1, C).

Midrib (Fig. 2, A and B). The upper epidermis, which is covered by a fairly thick and finely striated cuticle, consists of subrectangular or elongated cells with straight anticlinal walls; these cells are smaller than the epidermal cells of the lamina, measuring about H 13 μ, Lev B 11 to 18 μ and Lev L 18 to 39 μ; stomata are absent. Covering trichomes are quite numerous; they are conical, small, measuring from 18–**36**–50 μ in length and 11 to 25 μ in diameter at the base; the walls are warty, thickened and unlignified, and the lumen is wide at the base, narrowed in the limb and frequently occluded at the apex (Fig. 2, D, t_1).

The lower epidermis consists of tabular cells similar in size and appearance to those of the upper epidermis; stomata are absent; short, conical trichomes similar to those on the upper epidermis are abundant. Also present are larger, linear trichomes measuring 205–**365**–792 μ long and about 29 to 42 μ wide, which project from the midrib and lateral veins into the angle between them; these are unicellular but often contain 1 to 10 thin, transverse, lignified septa; limb straight or slightly curved, tapering, with an acute apex; walls evenly thickened, smooth and lignified and pitted at the base (Fig. 2, D, t_2).

A hypodermis is present beneath both epidermises and usually consists of a single layer of tetragonal to pentagonal thin-walled cells measuring about H 18 μ and Lev B 14 to 25 μ. The outer cortex consists of 3 to 5 layers of collenchyma situated immediately beneath each hypodermis; individual cells measure about H 18 to 32 μ and Lev B 11 to 43 μ. The inner cortex is composed of round, thin-walled parenchymatous cells measuring about H 18 to 50 μ and Lev B 36 to 54 μ with frequent and scattered idioblasts containing sandy crystals of calcium oxalate.

The *meristele* consists of a cylinder of xylem surrounded by a complete band of phloem; inside this cylinder are groups of supernumerary phloem intermixed with small groups of vessels, fibres and lignified parenchyma thus forming a kind of network replacing most of the central medulla. In some leaves pericyclic fibres are present; these form peripheral groups or, very occasionally, an almost complete cylinder surrounding the vascular tissue; individual fibres measure 324–648 μ long and 14 to 28 μ wide; the walls are fairly thick, pitted and slightly lignified. The *phloem* consists of groups of sieve tubes each measuring about 7 to 14 μ in diameter, frequently accompanied by large parenchymatous cells measuring H 11 to 25 μ and Lev B 14 to 21 μ and traversed by medullary rays which are usually one or two cells wide. The *xylem* consists of 3 to 4 rows of radially-arranged vessels measuring from 14 to 36 μ in diameter with spirally or annularly thickened walls, and groups of fibres and parenchyma; the medullary rays are usually lignified. The network of lignified elements inside the xylem cylinder consists of vessels, fibres and

lignified parenchyma; the parenchymatous cells are round to ovoid with fairly thick, pitted walls and measure H 14 to 54 μ and Lev B 18 to 54 μ; the vessels are similar to those of the main xylem cylinder, but they are smaller, measuring 14 to 25 μ in diameter, and occur singly or in small groups; the fibres are scattered throughout but are particularly numerous in the regions bordering the supernumerary phloem; they are smaller than the pericyclic fibres, measuring only 7 to 14 μ in diameter. The *supernumerary phloem* is composed of elements similar to those of the phloem of the main cylinder, but frequently the cells are collapsed. The *medulla*, which is much reduced, consists of thin-walled parenchyma, the cells of which are fairly large, round to ovoid, measuring about 18 to 39 μ in diameter and occasionally containing sandy crystals of calcium oxalate.

Lateral veins and veinlets. The lateral veins and veinlets are transcurrent, the palisade tissue above and the spongy mesophyll below being replaced by a few rows of small, thin-walled parenchymatous cells measuring about 14 to 22 μ in diameter; fibres occur singly or in small groups in the periphery of the bundle. Small, conical trichomes similar to those occurring on the midrib (Fig. 2, D, t_1) are present scattered on the lower epidermis of the lateral veins.

(ii) Stems

MACROSCOPICAL CHARACTERS

The young stems and branches of *Uncaria gambier*, which are used in the preparation of catechu, are somewhat angular and measure from 0·1 to 0·4 cm in diameter. The nodes, which occur at intervals of 3·5 to 6·5 cm, are slightly enlarged. The surface varies from light to reddish brown in colour and is smooth or very finely striated longitudinally; the phyllotaxis is opposite and decussate.

The transversely cut surface of the internode is square or oblong in outline with rounded corners, and slightly concave on each side. The opposing sides, which bear the hooks and leaves at the next node up the stem, usually show a deeper concavity than the other two sides, which bear the scars of the interpetiolar stipules. Since the arrangement of the leaves is opposite and decussate, this arrangement of the concavities alternates by 90 degrees at each node.

The transverse section shows a layer of brown cork externally and in the centre a large, whitish-yellow pith, which occupies about half to two-thirds of the diameter. The pith is separated from the narrow secondary phloem and cortex by a band of secondary xylem which is brown in colour (see Fig. 3, A).

HISTOLOGICAL CHARACTERS

The *epidermis*, which is covered by a thin, finely striated cuticle, is composed of polygonal cells measuring about R 7 to 14 μ, T 10 to 21 μ and L 18 to 58 μ, with straight, evenly thickened anticlinal walls; stomata are few and paracytic; small, conical covering trichomes, similar to those found on the midrib, are fairly numerous (Fig. 3, C; Fig. 2, D). *Cork*

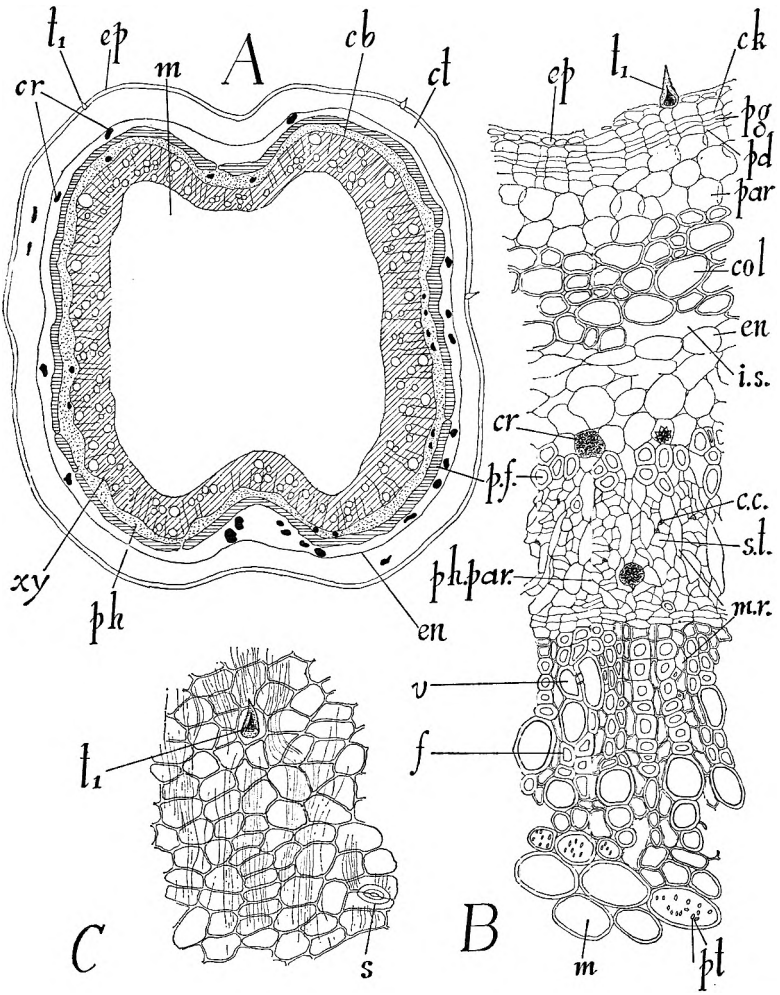

FIG. 3. Stem of *Uncaria gambier* Roxb. *A*, diagram of a transverse section of a young stem. ×40; *B*, details of a transverse section of an older stem; *C*, epidermis in surface view; *B* and *C*, ×200; *cb*, cambium; *c.c.*, companion cell; *ck*, cork; *col*, collenchyma; *cr*, crystals of calcium oxalate; *ct*, cortex; *en*, endodermis; *ep*, epidermis; *f*, fibres; *i.s.*, intercellular space; *m*, medulla; *m.r.*, medullary rays; *par*, parenchyma; *pd*, phelloderm; *p.f.*, pericyclic fibres; *pg*, phellogen; *ph*, phloem; *ph.par.*, phloem parenchyma; *pt*, pits; *s*, stoma; *s.t.*, sieve tube; t_1, covering trichomes; *v*, vessel; *xy*, xylem.

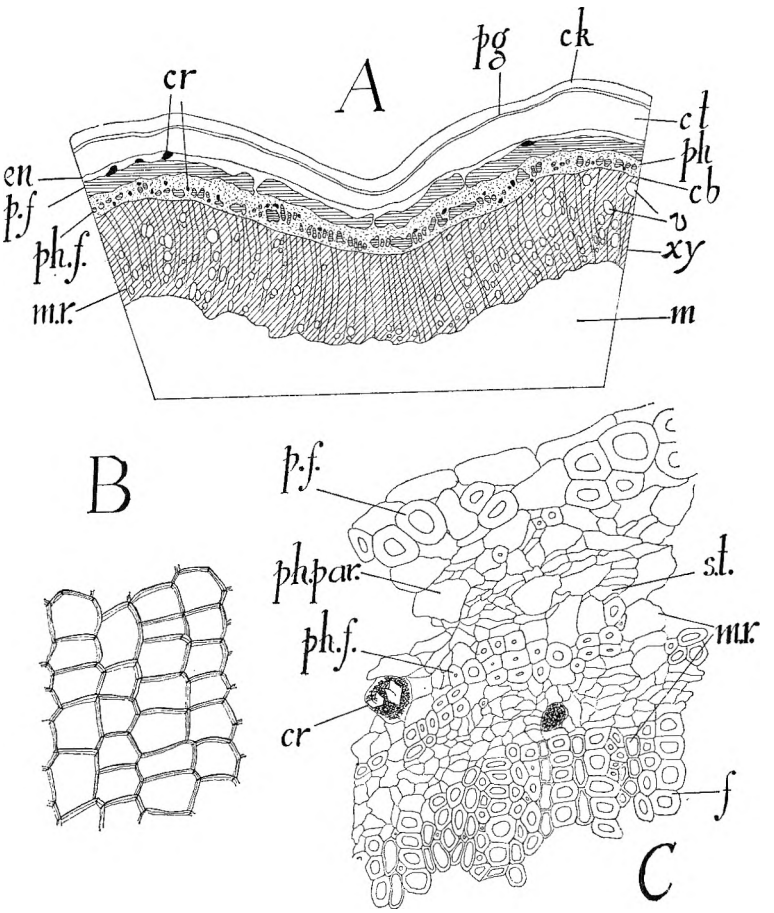

FIG. 4. Stem of *Uncaria gambier* Roxb. *A*, diagram of part of a transverse section of a mature stem showing the presence of phloem fibres, ×40; *B*, cork in surface view; *C*, details of a transverse section in the phloem region; *B* and *C*, ×200. *cb*, cambium; *ck*, cork; *cr*, crystals of calcium oxalate; *ct*, cortex; *en*, endodermis; *f*, fibres; *m*, medulla; *m.r.*, medullary rays; *p.f.*, pericyclic fibres; *pg*, phellogen; *ph*, phloem; *ph.par.*, phloem parenchyma; *s.t.*, sieve tube; *v*, vessels; *xy*, xylem.

is present in older stems, developed from a phellogen which arises in the hypodermis; it consists of 2 to 4 layers of polygonal cells measuring R 7 to 14 μ, T 15 to 46 μ and L 18 to 54 μ with dark brown contents and thin, suberised and occasionally lignified walls (Fig. 3, B; Fig. 4, B). Phelloderm is not well developed and forms the outer *cortex*, consisting of 2 to 4 layers of thin-walled, rounded or tangentially elongated parenchymatous cells with small intercellular spaces; the remainder of the cortex is collenchymatous and consists of 3 to 4 layers of cells measuring about R 14 to 25 μ, T 14 to 36 μ, with occasional large, intercellular spaces and scattered idioblasts containing sandy crystals of calcium oxalate. The endodermis is composed of a layer of thin-walled cells somewhat compressed radially, with no intercellular spaces; starch is absent.

The outer part of the *pericycle* is composed of 1 or 2 layers (increasing to about 4 in the concavities) of parenchymatous cells with large intercellular spaces; calcium oxalate occurs in some of the larger cells in the form of cluster crystals measuring about 16 μ in diameter or small sandy crystals or, occasionally, single cluster crystals embedded in a mass of sandy crystals. The inner part of the pericycle consists of an almost continuous band of from 1 to 3 layers of thick-walled, slightly lignified fibres; individual fibres measure from 10 to 36 μ in diameter and are very long, measuring 792 to **2,700** to 4,640 μ, with a few reaching a length of 5,000 μ (Fig. 5, D).

The primary phloem is not clearly distinguishable. The *secondary phloem* is well developed and from 6 to 9 rows in depth, or up to 12 rows at the concavities; the tissue is extremely soft and the cells are frequently broken or collapsed; some ceratenchyma is also present. The functional phloem is composed of sieve tubes and phloem parenchyma, traversed by numerous medullary rays (Fig. 3, B; Fig. 5, C). The sieve tubes measure about 6 to 14 μ in diameter and are accompanied by very small companion cells; the parenchymatous cells measure 14 to 25 μ in diameter and frequently contain crystals of calcium oxalate similar to those in the pericycle; the medullary rays are from 1 to 2 cells wide and are composed of large thin-walled parenchymatous cells which, in the outer part of the phloem, are radially elongated; individual cells measure about R 14 to 43 μ, T 7 to 29 μ and L 55 to 108 μ. Phloem fibres are only present in older stems in which cork formation has taken place (Fig. 4, A and C); they occur singly or in groups of up to 6 or in discontinuous bands of up to 4 layers, in the inner region of the phloem adjacent to the cambium; they are shorter and narrower than the pericyclic fibres, measuring 7 to 21 μ in diameter and up to 1,536 μ in length, and the walls are thicker and bear numerous, conspicuous slit-shaped pits (Fig. 4, C; Fig. 5, D).

The *cambiform tissue* consists of 2 to 3 layers of thin-walled, somewhat radially-compressed cells measuring R 4 to 7 μ and T 14 to 18 μ.

The *secondary xylem* consists of scattered vessels and tracheidal vessels and large amounts of fibres and xylem parenchyma. The vessel elements (Fig. 3, B; Fig. 5, A) measure about 28 to 72 μ in diameter and 390 to 972 μ in length; the side walls bear numerous small, spirally-arranged bordered pits and the end walls are oblique. The tracheidal vessels (Fig. 5, A)

are narrower, measuring about 14 to 29 μ in diameter and 432 to 936 μ in length; the walls bear numerous small, bordered pits and the perforations appear as oval or rounded openings near the ends, which are tapering and occasionally forked. The fibres are numerous and are arranged in radial rows of from 8 to 20 cells; they are identical in structure to the phloem fibres (Fig. 5, D).

The xylem parenchyma is lignified and consists of rectangular cells measuring R 7 to 29 μ, T 4 to 14 μ, arranged in radial rows of 8 to 20 cells between the groups of fibres and also accompanying the vessels; the walls are only slightly thickened and bear numerous bordered or simple pits (Fig. 5, B). The secondary medullary rays are also lignified and in transverse section the structure and arrangement of the constituent cells are very similar to those of the xylem parenchyma; they are, however, readily distinguished by their reddish-brown colour.

The *pith* is composed of parenchymatous cells with fairly thick, slightly lignified and pitted walls; the cells vary in size and shape depending on their position, those towards the periphery being round or ovoid measuring about 36 to 60 μ in diameter, whereas those in the central region are polygonal and measure up to 240 μ wide (Fig. 3, B).

Calcium oxalate is absent from all tissues internal to the cambium.

(iii) Hooks

MACROSCOPICAL CHARACTERS (Fig. 6, A)

One of the most characteristic features of *Uncaria gambier* and other tropical climbers of the same genus is the presence of a "hook" in the leaf axis after the inflorescence has fallen off; this is formed from the remnant of the axillary peduncle and is used by the plant for climbing. It is woody, about 1 to 2 cm long, curved downwards towards the stem axis, tapering towards the apex and laterally compressed; the surface is reddish-brown to dark brown and longitudinally furrowed; the basal region appears glabrous, but towards the apex the surface becomes densely pubescent and the hook very often terminates with a tuft of fine, silky hairs.

HISTOLOGICAL CHARACTERS

The epidermal cells are similar in shape, size and structure to those of the stem, except in the apical region where they are thinner-walled and smaller, measuring R 7 to 11 μ, T 7 to 25 μ and L 11 to 36 μ (Fig. 6, C_2); paracytic stomata occur infrequently. In the basal region the covering trichomes are similar in form and distribution to those on the stem epidermis (Fig. 6, C_3), but as the apex is approached they become longer, thinner walled, more tortuous in shape and progressively greater in number until, at the extreme apex, nearly every epidermal cell is developed as a covering trichome (Fig. 6, C_1).

The structure and arrangement of the remainder of the tissues are essentially the same as those of the stem. Larger vessels, however, are not found in the secondary xylem, and pericyclic fibres do not form a

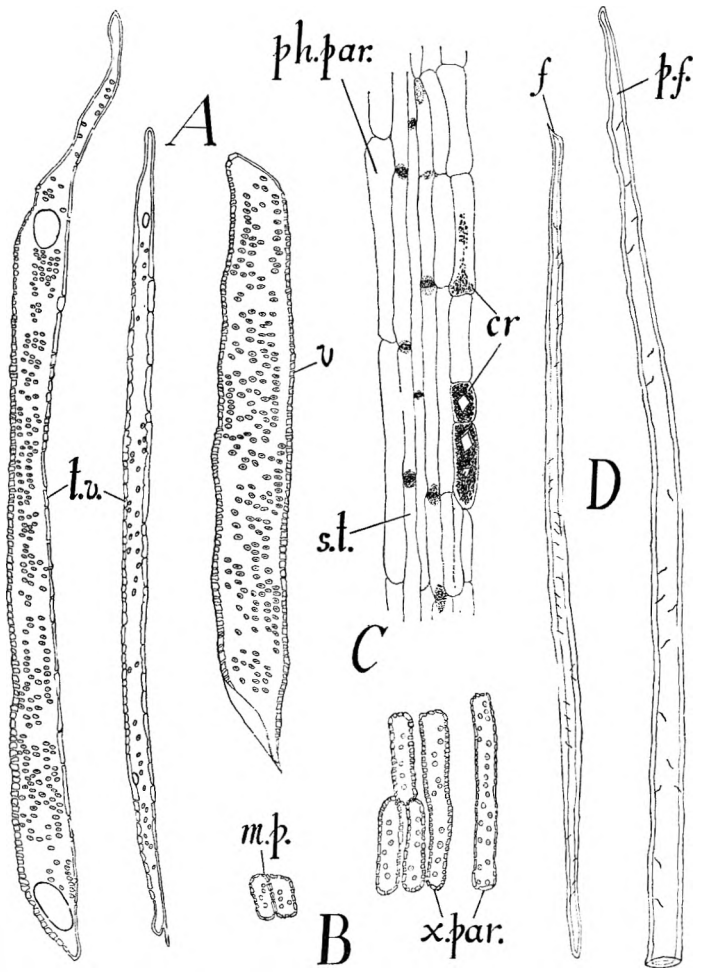

FIG. 5. Stem of *Uncaria gambier* Roxb. *A*, isolated vessel (*v*) and tracheidal vessels (*t.v.*) from the xylem; *B*, xylem parenchyma (*x.par.*) and medullary ray parenchyma (*m.p.*); *C*, part of the phloem in longitudinal section; *s.t.*, sieve tube; *cr*, calcium oxalate crystals; *ph.par.*, phloem parenchyma; *D*, isolated fibres from the xylem or phloem (*f*) and pericycle (*p.f.*). All ×200.

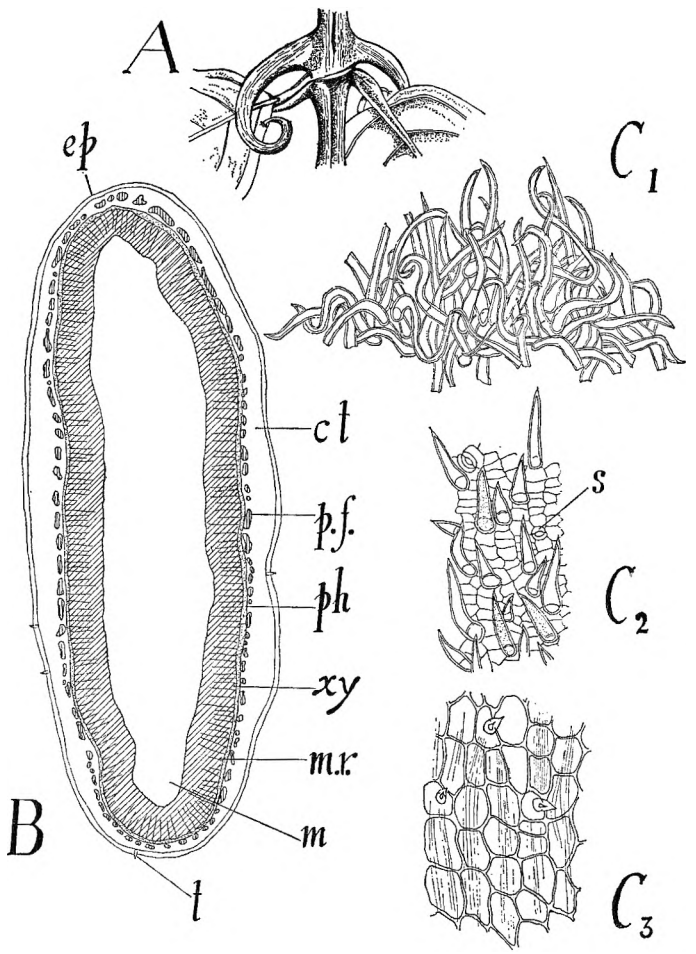

FIG. 6. Hook of *Uncaria gambier* Roxb. *A*, drawing of a node, showing the arrangement of the leaves and hooks, ×1·5; *B*, diagram of a transverse section cut near the base, ×25; C_1, C_2 and C_3, portions of the epidermis in surface view, all ×200; C_1, from the apex showing details of part of the tuft of hairs; C_2, from near the apical region and C_3, from near the base; *ct*, cortex; *ep*, epidermis; *m*, medulla; *m.r.*, medullary rays; *p.f.*, pericyclic fibres; *ph*, phloem; *s*, stoma; *t*, covering trichome; *xy*, xylem. (*A*, after Bentley and Trimen, modified.)

complete band surrounding the vascular tissues but occur in groups, separated by thin-walled parenchyma; the cortex is also narrower and its constituent cells are somewhat radially-compressed (Fig. 6, B).

(iv) Stipules

MACROSCOPICAL CHARACTERS (Fig. 7, A and B)

The paired interpetiolar stipules are generally only found intact on the apical buds and the nodes in the upper region of the young twigs as they fall off early, leaving two linear scars at each node. They are ovate, about 6 to 10 mm long and 4 to 6 mm wide; vascular strands, of which there are about 15 to 20, run almost parallel to one another from the base to the apex, and these are joined by a fine network of smaller veins; the upper surface (Fig. 7, A) is densely pubescent, particularly near the margin, but only scattered hairs occur on the lower surface.

HISTOLOGICAL CHARACTERS

The *upper epidermis* is covered with a thin, faintly striated cuticle and consists of thin-walled cells measuring Lev L and B 10 to 43 μ and H 7 to 11 μ, with very occasional, paracytic stomata. Covering trichomes are very numerous, particularly near the margins; they are unicellular, conical to linear measuring about 54 to 144 μ in length and 7 to 18 μ in diameter at the base; the walls are thin and may be warty or smooth (Fig. 7, E). The *lower epidermis* is also covered with a finely striated cuticle but the cells are smaller, measuring Lev L and B 9 to 32 μ and H 7 to 11 μ; paracytic stomata occur frequently and a few covering trichomes are present, similar to those on the upper epidermis (Fig. 7, D). The *mesophyll* is, in the main part, undifferentiated, and is composed of rounded or slightly elongated thin-walled cells measuring about 14 to 43 μ in diameter, with intercellular spaces; some differentiation occurs near the margins where the cells of the hypodermis and one or two adjacent layers are collenchymatous. Crystal-idioblasts are absent (Fig. 7, C). There is no midrib, and in transverse section the nearly parallel veins are seen at intervals cut transversely or somewhat obliquely; each vascular strand consists of from 7 to 10 xylem vessels with a small amount of sieve tissue and a few fibres (Fig. 7, F).

(v) Calyx

MACROSCOPICAL CHARACTERS (Fig. 8, A)

The calyx is green in colour and measures about 0·7 cm long and about 0·3 cm wide; sepals 5, joined, forming a tube at the base which is united with the inferior ovary, and at the top forming 5 triangular lobes which are somewhat thickened at the apex; outer surface densely pubescent with a ridge along the midrib of each sepal; inner surface glabrous except on the lobes, which are covered with silky hairs.

HISTOLOGICAL CHARACTERS

The *outer epidermis* is covered by a thin, very faintly striated cuticle; the cells on the lobes polygonal, measuring about Lev L and B 11 to 36 μ, with straight or slightly sinuous anticlinal walls, those on the tube smaller with slightly more sinuous anticlinal walls; paracytic stomata numerous; unicellular covering trichomes very numerous, those on the lobes and the upper part of the tube conical, somewhat sinuous, measuring about 72 to 180 μ long and 10 to 25 μ wide with thin, unlignified walls and occasionally containing a single septum (Fig. 8, C); those near the base of the tube longer, conical to linear, measuring about 198 to 414 μ long and 10 to 18 μ wide with fairly thin, usually lignified walls and with the limb bent at right angles to the finely pitted base.

The cells of the *inner epidermis* are similar to those of the outer but they are larger, measuring Lev B 14 to 36 μ and Lev L 18 to 50 μ; stomata are absent. Covering trichomes are present on the apex of the lobes only and they are similar to those occurring on the outer epidermis in this region.

(vi) Corolla

MACROSCOPICAL CHARACTERS (Fig. 8, A and B)

The corolla is pale purple in colour and measures about 1·5 cm long and 0·8 cm wide at the distal end, and 0·1 cm wide at the proximal end; petals 5, united for the greater part of their length to form a narrow tube and opening out at the top into 5 ovate, spreading lobes (Fig. 8, A); outer surface densely pubescent, inner surface glabrous except for a small tuft of long silky hairs at the base of each lobe; 5 epipetalous stamens alternating with the lobes of the corolla (Fig. 8, B).

HISTOLOGICAL CHARACTERS

The *outer epidermis* is covered by a thin, smooth cuticle; the cells on the lobes polygonal, fairly large, measuring Lev L and B 18 to 46 μ, those on the tube somewhat rectangular and elongated longitudinally measuring Lev B 8 to 22 μ and Lev L 40 to 126 μ; stomata paracytic, rare, present only on the lobes. Covering trichomes, similar to those found at the base of the calyx tube, are very numerous on the lobes (Fig. 8, G) but only occur scattered on the tube (Fig. 8, E), those on the lobes measuring 144 to **270** to 432 μ long and 10 to 18 μ wide at the mid-part of the limb, but those on the tube shorter, measuring 90 to 234 μ long and 10 to 14 μ wide at the mid-part of the limb. The cells of the *inner epidermis* are similar to those of the outer in the tubular region, but differ on the lobes, those on the basal region being larger than those on the corresponding part of the outer surface, measuring Lev L and B 18 to 54 μ while those on the central and marginal regions are much smaller, measuring Lev L and B 18 to 25 μ, and have unevenly thickened walls; stomata are absent. Covering trichomes only occur at the base of the lobes; they are unicellular, long and stout, measuring 252 to 576 μ long,

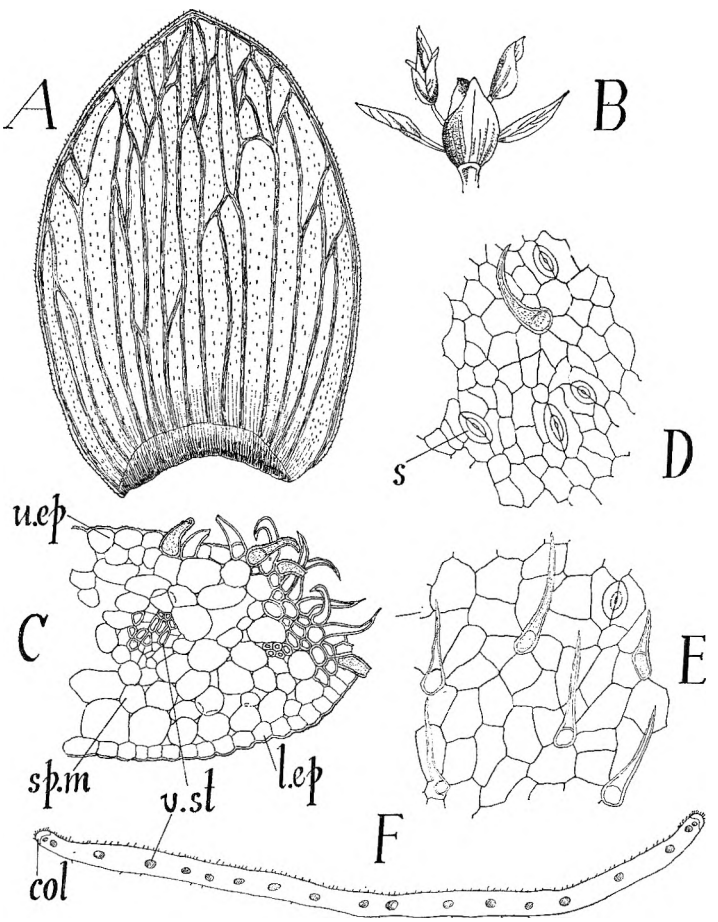

FIG. 7. Stipule of *Uncaria gambier* Roxb. *A*, an entire stipule, ×4; *B*, the extremity of a young twig to show the position of the stipules, ×1·5; *C*, details of part of a transverse section cut to include the margin; *D*, lower epidermis in surface view; *E*, upper epidermis in surface view; *C*, *D* and *E*, ×200; *F*, diagram of a transverse section, ×8; *col*, collenchyma; *l.ep*, lower epidermis; *s*, stoma; *sp.m.*, spongy mesophyll; *u.ep.*, upper epidermis; *v.st.*, vascular strands. (*B*, after Bentley and Trimen.)

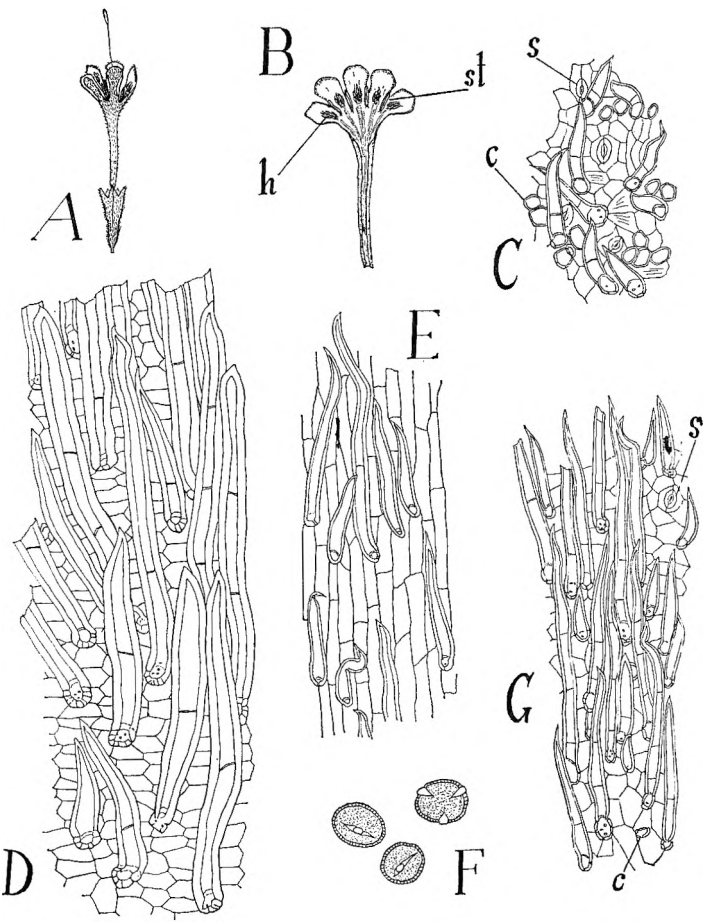

FIG. 8. Calyx and corolla of *Uncaria gambier* Roxb. *A*, entire flower, ×1·5; *B*, corolla, opened out to show the epipetalous stamens (*st*), and the tuft of hairs (*h*) at the base of each corolla lobe, ×2; *C*, outer epidermis from the lobe of the calyx; *D*, inner epidermis from the basal region of the lobe of the petal; *E*, outer epidermis of the corolla tube; *F*, pollen grains; *G*, outer epidermis from the lobe of the petal; *D* and *G*, ×130; *C* and *E*, ×180; *F*, ×350; *c*, cicatrix; *s*, stoma.

21 to 39 μ wide at the base and up to 43 μ wide in the limb; walls thick and lignified, pitted at the base; the limb frequently bent at right angles to the base and containing 1 to 3 thin, transverse, lignified septa (Fig. 8, D).

POLLEN GRAINS

Owing to the presence of epipetalous stamens, numerous pollen grains are found on the lobes of the corolla; they are sub-spherical, very small, measuring 11 to 18 μ in diameter with 3 furrows, each furrow having a central pore; exine covered with minute pits in a scattered arrangement (Fig. 8, F).

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Observations on the anti-anaphylactic activity of hydrocortisone and related steroids

P. GOADBY AND W. G. SMITH

Hydrocortisone and some synthetic analogues had negligible anti-anaphylactic activity in actively sensitised guinea-pigs when administered alone but they potentiated the protection afforded by mepyramine. Hydrocortisone did not affect the yield of histamine from sensitised guinea-pig lung subjected to anaphylaxis *in vitro* but reduced the amount of SRS-A produced under these conditions.

SEVERAL authors have been unable to prevent death from experimental anaphylactic shock by pretreating animals with cortisone or ACTH (Leger, Leith & Rose, 1948; Dworetsky, Code & Higgins, 1950; Friedlander & Friedlander, 1950; Malkiel, 1951.)

Herxheimer & Rosa (1952) showed that a single injection of cortisone given before exposure of actively sensitised guinea-pigs to aerosolised antigen did not increase the time for onset of dyspnoea and cough. However, Feinburg, Malkiel & McIntire (1953) found that cortisone increased the time of production of dyspnoea and cough in passively sensitised animals when the drug was administered 18 hr before exposure to aerosolised antigen.

The anti-anaphylactic activity of hydrocortisone and related steroids in actively sensitised guinea-pigs exposed to aerosolised antigen is here reported.

Methods

ANAPHYLACTIC SHOCK *In Vivo*

Groups of 10 guinea-pigs were sensitised to commercial egg albumin by the intraperitoneal injection of 100 mg as a 5% solution in normal saline. Three weeks later the animals were subjected to anaphylactic shock using the technique of Herxheimer (1952) as modified by Smith (1961). Each animal was placed in a glass vessel and exposed to an aerosol of antigen produced by applying air at 10 lb/in^2 to a Riddostat inhaler (Riddell Products, London) containing a 1% w/v solution of egg albumin in distilled water. The animals were removed from the chamber at the onset of dyspnoea and cough. The time for onset of dyspnoea and cough was measured at weekly intervals and was relatively constant for each animal. The mean of the last two exposures was termed the "normal collapse time" (Smith, 1961). Table 1 shows the times to onset of dyspnoea and cough for a sample group with the calculated "normal collapse time" of each animal.

Drug pretreatment was carried out before the fourth weekly exposure to antigen. An increase in the time to onset of dyspnoea and cough on exposure to antigen following treatment ("treated collapse time") indicated a protective effect. The protection was expressed as a "protection

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TABLE 1. EVALUATION OF "NORMAL COLLAPSE TIMES" AND "PROTECTION RATIOS"

Animal No.	Collapse time (sec)			Normal collapse time (calculated from 2 and 3 weeks)	Treated collapse time Week 4	Protection ratio (calculated from cols. 5 and 6)
	Week 1	Week 2	Week 3			
1	40	38	34	36	63	1·75
2	34	39	55	47	80	1·70
3	22	34	30	32	69	2·16
4	30	49	49	49	62	1·27
5	28	45	41	43	105	2·44
6	15	20	18	19	48	2·53
7	31	36	42	39	138	3·53
8	20	22	30	26	40	1·54
9	33	27	41	34	75	2·20
10	26	30	42	36	82	2·28

Group Mean Protection Ratio = 2·14. Standard deviation = 0·64. Treatment = 50 mg hydrocortisone sodium hemisuccinate intramuscularly 18 hr before shock.

ratio", this being the ratio of the "treated collapse time" to the "normal collapse time". It was found that animals which tolerated antigen for more than twenty times their "normal collapse time" did not exhibit signs of dyspnoea and cough even though exposure to antigen was continued for much longer. Therefore an animal showing a protection ratio of 20 was considered to be fully protected from the effects of anaphylaxis.

PHARMACOLOGICAL ACTIVITY ON GUINEA-PIG ILEUM

The terminal ileum was removed from a freshly killed guinea-pig. The last 2 cm adjacent to the caecum were rejected and suitably sized segments of the remaining ileum were suspended in aerated Tyrode solution at 37° in a 2 ml isolated tissue bath. The drugs were applied as solutions in Tyrode solution.

The SRS–A (slow reacting substance of anaphylaxis) was prepared by the method of Brocklehurst (1960) and standardised by comparison with a laboratory standard (see Marquis & Smith, 1963).

The hydrocortisone was applied 30 sec before the addition of the stimulant drug. Histamine was allowed 30 sec contact with the tissue and SRS–A was allowed 90 sec contact.

PHARMACOLOGICAL ACTIVITY ON GUINEA-PIG TRACHEAL CHAIN

Tracheal chains from the guinea-pig (Castillo & de Beer, 1947) were mounted in Krebs Hensleit solution (1932) in a 15 ml isolated tissue bath. The hydrocortisone was added 1 min before the stimulant drug. Histamine was allowed 5 min contact with the preparation and SRS–A 10 min contact.

ANAPHYLACTIC SHOCK *In Vitro*

Twelve sensitised guinea-pigs whose "normal collapse times" had been determined as described earlier were divided into two groups of approximately equal sensitivity to antigen.

One week after the third exposure to antigen, the animals of one group were injected intramuscularly with 50 mg of sodium hydrocortisone hemisuccinate as a 5% solution in Water for Injection B.P.

The animals of the second group received an equal volume of normal saline by the same route. Eighteen hr later the animals were killed, their lungs excised, perfused with Tyrode solution and subjected to anaphylaxis *in vitro* as described by Brocklehurst (1960).

The released histamine and SRS–A were assayed on segments of guinea-pig ileum (Marquis & Smith, 1963). The assays of SRS–A were made in Tyrode solution without sodium bicarbonate since this increased the sensitivity of the ileum to SRS–A (Firth, unpublished).

DRUGS

Cortisone acetate, hydrocortisone, prednisolone and triamcinolone were administered as fine suspensions and hydrocortisone sodium hemisuccinate as a solution in Water for Injection B.P. The dosage used throughout was 50 mg per animal intramuscularly 18 hr before exposure to antigen. (Feinberg & others, 1953.)

Mepyramine: 1 mg/kg, given intramuscularly 1 hr before shock was used in the *in vivo* experiments. This dose gave the peak effect against the symptoms of anaphylaxis in the study reported by Smith (1961).

Results

PROTECTIVE EFFECT OF CORTICOSTEROID DRUGS AGAINST ANAPHYLAXIS

In Vivo

Each drug was administered to a group 18 hr before the fourth weekly exposure to antigen. Table 2 shows the mean "protection ratio" obtained for each drug using groups of 10 guinea-pigs. It can be seen that none of the compounds tested had marked anti-anaphylactic activity. The greatest activity was shown by hydrocortisone sodium hemisuccinate, the only soluble derivative used.

TABLE 2. ANTI-ANAPHYLACTIC EFFECT OF CORTICOSTEROID DRUGS (50 MG) GIVEN INTRAMUSCULARLY 18 HR BEFORE SHOCK

Drug	Group Protection Ratio	Standard deviation
Cortisone acetate	1·58	0·52
Hydrocortisone	1·38	0·19
Prednisone	1·36	0·42
Prednisolone	1·60	0·41
Triamcinolone	1·48	0·48
Hydrocortisone sodium hemisuccinate	2·14	0·64

POTENTIATION OF ANTI-ANAPHYLACTIC EFFECTS OF MEPYRAMINE BY CORTICOSTEROIDS

Each drug was tested in a group of ten animals. The protective effect of mepyramine administered 1 hr before anaphylaxis *in vivo* was determined for each group. One week later the animals were exposed to antigen without pretreatment to ascertain that the mepyramine had had no lasting effects and then another week was allowed before the determination of the effects of the double pretreatment with mepyramine (1 hr) and the corticosteroid drug (18 hr).

TABLE 3. COMPARISON OF ANTI-ANAPHYLACTIC ACTIVITY OF CORTICOSTEROIDS (50 MG) GIVEN 18 HR BEFORE SHOCK PLUS MEPYRAMINE (1 MG/KG) GIVEN 1 HR BEFORE SHOCK

Drug	Mepyramine only		Drug plus mepyramine		
	Group Protection Ratio	Standard deviation	Fully protected animals	Group Protection Ratio of remainder	Standard deviation
Cortisone acetate	4·42	1·44	3	7·13	2·93
Hydrocortisone sodium hemisuccinate	2·47	1·14	3	5·35	1·96
Prednisolone	4·53	1·55	2	6·44	1·41
Prednisone	4·02	1·38	2	5·98	2·13
Triamcinolone	3·41	1·02	1	5·20	2·13
Hydrocortisone	3·42	1·06	1	4·36	1·39

Table 3 shows the mean "protection ratio" obtained with mepyramine in each group, together with the mean "protection ratio" obtained after pretreatment with steroid and mepyramine.

Most of the compounds potentiated the effects of mepyramine. Those showing the greatest activity were cortisone acetate and hydrocortisone sodium hemisuccinate.

DETERMINATION OF OPTIMUM TIME BETWEEN PRETREATMENT WITH HYDROCORTISONE SODIUM HEMISUCCINATE AND EXPOSURE TO ANTIGEN

Twenty animals whose "normal collapse times" had been determined, were divided into five groups of four so that sensitivity to antigen was approximately the same for each group. The groups were pretreated with hydrocortisone sodium hemisuccinate 1, 6, 12, 18 and 24 hr respectively before exposure to antigen. All animals were also treated with mepyramine. The mean "protection ratio" for each group was respectively 4, 5·2, 7·4, 9·5 and 4·2. Thus the potentiation of mepyramine reached a maximum in animals pretreated with hydrocortisone 18 hr before exposure to antigen.

ANTAGONISM OF HISTAMINE AND SRS–A BY HYDROCORTISONE SODIUM HEMISUCCINATE

The effects of hydrocortisone sodium hemisuccinate on the responses of the guinea-pig ileum to histamine and SRS–A are shown in Figs 1 and

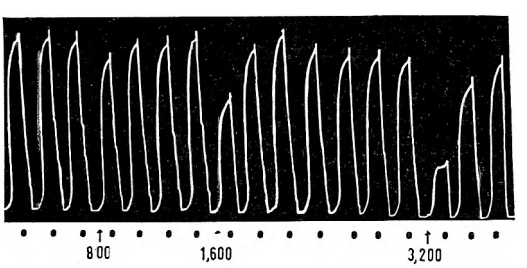

FIG. 1. Antihistaminic activity of hydrocortisone sodium hemisuccinate on guinea-pig ileum. Bath volume, 2 ml. The standard dose of histamine (●) was 0·2 μg The amounts below arrows are μg/ml of hydrocortisone sodium hemisuccinate added 30 sec before the next dose of histamine. Contact time for histamine = 30 sec and for hydrocortisone = 60 sec. Drum speed = 16 mm/min. Dose interval = 3 min.

2. The inhibitory effects of hydrocortisone sodium hemisuccinate on the responses to histamine and SRS–A of the guinea-pig tracheal chains are shown in Figs 3 and 4.

From the graphical relationship of the dose and the percentage inhibition of a reproducible response it was concluded that on the ileum there were indications of a preferential antagonism of SRS–A. There was no such preferential antagonism on the tracheal chain preparations.

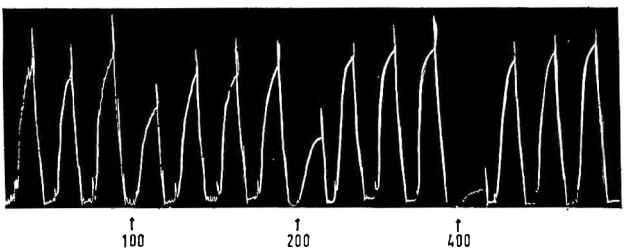

FIG. 2. Anti-SRS-A activity of hydrocortisone sodium hemisuccinate on guinea-pig ileum. The standard dose of SRS-A was two units. The amounts below arrows are mg/ml of hydrocortisone added 30 sec before the next dose of SRS–A. Contact time for SRS-A = 90 sec and for hydrocortisone = 120 sec. Drum speed = 8 mm/min. Dose interval = 5 min.

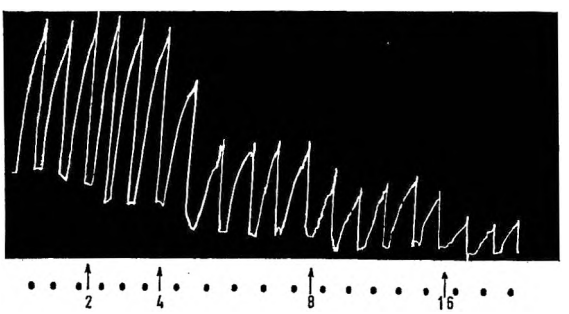

FIG. 3. Antihistaminic activity of hydrocortisone sodium hemisuccinate on the guinea-pig tracheal chain. Standard dose of histamine = 2 μg. The amounts below arrows are mg/ml of hydrocortisone added 1 min before the next dose of histamine. Contact time for histamine = 5 min and for hydrocortisone = 6 min Drum speed = 2 mm/min. Dose interval = 10 min.

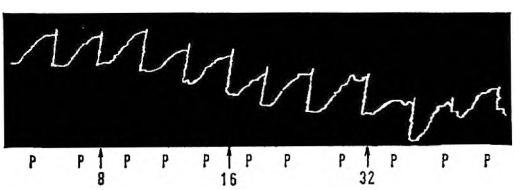

FIG. 4. Anti-SRS-A activity of hydrocortisone sodium hemisuccinate on the guinea-pig tracheal chain. P = 80 units of SRS–A. Amounts below arrows are mg/ml of hydrocortisone added 1 min before the next dose of SRS-A. Contact time for SRS-A = 10 min and for hydrocortisone = 11 min. Drum speed = 1 mm/min. Dose interval = 30 min.

EFFECTS OF HYDROCORTISONE SODIUM HEMISUCCINATE ON RELEASE OF CHEMICAL MEDIATORS OF ANAPHYLAXIS

The mean yields of histamine and SRS–A of the control and pretreated subgroups are shown in Fig. 5.

Pretreatment with hydrocortisone sodium hemisuccinate did not alter the amount of histamine but the same pretreatment reduced the amount of SRS–A released during anaphylaxis *in vitro*.

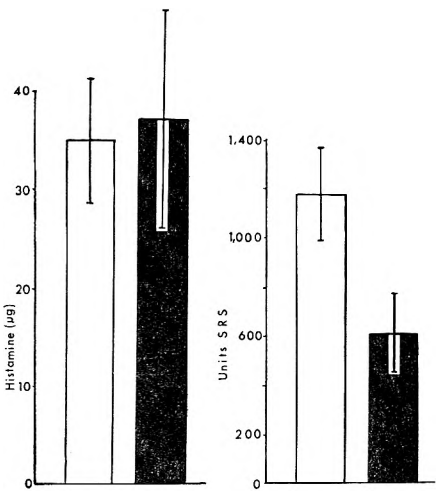

FIG. 5. Effect of pretreatment with hydrocortisone sodium hemisuccinate on the release of chemical mediators of anaphylaxis. The columns represented the mean yields of histamine and SRS-A from sensitised guinea-pig lungs shocked *in vitro*. Open columns, mean yields from untreated animals. Shaded columns, mean yields from animals pretreated 18 hr before shock with 50 mg/animal of hydrocortisone sodium hemisuccinate intramuscularly. The standard deviation of each mean is shown.

Discussion

The observation that corticosteroid drugs have little anti-anaphylactic activity when administered as a single injection to actively sensitised guinea-pigs before exposure to aerosolised antigen is in agreement with the findings of Herxheimer & Rosa (1952) for cortisone. However these results do not invalidate those of Feinberg & others (1953) who elicited a less severe reaction and who expressed results in a manner which emphasised a small degree of protection. Since the time at which maximum potentiation of the effects of mepyramine by hydrocortisone coincides with the optimum time of administration of cortisone as found by Feinberg & others, it is possible that mepyramine may magnify a reaction which is already present.

None of the drugs used in this investigation produced a greater level of protection than cortisone despite reports of greater anti-inflammatory potency. Thus it would appear that anti-inflammatory potency is not related to the anti-anaphylactic activity of the drugs. In view of the

difference between hydrocortisone administered as a fine suspension and as an aqueous solution of its sodium salt, water solubility would seem to be an important factor influencing anti-anaphylactic activity as studied under these conditions.

Although hydrocortisone can antagonise the smooth muscle stimulant actions of both histamine and SRS–A, large doses are needed and it is unlikely that its anti-anaphylactic activity observed 18 hr after intramuscular administration can be the result of such an antagonism.

The same dose of hydrocortisone as that used *in vivo* reduced the release of SRS–A during subsequent anaphylaxis *in vitro* to a level corresponding to about 50% of that found in the untreated animals without influencing the amount of histamine released. This observation may provide an explanation for the *in vivo* results, if it is assumed that the depression of SRS–A release was not apparent until the actions of histamine had been suppressed with mepyramine.

That the anti-anaphylactic action of hydrocortisone reached a maximum 18 hr after intramuscular injection suggests that the action may be due to an alteration in tissue metabolism. Goadby & Smith (1962) have reported that hydrocortisone, in the same dosage and under the *in vivo* conditions quoted in this paper, protected actively sensitised guinea-pigs from changes in the lipid metabolism of their lung tissue which were normally induced by anaphylaxis. Thus it is possible that the depression of the release of SRS–A may be related to such a metabolic action.

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Further pharmacological screening of some West Indian medicinal plants

P. C. FENG, L. J. HAYNES, K. E. MAGNUS AND J. R. PLIMMER

Tabulated results are presented for the systematic pharmacological examination of a further 61 Jamaican medicinal plants.

IN an earlier paper (Feng, Haynes, Magnus, Plimmer & Sherratt, 1962) the results were given of a systematic pharmacological examination of aqueous extracts of 55 Jamaican plants, most of which had a reputation as medicinal substances. In continuation of this programme of examination of medicinal plants we now present results of the pharmacological examination of a further 61 plants.

The experimental procedure for the preparation of the extracts follows that given by Feng & others (1962) as does the procedure for pharmacological testing, except that the tests on rabbit heart and duodenum and rat stomach and diaphragm were omitted and a test on toad rectus was added.

Experimental

Extracts A and B were prepared and routine pharmacological tests were applied as described previously (Feng & others, 1962).

The following additional test was performed.

FROG RECTUS ABDOMINIS MUSCLE

A piece of rectus muscle from the Jamaican Toad (*Bufo marinus*) was suspended in frog Ringer's solution. The extract was introduced to test for its stimulating effect and for inhibition of spasm induced by acetylcholine.

TABLE I. RESULTS OF INVESTIGATIONS

Plant No.	Family	Botanical name	Toxicity mice i.p.	Guinea-pig ileum	Rat uterus	Toad rectus	Rat limb flow	Dog B.P.
56	Acanthaceae	*Barleria cristata* L.	+	++	–	–	– – – –	P
57	,,	*Thunbergia grandiflora* Roxb.	0	0	0	0	0	
58	Anacardiaceae	*Mangifera indica* L. var. "Black"	0	0	0	0		D
59	Annonaceae	*Annona squamosa* L.	+	– –	0	0	– – –	D
60	Araceae	*Xanthosoma sagittifolium* Schott.	0	0	0	0	0	D
61	Asclepiadaceae	*Asclepias curassavica* L.	0	0	–	0	+ + +	O
62	,,	*Calotropis procera* R.Br.	++	0	0			D
63	Bignoniaceae	*Catalpa longissima*	0	0	–	0	+ + +	O
64	,,	*Tecomaria capensis* Fenzl.	0	0	– –	0	++	D
65	Boraginaceae	*Cordia brownei* (Friesen) Johnson	++	0	– –	–	+ + + +	D
66	,,	*Heliotropium angiospermum* Murray	0	+	+	0	– –	O
67	,,	*Heliotropium indicum* L.	0	0	0	0	– –	D
68	,,	*Tournefortia hirsutissima* L.	+	+	+	–	– – –	D

From the University of the West Indies, Kingston 7, Jamaica.

Plant No.	Family	Botanical name	Toxicity mice i.p.	Guinea-pig ileum	Rat uterus	Toad rectus	Rat limb flow	Dog B.P.
69	Cactaceae	*Harrisia gracilis* Britt.	0	+++	+++	0	−−−	D
70	,,	*Opuntia tuna* Mill	0	++	0	0	++	D
71	Caesalpiniaceae	*Bauhinia divaricata* L.	0	0	0	0	0	O
72	,,	*Bauhinia galpini* N.E.Br.	+++	0	+++	0	−−−−	P
73	,,	*Caesalpinia bonducella* Flenn.	+	0	−−	+	−−−−	D
74	,,	*Cassia emarginata* L.	0	0	0	0	+++	D
75	,,	*Cassia fistula* L.	0	+++	0	0	+++	D
76	,,	*Hymenaea courbaril* L.	+	0	0	0	++	D
77	Cappardiaceae	*Cleome viscosa* L.	+	0	−	0	0	P
78	Celastraceae	*Schaefferia frutescens* Jacq.	0	0	0	0	−−	D
79	Combretaceae	*Terminalia catappa* L.	++	0	+++	0	0	D
80	Compositae	*Parthenium hysterophorous* L.	0	+	0	0	0	D
81	,,	*Tithonia diversifolia* Gray	0	0	++	−	++++	D
82	Convolvulaceae	*Ipomea fistulosa* Mart.	++	0	0	0	−−	D
83	Cucurbitaceae	*Cucumis anguria* L.	0	++	0	+	++	D
84	,,	*Luffa cylindrica* M.Roem.	0	+	++++	+++	0	D
85	Euphorbiaceae	*Pedilanthus jamaicensis* Millsp. & Britton	0	+	−	0	++	D
86	,,	*Ricinus communis* L.	0	0	+	+	++	D
87	Flacourtiaceae	*Casearia hirsuta* Sw.	0	0	−	0	++	P
88	,,	*Laetia thamnia* L.	++	0	0	−	0	D
89	Gramineae	*Cymbopogon citratus* DC.	0	+	0	0	−−−−	O
90	,,	*Melocanna bambusoides* Trin.	0	0	−	+	−−	O
91	,,	*Panicum maximum* Jacq.	0	0	++++	0	0	P
92	Iridaceae	*Aristea compressa* Buch.	++	0	0	0	−−	D
93	Liliaceae	*Sansevieria* spp.	++	0	−−	−	−−−	O
94	Lythraceae	*Lagerstroemia indica* L.	+	0	0	0	−−	D
95	Malvaceae	*Hibiscus rosa-sinensis* L.	0	+	0	0	0	O
96	Meliaceae	*Trichilia hirta* L.	0	0	0	0	−−−	D
97	Mimosaceae	*Acacia lutea* Hitch.	0	0	0	0	++	O
98	,,	*Albizzia lebbeck* Benth.	+	0	0	0	0	D
99	Myrtaceae	*Eucalyptus Spp.*	0	+	0	0	−−−−	O
100	Nyctaginaceae	*Pisonia aculeata* L.	++	0	0	+	++	P
101	Papilionaceae	*Brya ebenus* DC.	0	0	0	0	++	P
102	Phytolaccaceae	*Petiveria alliacea* L.	0	+	+	0	0	D
103	Plumbaginaceae	*Plumbago capensis* Thunb.	++	++++	+++	0	−−	D
104	Polygonaceae	*Antigonon leptopus* Hook & Arn.	++	0	+++	0		D
105	,,	*Polygonum chinense* L.	+	0	−−	0	0	D
106	Rhamnaceae	*Ziziphus mauritiana* Lam. (*Ziziphus jujuba* Lam.)	+	0	++++	0	++	D
107	Rubiaceae	*Randia aculeata* L.	0	−−	−	0	0	O
108	Sapindaceae	*Meliococca bijuga* L.	0	0	0	0	0	D
109	Sapotaceae	*Chrysophyllum cainito* L.	0	0	0	0	−−−−	D
110	Solanaceae	*Capsicum frutescens* L.	0	0	++	0	−−−−	D
111	,,	*Solanum verbascifolium* L.	+++	0	+	+	0	D
112	Verbenaceae	*Citharexylon Spp.*	0	0	−	0	+++	P
113	,,	*Clerodendrum fallax* Lindl.	0	0	−	0	++	D
114	,,	*Verbena bonaviensis* L.	0	+	0	0	0	O
115	Vitaceae	*Cissus sicyoides* L.	0	0	++++	0	++	D
116	Zygophyllaceae	*Kallstroemia maxima* Terr. et Gr.	+	0	0	0	+++	D

Results

The results obtained for extracts A are tabulated in Table I: the results for extracts B are omitted as, in general, no further information was provided by examination of these extracts. The numbering of the plants is a continuation of that given in the earlier paper and the same system of expression of results has been used.

Acknowledgements. We thank the Botany Department of the University of the West Indies for plant identification. This investigation was supported by the Tropical Products Institute of the Department of Scientific and Industrial Research, U.K.

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The expectorant action of cephaeline, emetine and 2-dehydroemetine

ELDON M. BOYD AND LOIS M. KNIGHT

Cephaeline, emetine and 2-dehydroemetine were administered, as the dihydrochlorides, in doses from 0·1 to 81 mg/kg body weight, orally and subcutaneously to 135 rabbits and 92 cats arranged for the collection of respiratory tract fluid. To the extent that comparisons were made, the effects of the three alkaloids were identical. The volume output of respiratory tract fluid was increasingly augmented by doses of from 0·1 to 1·0 mg/kg. No further increase followed administration of higher doses up to the highest non-lethal dose. Doses of from 9·0 to 81 mg/kg were increasingly lethal and increasingly depressed the output of respiratory tract fluid, probably through their cardiotoxic action. Lethal doses were similar for all three alkaloids and by both routes of administration. It is concluded that the expectorant action of ipecacuanha is due in whole or in major part to its content of emetine and cephaeline. The expectorant action of synthetic 2-dehydroemetine is essentially similar to that of emetine and cephaeline.

IPECAC syrup, U.S.P. XVI (1960), is classified as an emetic and expectorant. Boyd (1954) has defined an expectorant as a drug which augments the output of respiratory tract fluid. Oral administration of ipecacuanha has been reported to augment the output of respiratory tract fluid in cats (Van Dongen & Leusink, 1953), rabbits and cats (Perry & Boyd, 1941), and albino rats (Boyd, Palmer & Pearson, 1946), and to lower its viscosity (Basch, Holinger & Poncher, 1941). These results indicate that ipecacuanha could be of expectorant value in the treatment of cough due to irritation of the respiratory airway below the epiglottis from insufficient production of demulcent respiratory tract fluid (Boyd, 1954; Beckman, 1961).

Effects of the alkaloids of ipecacuanha upon the output of respiratory tract fluid have not been previously reported. Assuming that augmentation of the output of respiratory tract fluid by galenical preparations of ipecacuanha was due, in whole or in part, to their content of emetine and cephaeline, it may be estimated from the data of Perry & Boyd (1941) and of Boyd, Palmer & Pearson (1946), that doses of these alkaloids of 1 to 20 mg/kg could have a similar effect. The higher doses in this range could also inhibit the output of respiratory tract fluid through their cardiotoxic and hypotensive actions (Boyd & Scherf, 1941).

We have set out to determine the effect of administration of cephaeline and emetine, two natural alkaloids of ipecacuanha and 2-dehydroemetine (2,3-dehydroemetine, Mebadin), a synthetic alkaloid, prepared by Brossi & others (1959) as the racemic mixture, upon the output of respiratory tract fluid in rabbits and cats.

Methods

The animals used were healthy CBL female cats and male rabbits of 2 to 3 kg weight. Food was withdrawn for 16 hr before intragastric, but not subcutaneous, administration of the alkaloids. The animals

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were anaesthetised with urethane at an average dose of 1·00 g/kg, given intraperitoneally as 4·00 ml/kg of a 25% (w/v) solution in distilled water, with supplementary local procaine as required during the tracheotomy.

The animals were arranged for the collection of respiratory tract fluid by a modification of the technique of Boyd & Perry (1963). The modification consisted of enlarging to 1·25 c.ft the reservoir of conditioned air. This eliminated temporary fluctuations in the temperature and humidity of air inhaled by the animals under study. Air in the reservoir was maintained at 100° F and 99 to 100% relative humidity as read from a built-in wet and dry bulb thermometer. Maintenance of conditioned air was arranged through a thermostat connected with a heater under a vessel partially filled with distilled water, the warm vapour from which was piped into and circulated through the reservoir by a blower.

The volume output of respiratory tract fluid was recorded hourly for a period of 3 hr before, and 4 hr after, drug administration. The output was measured in ml and expressed as ml/kg body weight per 24 hr. Output during the 2 hr immediately preceding drug administration was considered as the control output. This was subtracted from the output each hr after drug administration to obtain a difference. The mean and standard error of these hourly differences were calculated and the mean difference subjected to a "*t*" test of significance (Croxton, 1953).

The three alkaloids were used as dihydrochlorides. Cephaeline, emetine and 2-dehydroemetine were each given by intragastric tube to rabbits in doses of 0·0, 0·1, 0·3, 1·0, 3·0, 9·0, 27·0 and 81·0 mg/kg weight, using 4 to 11 animals per dose and 135 rabbits in all. The same range of doses of 2-dehydroemetine and doses of 0·1 and 0·3 mg/kg of emetine were given orally to 62 cats. An additional 30 cats and 30 rabbits received 2-dehydroemetine subcutaneously in doses of 0·0, 1·0, 3·0, 9·0, 27·0 and 81·0 mg/kg weight. Each dose was dissolved in distilled water and administered in a volume of 1·00 ml/kg except for the highest dose (81 mg/kg) which was given in a volume of 4·00 ml/kg. In animals given distilled water containing no drug, the volume used was 1·00 ml/kg.

Results

All three alkaloids augmented the output of respiratory tract fluid when given by mouth to rabbits in the lower range of doses. Representative data from doses of 1·0 mg/kg of emetine and cephaeline and 3·0 mg/kg of 2-dehydroemetine are illustrated in Fig. 1. The responses to doses of 0·1, 0·3, 1·0 and 3·0 mg/kg did not differ, per dose, amongst the three alkaloids. The mean percentage augmentation of output of respiratory tract fluid by all three alkaloids has been plotted against dosage in Fig. 2. There was a significant positive correlation with log dose over this range.

Oral doses higher than 3·0 mg/kg did not produce a further increase in the output of respiratory tract fluid in the rabbit but rather produced a plateau effect. This extended to and included a dose of 9·0 mg/kg of cephaeline and 2-dehydroemetine and of 3·0 mg/kg of emetine. Higher

doses were within the lethal range and depressed the output of respiratory tract fluid. The regression of effect over the complete range of doses, therefore, was quadratic and represented by the equation

$$Y = a + bX + cX^2,$$

where b is plus and c is minus.

Oral administration of 2-dehydroemetine to cats produced an increase in the output of respiratory tract fluid which was statistically significant at a dose of 1·0 mg/kg (Fig. 3), but not at higher doses. Doses in the lethal range depressed the volume output. Emetine significantly ($P = 0{\cdot}02$) augmented the output of respiratory tract fluid in cats at a dose of 0·3 mg/kg by mouth.

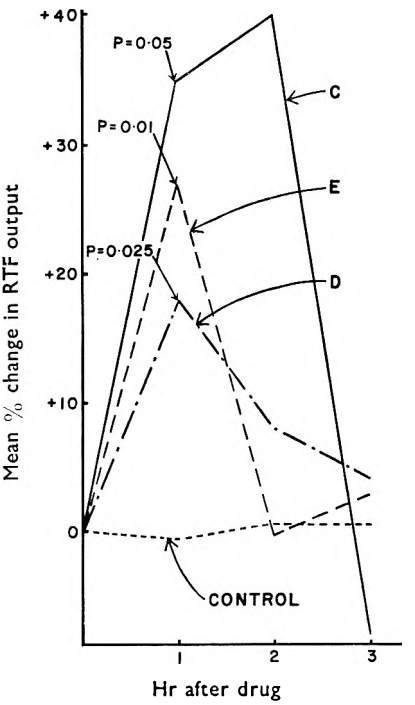

FIG. 1. The effect of representative low oral doses of ipecacuanha alkaloids on the output of respiratory tract fluid in rabbits. C, cephaeline, 1 mg/kg. D, dehydroemetine, 3 mg/kg. E, emetine, 1 mg/kg.

Subcutaneous injection of 2-dehydroemetine augmented the output of respiratory tract fluid at doses of 1·0 and 3·0 mg/kg as shown in Fig. 3. Larger doses had either no significant effect or produced a decrease.

The effect of lethal doses upon the output of respiratory tract fluid was similar for all three alkaloids given orally or subcutaneously to rabbits or cats. The results were averaged and are shown in Fig. 4. The mortality rate from a dose of 9·0 mg/kg was 4%. This figure was due to the death of 1 rabbit at 4 hr after receiving emetine at a dose of 9·0 mg/kg which significantly depressed the volume output of respiratory tract fluid

in the remaining rabbits. A dose of 27 mg/kg killed 42% of animals at 3 to 4 hr and death was preceded by a significant fall in the output of respiratory tract fluid. A similar result was obtained from a dose of 81 mg/kg which killed 75% of animals at 2 to 4 hr after administration.

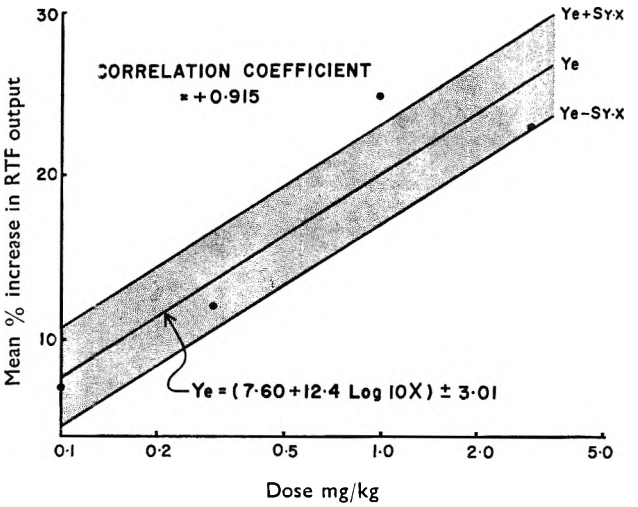

FIG. 2. The regression on dose of mean percentage increases in the output of respiratory tract fluid at 1 hr after oral administration of ipecacuanha alkaloids to rabbits.

The immediate cause of death in animals so observed was cardiac arrest, preceded by tachypnoea, tremors and struggling. Death rates have been collected in Table 1. The incidence of deaths from 2-dehydroemetine given orally to cats and rabbits was essentially similar to that given subcutaneously, the median lethal dose lying between 27 and 81 mg/kg.

TABLE 1. DEATH RATES (% MORTALITY) FOLLOWING ADMINISTRATION OF LETHAL DOSES OF IPECACUANHA ALKALOIDS

Alkaloid	Dose (mg/kg)	Species	Route	Mortailty %
Cephaeline	27	Rabbit	oral	33
Emetine	9	Rabbit	oral	25
Emetine	27	Rabbit	oral	50
Emetine	81	Rabbit	oral	100
2-Dehydroemetine	27	Rabbit	oral	75
2-Dehydroemetine	81	Rabbit	oral	100
2-Dehydroemetine	81	Cat	oral	50
2-Dehydroemetine	27	Rabbit	s.c.	50
2-Dehydroemetine	81	Rabbit	s.c.	75
2-Dehydroemetine	27	Cat	s.c.	25
2-Dehydroemetine	81	Cat	s.c.	50

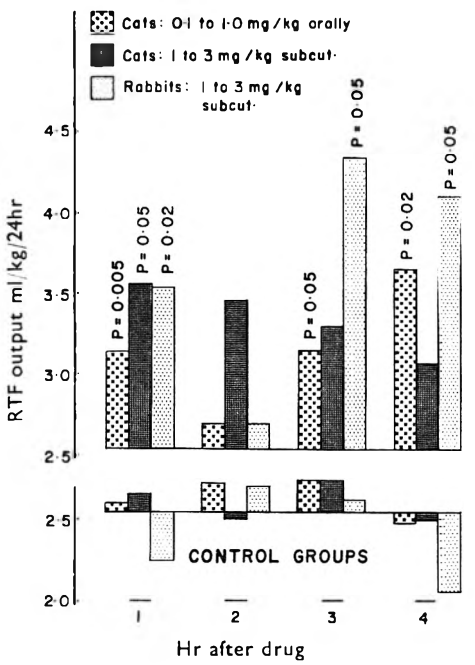

FIG. 3. The mean volume output of respiratory tract fluid after administration of 2-dehydroemetine dihydrochloride to cats and rabbits. The results are shown as changes from a common weighted mean output before drug administration. Mean changes significant at $P = 0{\cdot}05$ or less are so indicated. Controls received distilled water only.

Discussion

Perry & Boyd (1941) reported that powdered ipecacuanha, given by stomach tube as a suspension, produced an increase in the output of respiratory tract fluid after administration in a dose of 1 g/kg to cats under urethane anaesthesia. This dose corresponded to 20 mg/kg of total alkaloids calculated as emetine. Doses up to 3·9 mg/kg of the three alkaloids of ipecacuanha herein studied were found to augment the output of respiratory tract fluid. The expectorant action of ipecacuanha, therefore, is due in whole or in part to its content of cephaeline and emetine.

The three alkaloids, after oral administration, had almost identical ability to augment the output of respiratory tract fluid. Over the dosage range 0·1 to 3·0 mg/kg, increase in the output of respiratory tract fluid was positively correlated with log dose. The smallest and largest doses producing an individually significant increase in rabbits were 1 and 9 mg/kg for cephaeline, 3 and 9 for 2-dehydroemetine, and 1 and 3 for emetine. Radomski & others (1952) reported that cephaeline was slightly more potent than emetine as an emetic in dogs, while Herrero & others (1960) found 2-dehydroemetine slightly less potent than emetine in similar

circumstances. The oral emetic dose of all three alkaloids in dogs is about 1 mg/kg, which is similar to the dose which augmented output of respiratory tract fluid in rabbits. Boyd, Daicar & Middleton (1956) have noted that vomiting in cats and dogs is not necessarily accompanied by an increase in the output of respiratory tract fluid. Schwartz & Rieder (1961) found that 2-dehydroemetine is eliminated from tissues of the rabbit more rapidly than emetine.

Doses of all three alkaloids within the lethal range decreased the output of respiratory tract fluid by decreasing the volume of the pulmonary circulation. Cephaeline in an oral dose of 27 mg/kg killed 33% of rabbits, emetine 50% and 2-dehydroemetine 75% with no statistically significant difference between the death rates. The oral MLD of emetine in rabbits has been reported to be between 15 and 20 mg/kg (Spector, 1956). The single intraperitoneal LD50 of cephaeline in the rat is similar to that of emetine (Radomski & others, 1952), while single oral, subcutaneous and intravenous median lethal doses of 2-dehydroemetine in mice are higher than those of emetine (Herrero & others, 1960).

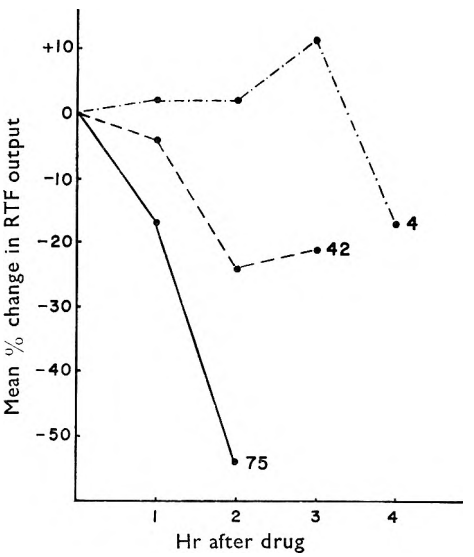

FIG. 4. The mean percentage change in the output of respiratory tract fluid in cats and rabbits after oral and subcutaneous administration of lethal doses of ipecacuanha alkaloids. Figures on the graph are % dead from 9 mg/kg (4%), 27 mg/kg (42%) and 81 mg/kg (75%).

Under the conditions of our experiments cats were more resistant than rabbits to the lethal effect of 2-dehydroemetine. For example, an oral dose of 27 mg/kg killed 75% of rabbits and no cats. The total mortality rate in 16 rabbits given doses of 27 and 81 mg/kg orally or subcutaneously was 75% and in 16 cats 31%, the difference being significant at $P = 0{\cdot}02$. Comparable data for emetine reviewed by Spector (1956) suggests that the rabbit and cat are about equally susceptible to its lethal action.

The death rate to 2-dehydroemetine given subcutaneously to rabbits and cats was insignificantly different from that when given orally. Spector (1956) indicated that the same relationship holds for emetine in frogs, guinea-pigs, rabbits, cats and dogs.

The cause of death from 2-dehydroemetine appeared to be similar to that from emetine, namely a cardiotoxic action. The cardiotoxic action of emetine has been related to an inhibition of oxidation of various substrates by heart homogenates (Marino & Magliulo, 1961). Heim, Froede & Erwin (1962), however, have provided the interesting information that emetine in lower doses, comparable to those which kill intact animals, actually stimulates the cardiac succinate oxidase system in a manner similar to that of thyroxine.

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A note on the electrically transmurally stimulated isolated trachea of the guinea-pig

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The isolated trachea of the guinea-pig was stimulated electrically transmurally to give a biphasic response. The initial contraction was abolished by atropine and was rapidly succeeded by relaxation which was blocked by low concentrations of dichloro-isoprenaline. The relaxation was also blocked by procaine, guanethidine, or by previous treatment of the animal with reserpine, but not with hexamethonium. It was concluded that the effects of transmural stimulation were mediated by post-ganglionic adrenergic nerves innervating beta receptors.

THE paired tracheal chain preparation from the guinea-pig (Foster, 1960) is useful in mode of action experiments with sympathomimetic drugs. Applied to the interaction between guanethidine or bretylium and catecholamines, it was seen that the potentiation slowly increased and reached a maximum only after about 3 hr. In an effort to see whether adrenergic neurone blockade by these drugs developed with the same time course in this tissue, the transmurally stimulated isolated tracheal preparation was developed. A description of this preparation was communicated to the joint meeting of the British and Scandinavian Pharmacological Societies, Copenhagen, 1960.

Methods

The apparatus in which the preparation was used is shown in Fig. 1.

The trachea was removed from a 600g guinea-pig which had been stunned and bled. A cannula was tied into each end of the trachea and a long platinum wire electrode was passed up through the lower cannula and tracheal lumen until its end lay in the upper cannula. The whole was fitted into a tissue bath containing Krebs solution at 38° bubbled with 95% oxygen and 5 % carbon dioxide. The lower cannula passed through a rubber sleeve, making a water-tight joint with the bottom of the bath, to a reservoir of Krebs solution. The upper cannula was connected to a graduated capillary tube through a three-way tap fitted with a short outlet tube. A second platinum electrode lay in the bath opposite the tracheal muscle.

The trachea was perfused with Krebs solution to wash out its lumen before being connected to the capillary tube of 1 mm internal diameter.

The fluid meniscus could be set at any starting position by opening the inlet from the reservoir and adjusting its height.

Any change in length of the tracheal muscle fibres produced a change in the volume of Krebs solution within the trachea and thus in the position of the meniscus in the graduated capillary tube.

Transmural stimulation was effected by a rectangular wave stimulator delivering pulses of 0·1 msec and 60 V for 30 sec; 3 pulses/sec was the

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frequency usually used. Stimuli were applied every 10 min and readings of the meniscus position were made at 2 min intervals or less. Three min after each stimulus the fluid in the bath was displaced by a larger volume of Krebs solution.

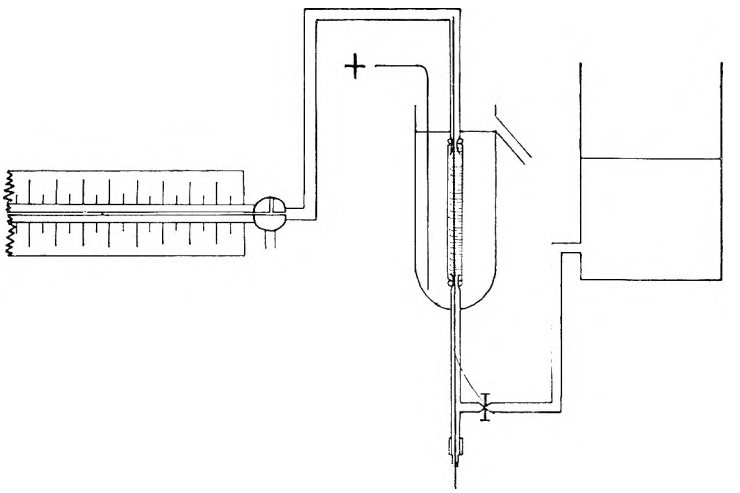

FIG. 1. The apparatus used to apply transmural stimulation to the tracheal muscle and record its effects. The apparatus for heating the tissue bath, displacing the Krebs solution in it, bubbling it with oxygen and carbon dioxide and applying stimulation across the electrodes is not shown.

The height of the reservoir, its inlet clip to the tissue bath and the three-way tap can be operated to perfuse the tracheal lumen or to set the starting position of the meniscus.

Results

Response of intact trachea to electrical transmural stimulation. Stimulation produced a biphasic response, initial contraction of the tracheal muscle being rapidly superceded by relaxation. Atropine, 0·4 μg/ml abolished the initial contraction and, since the relaxant response was the object of study, the alkaloid was included in all Krebs solution which came into contact with the trachea.

Analysis of mechanism of relaxation produced by electrical transmural stimulation. The relaxant response to transmural stimulation was not affected by hexamethonium, 10 μg/ml. It was blocked by procaine, 400 μg/ml, or by cocaine, 100 μg/ml, and was not seen using a trachea from a guinea-pig treated with reserpine, 5 mg/kg intraperitoneally, on each of the two preceding days. Concentrations of cocaine less than 25 μg/ml, increased the response. It was abolished by dichloroisoprenaline, 2 μg/ml, or by dichloronoradrenaline, 100 μg/ml.

Effect of frequency of stimulation on relaxant response. Frequency: response curves, over the range 1 to 12 pulses per sec, were linear or gently concave to the frequency axis.

The effect of bretylium on the relaxant response. The effect of bretylium is shown in Fig. 2. After the last two of a series of constant submaximal

responses, bretylium was kept in the bath continuously. Equilibrium was reached in about 45 min after the addition of bretylium when only 11% of the original response remained. Bretylium, 10 μg/ml produced a complete blockade in 20 min.

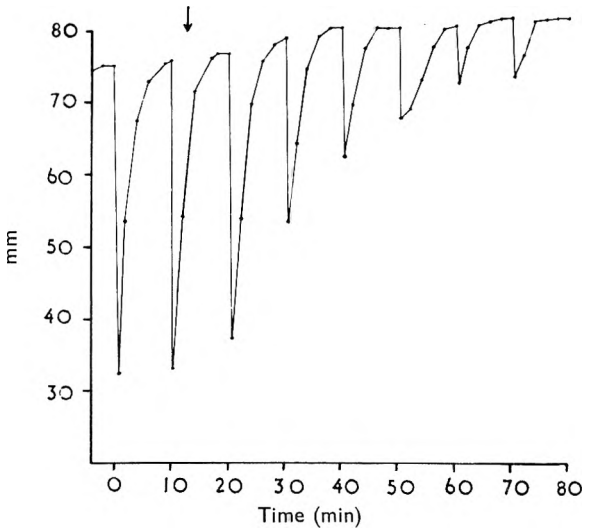

FIG. 2. The effect of bretylium, 2 μg/ml, kept in the bath after its addition at the arrow, on the response to transmural stimulation, applied at 10 min intervals, in the presence of atropine, 0·4 μg/ml. The scale position of the meniscus is plotted against time. Bretylium produces an increasing blockade of the relaxant response to stimulation.

The effect of guanethidine on the relaxant response and the interaction between cocaine and guanethidine. Fig. 3 shows the effects of concentration and time upon the blockade produced by guanethidine. The completeness of the blockade and also its rate of reaching equilibrium increased as the concentration of guanethidine was increased. Cocaine, 10 μg/ml in the presence of guanethidine 10 μg/ml altered the development of the blockade which was slower in onset but was no less complete than that achieved by guanethidine alone.

Discussion

The guinea-pig isolated trachea is a simple preparation which is rapidly set up and convenient to use.

Electrical transmural stimulation with the parameters used, in the presence of atropine produces relaxation. This effect is blocked by a low concentration of the β-blocking agent dichloroisoprenaline and by a far higher concentration of dichloronoradrenaline. The relaxation is therefore probably mediated mainly by β-adrenergic receptors. The blockade of the relaxant effect by local anaesthetic concentrations of cocaine and procaine suggests that the stimulation of nervous pathways is involved. This view

is confirmed by the blockade of the relaxant response by guanethidine, bretylium or by pretreatment of the guinea-pig with reserpine. The failure of hexamethonium to reduce the relaxant response suggests that the stimulation is effectively post ganglionic. The evidence strongly suggests that the effects of transmural stimulation were mediated by adrenergic nerves, apparently innervating β-adrenergic receptors.

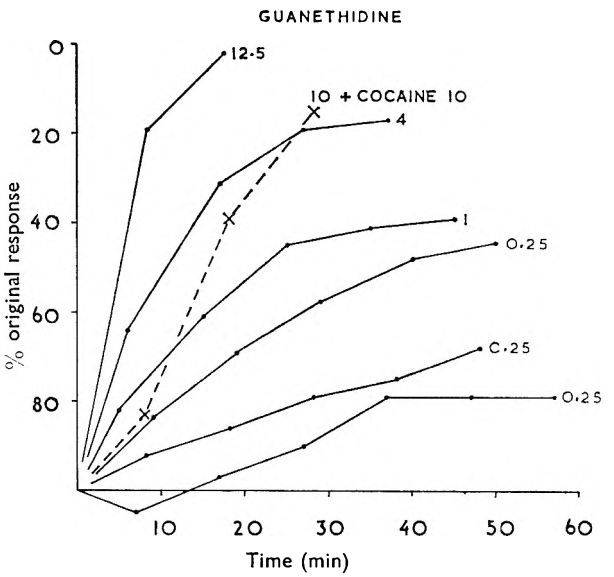

FIG. 3. The effect of guanethidine on the response to transmural stimulation in the presence of atropine, 0·4 μg/ml. The effect, as a percentage of original response to stimulation, is plotted against time of contact with guanethidine Each line represents the result of a separate experiment and the figure opposite the end of each line denotes the concentration of guanethidine used in μg/ml. The broken line is the result obtained using guanethidine and cocaine in equal concentrations 10 μg/ml.

This preparation has proved a simple and convenient one for use when an isolated, adrenergic nerve, effector cell preparation is required.

Carlyle (1964) has adapted it to a study of cholinergic neuro-effector junctions of the tracheal muscle.

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Letters to the Editor

The anaphylactoid reaction in rats

SIR,—The inflammatory anaphylactoid reaction produced in rats by the single intraperitoneal injection of dextran or egg-white is mediated chiefly through a release of 5-hydroxytryptamine and histamine (Parratt & West, 1957). Recently, Harris & West (1963) found that not all rats of the Wistar strain react to this injection although the concentrations of histamine and 5-hydroxytryptamine in the skin of rats not reacting are similar to those in the skin of those reacting. Non-reactivity has since been shown to be a genetically controlled character (Harris, Kalmus & West, 1963) and thus the problem is of wide application. We have re-examined the reactivity of Wistar rats to dextran and egg-white, and studied their responses to various dextrins as Veilleux (1963) has shown that some dextrins of relatively low molecular weight produce the anaphylactoid reaction in rats.

Wistar albino rats obtained from The Wellcome Laboratories, Beckenham and from the Agricultural Research Council's Field Station at Compton were used in all experiments. They were injected intraperitoneally with dextran (Intradex, Glaxo) according to the method of Harris & West (1963) and divided into two types: those which showed the anaphylactoid reaction (hereinafter called Reactors) and those which did not react (called Non-reactors). Both reactor and non-reactor rats were given various agents either intraperitoneally immediately after an intravenous dose of azovan blue dye (18 mg/kg), or intravenously together with the dye. The agents were dextran (Intradex, Glaxo) having a molecular weight of about 145,000, dextran (Rheomacrodex, Pharmacia) with a molecular weight of about 40,000, ovomucoid (L. Light & Co.), fresh hen's egg-white (50% v/v in normal saline), dextrin (Astra), and dextrin (Kerfoot). Fresh hen's egg-white previously boiled for 1 min was also used. The extent of the colloidal dye accumulation in the extremities (e.g. nose, ears, feet and tail) was estimated on a relative scale from 0 to +++. The results shown in Table 1 are the mean scores of the maximal responses from groups of 4 rats, except in the case of both egg-white preparations, where the highest intravenous dose was given to 16 non-reactor rats.

TABLE 1. RESPONSE OF THE AGENTS WHEN INJECTED INTRAPERITONEALLY AND INTRAVENOUSLY INTO REACTOR OR NON-REACTOR RATS, MEASURED ON A RELATIVE SCALE FROM 0 TO +++

Agent	Dose		Intraperitoneal route		Intravenous route	
	mg/kg	ml/kg	Reactors	Non-reactors	Reactors	Non-reactors
Fresh egg-white	—	3	++	0	++	0
	—	5	++	0	++	+
	—	12	+++	0	++	++
Boiled fresh egg-white	—	3	++	0	++	0
	—	6	++	0	++	0
	—	12	+++	0	++	+
Ovomucoid	200	—	+++	0	++	0
Dextran (Glaxo)	480	—	+++	0	+++	0
Dextran (Pharmacia)	480	—	+++	0	+++	0
Dextrin (Kerfoot)	1,250	—	0	0	0	0
	2,500	—	0	0	0	0
Dextrin (Astra)	1 250	—	+++	+++	++	+
	2 500	—	+++	+++	++	+++

Four interesting points may be deduced from the Table.

(1) Whereas with both samples of dextran given intraperitoneally or intravenously non-reactor rats failed to produce the anaphylactoid reaction, large *intravenous* doses of egg-white were effective.

(2) The component in egg-white responsible for the reaction shown by reactor rats was not destroyed by heat. In contrast, boiled egg-white in non-reactors gave a less intense reaction than did fresh egg-white.

(3) As egg-white was ineffective in non-reactor rats when given intraperitoneally but effective by the intravenous route, the active component when egg-white is given intraperitoneally may not reach the blood stream in concentrations sufficient to produce a response or it may be modified before absorption from the abdominal cavity.

(4) Whereas dextrin (Kerfoot) was ineffective by both routes in reactor and non-reactor rats, dextrin (Astra) when given intraperitoneally was equally effective in the two kinds of rat. However, the larger intravenous dose of dextrin (Astra) was less active in reactor rats than in non-reactors.

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Dexamphetamine and lipid mobilization in obesity

SIR,—We have studied the action of dexamphetamine on plasmatic free fatty acids (FFA) in rats. Albino Sprague-Dawley male rats were given amphetamine subcutaneously, 135 min before killing by decapitation. The drug induced a marked rise in levels of such a blood component (Table 1). The rise reached a maximum within 2–3 hr and lasted, at the highest dose levels, for more than 7 hr.

TABLE 1. CHANGES IN PLASMA FREE FATTY ACIDS (FFA) AFTER DEXAMPHETAMINE TREATMENT IN RATS

Dexamphetamine sulphate mg/kg	FFA % rise	P†
0·5	20 ± 9·9*	< 0·05
1·0	48 ± 2·3	< 0·01
2·0	101 ± 8·9	< 0·01
5·0	127 ± 4·7	< 0·01
10·0	69 ± 3·9	< 0·01

* Mean (6 animals) ± s.e.
† Statistical significance of difference from controls.
FFA were determined by the Dole (1956) method.

In our opinion, this increased mobilisation of lipids from adipose tissue may be important for the therapeutic use of amphetamine in obesity.

The value of sympathomimetic drugs such as amphetamine in the treatment of obesity in man, is generally ascribed to their anorexigenic effect, rather than to a peripheral increase of metabolic rate. The same effect of amphetamine in reducing bodyweight and desire for food, was demonstrated also in animals (Holm, Huus, Kopf, Möller Nielsen & Petersen, 1960).

The current view as to the mode of action of sympathomimetic drugs in producing anorexia, leans toward a central action. This central action would be not only a psychic or a generally exciting one, but a specific, direct influence on the mechanisms regulating food intake (Andersson & Larsson, 1961). In fact, a number of ring substituted α-methylamphetamines reduced food consumption in rats and lacked, at the same time, the motility-increasing effect of amphetamine. These results show that anorexigenic action is not necessarily linked to the same degree with stimulation of other central functions.

The weight loss of animals treated with such amphetamine-like compounds, was mainly due to reduction in body fat content. When instead, food intake was decreased by restriction, the weight loss was due to reduction in non-fatty dry matter. This suggested a mobilising effect of amphetamine-like compounds on depot fat (Holm, 1960).

Our results, showing that dexamphetamine has a striking specific action on fat metabolism, provide direct evidence for this hypothesis. The usefulness of amphetamine in the treatment of obesity appears then to be due not only to a central anorexigenic effect, but also to a metabolic action in peripheral tissues.

The question now arises whether the anorexigenic and the metabolic actions may somehow be connected.

Food intake is a centrally regulated mechanism. The existence of one or more "feeding centres" in the diencephalon, sensitive to a starvation state of the blood, was suggested as well. Thus far, the nature of the adequate feeding stimulus is not yet known. Heat production, dynamic action of food, glucostatic mechanisms, have been proposed as factors regulating food intake. It was also put forward that adjustment of feeding may be related to the amount of stored fat in the body: the central areas of the hypothalamus should thus be sensitive to the concentration of some metabolite in equilibrium with the stored fat (Andersson & Larsson, 1961).

According to the last hypothesis, the strong mobilising action of dexamphetamine on depot fat, or the consequent elevated concentration of plasmatic FFA, might possibly account for the anorexigenic effect of the drug.

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Vasopressor responses to kallidin, bradykinin and eledoisin in hypotensive rats

SIR,—There have been a few reports in the literature of pressor responses being obtained after injections of the normally vasodilator polypeptides bradykinin, kallidin and eledoisin. Thus, Croxatto & Belmar (1961) have shown that bradykinin is hypertensive in nephrectomised rats and in normal rats treated with the ganglion blocking drug pentolinium, whilst Erspamer & Glässer (1963) have reported hypertensive responses to eledoisin in decapitated chickens, in pithed rats and in rats pretreated with hexamethonium. The latter authors have produced evidence that the hypertensive actions of eledoisin are mediated through the adrenal medulla. Move direct evidence of the ability of bradykinin and kallidin to release adrenaline from the adrenal medula has also been provided recently by Feldberg & Lewis (1963). These authors injected the two plasma kinins into the coeliac artery of eviscerated cats and detected adrenaline in the venous affluent.

Bradykinin has recently been shown to be pressor in the cat during pregnancy (Parratt, 1964) and this observation has led to an examination of the vascular effects of the polypeptides bradykinin, kallidin and eledoisin in pregnant rats. The following is a summary of these observations.

Bradykinin and kallidin are initially purely hypotensive both in pregnant and in non-pregnant rats when injected intravenously in doses of 0·5–2·0 μg/kg. After repeated injections of these plasma kinins at intervals of 5 min however, this hypotensive response changes to a diphasic one, or is even purely hypertensive, provided the injections are continued for a period of from 1–3 hr. This evidence does not suggest any major difference in the vascular response of the rats to these polypeptides during pregnancy.

In both normal and pregnant rats with a blood pressure of 30–50 mm Hg, all three polypeptides raise the blood pressure when injected intravenously in doses of 0·5–5 μg/kg (for bradykinin and kallidin) or 0·25–1 μg/kg (for eledoisin). This is so whether the blood pressure has been lowered by ganglion blockade (pentolinium tartrate, 1 mg/kg intravenously), by acute haemorrhage, by overdose of the anaesthetic used (pentobarbitone sodium) or by injecting large doses of either polypeptide. These pressor responses are abolished by acute bilateral adrenalectomy, and are abolished or markedly reduced by pretreatment with reserpine (5 or 10 mg/kg, intraperitoneally 24 hr. previously) or by acute pretreatment with the α-adrenergic blocking drug rogitine in doses (1–2 mg/kg) which reverse the normal pressor response of the rat to adrenaline. On some occasions the pressor responses produced by kallidin and bradykinin were reversed by rogitine in a similar way to those of adrenaline. Pressor responses to all three polypeptides, and to adrenaline could be re-obtained after waiting for the effects of α-blockade to wear off.

These experiments suggest that kallidin, bradykinin and eledoisin are able, in hypotensive rats, to release catecholamines from the adrenal medulla. This may also contribute to the vascular effects of these polypeptides when they are injected into normotensive animals.

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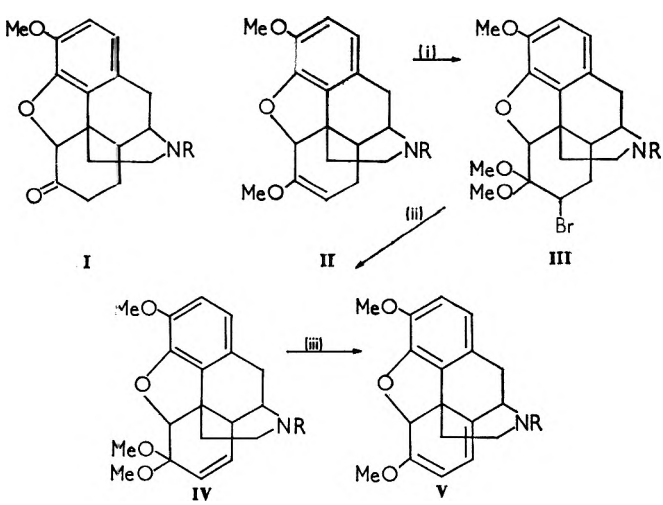
The synthesis of *N*-allylnorthebaine

SIR,—*N*-Allylnorthebaine (V; R = $CH_2:CH\cdot CH_2$) is of major importance in the study of the structure-activity relationships of the morphine alkaloids and their derivatives. We now report the synthesis of *N*-allylnorthebaine, *N*-cyclopropylmethylnorthebaine, *N*-(t-butoxycarbonyl)northebaine, and of northebaine (V; R = H) itself.

O-Methylation of *N*-benzyloxycarbonyldihydronorcodeinone (I; R = $PhCH_2O\cdot CO$) with methyl sulphate and sodium t-butoxide gave the enol ether (II; R = $PhCH_2O\cdot CO$), which on treatment with triethylsilane in the presence of palladium chloride and triethylamine, followed by addition of methanol (Birkofer, Bierwith & Ritter, 1961), gave Δ^6-dihydronorthebaine (II; R = H), m.p. 153–154°. This product was converted into northebaine (V; R = H) [salicylate, m.p. 192·5 – 193·5° (decomp.)] by the route illustrated, with is analogous to that employed by Rapoport and his co-workers in the synthesis (Rapoport, Reist & Lovell, 1956) of thebaine itself from Δ^6-dihydrothebaine (II; R = Me) (Homeyer, 1956). Alkylation of northebaine with allyl bromide, or, better, alkylation of the ketal (IV; R = H) followed by treatment with anhydrous toluene-*p*-sulphonic acid in chloroform, gave *N*-allylnorthebaine (V; R = $CH_2:CH\cdot CH_2$)(salicylate, m.p. 185–187°). An alternative synthesis started from the ketone (I; R = $Bu^tO.CO$), itself obtained on treatment of dihydronorcodeinone (I; R = H) with t-butyl azidoformate (Schwyzer, Sieber & Kappeler, 1959; the reagent was prepared according to Carpino, Giza & Carpino, 1959). Further stages, analogous to those already described, led to *N*-(t-butoxycarbonyl)northebaine (V; R = $Bu^tO\cdot CO$), which on further treatment with toluene-*p*-sulphonic acid gave northebaine.



(i) MeOBr (ii) $EtMe_2COK$ (iii) $p\text{-}Me\cdot C_6H_4\cdot SO_3H$

O-Methylation of *N*-cyclopropanecarbonyldihydronorcodeinone gave the enol ether (II; R = cyclopropanecarbonyl), which was reduced with lithium aluminium hydride to *N*-cyclopropylmethyl-Δ^6-dihydronorthebaine (II; R = cyclopropylmethyl). This compound was converted into *N*-cyclopropylmethylnorthebaine (V; R = cyclopropylmethyl) [salicylate, m.p. 197–198·5° (decomp.)] by methods already outlined.

The ketals (IV; R = H and R = $Bu^tO{\cdot}CO$) were surprisingly unstable, partial conversion to the corresponding thebaine analogues (V; R = H and R = $Bu^tO{\cdot}CO$) occurring during isolation. In contrast, the ketal (IV; R = cyclopropylmethyl) is stable in the absence of acid.

Satisfactory analytical and infra-red spectral data were obtained for all new compounds except the unstable ketal (IV; R = $Bu^tO{\cdot}CO$), which was characterised by the infra-red absorption of freshly-prepared material.

Additional spectral confirmation of structures was obtained for the new thebaine analogues (V) (ultra-violet absorption at 285 mμ in EtOH) and for Δ^6-dihydronorthebaine (II; R = H) and norcodeinone dimethyl ketal (IV; R = H) [nuclear magnetic resonance: $\tau(CDCl_3)$ 5·19 (5 — H), 5·31 (7 — H), 6·18 (3 — OMe) and 6·54 (6 — OMe), and 4·43 (7 — H + 8 — H), 5·28 (5 — H), 6·16 (3 — OMe), 6·55 (6 — OMe) and 6·89 (6 — OMe), respectively].

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Effects of smooth muscle stimulants and their antagonists upon potassium ion uptake and release in strips of guinea-pig ileum

SIR,—Born & Bülbring (1956), using the guinea-pig taenia coli, noted that spontaneous rhythmic activity was associated with increased outward flux of $^{42}K^+$. Stretching, histamine and acetylcholine had the same effect. Adrenaline, however, increased only the inward rate of movement of $^{42}K^+$ in 13 out of 24 preparations. Using a potassium-rich bath solution, Durbin & Jenkinson (1959, 1961) found that in the guinea-pig taenia coli, carbachol increased both inward and outward fluxes of $^{42}K^+$; both effects were abolished by atropine. Hurwitz (1960), using pilocarpine, found that $^{42}K^+$ release was increased and its uptake reduced. The effect on efflux was blocked by cocaine. Using the longitudinal smooth muscle layer of the guinea-pig ileum, Weiss, Coalson & Hurwitz (1961) showed that pilocarpine, acetylcholine and potassium-rich Tyrode's solution increased $^{42}K^+$ release. The two former also decreased $^{42}K^+$ influx. Calcium-free Tyrode's solution eliminated the contractile response to

pilocarpine, but not the increased $^{42}K^+$ release, while cocaine reduced both contractile response and $^{42}K^+$ efflux, due to potassium-rich bath fluid. Chujyo & Holland (1962) have shown that pilocarpine decreased $^{42}K^+$ uptake and increased its release in tubular segments of guinea-pig ileum. In 1956, Lembeck & Strobach showed that in the cat small intestine acetylcholine increased potassium (K^+) release: an effect counteracted by atropine and papaverine, but not by cocaine. Histamine and stretching had no effect but neostigmine and

TABLE 1. DRUG EFFECTS UPON $^{42}K^+$ UPTAKE AND RELEASE IN STRIPS OF GUINEA-PIG ILEUM

Drug	Dose (per ml)	Effect on $^{42}K^+$ release*	Effect on $^{42}K^+$ uptake*	Change in residual content of potassium*
Acetylcholine chloride	0·1 mg	Increased (8) $P < 0.001$	Decreased (16) $P < 0.001$	Decreased (8) $0.001 < P 0.01$
Carbachol	3 µg	Increased (8) $P < 0.001$	Decreased (16) $P < 0.001$	Decreased (8) $0.02 < P < 0.05$
Histamine acid phosphate	10 µg	Increased (8) $P < 0.001$	Decreased (16) $P < 0.001$	Decreased (8) $0.02 < P < 0.05$
5-Hydroxytryptamine creatinine sulphate	10 µg	Increased (8) $P < 0.001$	Decreased (16) $P < 0.001$	No change (8) $0.60 < P < 0.70$
Barium chloride	1 mg	Increased (8) $P < 0.001$	Decreased (16) $P < 0.001$	No change (8) $0.05 < P < 0.10$
Papaverine sulphate	0·2 mg	Decreased (8) $P < 0.001$	Decreased (16) $P < 0.001$	No change (8) $0.90 < P$
Lysergic acid diethylamide	10 µg	No change (8) $0.60 < P < 0.70$	No change (16) $0.80 < P < 0.90$	No change (8) $0.70 < P < 0.80$
Atropine sulphate	10 µg	No change (8) $0.40 < P < 0.50$	No change (16) $0.30 < P < 0.40$	No change (8) $0.80 < P < 0.90$
Mepyramine maleate	10 µg	No change (8) $0.50 < P < 0.60$	No change (16) $0.60 < P < 0.70$	No change (8) $0.80 < P < 0.90$
Adrenaline hydrogentartrate	0·5 mg	No change (8) $0.80 < P < 0.90$	No change (16) $0.05 < P < 0.10$	No change (8) $P = 0.80$
Acetylcholine chloride	10 µg	Depression of Acetylcholine increased release (9)	—	No change (9)
Atropine sulphate + Acetylcholine chloride	2 µg + 10 µg	$P < 0.001$		$0.40 < P < 0.50$
Histamine acid phosphate	1 µg	Depression of Histamine-increased release (9)	—	No change (9)
Mepyramine maleate + Histamine acid phosphate	0·2 µg + 1 µg	$0.001 < P < 0.01$		$0.50 < P < 0.60$
5-Hydroxytryptamine creatinine sulphate	10 µg	Depression of 5-Hydroxytryptamine-increased release (8)	—	No change (8)
Lysergic acid diethylamide + 5-Hydroxytryptamine creatinine sulphate	2 µg + 10 µg	$0.01 < P < 0.02$		$0.70 < P < 0.80$
Barium chloride	1 mg	Depresssion of Barium Chloride-increased release (8)	—	No change (8)
Papaverine sulphate + barium chloride	0·2 mg + 1 mg	$P < 0.001$		$0.10 < P < 0.20$

* Figures in parentheses indicate the number of paired strips of guinea-pig ileum used.

tetramethylammonium also increased K^+ release. Pilocarpine, nicotine, suxamethonium and decamethonium also did so, but irregularly. The studies of Tobain & Fox (1956) and Headings & Rondell (1962) suggest that, in dog arteries noradrenaline causes both increased uptake and release of K^+.

Using strips of isolated guinea-pig ileum and Krebs' solution (Banerjee & Lewis, 1963), we have also noted that acetylcholine increases release and depresses uptake of $^{42}K^+$; carbachol, histamine, 5-hydroxytryptamine and barium chloride have similar effects. Adrenaline had no statistically significant effect on $^{42}K^+$ uptake or release, but out of 16 uptake experiments the test-preparation showed a higher count than the control in 13 cases. Of the drug antagonists only papaverine sulphate, which significantly decreased both $^{42}K^+$ uptake and release (Banerjee & Lewis, 1963), had an effect. Atropine depressed the increased release of $^{42}K^+$ due to acetylcholine. Mepyramine, papaverine and lysergic acid diethylamide respectively antagonised the increased $^{42}K^+$ release due to histamine, barium chloride and 5-hydroxytryptamine (Table 1). These results support the findings of the workers cited above on potassium release, differing from Durbin & Jenkinson (1959, 1961) who used depolarized muscle on the effects of carbachol on $^{42}K^+$ uptake.

The results indicate that, at the doses used, drug antagonists have no apparent effects upon release or uptake of $^{42}K^+$, but are able to prevent the characteristic effects of the agonist. When the potassium remaining in the tissue following the release experiments was estimated flame photometrically, it was found that acetylcholine-treated muscles showed a highly significant fall. Significant falls were also shown by tissues which had been treated with carbachol and histamine (Table 1).

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