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A STUDY OF TWO METHODS FOR TESTING LOCAL ANAESTHETICS IN MAN

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Several methods are available for assessing local anaesthetic activity. For terminal anaesthesia the most widely used quantitative method is probably that based on intradermal wheals on the shaved backs of guinea-pigs (Bülbring and Wajda, 1945). The first part of this paper reports results obtained by a similar method on man. These results were at least as accurate as those obtainable on the guinea-pig, but the activity ratios found in the two species were different.

In the second part of the paper a further method for comparing the activity of local anaesthetics is described, based on an observation by Bert (1885) that, although cocaine had no effect on normal skin, it readily anaesthetized the base of an excised blister. A blister is formed on the flexor surface of the forearm using a cantharidin plaster (Armstrong, Dry, Keele, and Markham, 1953). A number of solutions are tested in succession on this exposed area of dermis. By this method an assay can be carried out which is as accurate when used on a single subject as is the wheal method on 20 subjects. When the assay is repeated the results agree within close limits. The activities of cinchocaine, lignocaine (lidocaine), cocaine, butacaine, amethocaine and "ravocaine" (2-propoxyprocaine) relative to procaine have been tested by this method.

METHODS

Intradermal Wheal in Man.—Most of these experiments were carried out during the course of routine practical classes for Second M.B. students. Each student was injected on the flexor surface of the forearm with four or six wheals (0.2 ml.) containing different concentrations of two local anaesthetic drugs in saline. The students were tested in groups of six to nine and the results from a number of groups pooled. The doses were arranged in random order along the forearm to allow for differences in sensitivity. To avoid subjective errors of assessment, the wheals were identified with letters until testing had been completed. Each wheal was tested for anaesthesia by pricking six times in different places with a pin, using a stimulus just great enough to elicit pain on normal skin near the wheal. The number of pricks not felt gave a measure of the degree of anaesthesia. The test was repeated every five minutes for half an hour and the summated response used as a measure of activity. The combined results of tests done at a particular time after the injection were used to study the time course of anaesthesia.

Test on Exposed Dermis in Man.—In this method an exposed area of dermis on the flexor surface of the forearm was used for comparing the activity of local anaesthetics in man. The epidermis was conveniently removed by employing the vesicant action of cantharidin. A 0.3% cantharidin plaster was applied to the area for 6 to 8 hr. (Armstrong *et al.*, 1953). A blister is formed which appears to leave the network of nerve endings in the dermis relatively intact. Only a small area need be exposed for this test—about ¼ sq. cm. After excision of the blister the area was bathed alternately in a stream of Tyrode solution and local anaesthetic solution, using the arrangement shown in Fig. 1. The solutions flowed at a constant rate of about 10 ml./min. from reservoirs through a jacketed warming-tube on to the exposed area. A mechanical stimulus was applied with the spring-loaded camel-hair brush shown in the inset of Fig. 1. The bristles form a plunger which moves into the outer casing as the load on the brush is increased. The outer casing carries a scale, and the load on the brush is indicated by the position of the end of the plunger relative to this scale. The bristles were placed lightly on the exposed area and the load increased until a definite pricking sensation was felt as the individual hairs came into contact with the unanaesthetized dermis. The scale reading at this load was noted and was kept constant throughout the assay. The position of the brush was then changed slightly and the stimulus repeated twice at intervals of about 2 sec. This stimulus is the base-line from which anaesthesia is measured. It was repeated each time before an anaesthetic solution was applied. Certain precautions must be taken to ensure constancy of the stimulus throughout the assay : the brush must not

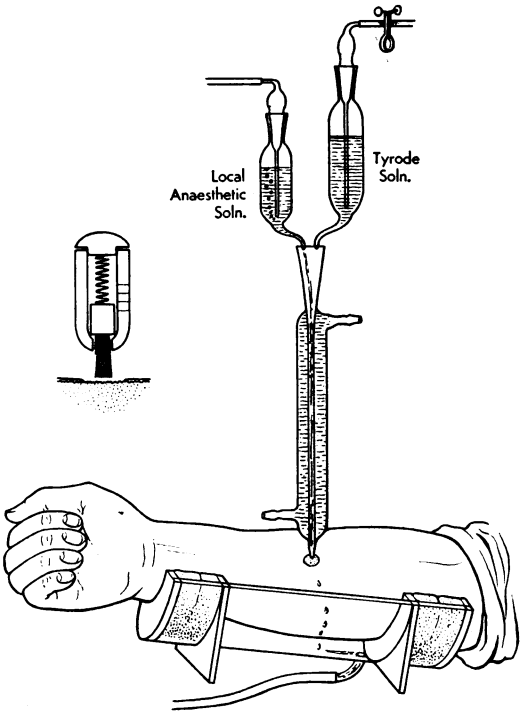

FIG. 1.—Arrangement of apparatus for applying local anaesthetic and Tyrode solutions to the area of exposed dermis. The inset shows the brush used for applying a stimulus to the area. For details, see text.

be moved once it has been brought into contact with the dermis, and must not touch the edge of the blister base; it should be dried between tests.

The degree of anaesthesia was assessed in terms of a three-point scale as slight, appreciable, and full anaesthesia (scored as 1, 2, and 3). The testing procedure consisted of two parts: calibration and assay.

For calibration full anaesthesia was first produced by applying a 0.2% procaine solution. (0.4% was sometimes needed on a freshly exposed area.) The degree of anaesthesia was such that after about 1 min. the standard stimulus could hardly be felt. This is a well-defined point on the scale, and it was usually necessary to determine it only once. Intermediate degrees of anaesthesia were produced by applying, repeatedly if the subject found it necessary, 0.025, 0.05, and 0.1% solutions of procaine. The standard stimulus was applied and the subject was asked to assess the response. Some guidance was given in allocating a score. Score 1 was allocated for a sensation in which the sharp pricking of the individual hairs of the baseline stimulus could no longer be distinguished, score 2 for one in which the edges of the brush could not be clearly felt but the stimulus was still easily perceived, and score 3 for full anaesthesia, in which the stimulus could only be detected by a slight perception of pressure.

The assay itself was carried out by a (2 + 2) design. It usually consisted of 20 "tests," each solution being tested 5 times. A test was carried out as follows. The local anaesthetic solution was allowed to flow over the exposed area; 30, 60, and 90 sec. after the test was begun the flow was interrupted and three stimuli were applied at intervals of about 2 sec. The anaesthetic solution was then replaced by Tyrode solution and a further 90 to 120 sec. were allowed for washing out the anaesthetic and checking the base line. The duration of a test cycle was thus 3 to 3½ min. Although it is desirable for the comfort of the subject to complete the testing within two hours, the area remained sensitive for many hours and was on one occasion used for a further assay on the second day. The assay was carried out as a "double blind" test in which neither subject nor tester knew which solution was used. In most assays the solutions were applied in randomized order in blocks of four, but in some instances a completely randomized design was adopted.

For the test on the exposed dermis the anaesthetic solutions were made up in Tyrode solution of *p*H 7.4. Tyrode solution is well buffered, and the presence of a local anaesthetic in a concentration below 0.1% did not alter its *p*H by more than 0.05 units.

Intradermal Wheal in the Guinea-pig.—These tests were carried out by a slight modification of the method of Bülbring and Wajda (1945), using four wheals (instead of two) on the back of each animal. In one experiment the intradermal wheal method in both the guinea-pig and in man was used in parallel assays. This experiment was carried out with solutions made up in M/5 phosphate at *p*H 7.0.

All drugs were used as the hydrochloride and all concentrations refer to the salt.

RESULTS

1. Intradermal Wheal Method

Fig. 2 shows a comparison of lignocaine and procaine on 52 subjects in a (2+2) assay with 0.1 and 0.4% lignocaine and 0.25 and 1.0% procaine solutions. Analysis of variance shows that the regression lines do not differ significantly from parallelism. The activity of lignocaine was calculated to be 2.6 times greater than that of procaine with limits of 2.2 to 3.1 for P=0.05.

Fig. 3 shows a comparison of cinchocaine and procaine on 46 subjects in a (3+3) assay; procaine 0.4, 1, and 2.5%; and cinchocaine 0.01, 0.025, and 0.062% was used. The detailed results are given in Table I. The mean scores were 14.5, 19.8, and 24.3 for the 3 solutions of procaine and 17.8, 21.0, and 26.4 for the 3 solutions of cinchocaine. There was no significant deviation of the regression lines from linearity or parallelism (Emmens, 1948). Cinchocaine was 49 times more active than procaine with limits of 39 and 61 for P=0.05. A further assay with only four test solutions (pro-

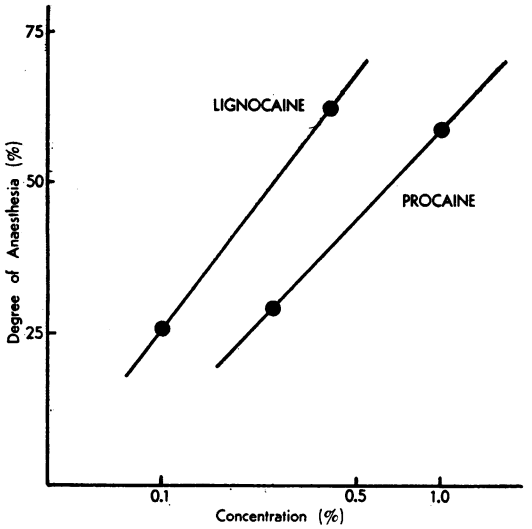

FIG. 2.—Mean results of a comparison of the activity of lignocaine and procaine on 52 subjects in a (2+2) assay using the intradermal wheal method; lignocaine 0.1% and 0.4%, procaine 0.25% and 1·0%. Lignocaine was 2.6 times more active than procaine with limits of 2.2 to 3.1 for P=0.05.

caine 0.5 and 2.5% ; cinchocaine 0.01 and 0.05%) on 57 different subjects gave an activity ratio of 50, with limits of 43 and 59 for P=0.05.

Time Course of Anaesthesia.—Fig. 4 shows the time course of anaesthesia with procaine and cinchocaine. The records were obtained by plotting against time the mean degree of anaesthesia for each of the six successive tests. The time course of anaesthesia varies with the drug used and its concentration. With 0.4% procaine full anaesthesia lasts for only a few minutes, whereas with 2.5% procaine anaesthesia is complete for 10–15 min. The rate at which anaesthesia wears off is independent of the initial concentration injected; anaesthesia of the wheal injected with 2.5% procaine decreases just as quickly as anaesthesia of the wheal injected with 0.4% procaine. The rate of decrease of anaesthesia with cinchocaine is appreciably slower than with procaine.

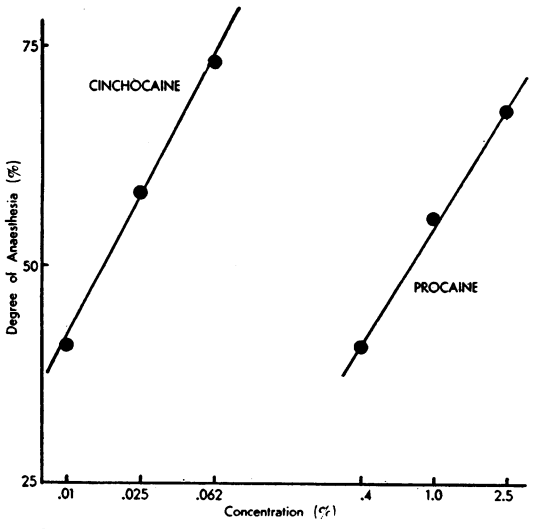

FIG. 3.—Mean results of a comparison of cinchocaine and procaine on 46 subjects in a (3+3) assay using the intradermal wheal method; cinchocaine 0.01%, 0.025%, and 0.062%, procaine 0.4%, 1.0%, and 2.5%. Cinchocaine was 49 times more active than procaine with limits of 39 to 61 for P=0.05.

TABLE I

(3+3) ASSAY OF CINCHOCAINE AGAINST PROCAINE: INDIVIDUAL RESPONSES OF 46 SUBJECTS TESTED IN 6 GROUPS

Response for total anaesthesia=36. The regression lines are linear and do not deviate significantly from parallelism. Cinchocaine is 49 times more active than procaine, with limits of 39 to 61 for P=0.05.

Expt. No.	Procaine			Cinchocaine		
	0·4%	1·0%	2·5%	0·01%	0·025%	0·062%
1	22	23	22	22	22	26
	23	23	27	23	21	33
	18	18	23	17	23	30
	23	27	29	25	30	30
	14	21	22	8	19	24
	15	29	21	17	28	22
	12	28	30	16	36	32
	14	31	31	14	34	29
2	27	24	26	12	20	31
	11	28	20	19	11	34
	27	30	25	24	31	36
	22	22	24	27	28	24
	13	24	34	20	17	28
	12	17	26	14	24	32
	9	16	23	7	17	30
	13	24	31	19	21	32
	11	31	24	15	30	33
3	12	17	25	10	24	27
	8	20	17	10	15	26
	10	10	29	19	19	31
	20	20	33	17	22	27
	11	10	28	10	20	27
	10	11	19	11	16	27
	13	20	21	10	23	22
	16	20	33	20	21	30
4	11	18	19	8	16	22
	9	13	19	16	19	20
	12	21	25	13	22	24
	16	27	18	15	21	23
	14	18	26	9	22	30
	22	24	29	23	20	33
5	14	13	25	16	19	27
	11	16	25	16	19	26
	11	16	24	12	16	22
	12	17	19	6	18	8
	10	16	23	1	24	19
	11	17	29	12	20	28
	15	14	25	12	16	21
6	23	23	32	16	14	26
	12	12	28	16	12	28
	18	16	15	9	16	22
	10	20	21	12	16	20
	16	19	18	20	13	18
	17	13	21	14	26	26
	9	12	12	14	20	23
	10	23	22	16	27	23
Means	14·5	19·8	24·3	14·8	21·0	26·4

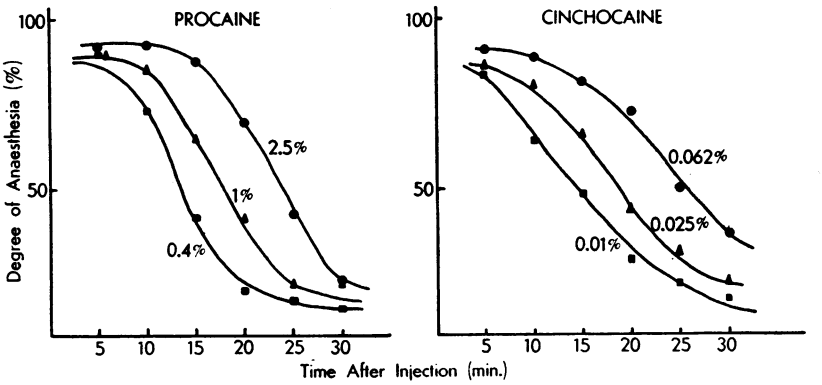

FIG. 4.—Time course of anaesthesia in intradermal wheals using 0.01%, 0.025%, and 0.062% cinchocaine, and 0.4%, 1.0%, and 2.5% procaine. The rate at which the anaesthesia wears off is slower with cinchocaine than with procaine.

The average time for a decline of anaesthesia from 80% to 20% is 14 min. for cinchocaine, and 10 min. for procaine.

Comparison of Human and Guinea-pig Wheal Test.—The value of 50 found for the activity of cinchocaine relative to procaine when tested on the human wheal is 2 to 5 times greater than published values for tests done on the guinea-pig wheal: Bülbring and Wajda (1945) found a value of about 23; Summers and Edge (1947), using a slightly modified version of the same method, found cinchocaine to be 24 times more active, whilst Elio (1948), using the original method, estimated the relative activity to be only 10. In unpublished class experiments with guinea-pigs we obtained an activity ratio of 21.

This species difference was confirmed by carrying out parallel assays in the guinea-pig and in man with solutions made up in M/5 phosphate buffer. Each assay consisted of 12 groups. The results are given in Table II. The presence of the phosphate buffer at *p*H 7.0 appeared to enhance the activity of the cinchocaine in both species, but the human result is again several times higher than that obtained on guinea-pigs.

TABLE II

COMPARISON OF GUINEA-PIG AND HUMAN WHEAL METHODS FOR ASSAY OF CINCHOCAINE AGAINST PROCAINE

Solutions in M/5 phosphate buffer at *p*H 7.0.

Test Solution	Degree of Anaesthesia (out of 36). Each Result is the Mean of 12 Tests	
	Guinea-pig	Man
Procaine, 0·1%	7·2	—
,, 0·5%	23·3	—
,, 0·4%	—	7·0
,, 2·0%	—	21·4
Cinchocaine, 0·01% ..	20·2	16·2
,, 0·05% ..	30·8	26·1
Relative activity (cinchocaine/procaine)	34	100
Species difference (man/guinea-pig) ..	2·9	

II. Exposed Dermis Method

Table III shows the responses obtained during a typical assay comparing cocaine solutions (0.005 and 0.02%) with procaine solutions (0.02 and 0.08%). The subject had previously been used for an experiment of this kind. The exposed area of dermis was about 0.3 sq. cm. Twenty tests (5 groups of 4) were done at 3 min. intervals. The responses elicited at 30, 60, and 90 sec. after the anaesthetic solution began to flow are given in detail to indicate the variation encountered. The test scores for the more concentrated solutions are about twice as great as those for a fourfold dilution; there is no overlap. Table IV shows the

TABLE III

DETAILS OF SCORES OBTAINED IN A TYPICAL ASSAY USING THE EXPOSED DERMIS METHOD

Each solution was tested five times. (Maximum score for each test=27.)

S=0·08% procaine. U=0·02% cocaine.

Group	Time (sec.)	U/4	S/4	U	S
1	30	0 1 1	0 0 1	2 1 3	2 2 3
	60	1 1 1	1 2 2	2 2 2	2 2 2
	90	0 1 1	2 1 2	2 1 2	2 2 3
2	30	1 0 0	1 2 2	1 2 2	3 2 2
	60	1 1 2	1 1 1	2 2 2	3 3 3
	90	1 1 2	1 1 2	2 3 3	3 3 3
3	30	1 1 2	1 1 2	2 2 2	2 3 2
	60	1 1 2	2 2 3	2 3 2	3 3 3
	90	1 2 2	3 2 2	2 3 2	3 3 3
4	30	0 1 1	1 2 2	2 3 3	3 2 3
	60	0 0 1	2 3 2	2 3 2	3 3 3
	90	1 1 1	1 1 2	3 3 3	3 3 3
5	30	2 1 2	2 1 1	2 3 2	2 3 3
	60	1 2 1	2 1 2	2 3 2	3 3 2
	90	1 2 1	1 2 2	3 2 3	2 2 3

TABLE IV

TOTALS OF SCORES AND ANALYSIS OF VARIANCE OF A TYPICAL ASSAY USING THE EXPOSED DERMIS METHOD

(Maximum score for each test = 27.)

S = 0·08% procaine. U = 0·02% cocaine.

Group	U/4	S/4	U	S	Total
1	7	11	17	20	55
2	9	12	19	25	65
3	13	18	20	25	76
4	6	16	24	26	72
5	13	14	22	23	72
	48	71	102	119	340

Variation	Sum of Squares	Degrees of Freedom	Mean Squares	P
Groups ..	68	4	17	<0·01
Anaesthetics	80	1	80	<0·01
Regression	530	1	530	<0·01
Parallelism..	2	1	2	>0·2
Error ..	30	12	2·5	

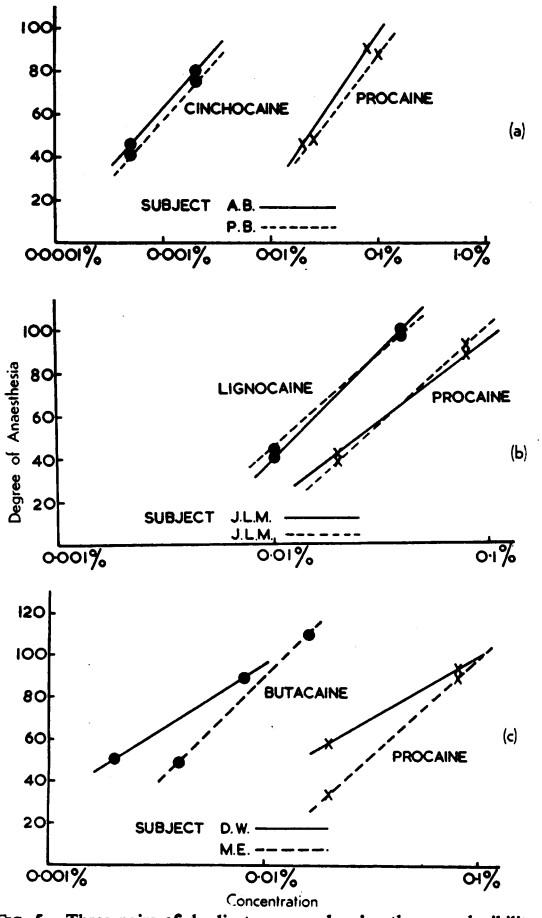

FIG. 5.—Three pairs of duplicate assays showing the reproducibility of assays done on the exposed dermis. The degree of anaesthesia is in terms of the test score. (Full anaesthesia = 135.)

total scores and the analysis of variance (Schild, 1942). The regression lines deviate significantly from parallelism and there is a highly significant difference between the scores for the two concentrations; the method is therefore valid for making quantitative comparisons. The accuracy of a comparison is surprisingly great in view of the fact that the subject had not been specially trained. By this test cocaine was 2.3 times more active (for infiltration anaesthesia) than procaine, with limits of error of 1.9 to 2.9 (P = 0.05).

The assays were reproducible. This is illustrated in Fig. 5, which shows three pairs of duplicate assays. One pair (Fig. 5b) was done on consecutive days on the same subject, using the same blister base. The other two pairs were done on four different subjects none of whom had been previously tested. The difference in slope of the regression lines on the two butacaine and procaine assays (Fig. 5c) reflects the difference of the assessment of the degree of anaesthesia. However, within each assay the regression lines for the two drugs were parallel.

Table V gives the relative activities of six local anaesthetics tested against procaine by the exposed dermis method. Each substance was tested twice; in five cases a different subject was used for the second test. Analysis of variance showed that there was no deviation from parallelism at the 1% level of significance.

TABLE V

ACTIVITIES OF LOCAL ANAESTHETICS RELATIVE TO PROCAINE MEASURED ON THE EXPOSED DERMIS OF THE FOREARM

Anaesthetic	Relative Activity		Limits of Error (P = 0·05)
	Duplicate Assays	Mean Result	
Cocaine	2·31 2·67	2·5	1·9– 2·9 2·1– 4·4
Cinchocaine ..	33·0 32·6	32·8	27–41 26–41
Lignocaine	2·28 2·30	2·3	1·8– 3·4 1·3– 4·2
Butacaine	7·86 8·06	8·0	4·1–15·1 4·7–13·7
Amethocaine ..	27·4 41·6	34·5	20–37 19–68
Ravocaine	9·55 6·02	7·6	4·2–17·4 4·2– 8·6

Duration of Test.—The question arises whether the duration of contact of the local anaesthetic solution with the exposed area of dermis is a significant variable in the assessment of the relative activity of two local anaesthetics. It was found that with all local anaesthetics tested except cinchocaine a continuous flow of solution over the area produced a degree of anaesthesia in 30 sec., which was 80 to 90% of the average value for the whole

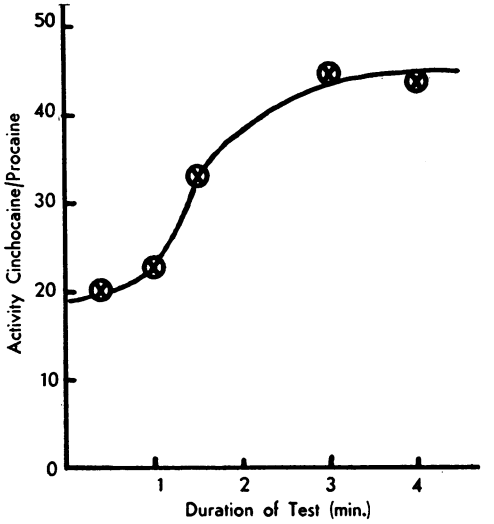

FIG. 6.—Effect of duration of test period on the activity of cinchocaine relative to procaine. The activity increases with time of contact for test periods of less than 3 min.

test. Cinchocaine is an exception; it acts more slowly than procaine and the activity ratio of the two drugs depends on the duration of the test. This is shown in Fig. 6, which gives the results of five assays of varying duration. Cinchocaine is about 20 times more active than procaine when tested after a contact time of 30 sec. but 45 times more active when tested after 3 or 4 min. contact. After this time the activity ratio reaches a steady value. The activity ratio obtained with the standard contact time of 90 sec. is thus 27% less than the steady value. Lignocaine, butacaine, amethocaine and ravocaine also appeared to act slightly more slowly than procaine, and cocaine more rapidly; but the differences are small compared with those shown by cinchocaine.

TABLE VI

PRECISION COEFFICIENTS OF ASSAYS

The value of λ is equal to the ratio standard error/slope of regression line and is an estimate of the variability from test to test

Substance		Design	Precision Coefficient
Lignocaine	Human intradermal wheal (class experiment)	2+2	0·36
Cinchocaine	,, ,, ,,	3+3	0·40
,,	,, ,, ,,	2+2	0·27
,,	Human intradermal wheal (research)	2+2	0·30
,,	Guinea-pig intradermal wheal (research)	2+2	0·38
Cocaine ..	Human exposed dermis	2+2	0·092 and 0·120
Cinchocaine	,, ,, ,,	2+2	0·092 ,, 0·102
Lignocaine	,, ,, ,,	2+2	0·166 ,, 0·180
Butacaine ..	,, ,, ,,	2+2	0·262 ,, 0·212
Amethocaine	,, ,, ,,	2+2	0·136 ,, 0·240
Ravocaine	,, ,, ,,	2+2	0·232 ,, 0·152

Accuracy of Tests

Table VI summarizes the precision of the various assays. Under class conditions the precision coefficient (λ) for assays by the intradermal wheal method in man averages 0.34; under research conditions it is 0.30. The corresponding value for the intradermal method with guinea-pigs is 0.38—i.e., the human subject is rather more discriminating than the guinea-pig. The coefficient for the exposed dermis method is consistently lower than for the intradermal wheal method. The average coefficient for the 12 assays in Table V is 0.155 (harmonic mean). This is about half the value of the intradermal wheal method. The exposed dermis method is thus considerably more precise: the same degree of accuracy can be obtained with one quarter the number of tests. A further advantage of the method is that many more tests can be done in one session. Five groups were usually done in one session as compared with only one group with the wheal method. In terms of numbers of subjects required for an assay of given accuracy the efficiency of the new method is therefore 20 times greater than that of the wheal method.

DISCUSSION

The design of a test for comparing the activity of local anaesthetics appears to be a compromise between accuracy and relevance to conditions of use. Probably the most precise method and the simplest of interpretation is that which measures depression of conduction in an isolated nerve trunk. However, the results of such measurements do not relate to practical conditions of use; factors such as diffusion to the nerve fibre and the buffering action of the surrounding tissue are ignored. On the other hand, *in vivo* tests on the intact animal are less accurate, but they do approximate to clinical conditions.

Despite the low precision of *in vivo* tests any desired accuracy can be obtained by increasing the number of tests. The three assays done by the intradermal wheal method on groups of about 50 subjects illustrate the degree of accuracy that can be achieved in this way. However, these assays are laborious and a method which gives similar accuracy with only 2 or 3 subjects is preferable.

The increase in precision obtained by using the exposed dermis method results from eliminating variations in the test area; all the solutions can be tested repeatedly on the same site under identical conditions. In this respect the exposed dermis method approaches the conditions of an isolated

nerve preparation, and the question arises whether the results obtained by it are as relevant to clinical use as those obtained by the intradermal wheal method. Although the site of action is similar in both methods, diffusion and buffering of the tissue probably play a smaller part in the exposed dermis. The contact time in the exposed dermis method is much shorter than in the wheal method (or in clinical practice) and this may bias the results when the rate of action of the two substances is appreciably different. For example, cinchocaine had only 35 times the activity of procaine with a contact time of 90 sec., but with 3 min. contact the value has risen to 45, approximating that of 50 for the wheal. Thus, when testing local anaesthetics with differing rates of action, the exposed dermis test of 90 sec. is likely to bias the result slightly against the slower acting compound. The rates of action of the five remaining compounds tested (Table V) do not differ much from that of procaine, and the two methods would therefore be expected to agree. This was so for lignocaine, which was tested against procaine by both methods. It is surprising to find that lignocaine does not act more rapidly on the exposed dermis than procaine. This observation is in contrast to the claims made from observations in clinical trials (Gordh, 1949; Crawford, 1953).

It may be possible to increase the precision of the exposed dermis method by further subdividing the scale of anaesthesia. This would involve using trained subjects. We considered it undesirable to ask a subject to submit to frequent testing even though the blisters are small and heal readily. Nine of the assays in Table V have been done on subjects who were entirely new to the test; the remaining three assays were done on subjects who had been tested only once before. The use of untrained subjects for the test does not produce misleading results. If the limits of error of the estimate of relative activity are not sufficiently close, the test can be repeated and the results combined until the desired accuracy has been reached. On the other hand, tests done on the guinea-pig, however accurately performed, cannot be directly applied to man, since there is a systematic species difference between guinea-pig and man.

SUMMARY

1. Two methods for studying the activity of local anaesthetics in man are described. One is an intradermal wheal method. The other uses a small area of exposed dermis on the forearm (exposed dermis method).

2. Cinchocaine and lignocaine were compared with procaine by the wheal method. In a (3+3) assay with 46 subjects cinchocaine was 49 times more active than procaine (39 to 61, $P=0.05$); in a (2+2) assay with 57 subjects it was 50 times more active (43–59, $P=0.05$). In a (2+2) assay with 52 subjects lignocaine was 2.6 times more active than procaine (2.2–3.1, $P=0.05$).

3. By the intradermal method the activity ratio of cinchocaine and procaine was 2 to 3 times greater in man than in the guinea-pig.

4. By the exposed dermis method, activities relative to procaine were: cocaine 2.5, cinchocaine 33, lignocaine 2.3, butacaine 8.0, amethocaine 34, and ravocaine 7.6.

5. By both methods cinchocaine acted more slowly than procaine.

6. The precision of the intradermal wheal and the exposed dermis methods has been compared. The latter was about 20 times more efficient in terms of the number of subjects required for an assay of given accuracy.

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