CONTRIBUTIONS TO THE STUDY OF HISTAMINE ANTAGONISTS IN MAN

(with additional papers)

Thesis submitted for the degree
of Doctor of Science in the University of Edinburgh
by

WILLIAM ALEXANDER BAIN
B.Sc.,Ph.D.,F.R.S.E.

From the Department of Pharmacology
University of Leeds.

November 1952.

PREFACE

The main part of this thesis consists, firstly, of a series of papers illustrating the development of the writer's quantitative approach to the study of the action of histamine antagonists in man; and, secondly, of a note concerning trials of antihistaminics, followed by the description of a pilot trial of one of these drugs. Some repetition has been unavoidable in the published accounts.

The supporting part of the thesis consists of two papers, in order of their publication. The first describes some early work on the mode of action of autonomic nerves, and is followed by a short addendum. The second, and more important contribution, deals with the inactivation of adrenaline - thought, at that time, to be the transmitter of adrenergic nerve effects. It is followed by a short addendum extending some of the observations to various mammals, including man. The war interrupted this work, but it was resumed, and extended to include noradrenaline, when that substance became available. A second addendum summarises some of the more recent observations.

This work, on histamine antagonists and on adrenaline and noradrenaline, is being continued. Some of the methods developed in it are being successfully applied to related fields, both by the writer and by some of his pupils.

PART I

CONTRIBUTIONS TO THE STUDY OF HISTAMINE ANTAGONISTS IN MAN

I.

SOME ASPECTS OF THE ACTION OF HISTAMINE ANTAGONISTS

Reprinted from THE LANCET, *Dec.* 18, 1948, *p.* 964

Reprinted from THE LANCET, *Dec.* 18, 1948, *p.* 964

SOME ASPECTS OF THE ACTION OF HISTAMINE ANTAGONISTS

W. A. BAIN
Ph.D. Edin., F.R.S.E.
PROFESSOR OF PHARMACOLOGY, UNIVERSITY OF LEEDS

F. F. HELLIER
O.B.E., M.A. Camb., M.D. Lond., F.R.C.P.
PHYSICIAN

ROBERT P. WARIN
M.D. Leeds, M.R.C.P.
REGISTRAR

DERMATOLOGICAL DEPARTMENT, GENERAL INFIRMARY AT LEEDS

THE reaction to an intradermal injection of histamine is modified if the injection is repeated at an interval after the oral administration of an adequate dose of a histamine antagonist. (Warembourg et al. 1944, Friedlaender and Feinberg 1946, Halpern et al. 1946a and b, Leavitt and Code 1947.) We present here the results of some preliminary observations in which the modification of the intradermal histamine reaction has been used to study the variation in effective dosage, duration of action, and other properties of various anti-histamine agents: 2-dimethylaminoethyl benzhydryl ether hydrochloride, introduced by Loew et al. (1945) and marketed as 'Benadryl' (Parke Davis); N(2-dimethylaminoethyl) N(*p*-methoxybenzyl) 2-aminopyridine, introduced by Bovet and Walthert (1944) as '2786 R.P.,' known in France and the U.S.A. as 'Neoantergan,' and now obtainable in this country under the name of 'Anthisan' (May & Baker); and N(dimethylamino-2-propyl) phenothiazine, first described by Halpern et al. (1946a and b) and known as '3277 R.P.' Administration of equal quantities of the different drugs was precluded by the wide differences in the ratio between the therapeutic and the toxic doses; ordinary clinical doses were therefore used.

The histamine reactions were produced on the volar surface of the forearm by the intradermal injection of 0·05 ml. of a solution of histamine acid phosphate 0·01% *w/v* in saline solution. Each injection thus contained 5·0 μg. of the acid phosphate, equivalent to 1·81 μg. of histamine base.

Whereas the flare component of the histamine response is clearly an area, the weal is three-dimensional and would be properly measured as a volume; but it was not found feasible to do this, and so both flares and weals were treated as areas. These can be determined by inking the outlines of the reactions on the skin, transcribing them to thin tracing-paper, and computing directly. With the dose of histamine used, however, the reactions were such that the areas as directly determined were virtually identical with those calculated from the mean radius of each reaction, as obtained from the average of two diameters, measured at right angles, in each instance. The labour involved in measuring the diameters was much less than in computing areas directly; so all the measurements on patients were made in this way, the average diameters of the weal and of the flare being determined 5–10 min. after the injection of each dose of histamine. It is these measurements which are given in tables I–IV and figs. 4–9, but in figs. 1–3 the areas calculated from these diameters are used. All the injections and measurements on patients were carried out by the same observer (R. P. W.).

In eleven men intradermal histamine reactions were measured before, and at intervals after, a single dose of benadryl 100 mg. by mouth. After a week's rest the observations were repeated with anthisan 200 mg. and, after a further week, with 3277 R.P. 50 mg. Other observations were made on twenty patients with chronic urticaria in whom the effect of anthisan on the modification of the intradermal histamine reaction and of the urticaria was compared.

RESULTS

Modification of Normal Histamine Reaction by Single Doses of Histamine Antagonists

Tables I–III give the results of these observations, together with their means, the differences in the means ($m_1 - m_2$), and the standard errors of the means (Em) and of their differences (Ed) calculated in the ordinary way. The paired t-test (Fisher 1944) has been applied to the estimate of t given in the lowest row of the tables. A diagrammatic representation of the mean results is given in fig. 1.

Judged from the mean maximal reduction of both weals and flares, the effects of anthisan 200 mg. and of 3277 R.P. 50 mg. were considerable and did not differ significantly, but the effects of benadryl 100 mg. were barely significant, and in the weal response alone, at three hours.

The differences in duration of action of each of the three drugs are also shown. Thus, though there was only a questionably significant reduction of the weal response 3 hours after administration of benadryl 100 mg., the effect of anthisan 200 mg. was still significant, both in weal and flare responses, at 27 hours, and of 3277 R.P. 50 mg. at 32 hours.

The frequency of our observations was not sufficient to enable us to get accurate information about any differences in the rate of establishment of the effect with each of the drugs, but it seems that anthisan and 3277 R.P. have some effect within an hour, and that the maximal effect is likely to be reached in 3–4 hours. The maximal effect persists longest with 3277 R.P.

TABLE I—EFFECTS OF SINGLE DOSES OF BENADRYL 100 MG. BY MOUTH ON INTRADERMAL HISTAMINE REACTION

Subject	Average diameters before benadryl (cm.)		Average diameters after benadryl (cm.)					
			1 hour		3 hours		10 hours	
	Flares	Weals	Flares	Weals	Flares	Weals	Flares	Weals
A	3·1	1·25	2·1	1·1	2·4	1·05	2·7	1·15
B	2·6	1·15	2·4	1·2	2·1	1·1	2·4	1·15
C	3·1	1·3	2·0	1·2	2·1	1·1	2·4	1·15
D	3·7	1·3	3·9	1·25	4·3	1·3	4·6	1·3
E	3·6	1·2	3·6	1·25	3·1	1·2	3·6	1·2
F	3·6	1·4	3·6	1·25	4·1	1·25	4·6	1·25
G	4·1	1·3	3·1	1·1	3·6	1·2	4·6	1·25
H	3·6	1·2	3·8	1·25	4·0	1·25	4·7	1·25
I	4·3	1·25	4·6	1·3	4·1	1·2	3·9	1·3
J	4·0	1·2	4·1	1·1	3·8	1·1	4·1	1·1
K	3·6	1·2	3·8	1·15	3·4	1·2	3·8	1·25
Mean	3·57	1·25	3·36	1·19	3·36	1·18	3·76	1·21
$m_1 - m_2$	..	..	−0·21	−0·06	−0·21	−0·07	+0·19	−0·03
Em	0·147	0·024	0·259	0·027	0·250	0·024	0·268	0·024
Ed	..	..	0·298	0·036	0·290	0·034	0·305	0·034
t	..	..	1·18	1·82	1·24	2·04	0·97	1·53

TABLE II—EFFECTS OF SINGLE DOSES OF ANTHISAN 200 MG. BY MOUTH ON INTRADERMAL HISTAMINE REACTION

Subject	Average diameters before anthisan (cm.)		Average diameters after anthisan (cm.)							
			2 hours		4 hours		11 hours		27 hours	
	Flares	Weals	Flares	Weals	Flares	Weals	Flares	Weals	Flares	Weals
A	3·5	1·3	3·5	1·3	1·0	0·5	2·0	0·6	2·0	1·0
B	3·0	1·3	2·5	1·3	1·0	0·6	1·5	0·6	1·5	0·9
C	3·0	1·3	2·5	1·0	1·0	0·5	1·4	0·6	1·5	1·0
D	3·6	1·3	2·5	0·75	2·4	0·7	3·5	0·6	3·5	1·0
E	3·5	1·0	1·5	0·55	1·0	0·5	1·8	0·6	3·0	0·9
F	3·5	1·6	3·0	0·8	2·5	0·7	2·5	0·8	3·5	1·05
G	4·0	1·3	2·5	1·0	1·0	0·5	2·0	0·55	3·0	1·05
H	4·5	1·2	3·5	1·2	1·5	0·6	2·9	1·0	4·0	1·2
I	4·5	1·2	3·5	1·1	2·7	1·1	2·5	0·8	3·5	1·0
J	3·8	1·2	3·5	1·0	2·0	0·55	2·8	0·8	4·0	1·0
K	3·1	1·0	2·0	0·8	2·0	0·6	2·1	0·8	3·0	0·8
Mean	3·63	1·25	2·77	0·98	1·63	0·62	2·27	0·70	2·91	0·99
$m_1 - m_2$	..	..	−0·86	−0·27	−2·00	−0·63	−1·36	−0·55	−0·71	−0·26
Em	0·160	0·051	0·206	0·069	0·208	0·054	0·19	0·042	0·274	0·033
Ed	..	..	0·261	0·086	0·262	0·074	0·248	0·066	0·317	0·061
t	..	..	2·66	2·38	3·01	3·02	2·94	3·98	2·47	2·83

TABLE III—EFFECTS OF SINGLE DOSES OF 3277 R.P. 50 MG. BY MOUTH ON INTRADERMAL HISTAMINE REACTION

Subject	Average diameter before 3277 R.P. (cm.)		Average diameter after 3277 R.P. (cm.)									
			1 hour		3½ hours		7½ hours		24 hours		32 hours	
	Flares	Weals	Flares	Weals	Flares	Weals	Flares	Weals	Flares	Weals	Flares	Weals
A	3·0	1·3	1·8	0·95	1·5	0·8	1·2	0·75	1·2	0·65	1·4	0·9
B	3·4	1·3	1·4	1·0	1·6	0·7	1·0	0·75	1·0	0·85	2·2	0·95
C	2·6	1·35	1·6	0·95	1·1	0·7	1·2	0·7	1·4	0·7	1·2	0·9
D	3·4	1·15	2·1	0·85	2·4	0·75	1·8	0·85	3·6	0·95	3·2	1·1
E	3·9	1·3	1·65	0·95	0·15	0·7	1·1	0·65	1·4	0·7	1·4	0·8
F	3·6	1·2	3·2	0·95	2·4	0·9	2·2	0·7	2·6	0·9	2·9	0·9
G	4·2	1·4	2·9	0·85	0·9	0·75	1·7	0·7	1·6	0·8	1·9	0·95
H	4·5	1·4	3·7	1·0	2·1	0·9	2·2	0·9	3·2	1·1	3·9	0·95
I	3·2	1·1	3·4	0·9	1·9	0·8	2·2	0·65	2·9	0·9	2·9	0·8
J	4·0	1·0	2·2	0·8	1·5	0·6	1·7	0·6	3·2	0·9	3·0	0·8
K	4·2	1·2	1·6	0·75	1·1	0·55	1·4	0·85	1·7	1·2	2·4	0·8
Mean	3·64	1·25	2·32	0·90	1·63	0·74	1·61	0·74	2·16	0·88	2·4	0·89
$m_1 - m_2$	..	..	−1·32	−0·35	−2·01	−0·51	−2·03	−0·51	−1·48	−0·37	−1·24	−0·36
*E*m	0·175	0·042	0·247	0·024	0·154	0·036	0·138	0·136	0·283	0·048	0·259	0·030
*E*d	..	..	0·303	0·087	0·233	0·055	0·223	0·055	0·333	0·064	0·312	0·051
t	..	..	2·73	3·22	2·897	3·09	3·04	3·08	2·64	2·72	2·73	3·06

Variation in Response to Histamine Antagonists

The response of different people to the anti-histamine drugs varied in the degree of maximal reduction of the erythema and wealing. When the maximal response of a person was poor with one of the drugs it was usually poor with the others also. Of the eleven persons studied, the degree of response in five (A, B, C, E, and G) was good ; in three (D, F, and I) poor ; and in the remaining three (H, J, and K) intermediate. Examples of good and poor responses taken from tables I–III are shown in fig. 2.

This variation in response did not seem to depend on the extent of the initial reaction to intradermal histamine ; nor was there any obvious correlation between the degree of specific response to the drugs, the toxic side-effects, and the weight or build of the person.

It would be expected, on general pharmacological grounds, that people who responded poorly to an ordinary dose of an anti-histamine drug would respond well if the dose were raised sufficiently. This was found to be so, and is demonstrated in fig. 3, where the effect of raising the dose in subjects D, I, and J is shown.

Apart from the differences already noted respecting the mean duration of action of each of the different anti-histamine drugs there is considerable variation from one person to another in this respect. But, as with the maximal effects, a person who showed a long duration of action with one of the agents showed, in most instances, a long duration of action with the other two. This is illustrated in subjects A, B, C, E, and G, all of whom showed a good maximal response (tables I–III), and it suggests a close correlation between these two aspects of the action.

Side-effects of Histamine Antagonists

In comparing the clinical value of anti-histamine drugs an important consideration is the ratio between the therapeutically effective dose and that which gives rise to side-effects. The assessment of the incidence and importance of the various side-effects is, however, complicated. The commonest symptoms are drowsiness and lassitude, and the reaction to these depends considerably on the sensitivity and type of the patient. Again, toxic symptoms are more common in ambulant persons than in those resting in bed. Thus seven ambulant medical workers on a single dose of anthisan 200 mg. all experienced mild symptoms, whereas of eleven men resting in bed on the same dose only one complained of a little drowsiness. When large clinical doses of the drugs were given, nearly all patients complained of some unpleasant side-effects. Such effects have occurred

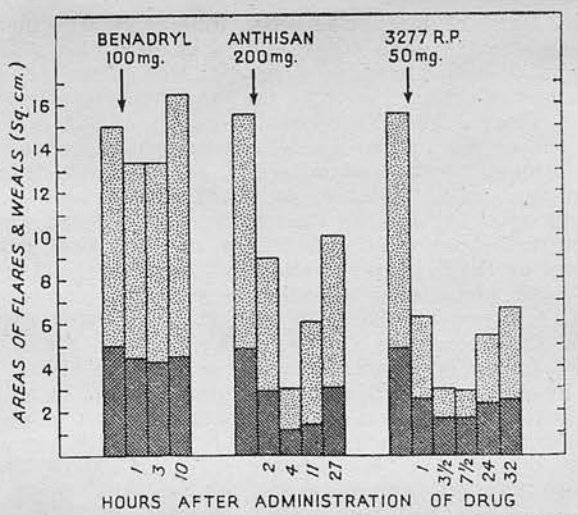

Fig. 1—Modification of intradermal histamine reaction by single doses of anti-histamine drugs (mean results from eleven people) : stippled area $1^1/_2$ times area of flares ; hatched areas 4 times area of weals.

with low doses in a few people, and in some people have been pronounced before an effective anti-histamine dosage could be reached.

Of our eleven subjects benadryl 100 mg. caused drowsiness and lassitude in four, anthisan 200 mg. in one, and 3277 R.P. 50 mg. in five. The results in this small series are in accord with observations on other people, though experience with 3277 R.P. has been limited. Anthisan certainly has a considerably higher therapeutic ratio than has benadryl, but we have insufficient information to reach any definite conclusion about 3277 R.P. in this respect. Vallery-Radot et al. (1947), however, agree that side-effects are commonly experienced with 3277 R.P., but state that, when the therapy is continued for a few days, they tend to pass off.

Dryness of the mouth has been noted with benadryl and 3277 R.P. but was commoner with anthisan, developing in about 20% of patients on an average dose. It was noted about 2 hours after ingestion of the drug and lasted $1^1/_2$–2 hours but did not cause serious discomfort or complaint. Average doses of anthisan in some patients caused mild colic, diarrhœa, nausea, and vomiting, which began about $1^1/_2$ hours after ingestion

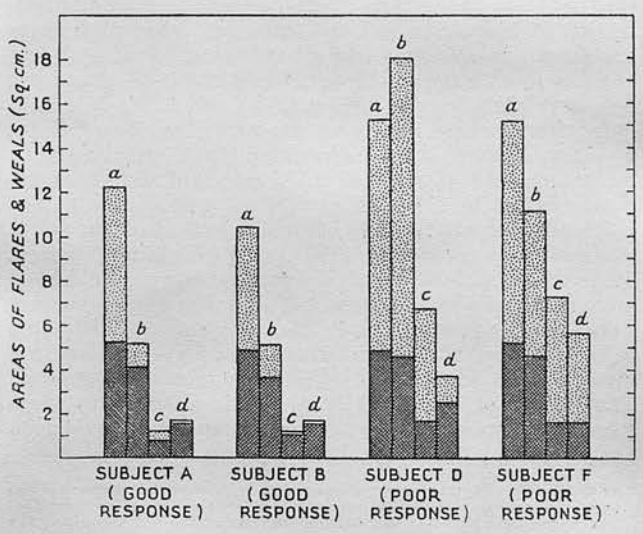

Fig. 2—Modification of intradermal histamine reaction by single doses of anti-histamine drugs in different people (A, B, D, and F) : *a*, before administration of anti-histamine drug ; *b*, after benadryl 100 mg. ; *c*, after anthisan 200 mg. ; *d*, after 3277 R.P. 50 mg. ; stippled and hatched areas as in fig. 1.

and cleared after 1–1½ hours. Since the precaution of administering the drug in the middle of meals was introduced we have had very little trouble from these effects.

Light-headedness, aching limbs, and ataxia have been experienced with 3277 R.P.

Observations on Patients with Chronic Urticaria

Similar observations to the foregoing have been carried out on twenty patients with chronic urticaria, the intradermal histamine reactions being noted at various times before, during, and after treatment with anthisan.

The weals were suppressed or considerably modified in all patients. Variation from one person to another in the response to the anti-histamine drug was shown as in the foregoing observations, and in three patients who required large doses of anthisan the side-effects outweighed the value of continuing the suppressive therapy.

In all cases the dosage necessary to reduce adequately, or to suppress, the urticaria was that necessary to modify appreciably the intradermal histamine reaction.

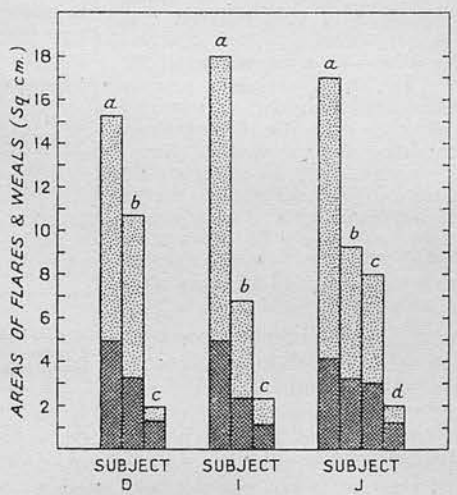

Fig. 3—Effect of increasing dose of anti-histamine drug in different people (D, I, and J) giving poor response to ordinary dose: *a*, before administration of anthisan ; *b*, after anthisan 300 mg. daily for three days ; *c*, after anthisan 600 mg. daily for three days ; *d*, after anthisan 900 mg. daily for three days ; stippled and hatched areas as in fig. 1.

Five of the patients had various degrees of factitious wealing. Measurements of the size of the weal and erythema provoked by different stimuli were made before, during, and after therapy. The stimuli used were weak and firm pressure with the stroke of a pin-point, and weak and firm pressure with the end of a cylindrical stick 2 mm. in diameter. The degree of reduction of weal and erythema by anthisan paralleled the reduction of the intradermal histamine reaction.

When the drug was stopped, urticaria recurred in most patients ; two complained that it was more severe than before treatment, but in these, as in the others, the intradermal histamine reaction was about the same as before therapy. In the five patients who showed factitious wealing the measurements of the weal and erythema, when the drug was stopped, were about the same as they were before therapy.

The action of anthisan in these patients followed in all respects the course to be expected from the results of the observations recorded above. Various points are illustrated by examples from the case-records. Figs. 4–8 illustrate variation of the effective dose of anthisan from one person to another. The following are examples of variation of duration of action of anthisan from one person to another.

Key to figs. 4-9 : (1) toxic symptoms : +, slight symptoms on questioning ; ++, complaint of mild symptoms ; +++, complaint of moderate symptoms ; ++++, severe incapacitating symptoms ; (2) urticaria : +, occasional weal ; ++, a few weals ; +++, moderate number of weals ; ++++, profuse weals.

Intradermal histamine reaction from 0·05 ml. of 0·01 % histamine acid phosphate. Mean diameters of flare (thin line) and weal (thick line) are recorded in cm.

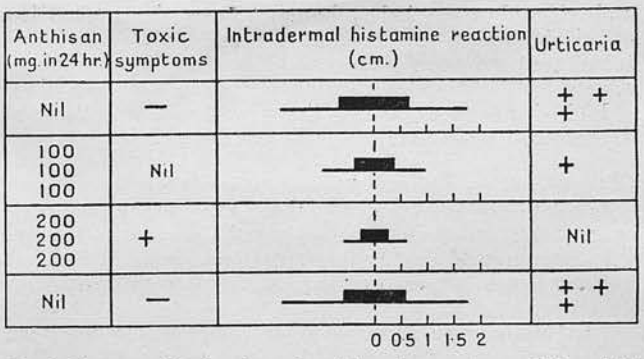

Fig. 4—Average effective dose of anthisan in a man, aged 69, weight 78 kg., with urticaria present for 3 months.

Short Duration.—In a woman, aged 38, weight 50·5 kg., with urticaria present for 6 months, the intradermal histamine reaction was modified by anthisan 200 mg. t.d.s. Coincidentally the urticaria was suppressed during the day but reappeared about 2 A.M. (the doses of anthisan were given at 7 A.M., 1 P.M., and 6 P.M.). Thus, in this patient an effective level of anthisan was maintained for only 8 hours after ingestion. A further dose at 11 P.M. relieved this early morning urticaria.

Long Duration.—In a woman, aged 23, weight 63·5 kg., with urticaria present for 4 months, who provided an example of a low effective dose (fig. 7), anthisan 100 mg. modified the intradermal histamine reaction, and, given as a single dose at 8 A.M., suppressed the urticaria for 24 hours. If anthisan was discontinued, urticaria reappeared only 36 hours after the last dose. The intradermal histamine reaction remained modified 24 hours after the dose.

An example of side-effects associated with a low effective dose is given in fig. 9. Drowsiness and lassitude were too severe for the patient to derive benefit from continued therapy. On the other hand, in a case cited as providing an example of a high effective dose of anthisan (fig. 5) toxic symptoms were not experienced until a dosage of 1200 mg. in 24 hours had been reached. Even then the symptoms were so mild that therapy was continued.

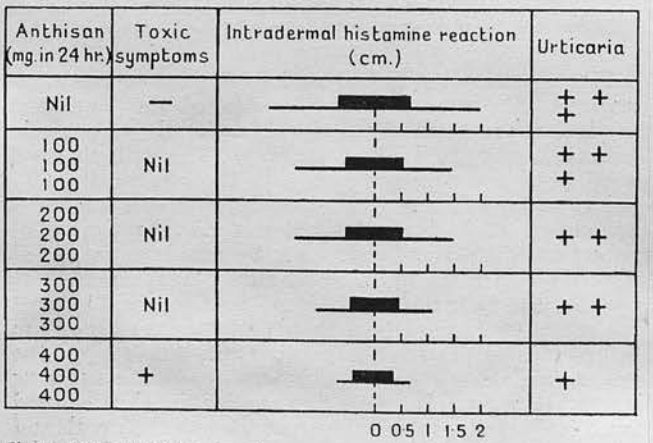

Fig. 5—High effective dose in a woman, aged 49, weight 55·5 kg., with urticaria present for $2^1/_2$ years.

Anthisan (mg. in 24 hr.)	Toxic symptoms	Intradermal histamine reaction (cm.)	Urticaria	Dermographism (cm.)
Nil	—		+ + +	
100 100 100	Nil		+ + +	
200 200 200	+ +		+	
300 300 300	+ + +		Nil	

0 0·5 1 1·5 2 — 0 0·5 1 1·5

Fig. 6—High effective dose in a man, aged 23, weight 65 kg., with urticaria present for 2 years associated with dermographism (mean of four measurements after use of different traumatising stimuli).

Anthisan (mg. in 24 hr.)	Toxic symptoms	Intradermal histamine reaction (cm.)	Urticaria
Nil	—		+ +
100 100 100	Nil		Nil
100	Nil		Nil

0 0·5 1 1·5 2

Fig. 7—Low effective dose in a woman, aged 23, weight 63·5 kg., with urticaria present for 4 months.

Anthisan (mg. in 24 hr.)	Toxic symptoms	Intradermal histamine reaction (cm.)	Urticaria	Dermographism (cm.)
Nil	—		+ + +	
100 100 100	Nil		Nil	

0 0·5 1 1·5 2 — 0 0·5 1 1·5

Fig. 8—Low effective dose in a woman, aged 20, weight 64 kg., with urticaria present for 2 years associated with dermographism.

Anthisan (mg. in 24 hr.)	Toxic symptoms	Intradermal histamine reaction (cm.)	Urticaria
Nil	—		+ +
100 100	+ + +		Nil

0 0·5 1 1·5 2

Fig. 9—Low effective dose associated with side-effects in a woman, aged 42, weight 58 kg., with urticaria present for 3 months.

DISCUSSION

The intradermal histamine response shows clearly that there are well-marked differences not only in potency but also in the mean duration of action of the different anti-histamine drugs. Benadryl is short-acting, anthisan and 3277 R.P. are long-acting, and the action of 3277 R.P. lasts longer than that of anthisan.

This factor of duration of action for a single dose merits more attention than it has received in the clinical assessment of anti-histamine drugs. Indeed, next to the therapeutic ratio it may be the most important feature in any new drug of this class.

The question immediately arises: what mechanisms determine duration of action in the different drugs? Are the anti-histamine drugs, for example, analogous to the long-acting and the short-acting barbiturates, where duration of action depends simply on the rate of break-down by the liver, or excretion by the kidneys, or both?

From preliminary experiments made by one of us (W. A. B.) in collaboration with P. B. Dews, it seems that the explanation is not so simple, and that one of the main factors may be variation in the degree and duration of fixation of the different drugs by the tissues on which they act. The subjects of these observations were medical students of either sex. In the first experiment it was intended to show the relative potencies of the three drugs by local administration to the skin. Each of thirty people had four intradermal injections, one of which contained 10 μg. of histamine base as control, and each of the others 10 μg. of histamine plus 100 μg. of the anti-histamine drug. The area of each weal was outlined in ink 5–10 min. after the injection, and the area determined from a tracing of the weal outline. Flares were disregarded because of the known local anæsthetic properties of the drugs. For convenience of presentation the results are expressed for each drug as the mean percentage reductions of the weal area below that of the control. The effects of simultaneous administration of histamine and the three anti-histamine drugs are shown in the first row of table IV. Benadryl and anthisan both gave significant reductions in the response, but, very surprisingly, 3277 R.P. did not.

TABLE IV—ACTIONS OF ANTI-HISTAMINE DRUGS ADMINISTERED LOCALLY TO SKIN

Time between giving anti-histamine drug and histamine (min.)	No. of people	Mean % reduction in area of weal response, ± standard error of mean		
		Benadryl	Anthisan	3277 R.P.
0	30	25·5 ± 5·7	32·6 ± 5·8	9·9 ± 7·3
10	10	24·3 ± 8·0	46·6 ± 6·3	46·6 ± 9·1
30	10	16·8 ± 8·4	33·1 ± 6·0	41·1 ± 5·6
100	10	7·7 ± 12·2	26·8 ± 7·9	43·3 ± 5·2

In view of these results we made further observations, in which areas of the skin were infiltrated with the anti-histamine drugs (0·2 ml. of 0·1% *w/v*) and the histamine was injected in different groups of people at different intervals thereafter. The control injection of histamine was made to an area of skin infiltrated with saline solution. The anti-histamine drugs in the concentration used caused a characteristic red reaction. The limits of the reaction were readily visible, and the subsequent histamine injection was given into this area. The results are given in the second and subsequent rows of table IV. The effect of benadryl was maximal at zero time (complete immediate fixation with maximal exclusion of histamine), began to pass off almost immediately, and was only questionably significant at 30 min. (light and short-lasting fixation). With anthisan the effect at zero time was not maximal (incomplete immediate fixation) but became so in a few minutes; there was still a significant effect at 100 min. (firm and lasting fixation). With 3277 R.P. there was no significant effect at zero time (slight immediate fixation), but the effect was maximal by 10–15 min. and was still at this level at 100 min. (firm and lasting fixation). In some subsequent observations on other subjects, but using a 1·5 μg. test dose of histamine, significant effects have persisted with anthisan to about 1000 min. and with 3277 R.P. to 3000 min. The standard errors of the observations in table IV give some idea of the variation from one person to another in both degree and duration of effect with the different anti-histamine drugs when administered locally to the skin.

It is thus evident that the differences in duration of action of the three anti-histamine drugs given by mouth have features analogous to those shown after their local application to tissues. It cannot therefore be supposed that the different durations of action observed clinically are due simply to differences in the rate of excretion or detoxication of the drugs, but are probably due, in part at least, to differences in the "firmness of fixation" of each drug by the tissues.

Besides these differences in the duration of action characteristic of each of the anti-histamine drugs, it was to be expected that there would be considerable variation from one person to another in the response to the drugs as indicated by the intradermal histamine reaction or by the modification of an urticaria. This is amply shown by our results, as is the further point that the response in any person is comparable for all three drugs, with the result that a person who is, for example, resistant to anthisan is also resistant to 3277 R.P.

It was to be expected, however, again on general grounds, that in people resistant to any anti-histamine drug (and in whom therefore the response to a given dose was less in degree and duration than the mean response), the degree and duration of the response could be made to approximate to the mean response for the particular drug by a suitable increase of the dose. This, in fact, is what happens. Thus, whereas the average daily dose of anthisan is 300 mg., people resistant to it

and those who show a small and short response to such a dose, may give an adequate response after 600, 900, or even 1200 mg. in 24 hours. On the other hand, though in our 20 patients treatment with anthisan suppressed or considerably modified the urticaria in all, in three of these who were resistant to the drug, and to whom therefore large doses had to be administered, therapy was discontinued because toxic symptoms outweighed the value of suppressing the urticaria. Therefore, though adequate anti-histamine effects can be obtained with the present anti-histamine drugs, in resistant people by a suitable increase of dose, the toxic effects which may derive from such a dosage are an important limiting factor in their usefulness.

In some people who are only moderately resistant to these anti-histamine drugs adequate clinical effects may be obtained with normal dosage during the day; but, the duration of effective action being shorter than normal, a subeffective level is reached between the last dose on one day and the first on the next. Three of our patients on anthisan t.d.s. had recurrences of the urticaria in the early hours of the morning, some 8–12 hours after the last dose. This was completely controlled by giving an extra dose, with a little food, last thing at night.

Nearly all the reports published on the clinical use of anti-histamine drugs in chronic urticaria have shown that some patients were not improved. This number has varied from one worker to another as follows:

Reference	*Drug*	*No. of cases treated*	*No. not improved*
Friedlaender and Feinberg (1946)	Benadryl	14	4
Friedlaender (1946)	Benadryl	11	2
Goldberg (1946)	Benadryl	15	3
Shaffer et al. (1945)	Benadryl	8	1
O'Leary and Farber (1947)	Benadryl	75	10
McGavack et al. (1947)	Benadryl	36	3
Curtis and Owens (1945)	Benadryl	17	4
Levin (1946)	Benadryl	9	3
Waldbott (1946)	Benadryl	20	4
Osborne et al. (1947)	Pyribenzamine	15	6
Arbesman et al. (1946)	Pyribenzamine	107	23
American Academy of Allergy (1946)	Pyribenzamine	97	21
Britton (1947)	Antistin	11	6
Hunter (1947)	Anthisan	8	—
Vallery-Radot et al. (1947)	3277 R.P.	47	1
Total		490	91

On the basis of our observations it is difficult to escape the conclusion that many of these failures were in people resistant to the drug, and that, if the dose had been suitably increased or a change made to a more powerful drug, the response in these people would have been satisfactory, as it was in our cases. In any event it is probable that, in previous work on anti-histamine drugs, even where attention was paid to differences in the potency and duration of action of the different drugs, there was little appreciation of the variation in response from one person to another to any one of them. It is clear, however, that this variation is important, and the methods described here are readily applicable to its assessment for clinical purposes.

SUMMARY

The quantitative modification of the intradermal histamine reaction by the oral administration of the anti-histamine drugs 'Benadryl,' 'Anthisan' ('Neoantergan'), and 3277 R.P. is described.

These drugs differ in their weight-for-weight effectiveness and in their duration of action, benadryl being classed as relatively weak and short-acting, and anthisan and 3277 R.P. as potent and long-acting.

There was considerable variation from one person to another in the degree and duration of the specific anti-histamine response to the drugs and in the incidence of side-effects. If the specific response to one drug was poor it was usually poor to the others also.

In people resistant to any of the anti-histamine drugs a "normal" response could be obtained by a suitable increase in the dose, the chief limiting factor being the incidence of unpleasant side-effects.

In 20 patients with chronic urticaria treated with anthisan, in all of whom the weals were suppressed or greatly modified, intradermal histamine reactions were recorded before and throughout treatment, and the modification of the reaction was found to parallel closely the response of the urticaria.

There was considerable variation from one person to another in the dose required to suppress the urticaria, and it is suggested that many of the reported failures in the treatment of urticaria with anti-histamine drugs were in patients resistant to the drugs, and that higher doses or more potent drugs might have produced satisfactory effects.

The results are discussed, with special reference to the importance of the differences in potency and duration of action of the different drugs and to the variation from one person to another in the response to any one. A possible factor in determining differences in duration of action is noted, and the significance of the variation in response from one person to another is emphasised.

Our thanks are due to Dr. J. T. Ingram for access to, and help with, the cases; Dr. B. N. Halpern for a supply of 3277 R.P.; Dr. W. R. Thrower for a supply of anthisan; and the Medical Research Council for an expenses grant to one of us (W. A. B.) to defray the cost of the work.

REFERENCES

American Academy of Allergy (1946) *J. Allergy*, **17**, 325.
Arbesman, C. E., Koepf, G. F., Lenzner, A. R. (1946) *Ibid*, p. 275.
Bovet, D., Walthert, F. (1944) *Ann. pharmaceut. franç.* suppl. no. 4.
Britton, C. J. C. (1947) *Lancet*, ii, 870.
Curtis, A. C., Owens, B. B. (1945) *Arch. Derm. Syph., Chicago*, **52**, 239.
Fisher, R. A. (1944) Statistical Methods for Research Workers. 9th ed., Edinburgh; p. 21.
Friedlaender, A. S. (1946) *Amer. J. med. Sci.* **212**, 185.
Friedlaender, S., Feinberg, S. M. (1946) *J. Allergy*, **17**, 129.
Goldberg, L. C. (1946) *J. invest. Derm.* **7**, 301.
Halpern, B. N., Hamburger, J., Debray, C. (1946a) *Bull. Soc. med. Hôp. Paris*, **62**, 607.
— Vallery-Radot, P., Mauric, G. (1946b) *C.R. Soc. Biol. Paris*, **140**, 480.
Hunter, R. B. (1947) *Lancet*, i, 672.
Leavitt, M. D. jun., Code, C. F. (1947) *J. Lab. clin. Med.* **32**, 334.
Levin, S. J. (1946) *J. Allergy*, **17**, 145.
Loew, E. R., Kaiser, M. E., Moore, V. (1945) *J. Pharmacol.* **83**, 120.
McGavack, T. H., Elias, H., Boyd, L. J. (1947) *Amer. J. med. Sci.* **213**, 418.
O'Leary, P. A., Farber, E. M. (1947) *J. Amer. med. Ass.* **134**, 1010.
Osborne, E. D., Jordon, J. W., Rausch, N. G. (1947) *Arch. Derm. Syph., Chicago*, **55**, 309.
Shaffer, L. W., Carrick, L., Zackheim, H. S. (1945) *Ibid*, **52**, 243.
Vallery-Radot, P., Hamburger, J., Halpern, B. (1947) *Pr. méd.* **55**, 661.
Waldbott, G. L. (1946) *J. Allergy*, **17**, 142.
Warembourg, H., Linquette, M., Machon (1944) *C.R. Soc. Biol. Paris*, **138**, 147.

II.

COMPARISON OF ANTHISAN (MEPYRAMINE MALEATE) AND PHENERGAN AS HISTAMINE ANTAGONISTS

Reprinted from THE LANCET, *July* 9, 1949, *p.* 47

Reprinted from THE LANCET, *July* 9, 1949, *p.* 47

COMPARISON OF ANTHISAN (MEPYRAMINE MALEATE) AND PHENERGAN AS HISTAMINE ANTAGONISTS

W. A. BAIN
Ph.D. Edin., F.R.S.E.
PROFESSOR OF PHARMACOLOGY IN THE UNIVERSITY OF LEEDS

J. L. BROADBENT
M.B. Leeds
DEMONSTRATOR IN PHARMACOLOGY IN THE UNIVERSITY

ROBERT P. WARIN *
M.D. Leeds, M.R.C.P.
REGISTRAR, DERMATOLOGICAL DEPARTMENT, LEEDS GENERAL INFIRMARY

THE histamine antagonist, N(2-dimethylaminoethyl) N(*p*-methoxybenzyl)2-aminopyridine maleate, was introduced as '2786 R.P.' by Bovet and Walthert (1944). Later known on the Continent as 'Neoantergan' and in this country as 'Anthisan' or mepyramine maleate, it was soon recognised as the most powerful and specific of the histamine antagonists. Halpern and Ducrot (1946) were responsible for a new series of anti-histamines, derivatives of phenothiazine, the most active of which was N(2-dimethylamino-*n*-propyl) phenothiazine hydrochloride or '3277 R.P.' Experiments on animals (Halpern et al. 1946) showed that this was a much more powerful histamine antagonist than anthisan, and after clinical trial it was marketed in France as 'Phenergan' and is now obtainable in this country under the same name.

Though some clinical reports on the use of phenergan in France have appeared (Vallery-Radot et al. 1947, 1948, Halpern and Hamburger 1948) and seem to indicate that it is the most effective histamine antagonist available, no detailed comparison of phenergan and anthisan has been made.

We give here the results of a comparison of these two drugs from both pharmacological and therapeutic studies in man.

PHARMACOLOGICAL OBSERVATIONS

While quantitative methods have been widely used in animals and in isolated animal tissues for comparing different histamine antagonists, comparative studies in man have been for the most part qualitative, though the desirability of applying quantitative methods in this field has been emphasised by Bain et al. (1948b). In communications to the British Pharmacological Society, Bain et al. (1948a) described methods they had developed for this purpose and some of the results obtained. That work will be published in detail elsewhere, but the salient points which are relevant to the subject of the present paper are summarised here.

Firstly, it was shown that, in any given person, if graded doses of histamine are injected into the skin, and the areas of the resulting weals or flares measured, the relationship between the logarithm of the dose and the effect is linear over at least a three-hundredfold range of dosage.

The volume of fluid injected was 0·05 or 0·1 ml., and the dose of histamine base contained in this volume varied from 0·01 to 100·0 μg.—i.e., a ten-thousandfold range. The linear relationship usually holds, for both flares and weals, over the range 0·01–3·0 μg.—though in some persons to as far as 10·0 μg. or more—and its limit is marked by a sharp discontinuity, from which point the slopes of the lines rise very steeply. This discontinuity will be discussed elsewhere.

* Now Dermatologist, United Bristol Hospitals.

BY APPOINTMENT
WINE MERCHANTS
TO THE LATE KING GEORGE VI.

BLOCK, GREY & BLOCK

INCORPORATING LIMITED

H. B. FEARON & SON

FOUNDED 1786

Wine Merchants - Cigar Importers

26, SOUTH AUDLEY STREET, LONDON, W.1

TELEPHONE : GROSVENOR 3355/6

Christmas 1952.

We have pleasure in suggesting as Christmas presents, the following cases which offer a fair selection of wines and spirits. We shall be happy to despatch any of these to you or your friends on receipt of your order.

Case No.		*Price per case* £ s. d.
1.	1 Bottle South African Sherry, *Pale Medium Dry* 1 Bottle **Parliament** Port. *Fine old Ruby*	1 16 0
2.	1 Bottle **San Sebastian** Sherry, *Fine Amontillado* 1 Bottle **Big Ben** Port, *Vintage wine matured in wood*	2 2 0
3.	1 Bottle **Raleigh** Sherry, *Pale Golden* 1 Bottle **Cocktail** Gin, 50° Proof	2 6 6
4.	1 Bottle **St. Vincent** Sherry, *Very Fine Amontillado* 1 Bottle Madeira, Bual 1878	2 10 0
5.	1 Bottle **Cognac,** *Very Superior Old Pale* 1 Bottle **Parliament** Port. *Fine Old Ruby*	3 0 0
6.	1 Bottle **San Sebastian** Sherry, *Fine Amontillado* 1 Bottle **Cocktail** Sherry, *Pale Fino Solera* 1 Bottle **Speaker** Sherry, *Oloroso Particular, Golden*	3 6 0
7.	5 Bottles Claret, Château Bellevue 1947 ½-Bottle **Armagnac,** Vintage 1936	3 8 0
8.	5 Bottles Burgundy, Cotes de Beaune Villages 1947 ½-Bottle **Cognac,** *Very Superior Old Pale*	3 12 0
9.	2 Bottles White Burgundy, Pouilly Fuissé 1949 2 Bottles Moselle, Piesporter Michelsberg 1950 2 Bottles Hock, Rüdesheimer Hauserweg Riesling 1949...	4 3 0
10.	1 Bottle **Charterhouse** Scotch Whisky 1 Bottle **Big Ben** Port, *Vintage wine matured in wood* 2 Bottles Claret, Cru Pins de Fleurs 1934	4 7 0
11.	2 Bottles Claret, Château Canon 1943 1 Bottle **San Sebastian** Sherry, *Fine Amontillado* 2 Bottles Champagne, **Duc de Belfort**	5 0 0
12.	1 Bottle Cognac, Grande Champagne 1929 2 Bottles Champagne, Veuve Clicquot 1942	6 0 0
13.	1 Bottle **Charterhouse** Scotch Whisky 2 Bottles Champagne, Pommery and Greno 1945 2 Bottles Vintage Port, Quinta do Noval 1934	7 10 0

PRICES INCLUDE CARRIAGE AND PACKING.

PLEASE SEE OTHER SIDE

Christmas 1952.

Below we offer a small selection of wines and spirits. In addition we hold extensive stocks of Vintage Port, Claret and Burgundy for laying down and present drinking, and also Champagne of all good Shippers, with adequate landing age. We have a wide selection of Hock and Moselle.

PORT.	*Bottle*
Big Ben, *Vintage wine matured in Lodge*	19/–
Palmerston, *Fine tawny of great age*	21/–
SHERRY.	
South African Sherry, *Pale Medium and Pale Selected Dry*	14/6
Cocktail, *Pale Fino Solera, Very Dry*	18/6
San Sebastian, *Fine Amontillado* ...	20/–
The Speaker, *Oloroso Particular Golden*	24/–
MADEIRA.	
Tears of Angels, *Very Old, Pale and Dry*	22/–
Very Choice Bual, 1878	23/–

CLARET.	*Bottle.*	*Half-Bottle.*
St. Julien	7/–	4/–
Château Bellevue, *Macau*, 1947	8/6	4/9
Château Nenin, *Pomerol*, 1945	11/6	—
Château Canon, *St. Emilion*, 1943	16/6	—
Château Latour, *Pauillac*, 1944	20/–	—
Château Langoa Barton, *St. Julien*, 1934... ...	20/6	—
WHITE BORDEAUX.		
Graves, *Light Dry* ...	6/9	3/10
Château Dumême, *Medium Dry*	8/6	4/9
Château Couhins, *Graves*, 1949	9/6	5/3
Château Grillon, *Barsac*, 1949	13/6	7/3
BURGUNDY.		
Beaujolais	8/6	4/9
Côtes de Beaune Villages, 1947	10/–	5/6
Beaune 1947	10/6	5/9
Nuits St. Georges, 1945 ...	16/–	8/6

WHITE BURGUNDY.	*Bottle.*	*Half-Bottle.*
Macon Blanc, 1950 ...	9/–	5/–
Pouilly Fuissé, 1949 ...	11/–	6/–
Meursault, Les Casse Têtes, 1947	14/6	—
HOCK.		
Niersteiner Domtal, 1949	12/–	6/6
Liebfraumilch Superior, 1949	15/–	8/–
Kallstadter Steinacker Riesling, 1949	16/–	8/6
Oestricher Gottestal Riesling, 1949	18/6	—
Rauenthaler Berg Spätlese, 1949	22/–	—
MOSELLE.		
Kaseler Lorenzberg, 1950...	9/6	5/3
Piesporter, 1949	11/–	6/–
Erdener Treppchen, 1950	12/6	6/9
Reiler Goldlay Spätlese, 1949	17/6	—
CHAMPAGNE.		
Duc de Belfort, *Extra Dry, Extra Quality*	23/–	12/3
SCOTCH WHISKY.		
Charterhouse, *Fine Old* ...	38/–	
IRISH WHISKEY.		
Ballagary, *Old Liqueur* ...	35/–	—
COGNAC.		
Good Quality	39/–	19/3
Very Superior Old Pale ...	43/–	22/3
Exhibition, *Fine Old Liqueur*	52/–	—
Grande Champagne, 1929	59/–	—

ALL CONSIGNMENTS OF 6 BOTTLES OR MORE ARE SENT CARRIAGE PAID. BY RAIL OR CARRIER.

BLOCK, GREY & BLOCK, Ltd., 26, South Audley Street, London, W.1

TELEPHONE: GROSVENOR 3355/6

Secondly, it was shown that, after the administration of an adequate dose of a histamine antagonist by mouth, the log-dose response curve to intradermal histamine shifts so that it occupies at any given time a new position such that, in the conditions of the experiments and so far at least as the weal responses are concerned, there is an approximately equal percentage reduction of weal area over the range of test doses from 0·1 to 10·0 μg.

It is clear that these two facts made it possible to undertake in man a more precise comparison of histamine antagonists than had hitherto been possible, and we have applied them in the first instance to a study of the relative weight-for-weight potencies and the relative durations of action of different drugs.

After the administration of a histamine antagonist by mouth the anti-histamine effect rises to a maximum and then tails off, the time relations depending on the conditions of the experiment, the drug used, and the individual. The extent of reduction of the intradermal weal response at the point of maximum action of any histamine antagonist depends, of course, on the conditions of the experiment, the dose used, and the individual.

By measuring in any person the maximum shift of the dose-response curve to intradermal histamine for each of several oral doses of a histamine antagonist, expressing the maximum shift for each oral dose as the mean percentage reduction in weal area, and plotting this as ordinate against the logarithm of the dose of antagonist as abscissa, the relationship between dose and effect for that antagonist in that person is determined.

By making such observations in a group of people, and taking the mean maximum percentage reductions of weal areas in the group for each oral dose of the antagonist, a mean dose-response curve for that drug is determined. By repeating such observations with other histamine antagonists in the same group of people mean dose-response curves for these other drugs are obtained, and from such data the mean relative weight-for-weight potencies of the various substances can be estimated.

The results of such a comparison of anthisan and phenergan are shown in fig. 1.

In these experiments the same six people were given, on different occasions, oral doses of 50, 100, and 300 mg. of anthisan and of 25, 50, and 100 mg. of phenergan, and the maximum reduction of weal area was determined for each dose of each drug by at least three intradermal test doses of histamine (10·0, 1·0, and 0·1 μg.) given before, and at intervals after, ingestion of the anti-histamine.

The areas of the weals were obtained by inking the weal outline on the skin 5–10 minutes after each injection, transferring to millimeter graph paper, and computing directly. The results were calculated from these measurements and not, as might have been done, from the regression lines most closely fitting the experimental points.

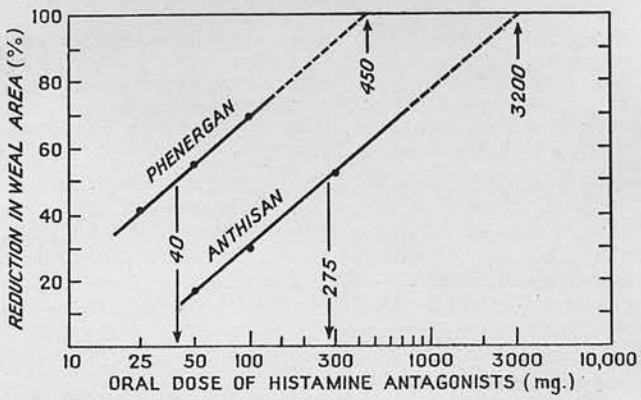

Fig. 1—Relationship between dose and maximum anti-histamine response for phenergan and anthisan. Mean results from six people. Mean doses to give 50 % reduction of weal area are shown on lower abscissa, and theoretical mean doses to produce 100 % reduction on upper abscissa.

As will be seen from fig. 1, phenergan is the more powerful drug. Thus, in our six people, whereas a 50% reduction of weal area was produced by about 40 mg. of phenergan, about 275 mg. of anthisan was required to produce the same mean effect. The mean doses theoretically required to abolish completely the intradermal histamine reaction were—assuming the dose-response graphs to be linear throughout their course—450 mg. of phenergan or 3200 mg. of anthisan.

We have compared other histamine antagonists by this method, but the results will be published elsewhere. It is sufficient to point out here, first, that phenergan is by far the most powerful of the drugs we have encountered and is thus a convenient standard against which the potency of others can be compared; and second, that such comparisons of potency are facilitated by the fact that the regression lines for the different drugs are parallel, so there is a simple ratio relating the mean dose of any two antagonists to give equal mean responses.

From the results shown in fig. 1 it is evident that about seven times the amount of anthisan is required to produce the effect of a given dose of phenergan; we can express this difference in potency by saying that, in relation to the mean maximum effect produced by a single dose, phenergan is on the average seven times more active, weight for weight, than anthisan.

Though the differences in potency among histamine antagonists are of considerable interest they are probably of much less practical importance than differences in the duration of action of the various drugs; for, whereas differences in potency can be allowed for by adjustment of the dose, it is on the duration of action that the frequency of administration will depend, and this is clearly an important factor in a class of drugs whose use is associated with a high incidence of unpleasant side-effects, which, when they occur, are usually most obvious for a period following the absorption of each successive dose.

We have therefore attempted to apply quantitative methods to the study of these differences, the problem being to obtain some measure of the relative durations of action such that the differences could be expressed in a fashion analogous to that which we used to express differences in potency. The difficulties in measuring the degree of anti-histamine effect when this has almost disappeared are obvious; so we have made our comparisons by estimating *the times taken for the maximum anti-histamine action of single approximately equi-effective doses of the different drugs to be reduced by 50%*.

Even the estimation of such "half-action" times presents difficulties, especially with the long-acting drugs, but the results of different experiments are remarkably consistent.

The results of these observations are represented diagrammatically in fig. 2. The experiments were made on groups of from four to eleven people, and the figures given for each drug are the average of three separate experiments in each of which the mean results have been used. The intradermal test-dose of histamine was either 1·0 or 3·0 μg.; and, before ingestion of the drug, the normal response to this dose was determined from at least three test injections. The drug was then taken with a cup of coffee two or more hours after a light breakfast. The onset and disappearance of the anti-histamine effect was determined by duplicate injections of the test-dose of histamine at suitable intervals—short at first, and

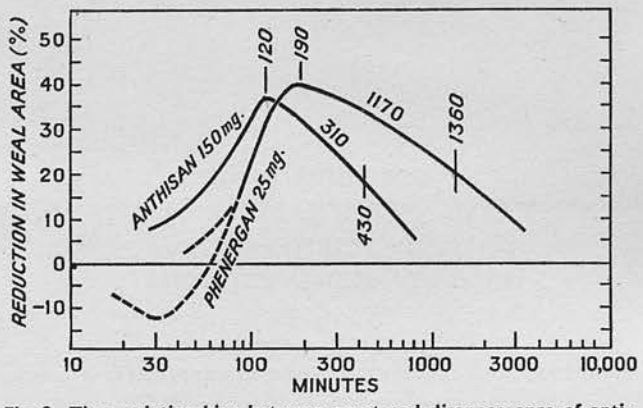

Fig. 2—Time relationships between onset and disappearance of anti-histamine effect with approximately equi-effective doses of phenergan and anthisan. Mean results of three experiments, each on four or more people, with each drug. Times to maximum and to half maximum action for each drug are marked.

longer in the later stages of the experiment. By this means it was possible to estimate graphically for each person the degree of maximum effect, the time for the establishment of this, and the time for the reduction of the maximum effect by half.

The great individual variation in the duration of action, as already pointed out by Bain et al. (1948b), is well shown in these experiments but will be dealt with in detail elsewhere. It is sufficient here to point out that, though the mean times from ingestion of the drugs to the point when half-action is reached are about 1360 minutes for phenergan and 430 minutes for anthisan, the range is 700–1800 minutes for phenergan and about 250–1000 minutes for anthisan.

But, in comparing the times taken for the different drugs to reach half-action, it seems desirable to take into account the well-marked difference in the rate of establishment of maximum action for each drug, and to state the time from maximum action to half-action rather than from ingestion to half-action. The average times for maximum action to be established in the conditions of our experiments were 190 minutes for phenergan and 120 minutes for anthisan. The mean times from full to half-action were thus 1170 minutes (19 hours 30 minutes) for phenergan, and 310 minutes (5 hours 10 minutes) for anthisan.

Hence the maximum effect of a single dose of phenergan takes over $3^3/_4$ times as long to be reduced by half as does the maximum effect of an approximately equipotent dose of anthisan, or the maximum effect of a single dose of anthisan is reduced by half in about a quarter of the time required for the same degree of reduction after an equipotent dose of phenergan.

It is thus clear that phenergan differs much from anthisan in both its potency and its duration of action. It is 7 times as potent, in the sense that to produce the same mean maximum effect as would be produced by a given dose of anthisan only a seventh of that amount of phenergan is required ; and its duration of action is $3^3/_4$ times greater than that of anthisan, in the sense that the mean time required for the reduction of the anti-histamine response from maximum to half maximum, after doses of the two drugs producing approximately equal mean maximum effects, is $3^3/_4$ times as long.

It is also evident from these results that both weight-for-weight potency and duration of action must be important in relation to the relative clinical effectiveness, and hence the intelligent therapeutic use, of histamine antagonists. It is likely too that a fair idea of the therapeutic possibilities of any new drug of this class could be obtained from experiments of the kind we have described. But such observations leave out of account the side-effects of the drugs, and these constitute, as Bain et al. (1948b) have pointed out, an important limiting factor in the usefulness of histamine antagonists. It might be argued that side-effects could be dealt with as " all or none " phenomena, but the number of people in our studies has generally been insufficient for this purpose. In any event the clinical significance of side-effects is not quantal but depends on their severity, the reactivity of the patient, and other such factors. The reactivity of the patient may vary with circumstances, depending, for example, on whether he is up and about or in bed, is mentally relaxed or doing work involving mental concentration ; it may also vary from day to day for no apparent reason—though seeming differences in this category may sometimes depend on how the questions are framed by the observer.

Though the assessment of the incidence and severity of side-effects is complicated, two facts are of outstanding practical importance : (1) some tolerance is often acquired to these when administration is continued for a few days ; and (2) with the long-acting drugs, such as phenergan, the specific anti-histamine effect of a single dose long outlasts the side-effects. This last observation, together with the long duration of action of the specific anti-histamine effects of phenergan, suggested the possibility of using phenergan clinically in a single daily dose given at night. In this way drowsiness would but contribute to sleep, and any other side-effects would pass unnoticed ; yet the specific histamine antagonism would continue through the following day.

THERAPEUTIC OBSERVATIONS

Twenty patients with chronic urticaria, who had been treated for various times with anthisan, were accordingly put on one dose of phenergan each night, and the dose was adjusted in an attempt to get clinical effects approximating as closely as possible to those obtained with anthisan. We have thus been able to make a controlled clinical comparison of these two drugs; to establish the approximate equivalent therapeutic doses; to assess the relative merits of equitherapeutic doses in relation to side-effects; and to show that the hypothesis which led to trial of the nightly dose routine was justified.

In these observations the degree of histamine antagonism was assessed partly by the response of the urticaria, partly by the dermographic reaction (when this was present), and partly by the reduction of weal areas in intradermal histamine tests. A close parallel has already been shown in the degree of response of all three phenomena to histamine antagonists (Bain et al. 1948b).

Since all the subjects were outpatients, it was impossible to make observations as often as was desirable, and it was usually 5–7 days after the control tests and the institution of therapy that the patients were seen again. Though most patients noted a pronounced improvement in their condition after 24 hours on anthisan, some noted little improvement with phenergan in the first 2 or 3 days. Thus, with phenergan given nightly the cumulative effect over some days was sometimes clearly necessary to establish an adequate therapeutic level. But once this level was reached there was little evidence of further cumulation. The initial lag in the onset of the therapeutic effect with nightly doses could presumably be obviated by giving a single high "loading" dose at the outset, or by giving ordinary doses three times a day until the desired action was obtained and following this with nightly "maintenance" doses. In the observations described below, however, we are concerned only with nightly doses of phenergan.

A summary of the results with the two drugs is given in table I, and a diagrammatic representation of the type of quantitative data obtained from some of the patients is given in fig. 3, which represents the results in case 1 (table I).

Drug mg. in 24 hr.	Side effects	Intradermal histamine reaction (cm.)	Urticaria	Dermographic reaction (cm.)
Nil	—	0 1 2	+ + +	0 1
Anthisan 100 100 100	Nil	0 1 2	+ + +	0 1
Anthisan 200 200 200	+ +	0 1	+	0
Anthisan 300 300 300	+ + +	0	Nil	0
Nil	—	0 1 2	+ + +	0 1
Phenergan 50	Nil	0 1	Nil	0

Fig. 3—Type of quantitative data obtainable in clinical comparison of histamine antagonists. The patient, a man aged 23, had urticaria for 2 years, associated with dermographism. Explanation of +s as in table I. Mean diameters of flare (thin line) and weal (thick line) in cm. Intradermal histamine reaction from 0·05 ml. of 0·01% w/v histamine acid phosphate. Dermographic reaction from different traumatising stimuli (mean of four measurements). In this patient 50 mg. of phenergan a day had effect of between 600 and 900 mg. of anthisan a day.

TABLE I—COMPARISON OF ANTHISAN AND PHENERGAN IN 20 PATIENTS WITH CHRONIC URTICARIA

Case no.	Sex	Age (yr.)	Pre-treatment urticaria		Anthisan therapy				Phenergan therapy				Preference of patient
			Duration	Severity	Divided dose (mg.)	Duration of treatment (weeks)	Urticaria	Side-effects	Nightly dose (mg.)	Duration of treatment (weeks)	Urticaria	Side-effects	
1	M	23	2 yr.	+++	100 t.i.d. 200 t.i.d. 300 t.i.d.	2 14 4	+++ + 0	Nil ++ +++	50	4	0	Nil	Phenergan
2	F	40	8 mos.	+++	100 t.i.d. 200 t.i.d. 300 t.i.d. 300 q.i.d.	1 1 10 2	++ ++ + 0	Nil Nil +* ++*	50 75 100	2 4 5	++ + 0	Nil Nil Nil	Phenergan
3	M	68	2½ yr.	+++	100 q.i.d. 200 q.i.d.	2 1	+ +	+ +	25 75 100	1 3 2	+ + +	+(½–1 hr. A.M.) +(½–1 hr. A.M.) ++(2–3 hr. A.M.)	Anthisan
4	F	30	7 mos.	+++	200 q.i.d.	6	+	++	50	5	0	Nil	Phenergan
5	F	38	13 mos.	++	100 t.i.d. 200 t.i.d. 300 t.i.d.	24 1 1	+ + 0	Nil + ++	25 50	1 6	+ 0	+(1–2 hr. A.M.) +(1–2 hr. A.M.)	Phenergan
6	F	29	10 mos.	+++	100 t.i.d. 200 t.i.d. 200 q.i.d.	7 12 5	+ + 0	+ ++ ++	75 100	1 5	+ 0	++(1–2 hr. A.M.) +(1–2 hr. A.M.)	None
7	M	30	7 mos.	+++	100 t.i.d. 100 q.i.d.	5 12	+ 0	Nil Nil	25	5	0	Nil	None
8	F	45	11 mos.	++	100 t.i.d.	1	++	++	75 100	2 3	+ 0	Nil Nil	Phenergan
9	F	39	3 yr.	+++	100 q.i.d.	8	0	Nil	25 50	1 6	0 0	Nil Nil	None
10	F	28	5 mos.	+++	100 q.i.d.	4	+	++	50 100 200	1 5 3	+ + 0	Nil +(½–1 hr. A.M.) +(½–1 hr. A.M.)	Phenergan
11	M	67	5 mos.	+++	100 t.i.d.	3	++	++	25	13	+	+(2–3 hr. A.M.)	Phenergan
12	F	39	8 mos.	++	100 t.i.d. 100 q.i.d.	4 16	+ +	Nil +	25	2	+	Nil	None
13	F	47	2 yr.	++	100 q.i.d.	11	0	+	25	6	0	Nil	Phenergan
14	F	50	12 mos.	++	100 q.i.d. 200 t.i.d. 200 q.i.d.	3 3 8	++ + +	Nil + +*	50 100 125	2 3 2	+ + 0	+(½–1 hr. A.M.) +(½–1 hr. A.M.) Nil	Phenergan
15	M	70	6 mos.	+++	100 b.i.d.	5	+	+	25	3	0	+(5–15 min. A.M.)	Phenergan
16	M	44	6 yr.	+†	100 t.i.d. 200 t.i.d.	24 2	0 0	Nil +	25	3	0	Nil	None
17	F	47	3 mos.	++	100 t.i.d.	4	0	+‡	25	2	0	Nil	Phenergan
18	F	19	8 mos.	+++	100 t.i.d.	6	++	Nil	50	4	0	Nil	Phenergan
19	F	68	5 mos.	++	200 t.i.d.	8	+	+*	100	3	0	Nil	Phenergan
20	F	54	4 mos.	++	100 b.i.d.	2	+	+	25	2	0	Nil	Phenergan

* Occasional diarrhœa and vomiting.
† Associated with attacks of giant urticaria every 2–3 days.
‡ Severe nausea and vomiting.

Severity of urticaria: + An occasional weal. ++ A few weals. +++ A moderate number of weals. ++++ Profuse weals.

Severity of side-effects: + Slight symptoms on questioning. ++ Complaint of mild symptoms. +++ Complaint of moderate symptoms. ++++ Severe incapacitating symptoms.

Table I shows that, whereas anthisan had to be given three or four times a day in all but 2 cases, a single nightly dose of phenergan controlled the urticaria in all the patients throughout 24 hours. Only 3 patients on anthisan were completely free from side-effects, against 14 on the nightly dose of phenergan. Many of the patients on phenergan felt a little dull and sleepy on waking during the first days of therapy—the symptoms taking from a few minutes to 4 hours to pass off—but in only 5 did this morning drowsiness persist throughout treatment. In any event this was the only side-effect noted with phenergan and is in contrast to the well-marked and persistent sleepiness and light-headedness which occur with anthisan and may be complicated, as it was in 4 of our patients, by occasional or persistent gastro-intestinal disturbances.

Of the 20 patients, only 1 preferred anthisan, 14 preferred phenergan, and 5 had no preference. The 14 patients who preferred phenergan were quite definite about their preference ; most had experienced side-effects with anthisan, and found none, or only the transitory morning sleepiness, with phenergan ; only 1 had been free from side-effects with both drugs. Of the 5 who had no preference, 1 had experienced mild side-effects with anthisan and had morning sleepiness with phenergan ; of the other 4 all were free from side-effects with phenergan, 3 of them had no side-effects with anthisan, and 1 had given evidence of slight symptoms with anthisan on questioning.

It is clear, then, that with phenergan in nightly doses the appreciated incidence of side-effects and the duration and severity of these when they develop are much less than with equitherapeutic doses of anthisan given, as it must be, two, three, or even four times a day ; and it follows that in patients intolerant of the necessary doses of anthisan adequate therapeutic effects can usually be obtained by easily tolerated nightly doses of phenergan. Whether equal freedom from side-effects could be attained by giving the daily amount of phenergan in divided doses remains to be seen, but the method we have adopted seems to have everything to recommend it, including the minor consideration that the patient may perhaps derive benefit rather than the reverse from the hypnotic effect of the drug taken at night.

By comparing the doses of anthisan and of phenergan required to produce approximately equal therapeutic results we can derive a measure of the relative therapeutic potencies of the two drugs. Table I shows that in some cases (4, 8, 10, 14, 15, 18, 19, and 20) the dose of phenergan used produced a greater therapeutic effect than did the dose of anthisan in the earlier period of treatment ; these, and cases 3 and 11, in which the urticaria was not abolished, have been ignored in this comparison.

In each of the remaining 10 cases the daily doses of anthisan and of phenergan which gave approximately equal therapeutic effects, and the ratios which these doses bear to one another, are shown in table II.

TABLE II—EQUIVALENT THERAPEUTIC DOSES IN 10 PATIENTS

Case no.	Equivalent daily doses (mg.)		Ratio anthisan/ phenergan
	Anthisan	Phenergan	
1	750	50	15
2	1200	100	12
5	900	50	18
6	800	100	8
7	400	25	16
9	400	25	16
12	400	25	16
13	400	25	16
16	300	25	12
17	300	25	12
..	..	..	Av. 14·0

It will be seen from the last column of table II that in these ten patients the therapeutic effect of a given dose of phenergan was produced by from 8 to 18 times, or by an average of 14 times, that dose of anthisan. Thus, on the average, 25 mg. of phenergan a day is the approximate therapeutic equivalent of 350 mg. of anthisan a day, or of three divided doses each of about 115 mg. Similarly, a daily dose of 50 mg. of phenergan is the therapeutic equivalent of about 700 mg. of anthisan a day, or of three divided doses of about 230 mg.

Thus, while phenergan is only about 7 times as potent as anthisan when compared in terms of the single doses required to produce the same intensity of effect it is almost 14 times as potent as anthisan when compared in terms of the relative doses per day required to maintain a similar

level of anti-histamine activity. The difference in the duration of action of the two drugs is clearly the main factor determining the difference between these two measures of potency.

The differences between anthisan and phenergan which we have already described are essentially quantitative, but it seems that there may also be a qualitative difference, which might conceivably be of clinical significance in some circumstances and, in any event, is worthy of mention.

In our first set of experiments on the duration of action of the different anti-histamines we were surprised to find that, though the histamine reaction was diminished by anthisan from the first, it was temporarily increased or potentiated by phenergan. Thus half an hour after ingestion of anthisan the mean reduction in the histamine response was 10%, whereas half an hour after ingestion of phenergan there was a mean increase of 10% in the response, the weal areas in all six persons showing some increase above normal. Half an hour later still the weal response was normal once more, after which it diminished rapidly to give the usual maximum anti-histamine effect 3 hours from the time the phenergan was taken. In subsequent experiments this phenomenon has been observed in only some of our subjects and has not shown on the mean curve ; we have consequently indicated in fig. 2 alternative routes for the onset of the anti-histamine effect of phenergan. A similar potentiating action of phenergan on the histamine response has often been seen in experiments where the drugs are administered locally to the skin in the manner briefly described in a previous paper (Bain et al. 1948b). The effect most commonly occurs when the phenergan and histamine are injected simultaneously.

Kapeller-Adler (1949) has shown that phenergan, but not anthisan, will inhibit histaminase in vitro ; so the potentiating action of phenergan which we have described may possibly be due to an inhibiting effect on histaminase. But to what total extent histaminase may be inactivated by therapeutic doses of phenergan, or how long the effect may persist, it is impossible to say from experiments of the type we describe, because the effect itself, manifested by the potentiation of the histamine weal response, becomes quickly masked by the establishment of the specific anti-histamine action of the drug. However, if phenergan is really potent as an inhibitor of histaminase, then small doses of the drug in persons relatively resistant to the anti-histamine effects might produce an exacerbation of any symptoms due to histamine release ; such untoward effects could presumably be countered by increasing the dose.

SUMMARY

The histamine antagonists 'Anthisan' (mepyramine maleate, 'Neoantergan,' '2786 R.P.') and 'Phenergan' ('3277 R.P.') are compared quantitatively as regards their weight-for-weight potencies, durations of action, and therapeutic efficacies in chronic urticaria.

The relative potencies of the drugs are compared by estimating the oral doses of each required to produce the same degree of reduction of the intradermal histamine weal response. Phenergan is shown to be about seven times as potent as anthisan. Thus 25 mg. of phenergan will produce, on the average, about the same maximum effect as 175 mg. of anthisan.

The relative durations of action are estimated from the times taken for the maximum effect of approximately equipotent doses of the drugs to be reduced by half. The mean half-action time for phenergan is $19^1/_2$ hours, and for anthisan $5^1/_6$ hours. Thus the maximum effect of a given dose of phenergan is likely to take $3^3/_4$ times as long to be reduced by half as is the effect of an equipotent amount of anthisan.

Anthisan and phenergan are compared therapeutically in twenty cases of chronic urticaria, anthisan having been given at the usual intervals but phenergan, because of its long duration of action, in a single dose at night.

Patients who prefer a nightly dose of phenergan to equi-effective doses of anthisan are usually those who experience less side-effects with phenergan than with anthisan, whereas those who express no preference have usually experienced no, or only very slight, side-effects with anthisan. Of these 20 patients, 14 preferred phenergan, and 5 had no preference.

The relative therapeutic potencies of anthisan and phenergan are estimated from the individual ratios between the daily dose of anthisan and the daily dose of phenergan required to produce the same therapeutic effect. Phenergan is, on the average, 14 times as potent as anthisan. Thus, a patient with chronic urticaria whose condition is controlled by 350 mg. anthisan a day, or 120 mg. three times a day, is likely to show a similar clinical response to 25 mg. of phenergan a day, given in a single dose at night. He is also likely to prefer this treatment.

The possibility that phenergan may exhibit an inhibiting effect on histaminase is noted, and a possible consequence of this action is pointed out.

Our thanks are due to Dr. J. T. Ingram and Dr. F. F. Hellier for access to, and help with, the patients; to Dr. G. Achari and Miss M. Robinson for help with the laboratory experiments, and to our willing subjects in these; to Dr. B. N. Halpern and Dr. W. R. Thrower for supplies of the drugs; and to the Medical Research Council for an expenses grant to W. A. B. which met the cost of the work.

REFERENCES

Bain, W. A., Broadbent, J. L., Robinson, M., Warin, R. P. (1948a) Communications to British Pharmacological Society, July, 1948.
— Hellier, F. F., Warin, R. P. (1948b) *Lancet*, ii, 964.
Bovet, D., Walthert, F. (1944) *Ann. pharmaceut. franç.* suppl. no. 4.
Halpern, B. N., Ducrot, R. (1946) *C.R. Soc. Biol. Paris*, **140**, 361.
— Hamburger, J. (1948) *Canad. med. Ass. J.* **59**, 322.
— — Debray, C. (1946) *Bull. Soc. méd. Hôp. Paris*, **62**, 607.
Kapeller-Adler, R. (1949) *Biochem. J.* **44**, 70.
Vallery-Radot, P., Hamburger, J., Halpern, B. N. (1947) *Pr. méd.* **55**, 661.
— — — (1948) *Sem. Hôp. Paris*, **24**, 655.

III.

The Quantitative Comparison of Histamine Antagonists in Man

[*Reprinted from* Discussion on Antihistamine Drugs, PROCEEDINGS OF THE ROYAL SOCIETY OF MEDICINE, *August*, 1949, *Vol. XLII, No.* 8, pp. 615-623 (*Section of Experimental Medicine and Therapeutics*, pp. 23-31).]

[*Reprinted from* Discussion on Antihistamine Drugs, PROCEEDINGS OF THE ROYAL SOCIETY OF MEDICINE, *August*, 1949, *Vol. XLII, No.* 8, pp. 615-623 (*Section of Experimental Medicine and Therapeutics*, pp. 23-31).]

The Quantitative Comparison of Histamine Antagonists in Man

Professor W. A. Bain (*University of Leeds*): *The Quantitative Comparison of Histamine Antagonists in Man.*

THIS work, done by a small team[1] at Leeds, is part of a general attempt to apply to pharmacological studies in man methods of a quantitative kind such as have hitherto been applied chiefly to animals and to isolated animal tissues. As a starting-point in this quantitative approach to human pharmacology histamine and the histamine antagonists seemed ideal drugs to use, since histamine produces easily measurable reactions in the most extensive and readily available organ of the body and these reactions, as is well known from much qualitative work, are readily modified by histamine antagonists. A further consideration influencing our choice was the practical desirability of developing methods for assessing the relative merits of antihistaminics: this has now become an urgent practical necessity in view of the bewildering rate at which new drugs of this class are being introduced and the inevitable confusion which this is causing.

Our first task was to determine the dose-effect relationship for histamine when the drug was injected into the skin; and the second to determine in what manner and to what extent this was modified by the oral administration of histamine antagonists. From such observations we were able to devise means for obtaining quantitative comparative information about three of the four most important practical aspects of the actions of these drugs—their relative weight-for-weight potencies, their relative durations of action, and their relative therapeutic efficacies. Only the hyoscine-like side actions were not amenable—or were not subjected—to quantitative study.

Our account is necessarily but a brief summary of what we have done. Results are given mostly as mean values: statistical treatment of the data is omitted and will be presented elsewhere. For information about the various drugs mentioned, and about previous work upon them, the reader is referred to the recent reviews by Halpern (1948) and by Hunter and Dunlop (1948).

The dose-response curve to intradermal histamine and its modification by histamine antagonists.—The two experimental facts which form the basis of the subsequent observations are these: First, if graded doses of histamine are given intradermally to any individual, and the areas of the resulting wheals or flares are measured when at their maximum, then the relationship between the logarithm of the dose and the effect is linear over at least a three-hundredfold dose range—usually from 0·01 μg. to at least 3·0 μg., and in many subjects to as far as 10 μg. or more—after which the slope of the graph increases suddenly. Second, after the oral administration of an adequate dose of a histamine antagonist the log-dose response curve to intradermal histamine shifts so that it occupies at any given time a new position such that, in the conditions of our experiments and as far at least as the wheal response is concerned, there is an approximately equal percentage reduction of wheal area for each of the test doses of histamine over the range from about 0·03 to at least 10·0 μg.

[1]The Author, with Dr. G. Achari, Dr. J. L. Broadbent, Miss M. Robinson, and Dr. R. P. Warin.

These facts are illustrated in fig. 1 which shows the mean results from a set of experiments on five subjects. The upper points show the mean wheal areas resulting from the intradermal doses of histamine indicated on the abscissa, and the graph is drawn through these points by eye. The lower points show the corresponding wheal areas three hours after the ingestion of 25 mg. Phenergan (3277 R.P.).

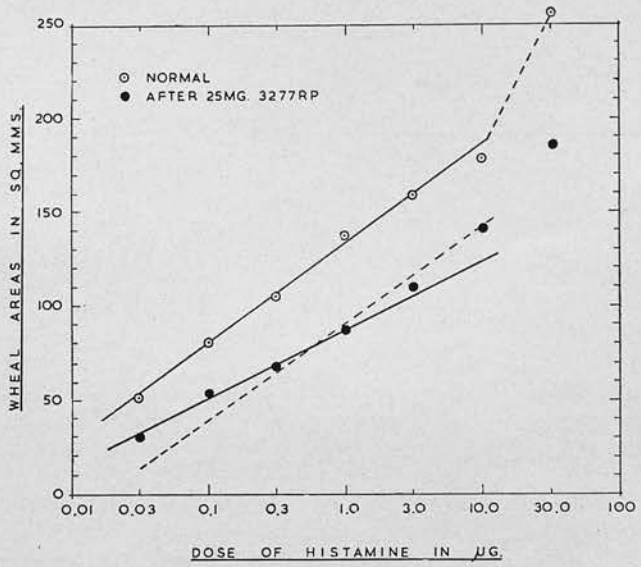

FIG. 1.—To show normal dose-response curve to intradermal histamine and its modification after the oral administration of a histamine antagonist. Mean results from five subjects. Abscissa—dose of histamine base in μg., logarithmic scale. Ordinate—wheal areas in sq. mm. The upper (open) points are normal: the lower (solid) ones were obtained three hours after the ingestion of 25 mg. Phenergan (3277 R.P.). For further explanation see text.

The average percentage reduction in wheal area for all the test doses of histamine was 35·0. The lower solid line is drawn to give throughout its course this same percentage reduction of the values represented by the normal dose-response graph. It fits the experimental points more closely than does the broken line which is parallel to the normal graph.

The comparison of weight-for-weight potencies.—When a histamine antagonist is taken by mouth the antihistamine effect, as gauged by the percentage reduction in the effect of an intradermal test dose of histamine, rises to a maximum and then tails off, the time relations depending on the conditions of the experiment, the drug used, and the individual. This is illustrated in fig. 2 which shows the mean results with various drugs in the same four subjects. It is clear that, other things being equal, the extent of the maximum reduction of the histamine response will depend on the dose of the antagonist, so that in comparing the relative potencies of different drugs it is essential to make the comparisons when the action of each drug is at its maximum, i.e. when the dose-response curve to intradermal histamine is maximally shifted.

If then, in any subject, the maximum shift of the dose-response curve to intradermal histamine is determined for each of several doses of an antagonist, and this maximum shift for each dose of the antagonist is expressed as the mean percentage reduction in wheal area and plotted as ordinate against log-dose antagonist as abscissa, then the relationship between dose and effect for that antagonist in that subject is determined. By making such observations in a group of subjects a mean dose-response curve for that antagonist is determined. By repeating such observations with other antihistaminics in the same group of subjects mean dose-response curves for these other drugs are obtained, and from such data, since the dose-response curves for the various drugs are parallel, the mean relative weight-for-weight potencies can be readily estimated.

Fig. 3 shows the results of such a comparison of Phenergan (3277 R.P.), Anthisan (Neoantergan, 2786 R.P.) and Antistin.

In these experiments the same six subjects were given, on different occasions, oral doses of 50, 100 and 300 mg. Anthisan, 25, 50 and 100 mg. Phenergan and 100, 200 and 300 mg. Antistin respectively, and the maximum reduction of wheal area was determined for each dose of each drug by at least three intradermal test doses of histamine (10·0, 1·0 and 0·1 μg.) given before and at intervals after ingestion of the antihistamine.

The areas of the wheals were obtained by inking the wheal outline on the skin five to ten minutes

after each injection, transferring to millimetre graph paper, and computing directly. The results were calculated from these measurements.

Phenergan is clearly the most powerful of these drugs and Antistin the least so. Thus, while a 50% reduction of wheal area was produced by about 40 mg. Phenergan, it required 275 mg. Anthisan or 600 mg. Antistin to produce the same mean effect. Furthermore, if

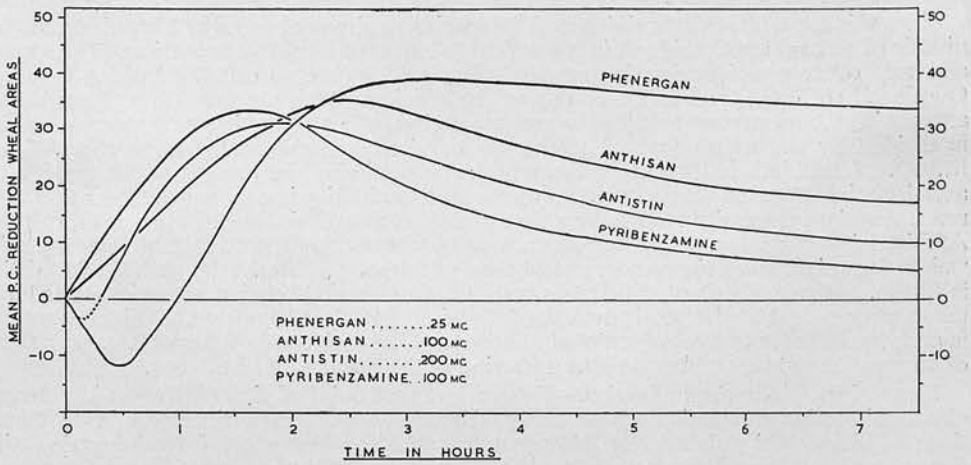

FIG. 2.—To show the variation in the rate of rise and fall of the antihistamine effect of single doses of various histamine antagonists. Mean results from four subjects. Abscissa—time in hours. Ordinate—mean percentage reduction below pre-drug level in wheal areas from intradermal test doses of histamine. The drugs and their doses are indicated on the figure. The individual points from which the graphs are constructed are omitted to avoid confusion.

we assume that the dose-response relationship remains linear throughout its course, then the mean doses of antihistamine theoretically required to abolish the intradermal histamine response are 450 mg. Phenergan, 3,200 mg. Anthisan and 7,000 mg. Antistin respectively—all of them quite intolerable doses.

Such comparisons of relative potency are, of course, facilitated by the fact that the regression lines for the different drugs are parallel, so that there is a simple ratio relating the mean dose of any two antagonists to give equal mean responses. Thus from the results shown in fig. 3 it is evident that about seven times the amount of Anthisan, or fifteen times the amount

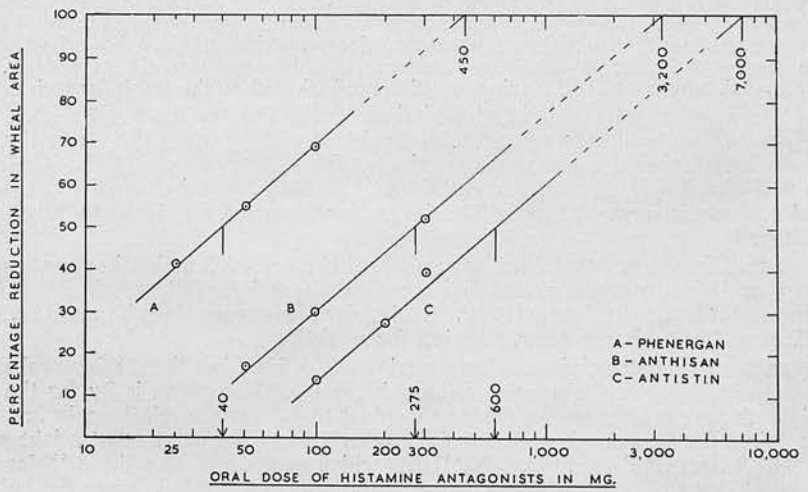

FIG. 3.—To show relationship between dose and maximum antihistamine response for Phenergan, Anthisan and Antistin. Mean results from six subjects. Abscissa—oral dose of drug in mg. on logarithmic scale. Ordinate—mean percentage reduction in wheal areas. Graphs A, B, and C are for Phenergan, Anthisan and Antistin respectively. Mean doses to give 50% reduction in wheal area are indicated on lower abscissa, and theoretical doses to produce 100% reduction on upper abscissa. For further explanation see text.

of Antistin, is required to produce the effect of a given dose of Phenergan. We can express these differences by saying that, in relation to the maximum effect produced by a single dose, Phenergan is seven times more active, weight for weight, than Anthisan, and fifteen times more active than Antistin. Phenergan, indeed, is by far the most powerful drug of this class which we have encountered and is thus a suitable standard with which the potencies of others can be compared.

Since this method of determining and comparing equi-potent doses of histamine antagonists is of general applicability it is convenient to express the relative potencies of Phenergan and any other antihistamine in terms of what we propose to call the "Mean Potency Quotient" (M.P.Q.), and to define this as the quotient obtained when, for any particular antagonist, the mean dose required to produce a given effect at the time of maximum action is divided by the mean dose of Phenergan required to produce the same effect. The M.P.Q. of Anthisan is thus approximately seven, signifying that, as far as the maximum antihistamine effect of single doses upon the skin capillaries is concerned, seven times as much Anthisan is required to produce the same effect as a given dose of Phenergan, or that Anthisan is weight for weight one-seventh as potent as Phenergan, or that Phenergan is weight for weight seven times more potent than Anthisan. Similarly, the M.P.Q. of Antistin is fifteen. It should be noted that this method of expressing relative potencies states how much more potent Phenergan is than the drug with which it is compared. This convention is adopted in order to avoid fractional quotients. The reciprocal of the M.P.Q. expresses, of course, the potency of any drug as a fraction of the potency of Phenergan.

Comparison of durations of action.—From a practical point of view differences in potency among histamine antagonists are probably of less importance than differences in the durations of action; for it is on this latter property that the frequency of administration will depend, and this is an important matter in a class of drugs the use of which is so often associated with unpleasant or disconcerting side-effects and where such effects are usually most evident for a period following the absorption of each successive dose. Fig. 2 gives some idea of the marked differences in the duration of action of some of these drugs; but our problem was to obtain a measure of the relative durations of action such that the differences could be expressed in a fashion analogous to that which we have used for expressing differences in potency. It is evidently impossible to estimate the relative times for the disappearance of the action and so our comparisons have been made by estimating the times taken for the maximum antihistamine effect of single approximately equi-effective doses of the different drugs to be reduced by 50%.

The experiments were carried out on groups of from 4 to 11 subjects. The normal response to intradermal histamine was determined by at least three injections of 1·0 or of 3·0 μg. histamine. The drug was then taken with a cup of coffee two or more hours after a light breakfast. The onset and disappearance of the antihistamine effect was determined by duplicate injections of the test dose of histamine at suitable intervals. It was thus possible to estimate graphically for each subject both the degree of maximum effect and the time for establishment of this, together with the time for its reduction by half. The graphs in fig. 2, already referred to, were obtained in this way. They are the average results in four subjects and represent a single experiment. Fig. 4 shows the mean results from three such experiments for the drugs Phenergan, Anthisan and Antistin respectively. In this the times to maximum and to half maximum action are indicated on the graph for each drug.

There is, of course, great individual variation among the results, but the mean values from different experiments are remarkably consistent. Thus while the mean times from ingestion to half action are about 1,360 minutes for Phenergan (1,375, 1,320 and 1,375 minutes) and about 430 minutes for Anthisan (400, 510, 390 minutes) the range among individual subjects is from 700 to 1,800 minutes for Phenergan and 250 to 1,000 minutes for Anthisan.

It will be seen from fig. 4 that Anthisan and Antistin reach full action in about two hours and Phenergan in just over three, and that the mean times from full to half action in these experiments are thus 1,170 minutes (19 hr. 30 min.) for Phenergan, 310 minutes (5 hr. 10 min.) for Anthisan and 210 minutes (3 hr. 30 min.) for Antistin.

As this method of determining and comparing the half-action times after oral administration is applicable to any histamine antagonist, and as Phenergan is by far the longest acting of the drugs we have studied, it is convenient to express the relationship between the half-action time for Phenergan and that for any other antagonist by what might properly be called the "Mean Half-action Quotient", but which we propose to call the "Mean Duration Quotient", or M.D.Q., which we define as the quotient obtained when the half-action time for Phenergan is divided by the half-action time for the drug with which it is compared. This relates the half-action time of any particular antagonist and Phenergan by expressing the half-action time of Phenergan as a multiple of the half-action time of the drug with which it is compared. This convention avoids fractional quotients.

Thus the M.D.Q. of Anthisan is 3·8, signifying that the maximum effect of a single dose

of Phenergan takes about three and three-quarter times as long to be reduced by half as does the maximum effect of an approximately equi-potent dose of Anthisan, or that the maximum effect of a single dose of Anthisan is reduced by half in about a quarter of the time required for the same degree of reduction after an equi-potent dose of Phenergan. Similarly, the M.D.Q. of Antistin is 5·6.

The remarkable similarity between the half-action times for Phenergan and Anthisan when administered orally, and the corresponding ones obtained when the drugs are infiltrated locally

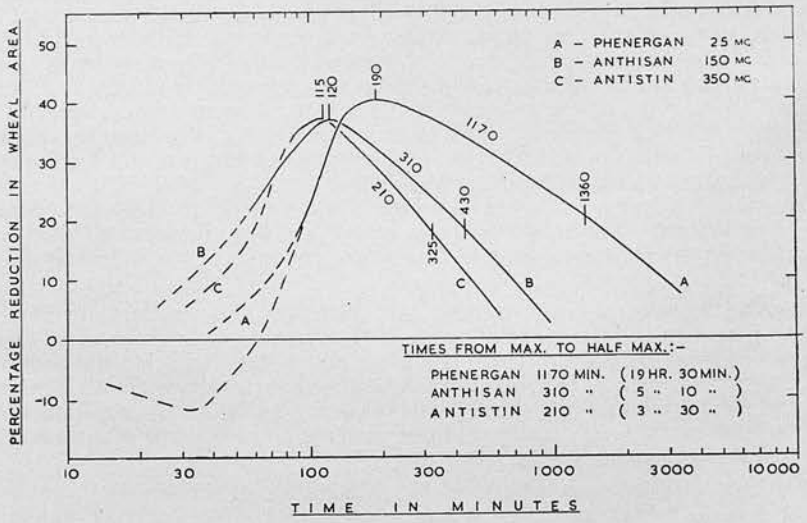

FIG. 4.—To show mean time relationships between onset and disappearance of antihistamine effect with approximately equi-effective doses of different antihistamines administered orally. Mean results from three experiments, each on four or more subjects, with each drug. Abscissa—time in minutes on logarithmic scale. Ordinate—mean percentage reduction in wheal area. Curves A, B, and C are for Phenergan, Anthisan and Antistin respectively. Times to maximum and to half maximum action are indicated for each drug. For further explanation see text.

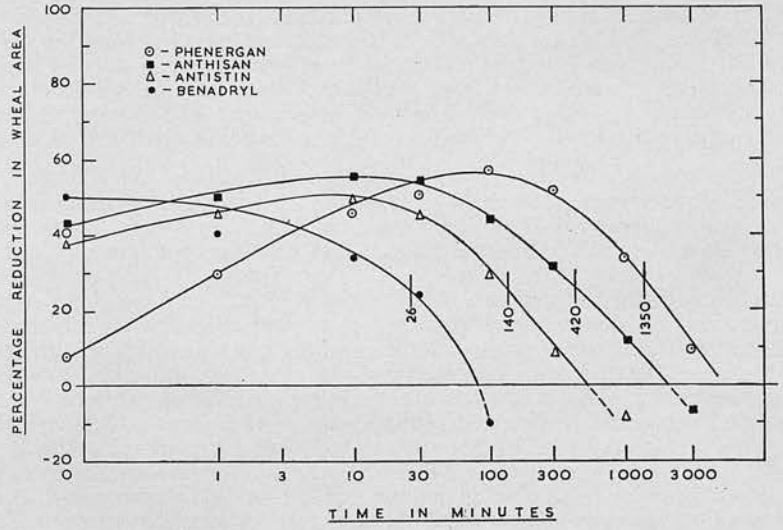

FIG. 5.—To show mean time relationships between onset and disappearance of antihistamine effect when the antihistamine drugs are administered locally to the skin and the test doses of histamine are injected to these infiltrated areas. Results from 14 subjects. Dose of antihistamine—0·2 ml. of 0·1% w/v. Test dose of histamine—1·5 μg. base in 0·05 ml. Injections at zero time—i.e. of histamine and the antagonist simultaneously, were of 0·2 ml. containing 0·1% w/v of antihistamine and 1·5 μg. histamine base. Indicated on graphs are the times from administration to half-action of the various drugs. For further explanation see text.

to the skin, in the manner briefly described in a previous paper (Bain, Hellier and Warin, 1948) and extended and reported on in more detail by Achari *et al.* (1948), is worthy of note. The results of these experiments are shown in fig. 5. It will be seen that the mean times for maximum action to be reached in these circumstances were about 100 minutes for Phenergan and 10 minutes for Anthisan, while the mean times from maximum to half-action were 1,250 minutes (20 hr. 50 min.) for Phenergan and 410 minutes (6 hr. 50 min.) for Anthisan. In view of the similarity of the figures derived from these two different types of experiment it is difficult to escape the conclusion that both the absolute and the relative durations of action of these two drugs are dependent mainly upon their duration of fixation by the tissues on which they act rather than upon, for example, differences in their rates of excretion. Some antihistaminics, however, give markedly discrepant results in the two types of experiment, indicating that such a view is not generally applicable to this class of drugs. Thus some may show a moderate half-action time when administered by mouth, and only a very short half-action time when administered locally. The most striking example is Benadryl, which has an oral half-action time somewhere between that for Antistin and that for Anthisan, but which, on local application (*see* fig. 5), has a half-action time of under 30 minutes. In such instances, where the drugs are not firmly fixed by the tissues, their duration of action must presumably depend mainly on their duration of sojourn in an active form in the extracellular fluid and thus ultimately on their rate of inactivation, or of excretion, or both.

The foregoing comparisons show clearly that histamine antagonists differ markedly in their relative potencies and durations of action. Thus Phenergan is about seven times more potent than Anthisan and about fifteen times more potent than Antistin: the time from full to half-action for Phenergan is about three and three-quarter times greater than that for Anthisan and over five and a half times greater than for Antistin. It is evident that both these factors must be taken into account in assessing the relative merits of both existing and proposed new histamine antagonists.

A further important factor is, of course, the relationship of side-effects to antihistamine activity, for it is the side-effects which at present constitute one of the chief limiting factors in the usefulness of these drugs. We have discussed elsewhere (Bain, Hellier and Warin, 1948; Bain, Broadbent and Warin, 1949) the difficulties associated with the assessment of the incidence and severity of side-effects, and have stated earlier in this paper that we have not dealt with these in a quantitative fashion. We hope, however, that it may be possible to devise a semi-quantitative treatment of side-effects by applying to them a system of "scoring". The "scores" obtained in the same group of subjects by different drugs could then be used to derive a "Mean Side-Effect" or "Mean Toxicity Quotient". Determined on equi-potent doses of different drugs this might indicate the relationship, if any, between the most interesting side-actions—those which are so similar to the effects of hyoscine—and the specific antihistamine effect itself. But from the clinical point of view it would also be important to compare equi-therapeutic as distinct from equi-potent doses and this would clearly give a different quotient, the difference depending to a large extent on the relative durations of action of the drugs compared. This will be evident from the observations about to be described.

Comparison of therapeutic potencies.—We have so far been able to make a therapeutic comparison of only two drugs—Phenergan and Anthisan. As details of this comparison are presented elsewhere (Bain, Broadbent and Warin, 1949) only a summary will be given here.

In 20 patients with chronic urticaria the reaction to intradermal test doses of histamine and, when present, the dermographic reactions to various stimuli, were measured by Dr. Warin before and at intervals after the institution of therapy with Anthisan, and compared with the progress of the urticaria. The Anthisan was given two, three, or four times a day, as was found necessary. After a rest period the control observations were repeated and the patients put on Phenergan. In view of the long duration of action of this drug it was given in a single dose at night. Given in this way we hoped that the drowsiness which it often causes would but contribute to sleep and that any other side-actions would pass unnoticed, whereas the antihistamine effect would continue throughout the following day. An attempt was made to adjust the dose of Phenergan so as to give the same therapeutic effect as with Anthisan in the earlier period of treatment: we hoped to be able in this way to establish the approximately equivalent therapeutic doses.

While Anthisan had usually to be given three or four times a day the nightly dose of Phenergan controlled the urticaria in all the patients throughout twenty-four hours. Only 3 patients were completely free from side-effects with Anthisan against fourteen on the nightly dose of Phenergan. The only side-effect noticed with Phenergan was morning drowsiness, but in only 5 patients did this persist throughout treatment. 14 patients preferred Phenergan, 5 had no preference and only one

preferred Anthisan ; the high preference for Phenergan was clearly based on the relative absence of side-actions with this drug. Thus with Phenergan in nightly doses the appreciated incidence of side-effects, and the severity of these when they occur, is much less than with equi-therapeutic doses of Anthisan given, as it usually has to be, several times a day : hence it should be possible to obtain adequate therapeutic effects by easily tolerated nightly doses of Phenergan in patients unable to tolerate the necessary divided doses of Anthisan.

A diagrammatic representation of the type of data obtained from some of the patients is given in fig. 6. In this case it is evident that the effect of 50 mg. Phenergan lies between that for 600 and for 900 mg. per day of Anthisan. In making the quantitative therapeutic comparison, however, 10 cases have had to be excluded; 8 because the dose of Phenergan used produced a greater therapeutic effect than did the Anthisan in the earlier period of treatment; and 2 because in them the urticaria was not abolished. In the remaining 10 cases the therapeutic effect of a given dose of Phenergan was produced by a total daily dose of Anthisan from eight to eighteen times greater: the average figure was fourteen. Thus, on the average, 25 mg. Phenergan per day is the approximate therapeutic equivalent of 350 mg. Anthisan per day, or of three divided doses each of about 115 mg. Similarly, a nightly dose of 50 mg. Phenergan is the equivalent of about 700 mg. Anthisan per day, or of three divided doses each of about 230 mg.

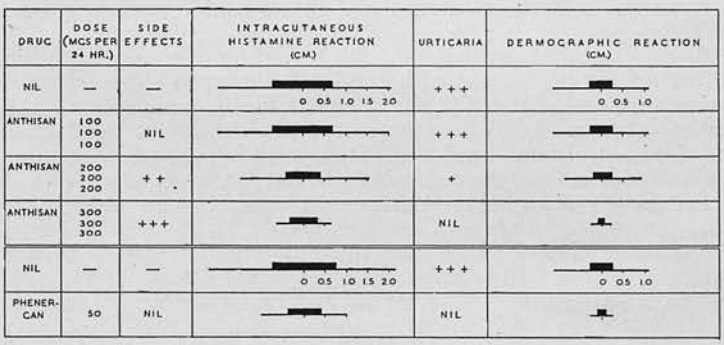

FIG. 6.—To show the type of result obtainable in the clinical comparison of histamine antagonists. Subject W. H., male, aged 23, urticaria present for two years, associated with dermographism. Urticaria: +, occasional wheal; ++, a few wheals; +++, moderate number of wheals. Side-effects: +, slight symptoms on questioning; ++, complaint of mild symptoms; +++, complaint of moderate symptoms. Mean diameter of wheals (thick lines) and flares (thin lines) in cm. Histamine reaction from 0·05 ml. of 0·01% w/v histamine acid phosphate. Dermographic reaction from various traumatizing stimuli (mean of 4 measurements). In this subject 50 mg. Phenergan per day had an effect between that of 600 and that of 900 mg. of Anthisan per day.

With such a relationship between these dose values it is perhaps justifiable to express the relative therapeutic potencies of Phenergan and Anthisan—and, when the information is available, of Phenergan and any other histamine antagonist—in terms of the average ratio between the equi-effective twenty-four hour doses. This "therapeutic ratio" cannot be so called because of the varied connotations which already attach to the expression, and we propose the clumsier "Mean Therapeutic Quotient" or M.T.Q. This can be regarded as stating in respect of the total dose in twenty-four hours, (1) how much more powerful Phenergan is than the drug with which it is compared, or (2) by how much, on the average, a given dose of Phenergan must be multiplied to find the daily dose of the other drug likely to produce the same therapeutic effect as the Phenergan.

The M.T.Q. of Anthisan is about 14. Thus while Phenergan is only about seven times more potent than Anthisan when compared in terms of the single doses required to produce the same intensity of effect (M.P.Q.), it is almost fourteen times more potent than Anthisan when compared in terms of the relative doses per day required to maintain a similar level of antihistamine activity (M.T.Q.). It is clearly the difference in the duration of action of the two drugs, expressed as the Mean Duration Quotient, which is the main factor determining the difference between the mean potency and mean therapeutic quotients.

Concluding remarks.—The differences so far noted among the various drugs are differences of degree. But in our first experiment on the duration of action of Phenergan (illustrated in fig. 2) the initial effect of the drug was to potentiate the intradermal histamine response in all four subjects. In subsequent experiments this phenomenon was seen only in some subjects, and the mean curve did not fall below the zero abscissa. These different results are indicated in fig. 4, where alternative routes for the onset of the antihistamine effect of Phenergan are indicated by broken lines. (No other histamine antagonism has exhibited this effect, with the

possible exception of Antistin: but with this the only evidence was indirect—the somewhat late rise of the curve (*see* fig. 2) from the zero line.) A similar potentiation of the intradermal histamine response has often been noticed—but again only in some subjects—when Phenergan and histamine have been injected to the skin in experiments of the kind illustrated in fig. 5. The effect was most commonly seen when the two drugs were administered simultaneously.

We sought to account for this phenomenon by the hypothesis that Phenergan might antagonize histaminase. Some support is perhaps given to this view by the work of Kapeller-Adler (1949) who has shown that Phenergan and Antistin inhibit the action of histaminase *in vitro,* but that none of the other histamine antagonists investigated do so.

If the primary potentiation of the intradermal histamine response with Phenergan is due to partial inhibition of histaminase then it is clear that the fixation of the drug by the enzyme occurs very quickly, in marked contrast to the rate of fixation by the capillaries. But to what total extent histaminase may be inactivated by therapeutic doses of Phenergan, or how long the effect may persist, it is impossible from our experiments to say, because the effect itself, manifested by the potentiation of the wheal response, becomes quickly masked by the establishment of the specific antihistamine action of the drug. However, if Phenergan is potent as an inhibitor of histaminase it is possible that therapeutic doses of the drug in persons relatively resistant to the antihistamine effects might produce an exacerbation of any symptoms due to histamine release; such untoward effects could presumably be countered by increasing the dose.

It is generally assumed that histamine antagonists act by receptor competition—that they become fixed, in varying degree and with varying firmness, to receptors on the tissues on which histamine acts, and, by this preferential fixation or competition, partially exclude or block the access of histamine to these receptors (Gaddum, 1948). The failure of histamine antagonists to diminish histamine-induced gastric secretion may be due to a difference in the histamine receptors in the gastric glands such that the antagonists fail to become fixed to them. Of the actions of histamine which are influenced by histamine antagonists those due to injected (exogenous) histamine are usually held to be more readily affected than those due to histamine released by the tissues (endogenous histamine). Furthermore, Dale (1948) distinguishes between histamine which is released by the cell on which it reacts and that which when released acts only upon more remote structures: the former he calls intrinsic and the latter extrinsic histamine. In man the antihistamine drugs are usually much less effective against intrinsic than against extrinsic actions of endogenous histamine. They are—assuming the role of histamine in these conditions—less effective, for example, in bronchial asthma, where the histamine is liberated by the reacting structure, than in chronic urticaria where it is not. But it may be that the relative failure of histamine antagonists to block intrinsic actions is due simply to the difficulty, with the agents at present available, of reaching an effective drug level at the site of histamine release with doses of the drugs which are tolerable. In chronic urticaria, on the other hand, the histamine does not act upon the cells from which it is liberated but upon the capillaries with which it subsequently comes in contact through humoral channels. Histamine antagonists are thus regarded as effective in urticaria because the liberated histamine acts in a fashion analogous to that of histamine injected from outside.

Nevertheless it may be wondered how antihistamine drugs can abolish urticaria when, according to the pharmacological data summarized earlier in this paper, very large doses may be required to produce a 70% reduction in the intradermal histamine wheal response and quite intolerable doses are theoretically required to abolish it. Nor is it only when histamine antagonists are administered orally that we have failed to abolish the intradermal histamine response, for we have failed equally to do so when the drugs are administered locally to the skin. The average maximum percentage reduction obtained by local administration, even of Phenergan, was about 60% (*see* fig. 5) and the maximum individual reductions seldom exceeded 80%.

Chronic urticaria is in fact abolished, however, when the area of the intradermal wheal response is reduced by, on the average, about 50% with a range of individual values from 30% to about 80%. On the other hand, the average percentage reduction in the dermographic wheal response at the stage when urticaria is cleared is of the order of 90% (range 86–96%). This type of difference is illustrated in fig. 6 where, with the 50 mg. dose of Phenergan, the dermographic wheal response is reduced by about 90% and the intradermal histamine wheal response by 70%. Unfortunately we have but few observations of this kind on the dermographic response. Nevertheless if this sort of result can be confirmed and the quantitative differences between the effects of the drugs on injected and on liberated histamine respectively can be shown not to be due to the different circumstances attending the production of the two types of reaction, then it will be difficult to escape the conclusion that, in the relief of chronic urticaria the histamine antagonists may be exhibiting a dual modality of action—

by antagonizing the action of histamine on the capillaries, by a receptor competition mechanism, on the one hand, and by diminishing the release of histamine from the tissues, by some unknown mechanism, on the other. We have evidence, indeed, from other lines of work that histamine antagonists may in certain circumstances act in this latter fashion. But much further work is needed before any discussion of the various possibilities concerning the mode of action of histamine antagonists can be justified on the basis of experimental work on man; the view just expressed is therefore to be regarded as little more than a speculation.

Summary

(1) Methods of comparing the weight-for-weight potencies, relative durations of action, and relative therapeutic efficacies of histamine antagonists are described, but statistical treatment of the data is omitted.

(2) The outstanding potency and duration of action of Phenergan (3277 R.P.) suggests its suitability as a standard against which these properties of the others can be compared.

(3) Relative weight-for-weight potencies are expressed in terms of the "Mean Potency Quotient" obtained by dividing the mean dose of the drug under test which produces a given mean antihistamine effect, by the mean dose of Phenergan which produces the same effect. The quotient states how much more powerful Phenergan is than the drug with which it is compared, or by how much the dose of the drug under test must be multiplied to give the same effect as a given dose of Phenergan. The M.P.Q. of Anthisan is about 7 and of Antistin about 15.

(4) Relative durations of action are expressed in terms of the "Mean Duration Quotient", obtained by dividing the time from maximum to half-maximum action for Phenergan by the corresponding time for an approximately equi-effective dose of the drug under test. This expresses the half-action time for Phenergan as a multiple of the half-action time of the drug with which it is compared. The M.D.Q. of Anthisan is 3·8 and of Antistin 5·6.

(5) Relative therapeutic efficacies in chronic urticaria may be expressed in terms of the "Mean Therapeutic Quotient", obtained by dividing the daily dose of the drug under test by the daily dose of Phenergan required to produce an equal therapeutic response. This expresses the therapeutic potency of Phenergan as a multiple of the potency of the drug with which it is compared, or states by how much the daily dose of Phenergan must be multiplied to give the equivalent daily dose of the other drug. The M.T.Q. of Anthisan in chronic urticaria is about 14.

(6) A possible qualitative difference between Phenergan and the other drugs is noted, and some aspects of the probable mode of action of histamine antagonists are briefly discussed.

The cost of this work was met by a grant from the Medical Research Council.

REFERENCES

ACHARI, G., BAIN, W. A., BROADBENT, J. L., and ROBINSON, M. (1948) Communication to British Pharmacological Society, July.

BAIN, W. A., BROADBENT, J. L., ROBINSON, M., and WARIN, R. P. (1948) Communications to British Pharmacological Society, July.

——, ——, and WARIN, R. P. (1949) *Lancet* (ii), 47.

——, HELLIER, F. F., and WARIN, R. P. (1948) *Lancet* (ii), 964.

DALE, H. H. (1948) *Brit. med. J.* (ii), 281.

GADDUM, J. H. (1948) *Brit. med. J.* (i) 867.

HALPERN, B. N. (1948) *in* Acquisitions Médicales Récentes. Flammarion, Paris, p. 251.

HUNTER, R. B., and DUNLOP, D. M. (1948) *Quart. J. Med.*, **17**, 271.

KAPELLER-ADLER, R. (1949) *Biochem. J.*, **44**, 70.

IV.

The Evaluation of Drugs in Man, with Special Reference to Antihistaminics

Reprinted from THE ANALYST, *the Journal of the Society of Public Analysts and Other Analytical Chemists, October,* 1951, *Vol.* 76, *No.* 907, *pp.* 573-579

Reprinted from THE ANALYST, *the Journal of the Society of Public Analysts and Other Analytical Chemists, October,* 1951, *Vol.* 76, *No.* 907, *pp.* 573-579

The Evaluation of Drugs in Man, with Special Reference to Antihistaminics

BY W. A. BAIN

THE evaluation of drugs in man involves essentially the application to man of those principles of quantitative pharmacological investigation that have hitherto been applied chiefly to biological assay and standardisation procedures on animals and isolated animal tissues. It was, indeed, largely for the solution of the problems of biological standardisation that these methods were first developed. In general they are methods that permit, among other things, the establishment of dosage - response relationships.

The pharmacologist has two different types of data that can be used for the determination of such relationships. The first is got by measuring the degree of effect produced by a drug (graded or quantitative response), the second by noting the presence or absence of a drug effect ("all or none" or quantal response). Both are important. The quantitative method is capable of yielding much more information than the other and is preferable when it can be applied. It has the further advantage that, if the same subjects are used for all the comparisons, only small numbers need be used. The quantal method, besides yielding less information, involves larger numbers of subjects. In man, however, because of subjective or other effects that can be arbitrarily graded, responses that would be quantal in animals can often be made to yield data that are essentially quantitative and can be dealt with as such. Further, there is usually more than one effect or manifestation of a particular drug that can be recognised and may be of importance in man: consequently, even when the different effects are strictly "all or none," there is more information to be extracted from the quantal type of response in man than from the corresponding type in animals.

It seems that the future advancement both of human pharmacology itself and of rational pharmacotherapeutics must depend largely on how far such methods can be developed and applied to the investigation of drug actions in man. The extent to which they can be developed will itself depend largely on the success achieved in recognising or establishing measurable criteria of the effects of the drug under test.

Within a class of drugs the short-term evaluation in man should involve not only the determination of relative weight for weight potencies when the drugs are administered by their usual routes, but also the determination of relative rates of onset and duration of action and the relative immediate toxicities. This pharmacological evaluation can sometimes be

carried out on normal people, but at other times only on patients suffering from some particular condition. In either event, since the object of obtaining the pharmacological information is to make possible its rational application in pharmacotherapeutics, a therapeutic evaluation of the most promising drugs of the class should succeed the pharmacological one. Often, however, the pharmacological evaluation on patients will constitute, by its nature, the short-term therapeutic evaluation as well.

Quantitative comparisons of drugs in man are subject to the same variables as are comparisons in animals and animal tissues, but additional difficulties and limiting factors immediately present themselves. Thus, in obtaining dosage - response data in animal experiments, an objective record of the whole effect of a given dose can often be obtained, leaving no doubt about the maximum effect. Even where no objective record is possible, the number of observations yielding the required data for a given dose can be made sufficiently great to give a smooth curve of response. Such curves are clearly necessary for dosage - response data in man and to determine differences in duration of action of different drugs, but continuous and objective records of the drug effect can seldom be obtained, and the number of observations that can be made to determine the rise and fall of the effect is limited. Further, the dose-range used in animals can be as great as desired, but is necessarily limited in man. The element of personal danger is never entirely absent in human experiments, and particular caution has to be exercised in investigating new or hitherto untried compounds. Finally, since only small numbers of subjects are usually available for such tests, all comparisons have to be made on the same people, who, if the experiments are numerous and of long duration, begin, sooner or later, to find them exceedingly tedious.

The illustrations in this paper are simple examples of the application of a graded response method to the comparison of antihistaminics in man.

The antihistaminics are drugs of varied structure, whose most important action is to antagonise, presumably by receptor competition, certain of the actions of histamine. Most antihistaminics have other actions, such as the hyoscine-like effects (both central and peripheral) seen on systemic administration and the anaesthetic effects produced by local application to suitable sites and lesions. The hyoscine-like effects, while sometimes useful therapeutically, more often constitute the most undesirable side-action of the drugs.

Since histamine itself, injected into the skin, gives characteristic effects—the wheal and the flare—that can be measured, the quantitative modification of either or both of these effects by antihistaminics can be used as the basis for an evaluation and comparison of different antihistaminic drugs in man.

The techniques used in our original experiments have been published[1,2] and need not be detailed here. Three drugs, promethazine, mepyramine and antazoline, were compared for relative potencies and durations of action, and the first two for their therapeutic efficacies. Comparisons of potency were made by determining, for each drug, under standard conditions, the maximum reduction of the intradermal histamine response in each of a group of six subjects to three different oral doses of the drug. The average maximum response to each dose of each drug was expressed as the average percentage reduction of wheal area. As the dose - response curves for the three drugs were parallel, a simple ratio expressed the potency differences. Promethazine was about seven times more potent, weight for weight, than mepyramine and about fifteen times more potent than antazoline.

Relative durations of action were determined by following the reduction of the effect of an intradermal dose of histamine, usually 3 μg, to and beyond its maximum after administering approximately equi-effective doses of the different antihistamine drugs to another group of subjects. Relative durations of action were expressed as the average times taken for the full effect of the drug to be reduced to half. For promethazine, mepyramine and antazoline these times were $19\frac{1}{2}$, $5\frac{1}{6}$ and $3\frac{1}{2}$ hours respectively. A therapeutic comparison of promethazine and mepyramine was then carried out on 20 patients with chronic urticaria. All these had first been treated with mepyramine and the daily dose to control the urticaria was determined. They were then taken off all therapy and, when the reactions to intradermal histamine had returned to normal, treated with promethazine. This drug, in view of its long duration of action, was given in a single dose at night, the dose being adjusted to give as nearly as possible the same effect as the mepyramine in the earlier period of treatment. The results showed that promethazine was, on the average, about fourteen times more potent therapeutically than mepyramine. In the pharmacological tests it was about seven times more potent. It is clearly to the prolonged duration of action of promethazine that this

difference between the pharmacological and therapeutic potencies was due. A further point brought out by this comparison was that the effective dose of promethazine produced fewer side reactions than the corresponding dose of mepyramine, so that 14 of the 20 patients preferred the promethazine treatment.

These first comparisons of potency and duration of action were made in separate experiments on different groups of people. The necessity for reducing the total number of observations to a minimum has led us to modify this procedure,[3] and now all the necessary

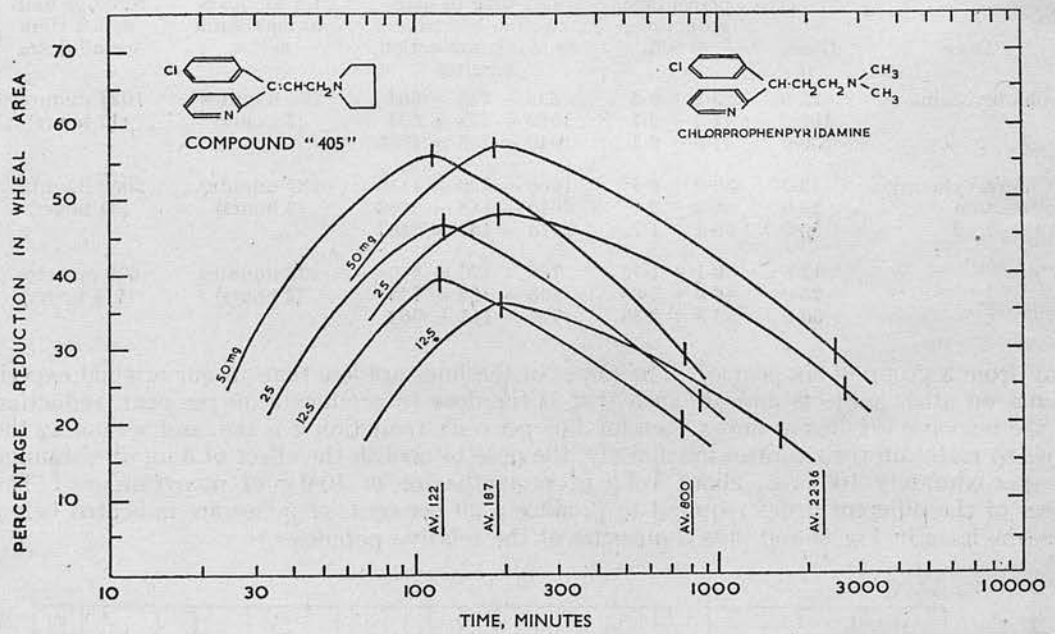

Fig. 1. Graphical representation of time - response data for compound "405" and chlorprophenpyridamine. Left-hand family of curves, effects of 12·5, 25 and 50 mg of "405"; right-hand curves, 12·5, 25 and 50 mg of chlorprophenpyridamine. Points of maximum action and of half-action are indicated on the graphs

information is obtained simultaneously from the same subjects. Each subject receives duplicate or triplicate intracutaneous injections of 3·0 μg of histamine, estimated as base, in 0·05 ml, and the resulting wheal areas are recorded 5 to 7 minutes later. The antihistaminic is then taken on an empty stomach. The test dose of histamine is injected half-hourly until the maximum effect has been reached, and then hourly or at longer intervals, until the effect has obviously passed its half maximum. By plotting the percentage reduction of wheal area against time for each subject, an approximate curve of action for the dose of the drug in each subject is determined. From each such curve is derived an estimate of the maximum effect, the time for the establishment of this and the time for it to be reduced to half. The individual results in the group are averaged. Three different doses of each drug are usually investigated.

Table I gives the main data abstracted from nine experiments of this kind on three different drugs. A graphic representation of the results with two of these, chlorprophenpyridamine, and a compound prepared by Dr. Adamson of the Wellcome Laboratories and known as "405," is shown in Fig. 1. The comparison of these drugs is of particular interest, as they both have the same potency but differ in the rate at which the maximum effect is reached and, more important, in the rate at which it falls to half. Compound "405" thus seems an exception to the general rule that the more potent an antihistaminic the longer its duration of action.

The averages of the individual maximum percentage reductions of wheal area for each dose of an antihistaminic (see Table I) constitute the data from which dose - response relationships are obtained. Fig. 2 shows such relationships for some drugs, derived in this

TABLE I

SUMMARY OF DATA ON RELATIVE POTENCIES AND DURATIONS OF ACTION FOR THREE ANTIHISTAMINICS

Drug	Dose, mg	Average of individual maximum percentage reductions ± S.E.	Times to maximum and to half-maximum action: Average of individual half-action times, *i.e.*, time to half-action less time to maximum action, minutes	Average time for all doses to maximum action	Average half-action time for all doses
Chlorcyclizine	25·0	23·2 ± 2·4	932 − 348 = 584	294 minutes (5 hours)	1024 minutes (17 hours)
	100·0	44·3 ± 2·7	1082 − 278 = 804		
	250·0	57·9 ± 2·3	1940 − 255 = 1685		
Chlorprophenpyridamine	12·5	36·0 ± 4·5	1600 − 190 = 1410	187 minutes (3 hours)	2050 minutes (34 hours)
	25·0	48·3 ± 2·1	2640 − 188 = 2452		
	50·0	56·7 ± 1·7	2470 − 183 = 2287		
"405"	12·5	39·1 ± 1·75	760 − 120 = 640	122 minutes (2 hours)	679 minutes (11½ hours)
	25·0	46·6 ± 2·66	863 − 128 = 735		
	50·0	55·8 ± 2·36	779 − 117 = 662		

way from a group of six people. The slopes of the lines are less than in our original experiments on other subjects and are such that if the dose to produce a 30 per cent. reduction in the response is taken as unity, then for a 65 per cent. reduction it is ten, and, assuming the lines to maintain their courses indefinitely, the dose to abolish the effect of 3 μg of histamine is approximately 100, *i.e.*, about 1·5 g of promethazine or 10·0 g of mepyramine! (The doses of the different drugs required to produce a 30 per cent. response are indicated below the abscissae in Fig. 2 and give a measure of the relative potencies.)

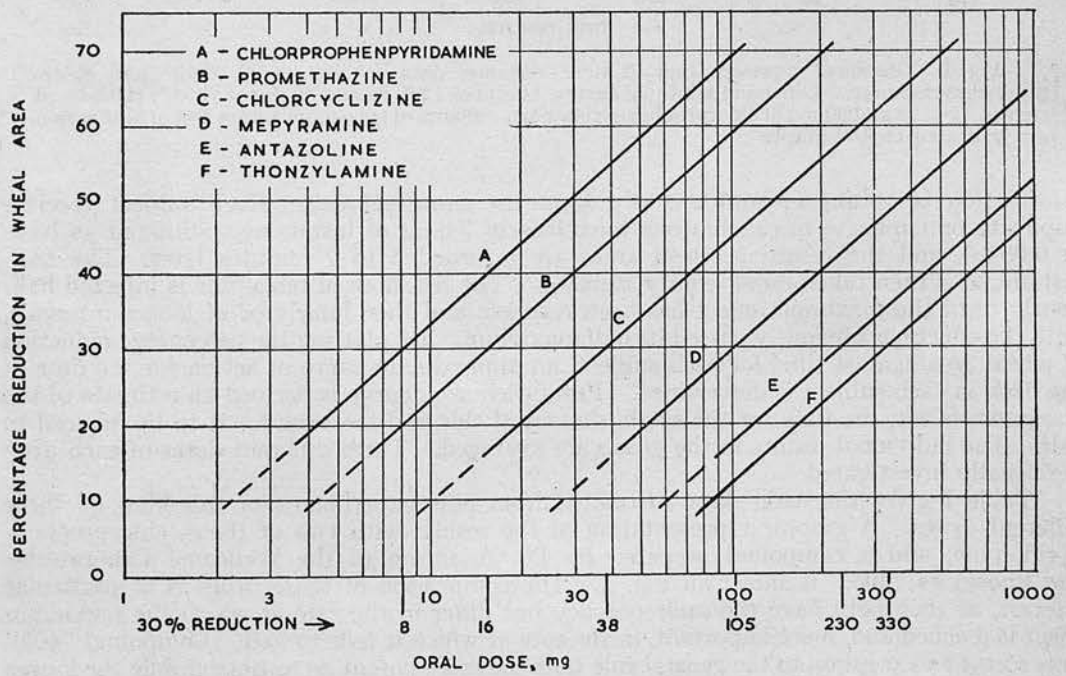

Fig. 2. Dose - response relationships for different antihistaminics. The dose of each producing a 30 per cent. reduction in wheal area is shown below the abscissa; these indicate relative potencies. Thus, taking promethazine as 1, the corresponding doses required to produce the same maximum effect are: chlorprophenpyridamine (and "405") ½, chlorcyclizine 2½, mepyramine 6½, antazoline 14½, thonzylamine 20

It is clearly impossible to abolish the effects of a 3-μg intracutaneous test dose of histamine with any of the antihistaminics at present available. Equally clearly, it is possible to prevent or abolish many of the effects of histamine liberated in the body by doses of these drugs that produce only a moderate reduction in the response to the 3-μg intracutaneous test dose. Clinical experience, indeed, especially with the longer acting drugs, indicates that the symptoms of some allergic conditions, *e.g.*, hay fever, can be controlled by a level of antihistaminic effect represented by no more than a 10 or 15 per cent. reduction of the 3-μg test wheal. How, then, can these percentage reductions of wheal area be interpreted so as to give a picture of how the antihistaminics produce their therapeutic effects?

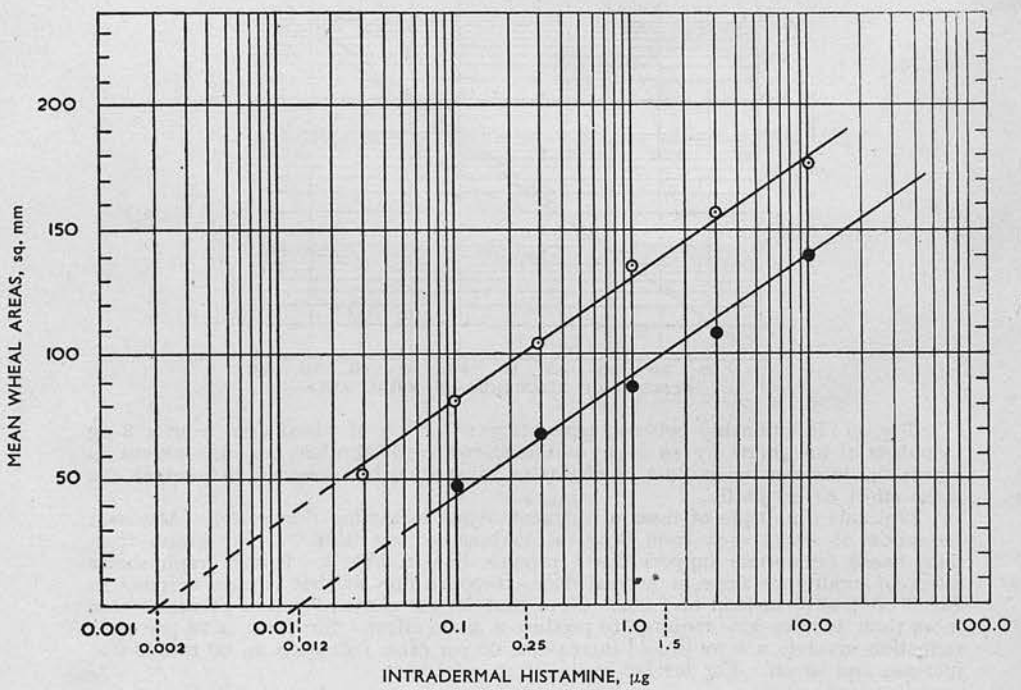

Fig. 3. Shift of dose - response curve for intracutaneous histamine by an antihistaminic. Experiment on five subjects. Upper line before and lower line after dosage of antihistaminic.
Assuming the regressions are parallel, the shift shown, representing a 25 per cent. reduction of the 3-μg test wheal, involves a six-fold increase in the dose required to produce any given effect. For further explanation see text

As the percentage reduction of wheal area that we measure is an expression of the shift of the dose - response curve to intracutaneous histamine, the following considerations provide a possible answer. The upper graph in Fig. 3 shows the log-dose - response regression for intracutaneous histamine in five of the subjects used in the foregoing experiments. The lower graph shows the shift of this 3 hours after ingestion of an antihistaminic. If it is assumed that the two regressions are parallel, the shift is such that a six-fold increase in dose is now required to produce the same effect as originally. Now the percentage reduction of wheal area to the 3-μg test dose in this experiment is about 25. Thus a 25 per cent. reduction of wheal area involves a six-fold increase in the dose of intracutaneous histamine required to produce any given original effect within the dose range represented on the graphs. By drawing parallel dose - response regressions through points representing different percentage reductions of the effect at the 3-μg dose level, the relationship between the percentage reduction of wheal area to this test dose, and the extent to which any dose of intracutaneous histamine must be raised to produce its original effect, is immediately established. This relationship, calculated from Fig. 3, is shown by the lower line in Fig. 4. If the slope of the dose - response curve to intracutaneous histamine is shifted slightly, so as to meet the abscissa at 0·002 instead of 0·0015 μg, the upper line of Fig. 4 results. Thus, though the exact relationship will depend on the slope of the dose - response regression, it is obvious that a relatively small percentage reduction in the intracutaneous wheal response represents a considerable

increase in the dose of histamine required to produce any given response, including a threshold response.

Thus it is not difficult to see how the effects of extrinsic histamine liberated in the body may be prevented or abolished by doses of antihistaminics that produce only a moderate reduction in the effect of an intracutaneous test dose, for this relatively small reduction may involve increasing many-fold the threshold for the production of extrinsic histaminic effects.

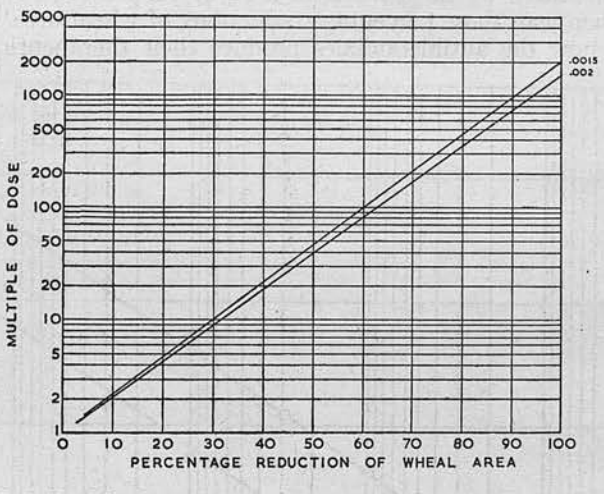

Fig. 4. Relationship between percentage reduction of wheal area from a 3-μg test dose of histamine by an orally administered antihistaminic, and the extent to which the intracutaneous dose of histamine must then be increased to produce the same effect as originally.

Ordinate: multiple of dose of intracutaneous histamine (log scale). Abscissa: reduction of wheal area from 3-μg intracutaneous test dose. Lower graph from data based on normal (upper) dose - response line in Fig. 3. Upper graph shows effect of modifying slope of normal dose - response line so that it cuts abscissa in Fig. 3 at 0·0015 instead of 0·002. Note that 10 per cent. reduction of wheal area more than doubles dose required to produce a given effect. Similarly, a 30 per cent. reduction involves a 9 to 10-fold increase, a 60 per cent. reduction an 80 to 100-fold increase and so on. For further explanation see text

This affords one way of resolving what many clinicians regard as an impossible discrepancy between pharmacological experiments and clinical experience.

In conclusion it should perhaps be pointed out that the methods very briefly outlined here, while probably capable of development to the same degree of precision as other quantitative biological methods, have been used simply as a better means than any other available of obtaining comparative information about antihistaminics in man. But no undue stress should be placed on the numerical results. Nor must it ever be forgotten how wide is the individual variation in response to these drugs[4] and how necessary it is, in their clinical use, to take account of this.

References

1. Bain, W. A., Broadbent, J. L., and Warin, R. P., *Lancet*, 1949, **ii**, 47.
2. Bain, W. A., *Proc. Roy. Soc. Med.*, 1949, **42**, 615.
3. Bain, W. A., Batty, J. E., and Broadbent, J. L., 1951, Communication to British Pharmacological Society, Jan., 1951.
4. Bain, W. A., Hellier, F. F., and Warin, R. P., *Lancet*, 1948, **ii**, 964.

V.a

TRIALS OF ANTIHISTAMINICS

Reprinted from the BRITISH MEDICAL JOURNAL
August 9, 1952, *vol. ii, p.* 337

Reprinted from the BRITISH MEDICAL JOURNAL
August 9, 1952, *vol. ii, p.* 337

TRIALS OF ANTIHISTAMINICS

[*To the* EDITOR *of the* BRITISH MEDICAL JOURNAL]

SIR,—Like Dr. H. Herxheimer (July 26, p. 228) I have a little experience with antihistaminics and, like him, too, thought it unfortunate that so much ingenuity and time should have been spent on a project (Sevitt *et al.*, July 12, p. 57) that did not and could not decide the question at issue. But I would like to go further than Dr. Herxheimer and make some remarks of a general kind about trials of antihistaminics—remarks that seem the more called for by reason of the very wide range of potencies possessed by the different drugs in this group, and the seeming unwillingness of people to take account of this.

It is, of course, desirable that any therapeutic trial should be well designed and executed and the data properly assessed and interpreted. While much more care is usually given to these matters now than formerly, there are two defects that may sometimes be evident in otherwise irreproachable trials. The first is a failure to state precisely the question that the trial is set to answer. The second is that the answer itself—especially if it is a negative one—instead of being set forth as a particular proposition, is often given a general form that is quite unwarranted. (And even if the investigator makes neither of these mistakes he may be misquoted by others and so made to seem guilty of one or both of them.)

Thus in a trial with a given antihistaminic a very relevant part of the question that should be posed at the outset is : " Does drug X (in dose *y*, *n* times a day) affect the course of the condition ? " And the most that the answer could show would be whether this seemed to be so or not. To generalize from the outcome of a single trial with one drug to the class of antihistaminics as a whole is of questionable validity when the results are highly favourable, but utterly indefensible if the drug seems to be of no value at all.

In a trial to test whether antihistamine action *per se* is of benefit in any particular condition a primary essential is to make sure that the drug chosen, its dose, and the spacing of this are such as will in fact produce an antihistamine effect (and, one might add, that the effect is maintained and is of adequate degree). There is little doubt that in some otherwise excellent trials this elementary condition has not been fulfilled. When the results of such trials are negative they are valueless, and if expressed in a general form

they are misleading as well. Indeed, the only negative evidence that would seem to justify a general negative conclusion would be that from several trials in each of which it was unquestionable that the drugs had been used in effective doses.

Nor is there much excuse to-day for investigators failing to use effective drugs in effective doses. Pharmacologists have gone to some trouble to determine, in man, the relative potencies and durations of action of different antihistaminics. This information is readily accessible, and it seems a pity that it should so often be ignored—especially when this may lead to the unwitting choice of the weakest available drug for an extensive and painstaking trial.

If antihistaminics are to be tested in a condition not known to respond to them, then, even if the available pharmacological information is appreciated and used, it is surely a good plan to find out by a preliminary or pilot trial whether a large-scale rigorous trial is likely to be worth while. If a small pilot trial, using one or more potent drugs in adequate doses, gives no evidence whatever of any benefit from the treatment, it is probably fair to say that an extensive rigorous trial is not worth while. If, on the other hand, the results of the pilot investigation are obviously favourable, then some of the best conditions applicable to the rigorous trial are already known, and others can be determined by further pilot observations, with consequent saving of time and effort in the design, conduct, and assessment of the main trial. It seems remarkable that pilot trials are not more popular, for their utility and value as a means of getting information that can be used in a large trial are often very great.

These various points may, indeed, be obvious ones; but they all seem, at one time or another, to have escaped the notice of some workers on antihistaminics, and so no apology is needed for drawing attention to them.—I am, etc.,

Department of Pharmacology, Leeds. W. A. BAIN.

V.b

PILOT TRIAL OF AN ANTIHISTAMINIC DRUG IN THE CONTROL OF "TETRYL" DERMATITIS

To be published in the British Journal of Industrial Medicine

PILOT TRIAL OF AN ANTIHISTAMINIC DRUG IN THE CONTROL OF "TETRYL" DERMATITIS

By W. A. Bain and Grizel H. Thomson

(From the Department of Pharmacology, University of Leeds, and a Royal Ordnance Factory)

The compound 2:4:6 trinitrophenylmethylnitramine, known commercially as "Tetryl", and in the services as C.E. (Composition Exploding), is an important intermediate detonating agent for high explosive charges. It has long been known that workers with "tetryl", in addition to becoming yellow about the hands and face, are prone to develop dermatitis and respiratory troubles (Smith, 1916; Cripps, 1917; Ruxton, 1917). When contact with "tetryl" is heavy, and workers are new to the job, the incidence of dermatitis is high - however carefully the workers are selected, and however stringent the application of preventive measures, such as protective clothing, barrier creams and the like. Of new people who go into training for work in heavy contact with "tetryl" only a small proportion completes the course and is available for production: of this a high proportion leaves work at the first onset of symptoms, - or even from fear of the substance and what it may do to them.

Heavy contact is suffered by those who press "tetryl" into pellets. The labour problem presented by this type of work is illustrated by Table I, which shows the turnover of trained pressworkers in a 6-month

period prior to the trial, together with an analysis of the reasons for the high rate of loss.

TABLE I.

LABOUR TURNOVER OF 'TETRYL' PRESSWORKERS IN A 6-MONTH PERIOD PRIOR TO THE TRIAL.

Total trained and entering presswork	49
Number on presswork at end of period	3
Number lost to presswork by end of period	46

Reasons for loss of pressworkers:-

PERSONAL	- staining of skin; fear of illhealth, including dermatitis; incipient dermatitis; other factors -	24
MEDICAL	- removed from presswork by medical officer for dermatitis or other medical reasons -	19
PRODUCTION	- transferred by Production Dept. to other work -	3
		46

It is clear from the table that, apart from transfers to other jobs by the Production Department, workers lost to the presses fall into two main categories. In the first are those who leave, often on very slight provocation, for personal reasons, - because of staining of the skin and clothing, the fear of skin trouble, the onset of symptoms of skin trouble, the respiratory irritation caused by "tetryl" dust, and other factors: these are the people who will not tolerate in peacetime the discomforts that they might

endure or ignore in time of war. In the second category - lost for medical reasons - are those willing to suffer discomfort, often of considerable degree, and who are only removed from heavy contact on the direction of the medical officer.

This high wastage of labour makes it difficult to maintain an adequate number of pressworkers. Any safe and effective means of treating or preventing the skin condition would not only reduce the losses from dermatitis itself but would be likely to lessen or abolish the fear of it and thus lower the losses from "personal" reasons.

Without going into detail it may be said that the dermatitis develops after 8 - 15 days' contact with "tetryl", and that people who have recovered from an attack can often return to a lesser, or, less commonly, to the same degree of contact, without developing further symptoms. Such are said to have "salted". Those who do not develop dermatitis at all - some ten per cent or less of those in heavy contact - are said to be "naturally immune" or "naturally salted".

Theoretical basis of trial.

As the premonitory symptoms are usually either itching of the face and neck - progressing to erythema and urticaria - or rhinitis with respiratory discomfort suggestive of mild asthma, it looked as if the onset of the condition was associated with the release of histamine. The fully developed dermatitis might thus be

the end result of a process in which such release played an important part. It might result from a sensitisation reaction to "tetryl" such as has been suggested by Gell (1944); or, alternatively, from "tetryl" acting as a simple histamine liberator. In either event it seemed possible that if adequate doses of a suitable antihistaminic were given by mouth, at a sufficiently early stage, the symptoms or signs might be modified or suppressed and "salting" occur - whether due to desensitisation in the strict sense, or to depletion of skin histamine, or both, - without the development of a frank and disabling dermatitis.

In view of the accident risk in work with "tetryl", and the known side-effects of antihistaminics that might well aggravate this, it was clear that before any large-scale controlled trial could be justified a preliminary or pilot trial would have to be conducted, in which the medical officer in charge knew exactly what was being given, and in what dose, and could be on the look-out for the sort of actions that might lower the standard of safety precautions. A pilot trial would show not only whether, on the basis of the therapeutic results, a large-scale trial was likely to be worth while, but it would also provide information of various kinds that would be useful in the design and conduct of a rigorous trial - as, for example, the drugs, and the doses of these, likely to be both safe and effective; the most satisfactory stage at which

to begin therapy; how long treatment should continue; and so on. It is such a pilot trial that is described here, in the belief that it affords a case for a rigorous controlled trial.

Plan and Conduct of Trial

Because of the small numbers available for test and the necessity of getting as much information as possible in the shortest time, - and for other reasons that need not be detailed - simultaneous controls were ruled out. It was therefore necessary to compare the results of the new treatment with those got by orthodox ("standard") methods in a period immediately preceding the trial. This defect in design is not so serious as it might seem, for a great deal of information was available about the incidence of dermatitis in press-workers and the results of "standard" treatment, so that if the results of the new treatment did not show a dramatic improvement over those of the old then the matter would obviously not be worth pursuing. A further defect of the trial, but a necessary one in view of the information wanted, was that the experimental treatment was started at various stages in different groups of subjects - sometimes when dermatitis was evident, and even severe; sometimes when only itching or erythema was present; and sometimes at the stage - usually preceding the itching - when respiratory trouble was first noticed. In the analysis of the results these differences have been ignored, though

the results themselves made it evident, as was to be expected, that the earlier treatment was started the better.

A careful and full record of the history and progress of each subject was kept. This involved the day to day recording of all information that could conceivably be relevant to the assessment and interpretation of the results. The collection of this information, by the medical and the training or production staff, was an onerous task; but, since no indication of how the data would be treated was divulged until after the end of the trial there was thus introduced into the final grouping of results an element of objectivity that might otherwise have been lacking. Though the records of the trial are extensive and complex the results are presented here in as simple a way as possible.

On all grounds it seemed desirable to use a powerful and long-acting drug in doses as low as were likely to be effective. A single dose at night was regarded as the ideal, with, if necessary, a smaller "boosting" dose of the same drug in the morning. In this way the maximum antihistaminic level would be reached during the night, and side effects be absent or minimal the next day (Bain et al., 1949). But should the antihistamine effect fall too low during the day it would be maintained by the smaller morning dose, which would be unlikely, by itself, to produce side

effects. This method of dosing was essentially a safety precaution, with which it was often possible to dispense once the reactivity of an individual to the drug had been determined.

Two drugs with the necessary potencies and durations of action were available - Promethazine Hydrochloride B.P. and Chlorcyclizine Hydrochloride A.N. (Bain 1949, 1951). But only a few observations were made with Promethazine, and these have not been included here. Chlorcyclizine is dl-1-(p-chlorobenzhydryl)-4-methylpiperazine monohydrochloride, and the preparation used was "Histantin" (B.W. & Co.). The original dosage scheme was 50 mg at night, followed, if necessary, by 25 mg the following morning. In view of the known variation in the response to antihistaminics (Bain et al., 1948) it was appreciated that the doses used would have to be adjusted to individual needs. This was accordingly done when necessary - but only after it was clearly safe to do so. Thus though the doses used were at first small, they were increased, as confidence in the safety of the procedure was established, to a maximum, in one or two cases, of 300 mg per day. It is still possible, of course, that some of the failures in the trial had inadequate doses, or did not take the full amount of the drug provided.

The possibility of having to use ephedrine or amphetamine sulphate to control sleepiness during the first day or two of chlorcyclizine treatment was

envisaged from the start, but this was seldom necessary: these sympathomimetic drugs, however, seemed more effective than the antihistaminic in controlling the respiratory symptoms associated with or premonitory to the dermatitis, and so were sometimes used along with the antihistaminic mainly for this purpose. Though this constituted a further defect in the trial it afforded information of some interest for subsequent work.

Before detailing the results it is desirable to describe the "standard" treatment used in the "control" group. This was started as soon as symptoms or signs developed, but most people who required it had to be removed from contact with "tetryl", since otherwise the dermatitis progressed. Preparations for local application to the affected areas were given. For erythema, papular rashes, and all cases of oedema without vesiculation, this was a calamine and lead lotion:-

Calamine Lotion	96
Strong Solution of Lead Subacetate	4

For all cases with exudation, or marked vesiculation, the use of this was preceded by the application of a lead, zinc and starch lotion:-

Strong Solution of Lead Subacetate	5
Zinc Oxide	2 5
Starch	2 5
Glycerine	1 25
Water	to 100

For subacute erythema, and during the recovery stage following the use of the calamine or starch lotion, a special calamine cream was used:-

Calamine	3 5
Kaolin	8
Glycerine	16
Emulsifying Wax	9
Perfume	q.s.
Solution of Bordeaux B.	q.s.
Water	63 5,

or a special oily calamine lotion:-

Calamine	5	5
Liquid Paraffin	50	
Oleic Acid		
Wool Fat	1	
Solution of Calcium Hydroxide to	100	

When sleep was disturbed by itching, Phenobarbitone (65 mg) was given at night.

Very severe cases of oedema, with or without vesiculation, were retained at rest in the surgery, and the affected areas were treated first with saline compresses, and then with the calamine and lead, or the lead, zinc and starch lotion.

These local applications were not witheld from patients in the antihistamine treated group if they wished to use them, or if the medical officer thought their use in any particular instance was desirable. But nobody on antihistaminic treatment was given phenobarbitone.

RESULTS

In the course of the trial twenty-eight patients had the antihistamine treatment and sixteen the "standard" treatment.

When a medical criterion of success or failure of treatment is used, the results are distributed as in Table II. The successes in this table are the people actually or potentially available for continued work in heavy contact with "tetryl". The failures are those who relapsed on return to presswork and so were removed from heavy contact by the medical officer. There were thus twenty-six successes out of twenty-eight in the antihistamine treated group, but only four out of sixteen in the "standard" treatment group.

TABLE II.

RESULTS OF TREATMENT ASSESSED ON A MEDICAL CRITERION:

(Successes are people actually engaged on, or potentially available for, presswork after treatment. Failures are people who relapsed on return to presswork.)

	Antihistaminic Treatment	Standard Treatment
Successes	26	4
Failures	2	12

Or, alternatively, of thirty successes from a medical point of view, twenty-six had received the new treatment and four the old; whereas of the fourteen failures only two had received the new treatment and twelve the old. The probability that such differences would occur by chance, in a rigorous experiment with random allocation of subjects to control and experimental groups respectively, is less than 0.001.

As there is a labour and production problem deriving directly from the medical or therapeutic problem presented by "tetryl" dermatitis, it was of interest to assess the results entirely on a labour and production criterion. This is done in Table III, in which the first row of the preceding table is modified, by the subtraction of losses for personal or production reasons, or for medical reasons other than "tetryl" dermatitis, and so includes only those medical successes, in each treatment group, who

continued to work as pressmen. The lower row remains unchanged as it includes only the medical failures who, by definition, could not continue to act as pressmen. Thus of twenty-one men continuing at work on the presses, nineteen had received the new treatment and two the old, whereas of fourteen who could not, for medical reasons, return to the presses, only two had received the new treatment and twelve the old.

TABLE III.

RESULTS OF TREATMENT ASSESSED ON A PRODUCTION CRITERION

	Antihistaminic Treatment	Standard Treatment
Continuing work as pressmen	19	2
Unable to continue work as pressmen	2	12

As before, the probability of such differences arising by chance in a rigorous experiment is less than 0.001.

(Twelve further patients were treated under pilot trial conditions subsequent to the trial period. Two of these were failures. If the twelve results are added to the first column of Table II the figures there become 36 and 4 respectively. These forty people will be referred to later.)

Though the aim of the pilot trial was to get evidence that would show whether a rigorous trial was likely to be worth while, the results set forth in Tables II and III might seem so overwhelmingly in favour of the antihistamine therapy as to make any

further trial superfluous. But it cannot be sufficiently stressed that the pilot trial suffered, by its very nature, and by some of the conditions and limitations necessarily imposed upon it, from defects of design and conduct, and from the liklihood of bias in the assessment and interpretation of the results, such as would make it quite unjustifiable to attribute the observed differences between the groups as due solely, or even mainly, to the differences in treatment. One factor, for example, that may have biassed the results in favour of antihistamine therapy was the greatly increased attention given by the medical officer to the people receiving this. And though those in the earlier control group, on "standard" treatment, had also an unusual amount of attention, this was not so great as in the antihistamine group, - nor was it reinforced by the swallowing of tablets.

While only a rigorous trial, with random allocation of subjects to "drug" and "control" groups respectively, could give reliable information on the contribution made by the drug itself, there can be no doubt that, in the circumstances of the present trial, people fared very much better with the new treatment than with the old - whatever factors may have been responsible for the difference.

If, however, the antihistamine therapy is as advantageous as the four-fold tables seem to indicate, then one would expect other evidence of this to have

been revealed by the trial. This was so. The first important advantage was that while people had usually to be removed from contact with "tetryl" at an early stage of the "standard" treatment - for otherwise the dermatitis progressed - they could usually remain in full contact for the whole period of antihistaminic treatment - with consequent benefit to the worker in earnings, and to production in output and costs. The second main advantage was the speed with which the new treatment controlled symptoms and signs. Some of these differences are indicated, for comparable groups of subjects, in Table IV.

The first column of the table describes each group of subjects: successes are those maintained in or returned to full contact; failures are those who relapsed on return to contact. The other columns, in order and for each group, give:- the average maximum degree of dermatitis experienced; the average number of days to control symptoms and signs respectively; the average daily dose of chlorcyclizine from the beginning of treatment to the clearing of symptoms or signs, whichever took the longer. Numerals in brackets indicate range of values. Further points of explanation are these:-

Degree of dermatitis. This was assessed, for each person, on the following five-point basis:

1. Erythema and itching.
2. Papular erythema, with or without itching.
3. Oedema or vesiculation.
4. Oedema plus vesiculation.
5. Marked oedema, vesiculation and exudation.

People in stages 1 and 2 are liable to leave work for "personal" reasons. Stage 3 is that before which a keen worker would probably not report to the medical department.

Average daily dose. This is calculated for each group from the average individual daily doses - that is, from the total drug ingested by each person, divided by the total treatment days - and gives no

TABLE IV

TREATMENT DAYS TO CLEAR SYMPTOMS AND SIGNS IN DIFFERENT GROUPS OF PEOPLE

(For full explanation see text)

Group	Av. degree of dermatitis.	Av. treatment days to clear:-		Av. daily dose of drug (mg)
		Symptoms	Signs	
Standard treatment out of contact. (15 patients - 1 success, 2 potential successes, 12 failures.)	3.2 (1 - 4.5)	17 (2 - 39)	18 (2 - 39)	nil
Antihistaminic treatment in contact. (15 patients - all successes.)	2.7 (1 - 4)	1.5 (0 - 6)	5 (1 - 16)	105 (25 - 175)
Antihistaminic treatment out of contact.				
a) Failures (4 patients)	4.6 (4 - 5)	9.5 (1 - 15)	12 (9 - 17)	110 (20 - 175)
b) Successes (4 patients)	4.5 (4 - 5)	2.5 (0.5 - 4)	7 (4 - 10)	110 (95 - 145)

indication of how the dose varied in individuals. But it may be said that while at the beginning of the trial, and at the start of treatment in each person, the dose was usually small, (25-50 mg), it was increased if need be up to as much as 300 mg per day in some cases.

The first row of Table Iv gives the data on fifteen patients who had "standard" treatment out of contact with "tetryl", and only one of whom was successfully returned to full contact. (Two "potential" successes in this group were transferred to other work.) The second row gives the corresponding information on fifteen patients who developed dermatitis of, on the average, slightly lesser degree than did the control series, but who had the new treatment while still in full contact and who were all subsequently maintained at presswork. While "standard" treatment out of contact cleared symptoms in seventeen days and signs in eighteen, the new treatment, - despite the continued contact with the offending agent, - cleared symptoms in under two days and signs in five. This is a remarkable difference between the two groups and affords additional presumptive evidence in favour of the chlorcyclizine therapy.

Of the forty people treated under pilot trial conditions, - twenty-eight in the trial itself, and twelve subsequently, - dermatitis was sufficiently severe in eight of them to demand treatment out of contact. Four were classified as failures and four were successfully returned to full contact. The last two rows of Table IV show the contrast between

these groups. The failures, though with more severe dermatitis than the control group with "standard" treatment, responded more rapidly, and were clear of symptoms in under ten days as against seventeen, and of signs in twelve as against eighteen. But the four successfully treated out of contact were free of symptoms in two and a half days and of signs in seven. Whether the failures might have been avoided by using larger doses of chlorcyclizine - or whether, indeed, these patients took all the drug supplied - cannot now, of course, be determined. It is noteworthy, however, that, in general, symptoms were controlled much earlier than signs by successful antihistamine therapy - in contrast to what happened with "standard" treatment and, indeed, though less markedly, in the failures with chlorcyclizine.

Altogether forty people who developed dermatitis had the antihistaminic treatment. An analysis of the position of these forty people three months after the end of the trial period is shown in Table V, which is similar to Table I and with which it should be compared. Of the nineteen people on presswork in Table III, six had by now been transferred by production and one had left for "personal" reasons, leaving twelve still at work when Table V was compiled. To these were added seven of the twelve later cases still at work, thus accounting for the nineteen pressworkers of Table V. In Table I the corresponding number is 3;

TABLE V.

LABOUR TURNOVER OF 'TETRYL' PRESSWORKERS TREATED FOR DERMATITIS WITH AN ANTIHISTAMINIC

Total entering presswork and developing dermatitis	40
Number on presswork at end of period	19
Number lost to presswork by end of period	21
Reasons for loss of pressworkers:-	
PERSONAL - discontent -	7
MEDICAL - dermatitis (4), other medical reasons (4)	8
PRODUCTION - moved to other jobs -	6
	21

---

but none of these three pressmen ever developed dermatitis - all were "naturally immune." Thus, as far as treatment results in relation to production are concerned, none out of forty-nine people were successfully treated and working as pressmen at the end of the "standard" treatment period, as against nineteen out of forty successfully treated and working as pressmen three months after the end of the antihistaminic treatment period. Other points are evident from the Table and need not be detailed.

While caution must be exercised in drawing conclusions from the marked differences between these two seemingly comparable groups, the differences are certainly suggestive, and can be taken as giving

additional evidence in favour of some further investigation of chlorcyclizine therapy.

Conclusion

Without committing ourselves to acceptance of any one of the several possible views concerning the mechanisms responsible for the skin reactions to "tetryl", or to whether the seemingly beneficial effects of the new treatment are to be accounted for by the chlorcyclizine - and, if so, to its antihistaminic action per se or not - all the results obtained seem to afford strong presumptive evidence in favour of the chlorcyclizine as against the "standard" treatment and constitute, therefore, a strong case in favour of a rigorous controlled trial.

Such a trial was accordingly designed, on the same lines as the large-scale M.R.C. trial of an antihistaminic in the common cold (Report of M.R.C. Committee, 1950), but with the important difference that the drug would be ingested in the presence of the medical officer and that the dose would be varied, up to a maximum of 350 mg per day, in accordance with the response of the patient. The trial was, in fact, started. But it came to an abrupt stop after only twelve people had entered it, for by then the labour problem had been solved - chiefly by the successes in the pilot trial - and as no further trainees were entering presswork no more subjects were available to us. This position has now been maintained for several months, thus not only postponing the chances of continuing the rigorous trial, but also, perhaps, raising the question of whether, on ethical grounds, its continuance would now be justified! It would be a pity, however, if it could not be continued, for, if the impressions already given by the pilot trial could be confirmed, the considerations that would then apply to "tetryl" dermatitis might prove to be of more general applicability, and might lead the way to a better understanding of the nature, and hence the possibility of control, of some other forms of industrial dermatitis, and even have important repercussions in a much wider field as well.

SUMMARY

The medical and labour problems presented by "tetryl" dermatitis, in press workers in heavy contact with the substance, are indicated.

As "tolerance" or "hardening" to the skin effects of "tetryl" commonly follows an attack of dermatitis, a solution to both problems would be afforded by a safe treatment that either shortened the period of disability from dermatitis, or enabled tolerance to be acquired, without the development of marked signs or symptoms, by men at work.

Since the primary manifestations of skin injury by "tetryl" are suggestive of histamine release, it seemed possible that antihistaminic drugs might serve these purposes. But before considering a rigorous trial to to test this view it was necessary to see from preliminary or pilot observations whether such a trial was likely to be worth while and, if it seemed to be so, to get further information for use in the rigorous trial.

A pilot trial with the drug chlorcyclizine hydrochloride ("Histantin" B.W. & Co.) is described. The results give ample justification for a rigorous trial, and provide much information that would be of use in it.

ACKNOWLEDGMENTS

We wish to thank Mr J.E. Jackson, O.B.E., Superintendent at a Royal Ordnance Factory, for the facilities he afforded us; Dr N. Langdon Lloyd and Dr A.D.K. Peters, O.B.E., of the Ministry of Supply, for their interest in the work; Dr F. Prescott, of the Wellcome Research Institution, for supplies of chlorcyclizine; the Medical Research Council for an expenses grant to one of us, and the Ministry of Supply for permission to publish this paper.

REFERENCES

Bain, W.A. (1949). The quantitative comparison of histamine antagonists in man. Proc. R. Soc. Med., 42, 615.

Bain, W.A. (1951). The evaluation of drugs in man, with special reference to antihistaminics. Analyst, 76, 573.

Bain, W.A., Broadbent, J.L. and Warin, R.P. (1949). Comparison of "Anthisan" and "Phenergan" as histamine antagonists. Lancet, ii, 47.

Bain, W.A., Hellier, F.F. and Warin, R.P. (1948). Some aspects of the action of histamine antagonists. Lancet, ii, 964.

Cripps, Lucy (1917). The properties of tetryl. Brit. J. Derm. and Syph., 29, 3.

Gell, P.G.H. (1944). Sensitisation to "tetryl". Brit. J. exp. Path., 25, 174.

M.R.C. (1950). Clinical trials of antihistaminic drugs in the prevention and treatment of the common cold. Brit. med. J., ii, 425.

Ruxton, W.L. (1917). An investigation into the cause and prevention of industrial diseases due to tetryl. Brit. J. Derm. and Syph., 29, 18.

Smith, Enid (1916). The prevention, symptoms and treatment of tetryl dermatitis. Brit. med. J., i, 618.

PART II

ADDITIONAL PAPERS

VI.

THE MODE OF ACTION OF VASODILATOR AND VASO-CONSTRICTOR NERVES.

Reprinted from the QUARTERLY JOURNAL OF EXPERIMENTAL PHYSIOLOGY, VOL. XXIII, 1933.

Reprinted from the QUARTERLY JOURNAL OF EXPERIMENTAL PHYSIOLOGY, VOL. XXIII, 1933.

THE MODE OF ACTION OF VASODILATOR AND VASO-CONSTRICTOR NERVES.

By W. A. BAIN. From the Department of Physiology, University of Edinburgh. (With five figures in the text.)

THAT "iterative" nerves produce their effects not directly but by the production of chemical substances at their terminations, and the subsequent action of these substances on the tissue, is now fairly well established. Direct proof of this has been given by LOEWI (1), and numerous others, in the case of amphibian cardiac nerves; by RIJLANT (2) for mammalian cardiac nerves; and by FINKLEMAN (3) and by CANNON and BACQ (4) for the sympathetic nerves to mammalian plain muscle. Indirect proof that mammalian parasympathetic vasodilator nerves produce their effects by a similar mechanism is afforded by the work of DALE and GADDUM (5).

The present paper gives a preliminary account of an attempt to demonstrate directly the production of specific chemical substances by stimulation of vasomotor nerves, the mode of demonstrating the existence of the substances being by their humoral transmission, from the site of production, to some test tissue.

For these experiments the dog's tongue was chosen. An organ of considerable size, it has a parasympathetic supply to the blood-vessels in the lingual nerve and a sympathetic supply in the vagosympathetic trunk, each of which can be readily isolated for stimulation. In principle the experiments consisted in perfusing one side of the tongue through the lingual artery by means of a Dale-Schuster pump, the perfusate being led from the lingual vein to a bath in which was immersed a short length of rabbit intestine as a test tissue.

METHOD.

A large dog is anæsthetised with chloralose, 0·1 grm. per kilo. A long skin incision in the mid-ventral line, reaching from 2 or 3 cm. behind the symphysis of the lower jaw to 5 or 6 cm. below the larynx, is made. The left common carotid is cleared for as great a distance as possible and strong ligatures placed in position round this. A strong ligature is also placed, ready for securing, round the left external carotid. On the right side the vagosympathetic trunk is cleared for a few centimetres. All the branches of the external carotid on the right side are

now successively identified and ligatured loosely, two ligatures being placed round the lingual artery. The lingual nerve is exposed and cleared as much as possible. Finally the right lingual vein is cleared and double ligatured, and a ligature placed round the anastomosing vein between the linguals of the two sides.

When all is ready, the ligatures round the left external carotid and the branches of the right external carotid are tightly tied, the animal moved to the experimental table and placed in position there. The centremost ligature round the right lingual artery is secured, the artery clipped distal to this, and a cannula attached to the output tube of the Dale-Schuster pump is inserted and secured by the second ligature. The vein is now ligatured and a short glass cannula inserted. The pump being in action, the blood is soon washed from the tongue by the Dale solution used for perfusion and a clear perfusate results. This drops on to the outside of a specially designed glass tube down which it rapidly passes, and so, with the minimum possible loss of time, arrives at the intestine bath. This last is of the type described by SHARPEY-SCHAFER (6) and has a capacity of about 15 c.c.

It should be noted that the output tube of the pump is connected with a mercury manometer as well as with the lingual artery. By this means the pressure of fluid applied to the tongue vessels can be read directly and can be recorded. Further, the rate of the pump can be altered by means of a resistance in the driving motor circuit, while the amount of fluid delivered by the pump can be altered instantaneously by means of an adjustment on the pump itself. Thus the pump can be made to maintain an artificial circulation in the tongue which imitates, in respect of pressure, flow, and frequency, the conditions which are maintained normally in the animal. Also, any change in the resistance to the flow applied through the tongue, whether brought about by spontaneous changes in the calibre of the blood-vessels, by changes imposed by stimulation of nerves, or by mechanical obstruction, will immediately show in the perfusion pressure record, which thus affords a criterion of the conditions obtaining as to the state of the blood-vessels at any given moment.

That the rate of flow of fluid through the tongue was maintained almost unchanged by the pump even when considerable changes in calibre of the tongue vessels were produced by nerve stimulation, is shown by the record of the outflow from the tongue. This was obtained by allowing the perfusate, as it overflowed from the intestine bath, to operate a tilter the movements of which are registered by electromagnetic signal: each mark shows 2 c.c.

From what has been said regarding the preparations for the experiment it is evident that the animal is intended to be alive during the preliminary observations: the purpose of this was to determine whether or not spontaneous changes in the calibre of the blood-vessels, brought

about by changes in the vasomotor centre itself, would produce effects upon the test tissue. This observation having been made, the animal was then usually killed by bleeding, and the lingual and vagosympathetic trunks prepared for stimulation. But sometimes the animal was kept alive during the whole experiment.

Results.

The results are immediately divisible into two categories, negative and positive, the former being always associated with the presence of blood in the perfusate. In no case where the procedures detailed above had been carried out and a bloodless perfusate failed to result were definite effects on the test tissue obtained, either as a result of vasomotor-centre activity or of stimulation of vasomotor nerves. The negative experiments demonstrate that the positive results about to be described are not due simply to changes in temperature of the fluid with which the test tissue is in contact, consequent upon the changed rate of flow through the tongue vessels. Indeed, as is easily shown, such temperature changes are negligible.

Spontaneous Changes in Vasomotor Activity (Traube-Hering Effect).

In the only case where Traube-Hering effects were exhibited coincidently with a blood-free perfusate the result shown in fig. 1 was obtained.

The top line shows the variations in perfusion pressure consequent upon the changes in the calibre of the tongue vessels; the next line shows the movements of the isolated strip of rabbit intestine used as the test tissue; the third line is the record of the outflow from the intestine bath, while the fourth and fifth lines are the signal and time markings respectively.

The animal was alive and had the left common carotid tied off but the right internal carotid patent.

As will be seen from the subsequent tracings, illustrating the results of nerve stimulation, the period between the onset of an effect in the tongue vessels and the appearance of the corresponding effect in the test tissue varies from 30 to 50 seconds.

In the present case about 40 seconds after the commencement of the first rise of perfusion pressure, which rise indicates increased activity of the vasoconstrictor nerves, the amplitude of the intestinal contractions has diminished very considerably. The amplitude then increases as the vasoconstriction passes off; once more diminishes as the perfusion pressure rises, and so on. In other words, the contractions of the intestinal muscle are inhibited by an increased activity of the

sympathetic nerves (and/or decreased activity of the parasympathetic) of the blood-vessels of the tongue, and stimulated by a decrease of

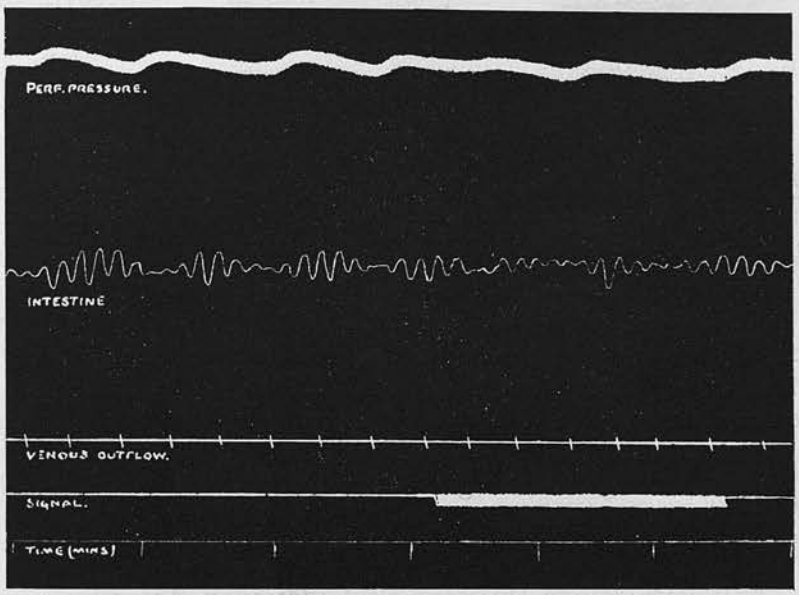

FIG. 1.—Humoral transmission of Traube-Hering effect. Top line, perfusion pressure recorded by Hg manometer in output of Dale-Schuster pump supplying tongue vessels ; second line, movements of isolated strip of rabbit intestine ; third line, output from tongue (recorded as overflow from intestine bath) ; fourth line, signal ; bottom line, time in minutes. (The above applies to all figures except fig. 4, where outflow recorder was not in operation.) For further description see text.

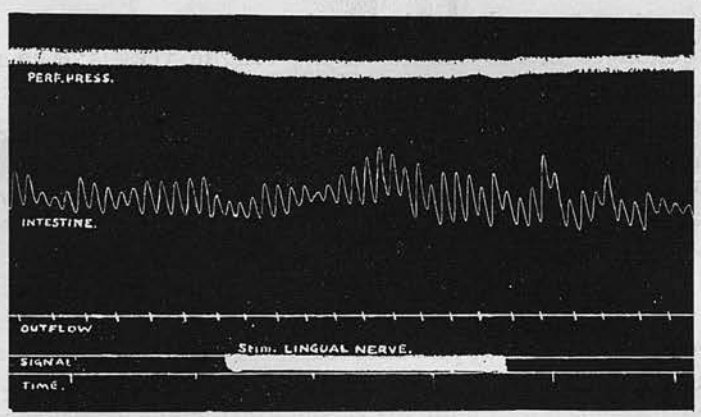

FIG. 2.—Humoral transmission of effect of lingual nerve stimulation. Lingual nerve stimulated during period shown by signal. Note increased tone of intestine strip and increased amplitude of contractions as a result of stimulating the tongue vasodilators. The effect on the tone appears about 30 seconds after commencement of stimulation, and the effect on amplitude of the contractions about 30 seconds later still.

sympathetic (and/or increase of parasympathetic) activity. The effects on the intestine having been humorally transmitted from tongue to test

tissue are thus presumably due to substances liberated in the tongue vessels by the activity of the nerves.

Stimulation of Lingual Nerve.

Fig. 2 is the record of an experiment in which the lingual nerve was stimulated. The perfusion pressure fell only slightly, and the output from the tongue is slightly increased. It can be seen that about 45

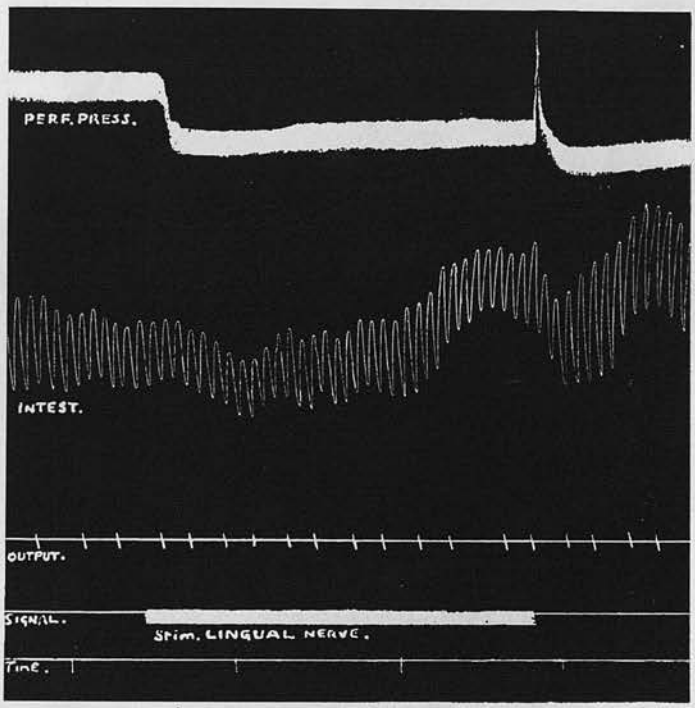

Fig. 3.—Humoral transmission of effect of lingual nerve stimulation. In this case the amplitude of the contractions is not much affected during the stimulation, but the tone of the intestine, after an initial reduction, is considerably increased. The sudden jump in the perfusion pressure record is due to a convulsive movement of the animal.

seconds after stimulation commenced the tone of the intestine rose considerably, and though this fell again towards the end of the period of stimulation the amplitude of the contractions was greater than before. The average amplitude, as measured on the original tracing, was 6 mm. before commencement of stimulation and 10 mm. during the last minute of stimulation. In this case the animal was killed just before the commencement of the experiment.

The next figure (fig. 3) shows the record of a similar experiment on the living animal, where the effect was more delayed and was preceded by a fall in tone of the intestine. In this case the amplitude of the intestinal contractions is not much affected during the stimulation, but

the rise in tone is considerable. Towards the end of the period of stimulation the animal gave a convulsive movement. The perfusion pressure rose momentarily, the intestinal tone fell, rose again, then rapidly returned to normal. The fall in perfusion pressure due to the vasodilatation brought about by stimulation is well marked here, and amounts to 20 mm. Hg.

Stimulation of Vagosympathetic.

In the experiment of which fig. 4 is a record, stimulation of the peripheral end of the vagosympathetic trunk caused an irregular rise

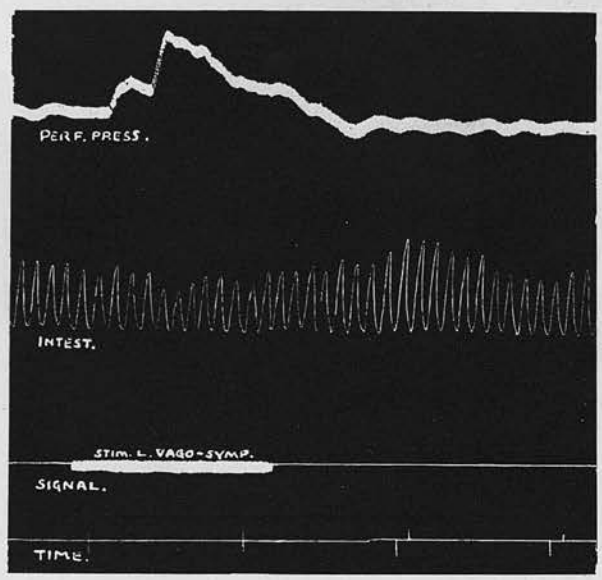

Fig. 4.—Humoral transmission of effect of vagosympathetic stimulation. Note the diminished amplitude of the intestinal contractions as a result of stimulation of the tongue vasoconstrictors. In this case there is, following the stimulation, an after-effect of increased amplitude of contractions. This soon passes off.

of perfusion pressure, the maximum extent of which was 30 mm. Hg. By 40 seconds after the commencement of stimulation the average amplitude of the intestinal contractions had become reduced from 12 to 9 mm. On cessation of stimulation the movements increased in amplitude to an average of 16 mm. and then reverted once more to their original height.

The last figure (fig. 5) is a record of a similar experiment where a certain degree of vasoconstriction is maintained after cessation of the stimulation, and continuance of sympathetic activity is shown in the behaviour of the intestine. The amplitude of the intestinal contractions at the beginning of the record averages 35 mm. This is reduced by sympathetic stimulation of the tongue vessels to a minimum of 18 mm.,

the tone of the intestine falling slightly at the same time. After cessation of stimulation the amplitude averages 27 mm.

While experiments along the line of those described above are being continued, the results here recorded afford some direct evidence in favour of the view that vasomotor nerves produce their effects, like the cardiac

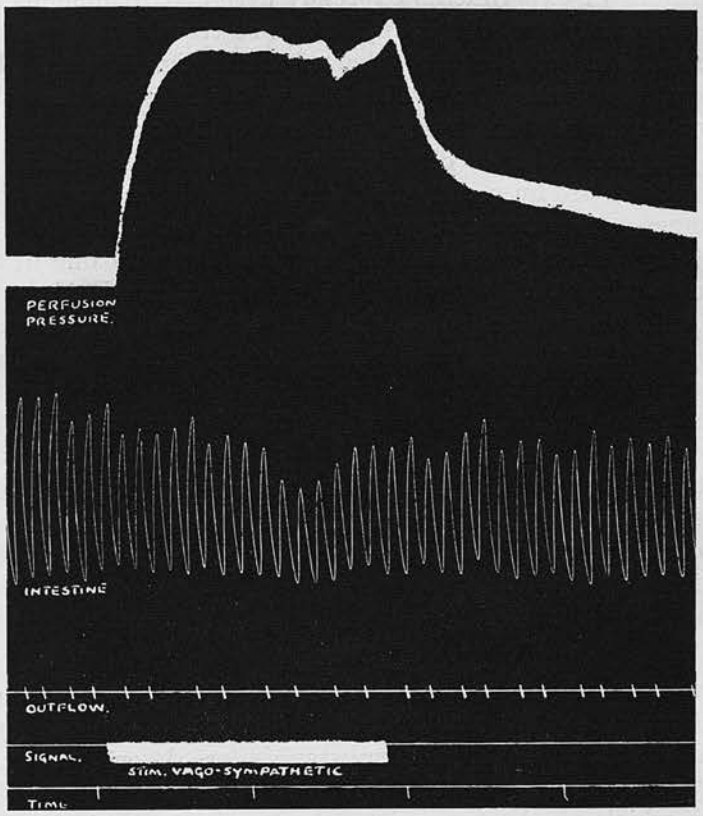

FIG. 5.—Humoral transmission of effect of vagosympathetic stimulation. Here the amplitude of the intestinal contractions is considerably diminished as a result of stimulation of the tongue vessels, and the tone of the muscle is slightly diminished for a short time. The vasoconstriction does not immediately pass off on cessation of stimulation—a further indication that the effect on the tongue vessels is due to a substance produced by the agency of the nerves.

nerves, by the production at their endings of specific chemical substances: vasodilators liberating a substance with actions similar to acetylcholine, and vasoconstrictors a substance with actions similar to adrenaline. Further, the fact that in no case where blood has entered the perfusate in appreciable amount have definitely positive results been obtained, tends to support the view that the substances produced by nerve stimulation are rapidly destroyed by the blood: indeed it may be that the substances are not even passed out of the tissues into the blood at all under normal circumstances.

In any case it seems unfortunate that the humoral transmissibility of the neuromimetic substances has ever been stressed because it has led a large number of physiologists to attach great physiological significance to this transmissibility for which there is no good evidence whatever. The effects of nervous stimulation are humorally transmissible under the conditions of experiments expressly designed to demonstrate this, but it is extremely doubtful if any effects so transmitted under physiological conditions are, or could be, of much significance. The main point about the work of LOEWI and his followers is not that the effects of nervous excitation are transmitted humorally but that these effects are due to production of chemical substances at the nerve endings. That the effects are humorally transmissible is the manner—indeed the only manner—of demonstrating directly that the nerves do act thus, and the very difficulty of such experiments is surely one of the main arguments against such transmissibility being of much, if any, physiological significance when compared with the effects produced by the substances at their site of production. Moreover, a mechanism exists which expressly tends to prevent such transmission being effective even if it takes place—the rapid destruction of the neuromimetic substances by the blood (7).

It is thus most probable that the substances produced by the activity of nerves exert their effects locally at the site of production and that any humoral transmission of these substances is, in normal circumstances, incidental and unimportant.

SUMMARY.

1. Experiments on the perfused tongue of the dog, which demonstrate directly that vasomotor nerves produce their effects by the peripheral liberation of chemical substances, are described.

2. It is shown that stimulation of the vasodilator nerves liberates a substance which can be transmitted humorally to an isolated strip of rabbit intestine and cause augmentation of the tone and/or contractions of this.

3. It is shown similarly that stimulation of the sympathetic supply to the blood-vessels liberates a substance which diminishes the tone and/or contractions of an isolated strip of intestine.

4. An experiment is described in which the effects of spontaneous changes in vasomotor activity (Traube-Hering effect) were humorally transmitted to the test tissue.

5. The effects described are only obtained when the perfusion fluid (Dale's solution) is uncontaminated with blood.

6. It is pointed out that the humoral transmission of the substances responsible for these effects is the method of demonstrating the existence of the neuromimetic substances, and that under normal circumstances such transmission is probably of little significance.

REFERENCES.

(1) Loewi, O., Pflügers Arch., 1921, clxxxix. 239.
(2) Rijlant, P., Compt. rend. soc. biol., 1927, xcvi. 204.
(3) Finkleman, B., Journ. Physiol., 1930, lxx. 145.
(4) Cannon, W. B., and Z. M. Bacq, Amer. Journ. Physiol., 1931, xcvi. 392.
(5) Dale, H. H., and J. H. Gaddum, Journ. Physiol., 1930, lxx. 109.
(6) Sharpey-Schafer, E., "Experimental Physiology," Longmans, Green & Co. (4th ed.), 1927, p. 17.
(7) Loewi, O., and Engelhart, Arch. internat. de pharmacodyn., 1930, xxxviii. 287.

[*From the Proceedings of the Physiological Society*, January 25, 1936.]
Journal Physiology, Vol. 86.

The mode of action of vaso-dilator nerves. By W. A. Bain.
(*From the Physiology Department, University of Leeds.*)

In a previous communication [Bain, 1932] it was shown that in the dog's perfused tongue stimulation of the chorda-lingual nerve conferred upon the perfusion fluid properties which rendered it excitatory to a piece of rabbit's isolated intestine, and it was concluded that these experiments gave direct support to the view that vaso-dilator nerves produce their effects by the production or liberation of a specific acetylcholine-like substance. A similar conclusion was come to independently and almost simultaneously by Feldberg, as a result of experiments on the tongue with its circulation intact [Feldberg, 1933].

This interpretation of our results is, however, open to the criticism [see *e.g.* Gaddum, 1935] that the acetylcholine-like substance may have been liberated not by the vaso-dilator fibres, as we had supposed, but by the secretomotor chorda fibres which are known to supply the numerous glands in the mucosa and submucosa of the tongue.

Experiments have accordingly been made in which the mucous membrane of the tongue has been removed as completely as possible, thus removing at least the greater number of the glandular elements of the organ, with the terminations of the postganglionic fibres which supply them.

The removal is most easily effected by light cauterization of the dorsal and lateral surfaces of the tongue, after which the mucous membrane is simply peeled off. Removal by dissection has also been tried, but this is both tedious and unsatisfactory. The eserinized dorsal muscle of the leech has been used as the test object for the transmitter of the chorda-lingual effects.

Removal of the mucous membrane usually leads to the appearance of an acetylcholine-like substance in the perfusate, and though the amount so liberated gradually disappears, it is often difficult, after the operation, to get a blank control. Nevertheless, a comparison of the results of chorda-lingual stimulation before and after removal of the mucous membrane, makes it apparent that even if, as seems almost certain, part of the active substance is liberated by the secretomotor chorda fibres, the greater amount by far is in fact liberated by the vaso-dilator components of the nerve. Whatever the source of the active substance, liberation of which results from the operative procedures incidental to the removal of the mucous membrane, these procedures do not appear appreciably to affect the functions of the vaso-dilator fibres as indicated by the vaso-motor response to nerve stimulation.

These new observations thus support our original contention that the vaso-dilator chorda fibres which accompany the lingual nerve transmit their effects—effects which are atropine resistant—by the peripheral liberation or production of a specific acetylcholine-like substance.

REFERENCES

Bain, W. A. (1932). *J. Physiol.* **77**, 3*P* (see also *Quart. J. exp. Physiol.* 1933, **23**, 381).
Feldberg, W. (1933). *Pflügers Arch.* **232**, 88.
Gaddum, J. H. (1935). *Ann. Rev. Biochem.* **4**, 323.

VII.

OBSERVATIONS ON THE INACTIVATION OF ADRENALINE BY BLOOD AND TISSUES *IN VITRO*

[*Reprinted from the Journal of Physiology*, 1937, Vol. **91**, No. 3, p. 233.]

[*Reprinted from the Journal of Physiology*,
1937, Vol. **91**, No. 3, p. 233.]
PRINTED IN GREAT BRITAIN

OBSERVATIONS ON THE INACTIVATION OF ADRENALINE BY BLOOD AND TISSUES *IN VITRO*

By W. A. BAIN, W. E. GAUNT and S. F. SUFFOLK
From the Pharmacological Laboratory, University of Leeds

(*Received* 31 *July* 1937)

Though knowledge relating to the chemical mediation of the effects of cholinergic nerve activity has advanced rapidly in the course of the last few years, the same progress has not been made with respect to our knowledge of adrenergic nerve activity. It appeared to us that one of the factors responsible for this latter state of affairs was a lack of information concerning the inactivation of adrenaline under experimental conditions resembling as closely as possible the conditions which obtain in the body.

The work recorded in this paper is an attempt to fill some of these gaps in our knowledge of adrenaline inactivation. It is necessarily incomplete and our interpretation of most of the phenomena described here for the first time must be considered tentative. Many fresh problems arose as the work proceeded and only a few of these have been solved: others we hope to deal with later.

We have worked for the most part from first principles. Our main object was to determine whether adrenaline is destroyed by blood and other tissues in a manner analogous to that in which acetylcholine is destroyed, and to determine whether cocaine produces its potentiating action on adrenaline effects, and on effects of adrenergic nerve stimulation, by preventing or delaying this destruction, in the same way as eserine potentiates the action of acetylcholine.

A chance observation, that the degree of inactivation of adrenaline in blood at the end of 10 hr. was no different from that at the end of 5, led us to investigate in more detail what happened to adrenaline when added to blood alone, and it is with this phase of our work that the second and major part of the present paper deals. The order of presentation of

our results may seem illogical in so far as our detailed description of what happens to adrenaline in blood follows our description of experiments with liver; but it is simpler to deal with the work in the order in which it was done and so, to this extent, we sacrifice logic to convenience.

Our experience of the bio-assay of adrenaline in the earlier experiments enabled us to develop a method of following the inactivation over periods of several hours, at such intervals, and with such a degree of accuracy, as to make the construction of inactivation-time curves possible. It is in the form of such graphs that most of our results are presented. Certain of our early and more or less qualitative experiments on the action of liver and cocaine have been repeated using this later technique, and the results are given in the same form as the others. Some of our results have been communicated to the Physiological Society [Bain & Suffolk, 1936; Bain *et al.* 1936].

The literature on adrenaline inactivation is scanty. For further information and references on the subject the reader is referred to the papers of Elliott [1905], Wiltshire [1931] and Blaschko *et al.* [1937].

Materials and methods

General details

In most of the experiments the medium in which we measured adrenaline inactivation was oxalated or defibrinated cat's blood, with or without added tissue slices or extracts. The blood was collected into clean or oxalated glass vessels from a cannula in the common carotid artery of a spinal or etherized cat. There was no apparent difference in the results with oxalated and with defibrinated blood, nor did it appear to matter whether the blood was obtained from spinal or from etherized animals. For experiments continued for 24 hr. or longer the blood was collected under conditions as nearly aseptic as possible, but usually no such precautions were taken.

The adrenaline used was the pure synthetic lævo base of British Drug Houses, Ltd. A concentrated stock solution of the hydrochloride was made by dissolving an amount of the base in a volume of $N/10$ HCl such that the solution contained 10 mg. adrenaline base per ml. From the concentrated stock solution we prepared, with distilled water, a dilute stock solution containing exactly 1 mg. of the base per ml. The experimental solutions in blood, in which inactivation was to be determined, were made up by adding measured quantities of the dilute stock solution to measured volumes of the blood. In experiments where several

different initial concentrations of adrenaline in blood were investigated, and in which therefore the volume of blood having a given concentration of adrenaline was small, the dilutions were effected by making up a certain volume of blood to an initial concentration of, say, 100γ/ml., and diluting measured portions of this to give the other desired and lower initial concentrations.

The standard solutions of adrenaline used for the assays were prepared from the dilute stock solution by further dilution with distilled water. The concentrations of adrenaline in the standard solutions were similar to the initial concentrations in blood, so that quantities of standard and experimental solutions of the same order were being measured during the assays, thus, to a large extent, obviating major errors of measurement. For injection of very small standard quantities of adrenaline in the late stages of an experiment some of the standard solution was still further diluted before use: such diluted standards were always checked against the original.

All the reactions described were carried out at a temperature of $38 \pm 1°$ C. except where otherwise stated. In most of the early experiments the experimental fluids were placed in test-tubes or hard glass flasks in a Wassermann bath and agitated at intervals to counteract sedimentation and promote full oxygenation. In all the later experiments the fluids were either in hard glass flasks or 25 ml. Jena glass vaccine bottles and were agitated continuously during the whole experiment.

Method of assay

The "adrenaline values" or "adrenaline equivalents" of the various experimental fluids were determined by blood pressure assay on cats, by what may be called the method of "continuous assay". In the earliest experiments we used both spinal and anæsthetized cats, but as the former proved much more sensitive to adrenaline all our later assays were made on spinal animals: all the results reported here refer to experiments on atropinized spinal preparations which had been "rested" for 2–3 hr. after transection of the spinal cord and destruction of the brain.

The arterial blood pressure was recorded from the left common carotid artery, and the anticoagulant fluid in the manometer system was a half-saturated solution of sodium bicarbonate. Injections were made into the right external jugular vein through a narrow metal cannula the proximal end of which fitted the nose of a record syringe: the cannula was provided with a stilette.

All the injections of experimental or standard solutions were made up to 1 ml. by dilution with Locke solution *immediately* before injection and were washed in with a further 0·5 or 1 ml. of Locke solution, making the volume of fluid injected at each observation 1·5 or 2 ml. according to the particular experiment. Control experimental fluids were treated in an exactly similar way. In some instances, where the amount of adrenaline left in the experimental fluids was small, or where the initial concentration was itself small, as much as 3 ml. of *undiluted* blood and adrenaline, or of control blood, had to be injected at an observation; but in general the total amount of undiluted experimental fluid measured out and subsequently diluted before injection was not more than 0·5 ml. and, in the early stages of an experiment, was usually less than 0·1 ml. These small amounts were measured into little conical glass dishes, and, a few seconds before the injection was due, were made up to volume with Locke solution from the injection syringe, rapidly mixed by barbotage, taken up and injected.

Injections were given at regular intervals of 3–6 min., the interval in any particular experiment depending chiefly on the time taken for the blood pressure to recover from a previous injection. After giving a number of injections of various doses of standard adrenaline, in order to determine the initial sensitivity of the animal, we continued with the injections of the standard doses within the range of greatest sensitivity. At convenient intervals a standard injection was replaced by an experimental one, the volume of experimental fluid taken up for dilution and injection being such as was calculated to bring the blood pressure rise between two of the standard rises, and as near to one of these as possible. Injections of standard and experimental fluids were continued in this way until the end of the experiment.

An important point about this continuous assay technique is that after 20 or 30 injections a large rise of blood pressure may be got with an amount of adrenaline which, at the beginning of the experiment, gave a small rise or no rise at all. This increase in sensitivity to adrenaline is always seen under the conditions of our experiments and usually occurs without any appreciable change in the resting blood pressure level. Sometimes the maximum increase may be only 80 p.c. for a given dose or it may be as much as 800 p.c. Whatever the extent of the increase it usually takes place smoothly, provided the injections are continued at regular intervals and that the heights of the blood pressure rises in response to the injections do not suddenly become much greater or much smaller, on the average, by marked alterations in the amounts of

adrenaline injected. The sensitivity generally reaches a maximum after 40 or 50 injections, may stay at this level for hours, and then decrease either gradually or suddenly. This decrease usually heralds the death of the animal, though in some instances there may be a fall after a few hours and the sensitivity persist at this lower level for a further period. The rate at which maximum sensitivity is attained seems to depend not so much on the number of injections given as on the blood pressure rises which they cause: the larger the individual rises of blood pressure the quicker is the rise of adrenaline sensitivity.

The importance of this change of sensitivity need not be stressed but it shows the absolute necessity for continued injections of standard adrenaline solutions throughout an experiment. It is also evident that if a high degree of sensitivity in the animal is desired before the assays proper are started, this can be achieved by giving a sufficient number of standard injections beforehand. For the type of work described here the increase of sensitivity is advantageous in that as the adrenaline disappears from our experimental solutions we are not compelled to increase the amount of such solutions injected to the same extent as would otherwise be necessary.

The standards to be injected are determined in the first place by the initial sensitivity of the animal and usually have to be modified later on: thus at the start of an experiment convenient standards may be 3, 3·5 and 4γ or more and, when full sensitivity is established, 0·5, 1 and 1·5γ or less.

Our method of arriving at the actual amount of adrenaline in the different volumes of experimental fluid injected is as follows: The blood pressure rises are measured and plotted on graph paper against time as abscissa. A line is drawn through each set of points representing rises for a particular dose of standard. The adrenaline value of any given experimental injection is then determined by observation or calculation.

As noted above, standards were not usually closer than 0·5γ, but in good experiments, where the sensitivity is high and the sensitivity curves are smooth, the adrenaline equivalent of the actual volume of fluid injected can be estimated to 0·1γ, especially if the position of intermediate points on the sensitivity curves is determined on the basis of a few injections and interpolated accordingly. In experiments where low concentrations of adrenaline had to be determined, standards differing by as little as 0·05γ were used.

A remarkable feature of these assay experiments is the large number of injections that can be given to a single spinal animal without causing

any appreciable change in the level of the resting arterial blood pressure, or in the accuracy of the adrenaline determinations. Most of our cats have had at least 60 injections and many well over 100, and it is very seldom that an experiment has had to be abandoned because of irregular responses at a late stage in the assaying. Bad cats are usually so from the start.

Results

Part I. Preliminary observations on inactivation by blood and tissues

In oxalated blood plasma and in blood serum at body temperature the inactivation of adrenaline proceeds to completion as it does in normal saline, Ringer or Locke solution. The inactivation proceeds in an almost

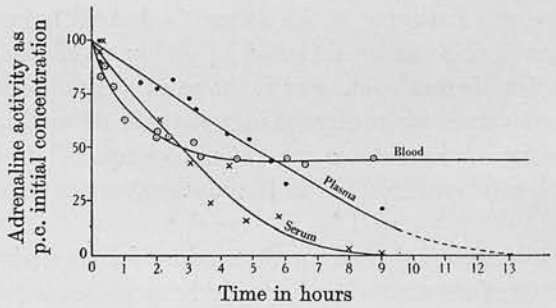

Fig. 1. To show rate and extent of inactivation of adrenaline at body temperature in (i) oxalated or defibrinated whole blood, (ii) blood plasma, and (iii) blood serum. Initial concentration of adrenaline 40γ/ml. in all cases. Data from Exps. A 47, 50, 58, 63 and 64.

linear manner during the greater part of its course and then tails off; it is more rapid in serum than in plasma. In oxalated or defibrinated blood inactivation of adrenaline does not proceed to completion: it proceeds in an exponential manner and ceases when an amount, which depends on the initial concentration of adrenaline, has disappeared. These facts are illustrated in Fig. 1, which shows the combined results from five typical experiments.

The difference between the behaviour of adrenaline in plasma and in serum seems to be chiefly one of rate. It is not accounted for by the absence of oxalate from the serum—since oxalated serum behaves in exactly the same way as normal serum—and it may be due simply to the difference in protein content of the two fluids. Comparison of the behaviour of adrenaline in whole shed blood, and in plasma or serum, shows that the difference here is of another order: it is a difference in kind. In shed blood, whether oxalated or defibrinated, it seems that inactivation

proceeds to what it is convenient to term an "equilibrium concentration" of adrenaline beyond which no further inactivation can be demonstrated.

It thus appears at the outset, from the marked contrast between the action of whole blood on adrenaline and its action on acetylcholine, that the hypothetical "oxidase" for adrenaline, corresponding to the known esterase for acetylcholine, either does not exist in the blood or, if it does, is incapable of exerting its full action under the conditions of our experiments. That we still get this equilibrium concentration even with full oxygenation of the blood and adrenaline shows that, apart altogether from the question of oxidizing enzymes, and apart, too, from the question of what has happened to the inactivated portion of the original adrenaline, full auto-oxidation itself is in some way prevented in whole blood.

Having failed to show complete inactivation of adrenaline by blood, we turned our attention to the possible effects of tissues added to blood and adrenaline *in vitro*.

Effects of tissues on adrenaline activation in blood. The tissues were removed from the animal used to supply blood for the experiment, cut into thin slices with a razor, rapidly washed to free them from blood and the excess moisture removed by filter papers, weighed, and added to the blood in the proportion of 1 g. of tissue/ml. blood. Adrenaline was then added to the mixture and to the control blood to give the same initial concentration per ml. of blood in each. Often the reverse procedure was adopted, i.e. 1 ml. of blood and adrenaline per g. tissue, mixed immediately beforehand, was added to the weighed liver slices. Control mixtures of tissue and blood were also set up and the different mixtures incubated in the usual way.

Of the tissues studied liver showed by far the greatest activity, and so attention was concentrated on it. Of the others, skeletal muscle was slightly active and kidney tissue intermediate between skeletal muscle and liver. Experiments on lung tissue proved fruitless because of the large amounts of an atropine-resistant histamine-like substance liberated during incubation.

That addition of liver slices to blood and adrenaline leads to the complete disappearance of adrenaline activity from the blood is shown in Fig. 2. This activity of liver is diminished by dipping the tissue in boiling water, and abolished by boiling for 2 min.

The activity of liver appears to be diminished by acid. This statement is based on two experiments (A 16 and 17) in which the activity of liver was so little in evidence as to lead us to suspect some error. It was found that the acid used in making up the stock adrenaline was *N* instead of

$N/10$. The activity of liver does not seem to be dependent on a simple catalysis of auto-oxidation, consequent upon contact of the tissues with metal in the course of their preparation. Even a bright copper strip or copper filings added to blood and adrenaline, or to blood, liver and adrenaline, does not affect appreciably the disappearance of adrenaline activity under conditions where neither the blood nor the liver has hitherto come into contact with metal. This is in direct contrast to what happens when metallic copper is added to a solution of adrenaline in Ringer's fluid, as first pointed out by Schild [1933]. The fact that the inactivating power of liver is abolished by boiling is also against the view that such an effect could be responsible.

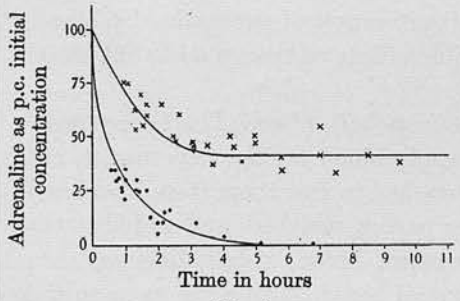

Fig. 2.

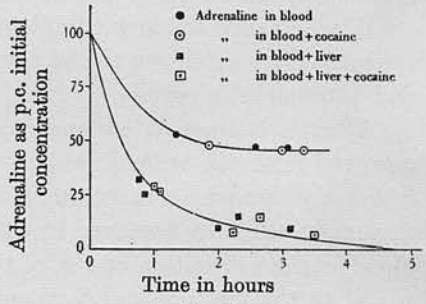

Fig. 3.

Fig. 2. To show contrast between inactivation of adrenaline in oxalated or defibrinated blood with and without addition of liver slices. Upper curve shows inactivation in blood alone: lower curve inactivation in blood to which liver (1 g./ml.) has been added. Initial concentration of adrenaline 20γ/ml. Data from Exps. A 30–34, 36, 37, 41–43, 51, 52, 56, 62, 67 and 68.

Fig. 3. To show that cocaine does not inhibit the inactivation of adrenaline in blood or in blood to which liver has been added. Initial concentration of adrenaline 20γ/ml. and of cocaine 10γ/ml. Data from Exps. A 61 and 62.

Liver ground with sand is active; but, when ground with sand in saline or Locke, filtrates from such concoctions, as free as possible from cells and from cell fragments, have proved inactive in our hands. Similarly, cell free extracts with $N/100$ HCl, with 25 p.c. glycerol and with acetone were inactive. Our results with extracts were thus disappointing [cf. Schütz, 1933; Blaschko *et al.* 1937] and we could only be sure of results when we used liver slices or the unfiltered concoctions got by grinding the material with sand. We preferred the slices.

Attempts to abolish the activity of liver by means other than heating were not satisfactory. We could diminish the activity considerably by withholding oxygen but we never succeeded in abolishing it completely

even by bubbling N_2 through the blood. Addition of KCN had no effect in the doses which we investigated (10–20γ/ml.) and formol, which we also tried, proved useless because of its own inactivating effect on adrenaline [Cramer, 1911], an effect quite evident with as little as 5γ/ml.

Effect of cocaine on adrenaline inactivation. Though the point was perhaps not yet proven, liver appeared to produce an effect on adrenaline which might provisionally, at least, be attributed to an enzyme (oxidase) and the last part of our first object, as explained in the introduction, was to determine whether cocaine had an inhibitory effect on this action and on the action of blood itself so far as this went. (For a review of the early literature relating to the action of cocaine on the effects of adrenaline and of sympathetic nerve stimulation see Trendelenburg [1924].)

In making up the experimental solutions cocaine hydrochloride was first added to a portion of the blood; liver slices were then added to a portion of the blood and cocaine, and to a portion of control blood; the adrenaline was added to control and to experimental fluids last of all. In some experiments the liver was added last of all. The particular procedure used made no difference to the results. The concentrations of cocaine used were from 5 to 20γ/ml., i.e. a total per ml. many times greater than that sufficient to produce a demonstrable effect on adrenaline sensitivity when injected intravenously into a cat.

In determining the adrenaline values of blood, etc., in which cocaine was present, it was found convenient to inure the test animal to the effects upon adrenaline sensitivity of further additions of cocaine by administering from 1 to 10 mg. of cocaine hydrochloride subcutaneously before the experiment started. Failure to do this resulted in highly inconsistent results due to the fleeting and/or step-like changes in adrenaline sensitivity induced by the minute amounts of cocaine in the experimental injections.

Fig. 3 illustrates the results of two such experiments. Cocaine, added to blood or to blood and liver, has no inhibiting effect on the inactivation of adrenaline under the conditions of our experiments, and thus, presumably, does not produce its well-known potentiating action on the effects of adrenaline, and on certain actions mediated by sympathetic nerves, by inhibiting adrenaline inactivation.

From the experiments so far described we see that the hypothetical analogy between the mechanisms responsible for the inactivation of adrenaline and of acetylcholine breaks down in one important respect, for while whole blood is extremely active in destroying acetylcholine this is not so with adrenaline. The hypothetical analogy between the actions

of eserine and cocaine, in so far as these substances affect the actions of acetylcholine and adrenaline and the actions of autonomic nerves, breaks down completely. Thus while eserine inactivates choline-esterase, cocaine fails to inactivate the inactivating principle (or principles) of tissues and so must produce its potentiating or "sensitizing" action to adrenaline and to adrenergic nerve stimulation in some other way.

Part II. Further observations on adrenaline inactivation in blood

That an "equilibrium", with respect to inactivation, is reached in blood was confirmed by many experiments. For any given initial concentration the equilibrium concentration varies somewhat from blood

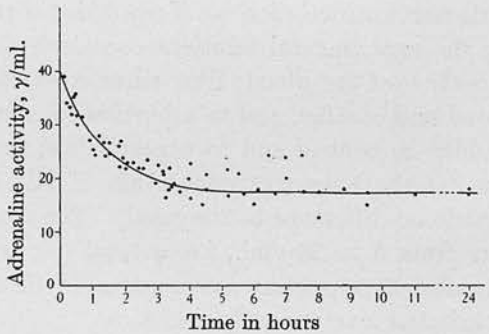

Fig. 4. To show variations of equilibrium value in different experiments with the same initial concentration of adrenaline (40γ/ml.). Data from Exps. A 47, 49, 51, 52, 53, 55, 57, 58, 63, 64, 67, 74 and 85.

to blood. This fact is evident from Fig. 4, which gives the graphed results of a number of experiments in which the initial concentration of adrenaline was 40γ/ml. In any individual experiment the equilibrium concentration is quite definitely established. The average value of the equilibrium concentrations for an initial concentration of 40γ/ml. was 17·8γ (13 experiments) and the individual values ranged from 14γ to 24γ/ml. (see Fig. 5*a*).

It is obvious that these individual variations may partly be due to errors and thus be insignificant; for if the initial concentration is more than that estimated by, say, 2γ/ml. and the standard solution used for assay less by 2γ/ml. than estimated, the assay (assuming there are no inherent errors in this) will give an equilibrium value 4γ/ml. higher than the actual; but the greatest care in measuring the blood and the adrenaline solutions has not diminished the variation, and we therefore consider it to be probably significant. As we shall see later, it may be related to differences in the corpuscle : plasma ratio in different samples of blood.

In addition to these individual variations for a given initial concentration there is a variation in the equilibrium concentration related to differences in the initial concentration of adrenaline. This relationship for different initial concentrations from 60γ downwards is shown in Fig. 5*a*. The points through which the graph is drawn represent the average values for that number of observations indicated for each initial concentration by the bracketed numerals on the right-hand side of the chart; while the small vertical lines, lateral to the main points, represent the range of the equilibrium values for each initial concentration. From

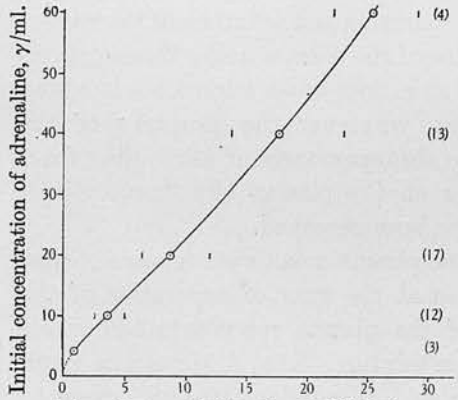

Fig. 5*a*.

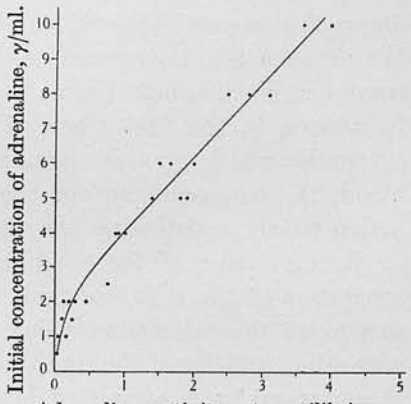

Fig. 5*b*.

Figs. 5*a* and 5*b*. To show variation in equilibrium value of blood and adrenaline with different initial concentrations. Fig. 5*a* shows the variation for initial concentrations from 60γ/ml. downwards. For description see text. Fig. 5*b* shows in greater detail the variations of the equilibrium value for initial concentrations from 10γ/ml. to 0·5γ/ml. Each point for a particular initial concentration on this chart represents a separate experiment. Data for Fig. 5*b* from Exps. A 8, 44, 64, 65, 66, 89 and 92.

a physiological point of view the most interesting part of this curve is that which relates to initial concentrations between, say, 10 and 0·5γ, and so that part of the curve is reproduced in greater detail as Fig. 5*b*. Unfortunately, however, it is with these small initial concentrations that our method tends to break down, for the simple reason that accurate estimates of the adrenaline activity at equilibrium involve the injection of relatively enormous quantities of blood (2–3 ml.). Nevertheless, the results, carefully controlled, are concordant so far as they go. But the experiments will have to be repeated using other techniques before we could feel justified in laying much stress on them. In the meantime

we can confine our attention to the results got with the relatively unphysiological initial concentrations of 20–40γ/ml. without prejudice as to what happens with very small initial concentrations.

The part played by the corpuscles in the inactivation of adrenaline by blood. The fact that adrenaline becomes completely inactivated in plasma and in serum but only partially in oxalated or defibrinated blood led us to consider the possible significance of the corpuscles in the phenomena exhibited by blood. We therefore made experiments in which oxalated blood, with added adrenaline, was allowed to come to equilibrium, the corpuscles then being spun off and the adrenaline value of the plasma determined as soon as possible after separation and at intervals thereafter. We soon saw that the adrenaline value of the plasma under these circumstances diminishes in the same way as it does when adrenaline is added to plasma in the first place. Thus, whatever the property of the corpuscles which prevents complete disappearance of adrenaline from blood, the corpuscles do not confer on the plasma any "protective" action which persists after they have been removed.

Extrapolation of the equilibrium plasma inactivation curve, after separation of the cells, indicates that at the time of separation of the corpuscles the adrenaline value of the plasma represents the whole adrenaline activity of the equilibrium mixture. Exp. A 57 may be given as an example: A sample of blood and adrenaline, 40γ/ml., came to equilibrium at 15γ/ml. Separation of cells from the plasma from 40 ml. of this equilibrium mixture gave 18 ml. of cells and 22 ml. of plasma. The adrenaline value of the plasma 30 min. after separation was 25γ/ml. and 2 hr. 50 min. after separation 18γ/ml. (average values for triplicate readings at 5 min. intervals). Extrapolation from these points, which lie in the linear part of the plasma inactivation time curve, gives the plasma adrenaline concentration at the time of separation as 26·5γ/ml. Now, if all the adrenaline of the equilibrium mixture (i.e. 15γ/ml.) is in fact present in the plasma, the adrenaline content of the separated plasma at the time of separation should be $(15/1 \times 40/22)\gamma$/ml., i.e. 27$\gamma$/ml. approximately, which is not significantly different from that found. Direct determination of the plasma or serum adrenaline immediately after separation confirms this type of result, and so we have the further important fact that all the adrenaline activity of an equilibrium mixture, as determined by bio-assay of the intact mixture, resides in the plasma or serum of the mixture.

It immediately appears, therefore, that the blood corpuscles play an essential part both in the establishment and in the maintenance of the equilibrium phenomena which we have described.

Adrenaline associated with the cells of an equilibrium mixture. Having shown that serum or plasma, freshly separated from an equilibrium mixture, appeared to contain an amount of adrenaline which accounted for the whole activity of the equilibrium mixture, it remained to confirm this by showing that the corpuscles from an equilibrium mixture showed by themselves no adrenaline activity. This was done. Whole corpuscles from an equilibrium mixture show no adrenaline activity when taken up in Locke solution and immediately injected into the test animal. If, however, the cells are left in Locke solution, or in fresh plasma or serum, the mixture soon acquires an adrenaline activity which gradually increases to a constant value, a fact which immediately suggests the survival of some of the missing adrenaline in reversible association with the corpuscles. This is dramatically demonstrated when corpuscles separated from an equilibrium mixture are laked either by the action of acid or by freezing: they then exhibit a marked adrenaline activity not shown by control laked corpuscles. From such observations it became apparent that the adrenaline equivalent of an equilibrium mixture, i.e. the equilibrium concentration, does not in fact represent the total amount of adrenaline in such a mixture, but that a further amount is present in or on the corpuscles, an amount incapable under ordinary circumstances of exhibiting its presence by an action on the test animal.

The next step was to determine the amount of adrenaline associated with the corpuscles of an equilibrium mixture. Two methods of doing this were open to us: One was to separate the cells from the mixture, determine the relative cell/plasma or cell/serum volumes, lake the cells and find their adrenaline value per unit volume; the other was to lake the equilibrium mixture with dilute hydrochloric acid and find the adrenaline value of the laked mixture. Both methods were used and some results are summarized in Tables I*a* and I*b*.

TABLE I*a*. Recovery of adrenaline from equilibrium mixtures. Plasma adrenaline, from separated equilibrium plasma, is calculated as adrenaline equivalent per ml. original whole blood. Corpuscle adrenaline, from separated equilibrium cells, is calculated as adrenaline equivalent per ml. original whole blood

Initial conc. (γ/ml.)	Adrenaline value plasma (γ/ml. whole blood)	Adrenaline value cells (γ/ml. whole blood)	Adrenaline value cells + plasma (γ/ml.)	Total adrenaline recovered (p.c. of initial adrenaline)
20	9·4	5·9	15·3	76·5
40	14·8	14	28·8	72
40	15·8	12·4	28·2	70·5
60	26·2	19·1	45·3	75
			Average	73·5

TABLE I*b*. Recovery of adrenaline from equilibrium mixtures. Corpuscle adrenaline by laking equilibrium mixture

Initial conc. (γ/ml.)	Equilibrium conc. (γ/ml.)	Adrenaline equivalent laked equilibrium mixture (γ/ml.)	Total adrenaline laked equilibrium mixture (p.c. original)
20	6·7	17·0	85
20	7·7	14·5	72
40	13·3	30·9	77
40	15·5	33·5	84
60	29·0	43·3	72
60	26·0	52·0	87
		Average	79·5

It will be seen that a somewhat greater "recovery" of adrenaline was got by laking the equilibrium mixture than by separating the cells from the serum or plasma. This difference may be accounted for by loss of cell-adrenaline in the experimental procedures necessary with the separation method. The average "recovery" or "total equilibrium" adrenaline from 10 equilibrium mixtures selected at random (initial concentration 40 and 20γ/ml.) represents approximately 80 p.c. of the original amount added. Thus while an average of 56 p.c. of the initial adrenaline (40 or 20γ/ml.) becomes physiologically inactivated by the time equilibrium is reached, all of this amount is not destroyed; a further 36 p.c. of the original amount can be recovered from the corpuscles in which situation it is ordinarily incapable of manifesting a pressor action on the test animal. We conclude therefore that the total irrecoverable adrenaline of an equilibrium mixture is actually only about 20 p.c. of the original, as against the apparent 56 p.c. of the original as given by the equilibrium adrenaline value.

If the procedure for obtaining an inactivation curve for blood and adrenaline is followed out and alternate samples of blood are laked immediately before assay two separate curves result, the normal ("apparent") inactivation curve, and what one might call for convenience the "virtual" inactivation curve. The uppermost graph in Fig. 6, constructed from the data of two experiments (A 63 and 64), is an example of such a "virtual" inactivation curve. It would seem from such results that the "irrecoverable" adrenaline is lost before equilibrium is reached, since the assay curve for the laked samples becomes horizontal before the normal inactivation curve does so.

Whether the irrecoverable adrenaline of an equilibrium mixture is destroyed by oxidation or otherwise, or is inactivated in the same way as the recoverable cell adrenaline but in a less easily reversible manner, or is inactivated by combination with some constituent of the blood,

irreversibly or otherwise, we are not at present in a position to say. Nor are we able to say whether the adrenaline recovered by laking an equilibrium mixture, or separated equilibrium corpuscles, is inactive because it is inside the corpuscles in a free state, or is adsorbed on the corpuscles, or both. Whatever be the truth it is certain that, so far as the recoverable adrenaline associated with the corpuscles is concerned, the mechanisms involved can be partially reversed under circumstances not involving destruction of the corpuscles. If, for example, cells from an equilibrium mixture are added to fresh serum or plasma this mixture acquires and shows a progressively increasing adrenaline value which ultimately becomes constant—that is, comes to equilibrium: the adrenaline value of the separated cells diminishes accordingly. Similarly, if fresh blood is added to an equilibrium mixture the equilibrium changes and in 3–5 hr. has a value near to that which it would have had if the initial adrenaline had been added to the final volume of blood in the first place, and the adrenaline value of the cells is diminished in consequence.

What is perhaps a more important point, and one which may be considered as giving evidence that the recoverable adrenaline associated with the cells is normally inactive simply because it is within them, or is adsorbed by them, and that the irrecoverable adrenaline may be lost in another way, is the fact that the *integrity* of the corpuscles does not appear to be necessary either for the loss of the irrecoverable adrenaline or for the persistence of that amount normally associated with the cells and normally recoverable after laking either the equilibrium mixture or the separated equilibrium cells. This is illustrated by the following experiment (A 85):

Oxalated blood, collected aseptically, was divided into two portions. One of these was laked.

In this experiment, and also in most of those in which we studied inactivation of low initial concentrations, the blood was laked by freezing with liquid air. 10 ml. or so of blood at a time were placed in a hard glass test-tube the bottom of which was dipped in liquid air until the blood froze solid. It was then rapidly thawed by running warm water over the outside of the tube. Often the whole process was repeated to ensure thorough laking.

Samples of each portion having been retained as controls adrenaline was added to each of the remaining portions to give an initial concentration of 40γ/ml. Experimental and control samples were then incubated with continuous mechanical agitation in stoppered sterile vessels. Immediate assay gave initial values of 39 and 41γ/ml. for the normal and the laked portions respectively. 24 hr. later the adrenaline value of the normal sample was 16·7γ/ml. and of the laked sample 29γ/ml. A portion

of the normal 24 hr. sample laked with liquid air had an adrenaline equivalent of 30γ/ml. The 24 hr. equilibrium value is thus well within the normal limits, as is the total recoverable adrenaline from this equilibrium mixture. The adrenaline value of the sample laked before the adrenaline was added to it 24 hr. previously is, however, not significantly different from the adrenaline value of the laked equilibrium mixture. It thus appears (1) that the irrecoverable adrenaline does not depend for its loss upon the integrity of the corpuscles, and (2) that the total recoverable adrenaline is the same whether the corpuscles are intact or not, that is, whether part of it is in a state in which it is incapable of exercising an effect on the test animal by reason of association with intact corpuscles, or is free in the plasma along with destroyed corpuscles, in which situation it is free to exercise its action on the test animal from the beginning.

Relative amounts of adrenaline associated with cells and with plasma or serum of equilibrium mixtures. A further interesting point relating to the corpuscle adrenaline is that at equilibrium the adrenaline equivalent of the corpuscles appears to be greater, volume for volume, than that of the plasma. If, for example, making use of the 4 : 6 corpuscle/plasma ratio determined by the hæmatocrite and the equilibrium and laked equilibrium values given in Table I*b*, we calculate the corpuscle adrenaline and plasma adrenaline concentrations respectively for each of the six blood samples of that table, we find the average values for each of the three sets of initial concentrations to be 21·4 and 12γ/ml. for the original 20γ/ml. samples, 44·5 and 24γ/ml. for the original 40γ/ml. samples, and 50·4 and 45·8γ/ml. for the original 60γ/ml. samples.

If we calculate the plasma and corpuscle adrenaline concentrations from the average equilibrium values for different initial concentrations (see Fig. 5*a*), assuming an 80 p.c. recovery and a 4 : 6 corpuscle/plasma ratio, the differences are not so striking as those just quoted; for an initial concentration of 10γ/ml. they are for corpuscles and plasma, in that order, 10·25 and 6·5γ/ml.; for 20γ, 18 and 14·7γ/ml.; for 40γ, 35·7 and 29·5γ/ml.; and for 60γ, 56·2 and 42·5γ/ml. respectively. The differences are, of course, reduced still further if the total recovery is reduced or the corpuscle/plasma ratio approaches nearer to unity. But that the corpuscle adrenaline concentration at equilibrium probably is significantly greater than the plasma adrenaline is confirmed by experiments in which the total recovery was accurately estimated and the corpuscle/plasma ratio determined at the outset by the addition of known volumes of spun cells to known and widely different volumes of separated plasma. In the

experiment which we quote, spun cells from oxalated blood were added to separated plasma in four different ratios; adrenaline was added to give an initial concentration of 20γ/ml. of the mixtures, and allowed to come to equilibrium. In addition to the equilibrium values, the total adrenaline recovery was determined in each instance; from these figures, together with the known corpuscle/plasma ratios, the plasma and corpuscle adrenaline concentrations were calculated. These results are set forth in Table II. (It must be clearly understood, however, that in speaking of

TABLE II. Equilibrium values, total recovery, and corpuscle and plasma adrenaline values in blood with altered corpuscle/plasma ratios. Initial concentration of adrenaline 20γ/ml. in all cases. Cell (or plasma) conc. $= \left(c \text{ [or } p\text{] amount} \times \frac{\text{total volume}}{c \text{ [or } p\text{] vol.}} \right)$

Ratio cells : plasma	Equilibrium value (i.e. plasma adrenaline) (γ/ml.)	Adrenaline equivalent laked equilibrium mixture (i.e. plasma and cell adrenaline) (γ/ml.)	Total adrenaline recovered (p.c. initial conc.)	Plasma adrenaline conc. (γ/ml. plasma)	Cell adrenaline conc. (γ/ml. cells)
2	3·9	15·3	76·5	11·7	17·1
4 : 6 (normal)	7·5	14·3	72·0	12·5	17·0
4 : 10	9·7	16·0	80·0	13·6	22·0
4 : 16	11·0	16·7	84·0	13·7	28·5

the "cell concentration" of adrenaline we do not infer that the adrenaline is in fact in simple solution within the cells at equilibrium: adsorption may well be the main, or indeed the exclusive factor at work. At the present time we cannot commit ourselves either one way or the other.)

In an earlier part of this paper it was pointed out that the individual variations in the equilibrium concentrations for different initial concentrations of adrenaline might be partly accounted for by differences in the corpuscle/plasma or corpuscle/serum ratios in the different samples of blood. The effects upon the equilibrium value of alterations in the corpuscle/plasma ratio, as in the experiment just quoted, show that this view is perhaps justified.

Experiments with low initial concentrations of adrenaline. We have hitherto dealt for the most part with results arrived at in the study of initial concentrations of 20 and 40γ of adrenaline per ml.; we revert finally to the question of much lower initial concentrations. We have already alluded to these (p. 243), and to the difficulties of investigating them by our method. The chief difficulty arises from the relatively enormous quantities of blood that have to be injected in the later stages of such experiments in order to get an estimate of the adrenaline present.

The number of injections is thus limited and the difficulties both of making an assay and of making adequate controls are further increased by this restriction. More serious, however, than the difficulties introduced by the simple volume factor is the fact that the controls often cause an atropine-resistant depressor effect which is greatly exaggerated when the blood is laked. Sometimes these presumably histamine effects are absent even with 5 ml. injections of blood: there is considerable variation either in the content of the depressor substance in different samples of blood and/or in the sensitivity of the test animals to it. In view of these difficulties, however, we regard our results with these low initial concentrations with caution until such time as the experiments can be repeated using other techniques.

Keeping this proviso in mind we present in Table III the results of an experiment (A 92) conducted under the best conditions, i.e. where the

TABLE III. To show equilibrium values, total recovery and relative corpuscle and plasma adrenaline concentrations with low initial concentrations. Figures in brackets are the percentages of the original concentration represented by the figures which precede them

Initial conc. (γ/ml.)	Equilibrium conc. (γ/ml.)	Adrenaline conc. laked equilibrium mixture (γ/ml.)	Cell conc. (γ/ml. cells)	Plasma conc. (γ/ml. plasma)
5·0	1·8 (36)	3·7 (74)	4·75	3·0
2·0	0·45 (22·5)	1·24 (62)	1·97	0·75
1·5	0·25 (16·6)	0·70 (50)	1·12	0·42
1·0	0·075 (7·5)	0·25 (25)	0·44	0·12
0·5	0·025 (5)	0·10 (20)	0·19	0·04

sensitivity of the test animal to adrenaline was extraordinarily high and the controls had no apparent histamine activity. The chief points of interest appear to be: (1) that as the initial concentration of adrenaline is lowered from 5 to 0·5γ/ml. the percentage of the original adrenaline represented by the equilibrium value falls from 36 to 5, and the total recovery from 74 to 20; (2) at equilibrium the "cell concentration", expressed as a multiple of the plasma concentration, rises from about 1·6 for an initial concentration of 5γ/ml. to about 4·7 at 0·5γ and this despite the fact that if histamine is interfering with the assays it would tend to diminish the observed cell concentration of adrenaline to a greater extent than the plasma concentration. If adsorption is the main factor at work in determining the phenomena which we have described in blood it may be that the comparatively low equilibrium values with the very small initial concentrations are due to more complete adsorption, and the low recoverable adrenaline to very incomplete reversal of the process, under these circumstances.

Concluding remarks

Detailed discussion of the results reported in this paper would be premature at this stage and must wait until some further facts are forthcoming. One or two observations of a general nature may, however, be permissible now. In the first place, we would point out the advantage of using blood as a medium in experiments on adrenaline inactivation. Despite the fact that Oliver & Schäfer in 1895 pointed out the feeble adrenaline inactivating power of blood, most studies of adrenaline inactivation since that time have been carried out using Ringer fluids as the medium. The rapidity of free oxidation in this medium, taken together with the rapid inactivation of adrenaline *in vivo*, appears to have led many, at least implicitly, to identify the processes in Ringer's fluid and *in vivo*, or if not going so far, at least to regard the process in Ringer's fluid as the standard against which inactivation by tissues or other factors is to be measured. This is seen, for example, in Miss Wiltshire's [1931] work. By taking blood as a medium and the inactivation of adrenaline in blood as a physiological standard the fallacious arguments and the contradictory interpretations which arise when other media are used would to a large extent be obviated.

The only general conclusion which we can safely draw from the work recorded here is that blood itself is probably but of small significance in determining the inactivation of adrenaline in the body and that it is in the tissues almost exclusively that the conditions for this inactivation are fulfilled. It is true that under the conditions of our experiments complete inactivation of 40γ adrenaline by 1 g. of liver—the most active of all the tissues we examined—takes some 4–5 hr. But this length of time does not in our view make it unlikely, as Bacq [1936] rather seems to suggest, that the same mechanism is in fact the one responsible for the rapid inactivation of adrenaline *in vivo*. In the first place, the conditions under which our experiments are carried out are manifestly much less favourable to the inactivating property of the tissues than are the conditions *in vivo*. In the second place, even if 1 g. of liver does take 4 hr. to inactivate 40γ of adrenaline, surely it is not too much to expect the 80 g. or so of cat liver *in vivo*, taken together with the lesser but very significant inactivating effect of many if not most of the other tissues of the body, to inactivate the same amount in a few minutes. Apart from such considerations, however, an examination of the inactivation-time curves for the different media we have investigated will immediately show how different are the inactivation *rates* in these media. Thus the

approximate times for 25 p.c. inactivation are in blood plasma 180 min., in serum 80 min., in blood 40 min., and in blood + liver 6 min. These facts are illustrated in Fig. 6 which summarizes most of our results. The relatively high initial rate of inactivation by liver is obvious from the graph, and the tailing-off may well be due partly to accumulation of oxidation products, a factor which might not operate to anything like

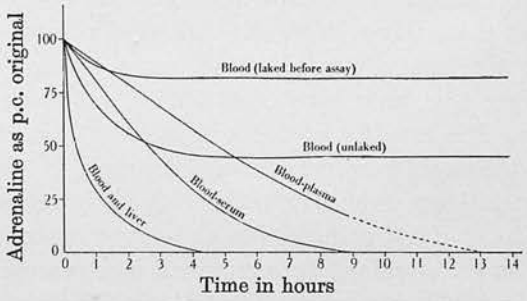

Fig. 6. To summarize the principal data given in Figs. 1, 2 and 3, and to show in addition the type of inactivation curve obtained by laking, immediately before assay, blood to which adrenaline has been added at zero time (see p. 246).

the same extent *in vivo*. But in a later paper one of us will revert to this topic and will show, among other things, that the activity of cat's liver in respect of the adrenaline inactivating principle is not nearly so great as is that of the liver of some other mammals, including man.

Summary

1. The methods used in studying the inactivation of adrenaline are described; all the experiments were made with cat's blood and cat's tissues and the adrenaline determinations made by blood pressure assay on spinal cats.

2. In oxalated blood plasma and in blood serum inactivation of adrenaline proceeds slowly to completion; it is more rapid in serum than in plasma.

3. In oxalated or defibrinated whole blood inactivation is never complete; it proceeds to what it is convenient to call an equilibrium value beyond which no further inactivation can be demonstrated.

4. Addition of tissue slices, or of concoctions of tissues, to blood and adrenaline leads to the complete disappearance of adrenaline activity from the blood. Many tissues show this activity to some extent but the most active, weight for weight, is liver.

5. The adrenaline inactivating power of liver is not diminished by cyanide or cocaine: it is diminished by increased acidity of the medium and by the withholding of oxygen; it is absent after the tissue has been boiled.

6. Further study of the inactivation of adrenaline by blood showed that the equilibrium concentration varies with the initial concentration and that there is also some variation, for a given initial concentration, from sample to sample of blood. These last variations are probably due to variations in the cell/plasma or cell/serum ratios.

7. The adrenaline value of an equilibrium mixture is represented exclusively by adrenaline in the plasma or serum, but a further amount of adrenaline is present in or on the cells of such a mixture, an amount which ordinarily is incapable of manifesting its presence by an action on the test animal.

8. This additional adrenaline is in reversible association with the cells and can be recovered in maximum amount by laking the separated cells or the equilibrium mixture, or, in lesser amount, by placing separated equilibrium cells in fresh plasma or serum or Locke solution. Over 80 p.c. of the original adrenaline (20–40γ) added to blood is recoverable by laking an equilibrium mixture.

9. The total adrenaline recoverable from blood seems to be independent of the integrity of the cells.

10. Whether the association of adrenaline with the cells is one of simple adsorption, or of passage of adrenaline within the cells, or both, is not clear. Nor is it clear whether the 20 p.c. of original adrenaline irrecoverable from such a laked equilibrium mixture is irrecoverable because it is destroyed, or is combined in some way with some constituent of the blood, or is held inactive in the same way as the recoverable cell adrenaline but in a manner which renders its association irreversible under the conditions of our experiments.

The expenses of this work have been partly met by a grant to one of us (W. A. B.) from the Government Grant Committee of the Royal Society.

REFERENCES

Bacq, Z. M. (1936). *J. Physiol.* **87**, 87 *P.*
Bain, W. A. & Suffolk, S. F. (1936). *Ibid.* **86**, 34 *P.*
Bain, W. A., Gaunt, W. E. & Suffolk, S. F. (1936). *Ibid.* **87**, 8 *P.*
Blaschko, H., Richter, D. & Schlossmann, H. (1937). *Ibid.* **90**, 1.
Cramer, W. (1911). *Ibid.* **42**, 36 *P.*
Elliott, T. R. (1905). *Ibid.* **32**, 401.
Oliver, G. & Schäfer, E. A. (1895). *Ibid.* **18**, 230.
Schild, H. (1933). *Ibid.* **79**, 455.
Schütz, F. (1933). *Biochem. Z.* **265**, 282.
Trendelenburg, P. (1924). *Heffter's Handbuch der experimentellen Pharmakologie*, **2**, 2, p. 1167.
Wiltshire, M. O. P. (1931). *J. Physiol.* **72**, 88.

VII.a

On the inactivation of adrenaline by mammalian liver *in vitro*.

[*From the Proceedings of the Physiological Society*, 2 July 1938.]
Journal Physiology, *Vol.* 93.

[*From the Proceedings of the Physiological Society*, 2 July 1938.]
Journal Physiology, Vol. 93.

On the inactivation of adrenaline by mammalian liver *in vitro*.

By W. A. Bain and Sylvia Dickinson. (*From the Pharmacological Laboratory, University of Leeds*)

A method has already been described for following the course of inactivation of adrenaline *in vitro* [Bain *et al.* 1937]. In the experiments here reported the inactivating power of different samples of liver, and of samples from different species, is compared. The technique used was the same as in the previous experiments except that the amount of liver used was less (1 g. per 5 ml. instead of 1 g. per ml.). The experiments were carried out at body temperature: the medium was defibrinated cat's blood; and the initial concentration of adrenaline was 40γ per ml. The results were recorded by plotting the amount of adrenaline left against the interval of time between its addition to the blood and liver and the taking of the sample. On an average, seven points were determined in each experiment. The results of forty-two experiments are summarized in Table I.

Table I. Approximate mean times in minutes for 50, 75 and 90 p.c. inactivation of adrenaline by livers of different species. Figures in round brackets are the shortest and longest times obtained. Figures in square brackets indicate number of experiments on each species.

P.c. inactivation	Guinea-pig [7]	Man [13]	Rat [7]	Cat [7]	Dog [4]	Mouse [4]
50	12 (8–17)	13 (10–16)	27 (24–31)	34 (21–50)	38 (30–44)	42 (28–48)
75	26 (15–35)	26 (22–31)	53 (49–60)	75 (55–107)	80 (67–100)	79 (53–93)
90	47 (31–61)	43 (35–50)	84 (78–96)	—	—	—

It will be seen that the rate of inactivation effected by the liver is different for different species. There is some variation in the curves obtained from livers of individuals of the same species. But in the guinea-pig, rat, and a set of thirteen samples of human liver, the results lie between fairly well-defined limits about a mean curve, the mean curve being characteristic of the species. It seems possible, therefore, to establish for each of these animals a normal "adrenaline inactivation curve" of liver to which the curve for any given individual will approximate. Wide

deviation from the characteristic curve would suggest an abnormality in the adrenaline inactivating power of the tissue.

In three human livers the adrenaline inactivating power was found to be diminished to such an extent, the average times for 50, 75 and 90 p.c. inactivation being 24, 43 and 70 min. respectively. Two of the subjects suffered from arterial hypertension of non-renal origin and the third had a diastolic pressure of 90 mm. Hg, but no other evidence of hypertension. On the other hand, the group of thirteen human subjects includes four cases of chronic nephritic hypertension, in each of which the adrenaline inactivation curve falls within normal limits. It thus seems possible that essential hypertension may be associated with a diminished adrenaline inactivating power of the tissues.

If adrenaline is the transmitter of adrenergic vasoconstrictor nerve activity the raised arterial tension in essential hypertension, and perhaps also in malignant hypertension, may be due to a potentiation of the effects of normal vasoconstrictor nerve activity resulting from the delayed inactivation of the transmitter. We hope to deal with this question in detail later.

REFERENCE

Bain, W. A., Gaunt, W. E. & Suffolk, S. F. (1937). *J. Physiol.* **91**, 233.

VII.b

On Adrenaline and Noradrenaline Inactivation by Human Liver *in vitro*.

From J. Physiol. 1952, 118, 13P.

On adrenaline and noradrenaline inactivation by human liver *in vitro*

By W.A. Bain and Jean E. Batty. Department of Pharmacology, University of Leeds.

Bain & Dickinson (1938) showed that, when liver slices from various mammals were incubated at 37°C in blood with 40µg/ml. adrenaline, the rate of adrenaline inactivation varied with the species and was fastest in the guinea-pig and man, slowest in the cat, dog and mouse, and intermediate in the rat.

In three out of sixteen human livers, however, the inactivation rate approximated to that of rat liver, and as two of the subjects had suffered from arterial hypertension of non-renal origin it was suggested that delayed inactivation of the transmitter might be responsible for such forms of raised blood pressure.

We have now compared, by the same methods as before, the inactivation rates of adrenaline and of of noradrenaline by twenty-eight samples of human liver. An average of six points was determined for each drug in each experiment. The mean regressions for all experiments are:

adrenaline, $\ln Y = \ln y_0 - 0.04474t$; noradrenaline, $\ln Y = \ln y_0 - 0.05655t$; the difference between them is highly significant ($P < 0.001$). The calculated mean half-inactivation times in minutes, and their

fiducial limits (P = 0.95), are: adrenaline, 15.5 (13.8 - 17.7); noradrenaline, 12.25 (11.1 - 13.7).

Inactivation of both drugs is unaffected by cyanide but is inhibited by octyl alcohol, so the enzyme concerned is amine oxidase (Blaschko, Richter & Schlossmann, 1937).

Though some livers seem to inactivate both drugs so slowly as to belong to a separate category - as in the previous observations with adrenaline - this is difficult to prove; and the clinical data available give no help one way or the other.

It is clear, however, that the hypothesis concerning delayed inactivation of adrenaline as a possible factor in causing certain forms of high blood pressure can now be extended to include noradrenaline. But any rigorous test of the validity of this hypothesis will require many more experiments than we have been able to do, and clinical information about the cases of a much more critical kind than we have been able to get.

REFERENCES

Bain, W.A. & Dickinson, S. (1938) J. Physiol. 93, 54P.

Blaschko, H., Richter, D. & Schlossmann, H. (1937). J. Physiol. 90, 1.