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MULTIPLE TOE-PINCH METHOD FOR TESTING ANALGESIC DRUGS

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In guinea-pigs and rats, an immediate squeak was one of the most consistent and readily observed responses to application of a light artery clip to the base of a toe. Morphine and related drugs suppressed this response. Squeak-responses from each toe of an experimental animal formed the basis of a technique for measuring activity of analgesic drugs. A statistical method was developed to analyse the correlated quantal observations obtained. It provided an estimate of the increase of information from several toes compared with one. Testing all toes of each animal yielded a substantial increase of information, because the correlation between responses of different toes was low. Among drugs having an analgesic action in man, 1-(β-diethylaminoethyl)-2-(*p*-ethoxybenzyl)-5-nitrobenzimidazole, methadone, morphine, pethidine and codeine (in descending order of potency) were active in this test in guinea-pigs. Acetylsalicylic acid, amidopyrine, amphetamine, chlorpromazine, 4-hydroxyisophthalic acid, lysergic acid diethylamide, mephenesin, nalorphine, pentetrazole, phenobarbitone, phencyclidine, phenytoin, salicylamide, strychnine and troxidone showed little or no activity. The time-courses of active drugs were estimated, and morphine had the longest action.

Haffner (1929) evaluated analgesic drugs by ability to suppress responses to a clip applied to the tail of a mouse or to the ear of a guinea-pig. Bianchi & Franceschini (1954) used a similar tail-pinch method in mice to investigate the potencies and time-courses of morphine, pethidine and methadone and to study the development of tolerance to these drugs.

We describe a method of measuring the activity of analgesic drugs in which a clip was applied, not to a single point, but to several comparable points on the same animal so as to increase the information yielded by each animal. The correlated quantal observations obtained required a novel statistical analysis. We have used mainly guinea-pigs to estimate the potencies and time-courses of some known analgesic drugs, testing all toes of each animal. Experiments with rats have shown that the multiple toe-pinch method can be used with a second toed species, and it seems suitable for others.

Warner & Collier (1960) have communicated an account of the statistical procedure involved in the multiple toe-pinch test. The pharmacological findings were reported to the British Pharmacological Society in January, 1961.

METHODS

Pharmacological methods

Male albino guinea-pigs, about 4 weeks old and ranging in weight from 150 to 300 g, were used, each animal being submitted to experiment once only. During experiments, animals were caged singly but within sight of one another. Responses were tested with an artery clip of light tension, the jaws of which were sheathed in polythene tubing. It was applied in random sequence to the bases of the 14 toes and, in untreated animals only, to both ears and to 4 places on the loose skin of the back (Fig. 1). In most experiments with analgesic

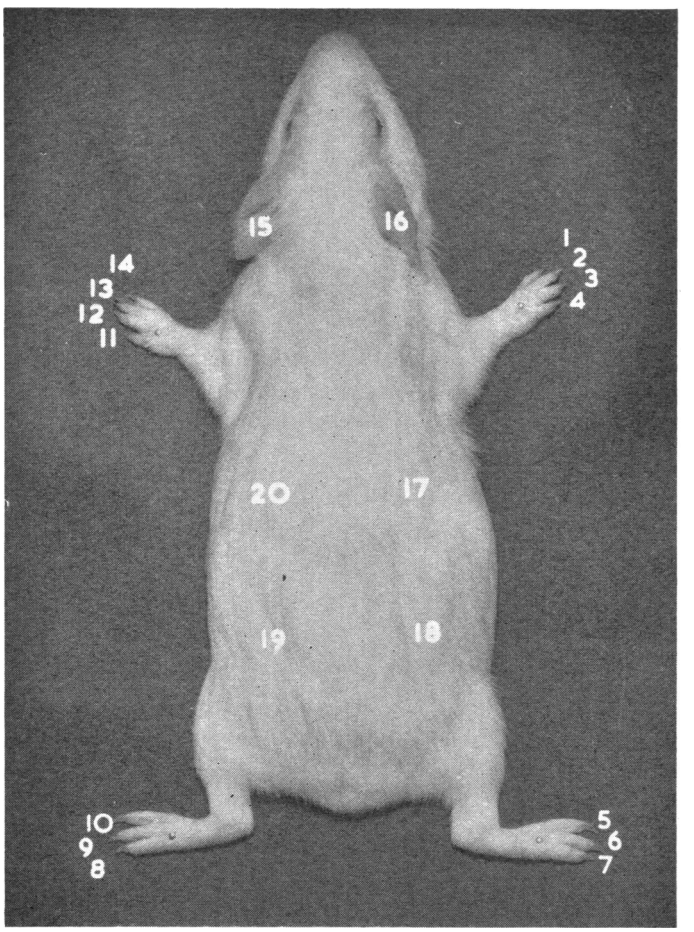

Fig. 1. Guinea-pig, showing points of application of the artery clip.

drugs the clip was applied once to each of the toes before administration of drug and at predetermined times afterwards, each round of 14 applications making one cycle of testing. In a few experiments a cycle consisted of 14 applications to toe 2 (see Fig. 1) at intervals of 5 sec. The clip was removed as soon as it was clear whether an immediate squeak (the squeak-response) was forthcoming, usually within 2 to 3 sec. Some experiments were performed with white rats, about 3 weeks old and weighing about 50 g.

Substances tested. Acetylsalicylic acid and its calcium salt, prepared by mixing 10 parts by weight of the acid with 3 of calcium carbonate and 1 of citric acid and dissolving in 0.9%

sodium chloride solution immediately before use; amidopyrine; amphetamine sulphate; chlorpromazine hydrochloride; codeine phosphate; 1-(β-diethylaminoethyl)-2-(*p*-ethoxybenzyl)-5-nitrobenzimidazole; 4-hydroxyisophthalic acid; lysergic acid diethylamide bitartrate; mephenesin; methadone hydrochloride; morphine sulphate; nalorphine hydrobromide; pentetrazole; pethidine hydrochloride; phenobarbitone sodium; 1-(1-phenylcyclohexyl) piperidine hydrochloride (phencyclidine; Chen & Weston, 1960); phenytoin sodium; salicylamide; strychnine sulphate; troxidone. Doses are expressed throughout as weights of active acid or base.

Statistical methods

Computations were based on the score which was taken as the proportion of applications of the clip which failed to elicit a squeak in one animal in one cycle of testing. The mean scores in dosage groups were subjected to the angular transformation (Finney, 1952b) and regression lines against log dose fitted by the usual iterative procedure, so that for any compound the ED50 (the dose for which the mean score was 0.50) could be estimated. To compare compounds, parallel lines were fitted at the times of maximal effect for each, and the ED50 values and relative potencies determined from these lines. To investigate the time-course of action of a drug, lines were fitted independently at each time at which observations were made. The 95% fiducial limits of the ED50 values and relative potencies were determined using Fieller's Theorem (Fieller, 1940) after estimation of the residual standard deviation.

Estimation of residual standard deviation. The computations differed from the usual quantal procedure in that the residual standard deviation had to be calculated from the results instead of being taken as unity. Suppose there are k dosage groups of n pigs, the score on the jth pig of the ith group is p_{ij}, and the mean score for the ith group is $\bar{p}_i$. Suppose also that the fitted angle for the ith group at the final stage of the iterative procedure is Y_i, corresponding to a probability of a positive response of P_i. The angular transformation is

$$P_i = \sin^2 Y_i$$

The residual sum of squares to be used in this analysis is

$$\sum_{i=1}^{k} \frac{\sum_{j=1}^{n} (p_{ij} - \bar{p}_i)^2}{P_i(1-P_i)} = 4 \qquad \sum_{i=1}^{k} \frac{\sum_{j=1}^{n} (p_{ij} - \bar{p}_i)^2}{\sin^2 2Y_i}$$

and this has $k(n-1)$ degrees of freedom. The left-hand side of the equation shows that the residual is a sum of ratios of the observed variance between scores in the same dosage group to the binomial variance that would be expected if only one quantal variate/guinea-pig was observed. The right-hand side was used in computation.

The computational procedure is illustrated in Table 1, using results of the experiment of Fig. 2. The notation is that of Finney (1952a, b). The first fitted line is obtained from empirical angles corresponding to the mean scores, $\bar{p}_i$. Two cycles of iteration are necessary to get successive values of Y_i differing by less than 1°, and the values of y_i and Y_i in the second cycle were used subsequently. The sums of squares and products, S_{xx}, S_{xy}, S_{yy}, were calculated giving a weight of one to each dose, so that the numerical factor appearing in the analysis of variance table is

$$5w = 5 \times \frac{4\pi^2}{(180)^2} = 0.0060925$$

where the 5 occurs because there are 5 pigs in each dosage group.

Information ratio. An analysis of 5 independent quantal responses at each dose would be the same as above except that the residual mean square would be unity. It follows that the reciprocal of the residual mean square is an estimate of the ratio of information obtained from several quantal observations to that expected from one. We have called this the information ratio, and, for the example in Table 1, it is 2.94.

TABLE 1

COMPUTATIONAL PROCEDURE FOR ESTIMATING ED50 VALUES, RESIDUAL STANDARD DEVIATION AND INFORMATION RATIO

Results were obtained in one experiment in which guinea-pigs were tested 1 hr after receiving morphine subcutaneously. The clip was applied to 14 toes and the score is the proportion of applications that failed to elicit a squeak. The information ratio is the ratio of information obtained using more than one toe to that expected from one

Group i	Dose of morphine (mg/kg)	x_i	Score for each guinea-pig p_{ij}	Mean score $\bar{p}_i$	Empirical angle	Y_i	1st cycle y_i	1st cycle Y_i	2nd cycle y_i	2nd cycle Y_i	$\sin^2 2Y_i$	$\Sigma(p_{ij}-\bar{p}_i)^2$
1	2·825	−2	0 0·36, 0 0 0	0·07	15·3	12·8	15·6	13·8	15·4	13·9	0·2175	0·1020
2	4·0	−1	0·57, 0·14, 0 0·43, 0·14	0·26	30·7	30·2	30·7	30·2	30·7	30·1	0·7531	0·2204
3	5·65	0	0·43, 0·07, 1·00, 0·71, 0·43	0·53	46·7	47·5	46·7	46·6	46·7	46·4	0·9976	0·4857
4	8·0	+1	0·86, 0·29, 0·57, 0·64, 1·00	0·67	54·9	64·9	53·7	63·0	54·2	62·6	0·6677	0·3020
5	11·3	+2	1·00, 1·00, 1·00, 1·00, 1·00	1·00	90·0	82·2	86·1	79·4	84·8	78·8	0·1452	0

$S_{xx}=10$, $S_{xy}=162{\cdot}3$, $S_{yy}=2742{\cdot}972$, $\bar{x}=0$, $\bar{y}=46{\cdot}36$, $b=S_{xy}/S_{xx}=16{\cdot}23$

Fitted line, $Y=y+b(x-\bar{x})=46{\cdot}36+16{\cdot}23\,x$

When $Y=45$, $x=-0{\cdot}0838$.

Hence ED50 $=5{\cdot}65$ antilog$_{10}$ $(-0{\cdot}0838 \log_{10}\sqrt{2})=5{\cdot}5$ mg/kg.

Nature of variation	Sum of squares	d.f.	Mean square	Variance ratio
Regression	$0{\cdot}0060925\ (S^2_{xy}/S_{xx})=16{\cdot}048$	1	16·048	47·2, $P \ll 0{\cdot}001$
Means about regression	$0{\cdot}0060925\left\{S_{yy}-\frac{S^2_{xy}}{S_{xx}}\right\}=0{\cdot}663$	3	0·221	<1, not significant
Residual	$4\sum_i \frac{\Sigma(p_{ij}-\bar{p}_i)^2}{\sin^2 2Y_i}=6{\cdot}808$	20	0·340	

95% fiducial interval for ED50 = 4·7–6·3 mg/kg. Information ratio $=(0{\cdot}340)^{-1}=2{\cdot}94$.

RESULTS

Responses of untreated guinea-pigs to the clip

A guinea-pig responds to external application of an artery clip in a number of ways that depend partly on the tension of the clip and on the site of application. Responses include squeaking, starting, flattening the ears, opening and closing the mouth, shaking or withdrawing the limb if the clip is applied to a toe and struggling. The squeak-response was chosen for experiments with analgesic drugs, since it occurred almost immediately and was readily observed as a quantal effect.

Table 2 gives the squeak-responses obtained from 3 untreated guinea-pigs when the artery clip was applied at 0, 30 and 60 min in random order to the 20 points illustrated in Fig. 1. This table shows that guinea-pigs nearly always responded

TABLE 2

SQUEAK-RESPONSES OF UNTREATED GUINEA-PIGS TO REPEATED APPLICATIONS OF AN ARTERY CLIP AT HALF-HOURLY INTERVALS

The points of application are shown in Fig. 1

Time of test (min)	Guinea-pig no.	No. of squeaks		
		14 toes	2 ears	4 skin points
0	1	14	1	0
	2	13	0	0
	3	11	0	1
30	1	14	1	0
	2	14	0	1
	3	13	0	1
60	1	14	1	1
	2	14	0	2
	3	14	0	1

to applications of the clip to their toes, but not to their ears or skin. Although a clip of greater tension might have evoked a consistent response from ears or skin, we used the toes because a light clip seemed less likely to damage tissues and because the toes presented multiple similar points for application.

Table 2 also shows that the proportion of squeak-responses increased with repeated applications of the clip to toes or skin. Since its second application to the toes elicited almost 100% squeak-responses, the clip was applied to every toe immediately before drug treatment.

Responses of treated guinea-pigs to the clip

Morphine in sufficient dose suppressed squeak-responses to all applications of the clip to the toes. Fig. 2 shows examples of dose-response lines obtained on different occasions with morphine by three different routes. The values plotted are those at the times of peak effect.

Table 3 gives the average values of the ED50, slope and information ratio, and the coefficient of variation between the ED50 values, obtained from 25 experiments in which morphine was given by one of three routes. The subcutaneous route was chosen for general use, because the average slope and information ratio were greatest and variation between estimates of the ED50 value least.

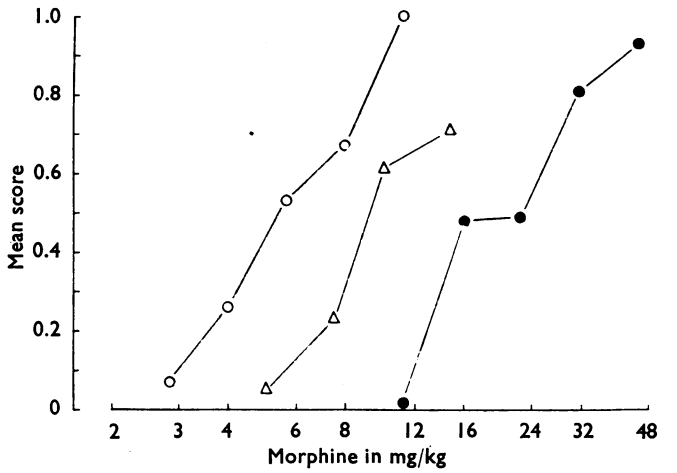

Fig. 2. Examples of log dose-response lines for morphine by three routes in guinea-pigs. For definition of mean score, see Table 1. ○ — ○ subcutaneous, 1 hr after treatment, 5 animals/dose. △— △ intraperitoneal, 1 hr after treatment, 4 animals/dose. ● — ● oral, 4 hr after treatment, 6 animals/dose.

TABLE 3

AVERAGE VALUES OF THE ED50, SLOPE AND INFORMATION RATIO, AND COEFFICIENTS OF VARIATION BETWEEN THE ED50 VALUES, OBTAINED IN 25 EXPERIMENTS WITH MORPHINE BY THREE ROUTES IN GUINEA-PIGS

For explanation of information ratio, see Table 1

Route of administration	No. of tests	Total no. of guinea-pigs	Average ED50 (mg/kg)	Average slope (°/log dose)	Average information ratio	Coefficient of variation between ED50 values (%)
Intraperitoneal	13	156	13·8	67	1·92	37
Oral	4	76	11·1	59	1·95	66
Subcutaneous	8	102	6·3	82	4·13	26

By this method, four morphine-like drugs—codeine, pethidine, methadone and 1-(β-diethylaminoethyl)-2-(*p*-ethoxybenzyl)-5-nitrobenzimidazole — gave dose-response lines from which potencies were estimated (Table 4). Consistent results were obtained in two independent experiments in each of which codeine and

TABLE 4

SOME ANALGESIC DRUGS IN GUINEA-PIGS. POTENCIES RELATIVE TO MORPHINE AND INFORMATION RATIOS

Relative potencies were determined from minimal values of ED50 obtained in the same experiment using the same route of administration. s.c.=subcutaneous; i.p.=intraperitoneal. 20684-Ba is 1-(β-diethylaminoethyl)-2-(*p*-ethoxybenzyl)-5-nitrobenzimidazole. For explanation of information ratio, see Table 1

Drug	Route of administration	Potency relative to morphine (95% fiducial interval)	Information ratio
Codeine	s.c.	0·19 (0·13–0·25)	5·6
	s.c.	0·23 (0·19–0·27)	2·7
	i.p.	0·38 (0·27–0·63)	2·7
Pethidine	s.c.	0·25 (0·18–0·35)	6·3
	s.c.	0·37 (0·31–0·45)	13·4
	i.p.	0·46 (0·30–0·74)	1·6
Methadone	s.c.	2·5 (1·9–3·3)	2·3
20684-Ba	s.c.	1,960 (1,650–2,460)	4·9

pethidine were given subcutaneously. The information ratios in Table 4 range between 1.6 and 13.4, showing that, with each drug, testing 14 toes yielded considerably more information than would have been obtained by testing only one.

Some drugs not of the morphine type but having or reported to have analgesic effects were also tested. Given subcutaneously, acetylsalicylic acid (800 mg/kg) and its calcium salt (400 mg/kg), amidopyrine (160 mg/kg), amphetamine (50 mg/kg), chlorpromazine (64 mg/kg), 4-hydroxyisophthalic acid (400 mg/kg), lysergic acid diethylamide (1 mg/kg), nalorphine (128 mg/kg), phencyclidine (16 mg/kg) and salicylamide (400 mg/kg) did not suppress the squeak-response. Although itself ineffective, nalorphine (32 mg/kg) antagonized the effect of morphine (8 mg/kg) given half an hour afterwards, both drugs being administered subcutaneously.

Some anticonvulsant drugs were given subcutaneously at doses which retarded but did not abolish the righting reflex. Of these, mephenesin (800 mg/kg), phenobarbitone (50 mg/kg) and troxidone (1 g/kg) did not affect the squeak-responses; but phenytoin (50 mg/kg) suppressed about one-third of these responses.

The convulsant drugs pentetrazole (50 mg/kg) and strychnine (2 mg/kg), when given subcutaneously in amounts just below the lethal dose, failed to suppress the squeak-response.

TABLE 5

TIME-COURSES OF SOME ANALGESIC DRUGS IN GUINEA-PIGS

s.c.=subcutaneous; i.p.=intraperitoneal; p.o.=oral. 20684-Ba is 1-(β-diethylaminoethyl)-2-(*p*-ethoxybenzyl)-5-nitrobenzimidazole

Drug	Route of administration	No. of guinea-pigs	Time after administration in hr	ED50 (95% fiducial interval) in mg/kg
Morphine	s.c.	20	0·5	6·5 (4·7–8·0)
			1	5·4 (4·4–6·6)
			2	5·1 (4·1–6·4)
			4	6·0 (4·7–7·5)
			6	11·8 (8·6–18·7)
Morphine	p.o.	30	0·5	36·7 (29·3–55·9)
			1	26·0 (18·9–42·2)
			2	22·3 (15·8–29·8)
			4	21·4 (16·9–26·4)
			6	28·6 (23·5–39·0)
Codeine	s.c.	15	0·25	25·8 (17·9–32·6)
			0·5	25·7 (18·8–31·6)
			1	40·4 (27·4–826)
			2	>50
Pethidine	s.c.	15	0·25	15·9 (13·6–18·0)
			0·5	16·1 (14·7–17·6)
			1	22·2 (18·8–28·2)
			2	⩾30
Pethidine	i.p.	20	0·25	26·4 (14·4–31·9)
			0·5	30·2 (22·2–39·8)
			1	46·0 (36·7–68·1)
			2	>64
Methadone	s.c.	12	0·25	3·6 (2·8–6·3)
			0·5	3·2 (2·5–4·7)
			1	>4·5
20684-Ba	s.c.	15	0·25	0·0037 (0·0018–0·0059)
			0·5	0·0037 (0·0028–0·0059)
			1	0·0049 (0·0034–0·0066)
			2	>0·0083

TABLE 6

NUMBERS OF SQUEAK-RESPONSES CLASSIFIED BY ANATOMICAL POSITION OF TOES AND BY TEMPORAL ORDER OF TESTING IN SIX EXPERIMENTS WITH ANALGESIC DRUGS IN GUINEA-PIGS

The positions of toes are shown in Fig. 1. Toes were tested in random sequence. Responses, at the observed time of peak effect, at all dose-levels within each experiment are pooled

		Total squeak-responses																									
		By anatomical position														By temporal order of testing													
Drug	No. of pigs	1	2	3	4	5	6	7	8	9	10	11	12	13	14	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Morphine	24	12	16	14	13	14	17	14	12	15	12	6	13	14	8	9	9	10	12	11	13	13	13	14	13	17	17	15	14
Morphine	18	9	10	6	8	7	7	11	6	9	11	6	5	9	10	3	5	7	7	7	10	9	8	8	7	9	11	11	12
Codeine	15	5	6	4	6	7	8	8	7	7	8	3	5	5	4	2	4	5	5	6	7	6	6	6	8	8	8	5	7
Pethidine	15	4	5	7	7	6	8	8	7	6	7	4	6	7	8	2	3	5	6	6	9	6	8	7	7	6	7	10	8
Morphine	18	10	6	8	6	8	6	7	6	4	6	4	3	8	6	2	1	6	4	5	6	7	6	7	9	8	10	7	10
Morphine	25	12	13	13	11	14	13	15	13	16	10	5	12	14	12	7	10	9	10	10	9	11	13	17	15	16	15	15	16
All	115	52	56	52	51	56	59	63	51	57	54	28	44	57	48	25	32	42	44	45	54	52	54	59	59	64	68	63	67

Time-courses of actions. The time-courses of active drugs were studied by testing the same animals at a number of times after treatment. Table 5 gives the time-courses of 5 drugs in terms of the ED50 values obtained at each time of testing. Of the drugs examined, morphine was by far the longest-acting, for its effect did not lessen appreciably until >4 hr after treatment by oral or subcutaneous routes.

Position and order of testing of toes. To examine how the anatomical position of toes and temporal order of testing affected the response, we analysed results from a number of experiments with analgesic drugs. The numbers of squeak-responses obtained in 6 experiments are classified by position and order in Table 6. Analysis showed that the numbers of responses were significantly different with respect both to anatomical position ($P<0.001$) and temporal order ($P<0.00001$). By an approximate test for outliers (Tukey, 1949), the toe in position 11 was significantly ($P<0.00001$) less responsive than the remaining 13 toes, which did not differ significantly ($P>0.20$) from one another. Although the differences were not significant, the two next least responsive toes (12 and 14) also occurred on the left forelimb.

In all experiments in Table 6, the animal was held with the left hand and the clip applied to its toes with the right. To investigate whether the unresponsiveness of toe 11 was due to a bias inherent in this way of handling, such as a difference in accessibility, two experiments were performed, in which the operator reversed the actions of left and right hands. In these, toe 11 was slightly more responsive than average, while toe 4, the corresponding toe on the opposite side, was slightly less responsive than average.

Table 6 also shows that, as the cycle of testing proceeded, responsiveness increased. Plotting total squeak-responses in Table 6 against order of testing gave a line that had significant slope ($P\ll0.00001$) and did not deviate from linearity significantly ($0.1<P<0.25$). In all experiments in this table, the 14 toes were tested in random sequence as quickly as practicable, the cycle taking about 1 min. To explore how time between testing of individual toes affected responsiveness, we carried out 2 experiments, in which toes were tested 1 hr after treatment with morphine, at intervals of 30 sec in one experiment and of 2 min in the other. In both of these, the proportion of squeak-responses increased with temporal order of testing.

TABLE 7

CORRELATIONS BETWEEN TOES BY ANATOMICAL POSITION IN TWO INDEPENDENT EXPERIMENTS IN GUINEA-PIGS

Average correlation between pairs of toes	No. of correlations	Expt. 1 (48 animals)	Expt. 2 (18 animals)
All possible	91	0·21	0·32
On different feet	73	0·20	0·31
On same foot	18	0·24	0·35
Adjacent on same foot	10	0·29	0·39
Non-adjacent on same foot	8	0·18	0·29
Corresponding on left and right feet	7	0·20	0·20

The product moment correlation coefficients between responses from pairs of toes, classified by anatomical position and temporal order of testing, were computed. The results for anatomical position from two experiments involving 48 and 18 guinea-pigs are summarized in Table 7. The average correlations between all

possible pairs of toes were low. As might be expected, average correlations between pairs of toes both on the same foot were higher than those between pairs on different feet and average correlations between adjacent toes were higher still, though all differences were small. The average correlations between corresponding toes on left and right sides were slightly lower than the overall average. The significance of these differences could not be tested. When toes were classified by temporal order of testing, correlations between pairs did not seem to depend on the number of intervening toes.

Information from different numbers of toes. In all experiments with guinea-pigs reported above, 14 toes were tested. By analysing separately results from selected toes, we have studied the relationship between number of toes tested and information gained. Information ratios were determined for even numbers of toes, chosen either by anatomical position or by temporal order. With either classification, comparable results were obtained. For example, using results from the larger experiment in

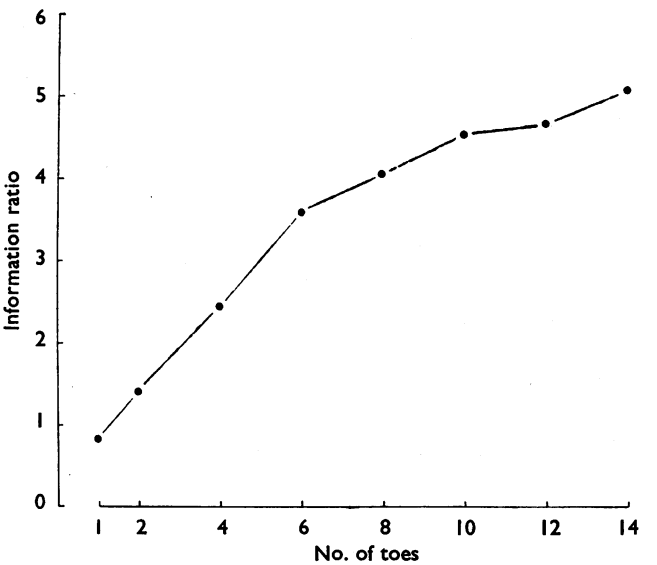

Fig. 3. Increase of information with number of toes. To calculate information ratios (for explanation, see Table 1), toes were taken in temporal order of testing. The results are from a comparison of morphine, pethidine, and codeine by the subcutaneous route in an experiment using 48 guinea-pigs.

Table 7, information ratios were calculated for different numbers of toes taken in temporal order. These are plotted in Fig. 3, which shows, as might be expected, that information increased with the number of toes tested, but the rate of increase declined.

Although increasing responsiveness with temporal order of testing did not change the shape of the curve in Fig. 3, it caused a rise in ED50 values as more toes were included. For example, the ED50 of morphine for the first 6 toes tested was 4.5 and for all toes 5.9 mg/kg.

Repeated testing of a single toe. In two experiments with morphine, a clip was applied 14 times to toe 2, and, in one reference experiment, the same clip was applied as usual once to each toe per test cycle. The results of these experiments are given in Table 8, which shows that morphine was considerably more potent when

TABLE 8

REPEATED TESTING OF A SINGLE TOE. ESTIMATES OF ED50, SLOPE AND INFORMATION RATIO 1 HR AFTER MORPHINE SUBCUTANEOUSLY

For explanation of information ratio, see Table 1

Form of test	Slope in °/log dose	ED50 (95% fiducial interval) in mg/kg	Information ratio
14 toes tested once	89	7·2 (5·5–9·2)	3·0
1 toe tested 14 times	28	1·2 (0·4–2·1)	2·1
	43	1·6 (0·7–3·6)	1·8

one toe was tested repeatedly, but the slope of the dose-response curve and the information ratio were lower. Another difference from our findings with the usual procedure was that the increase of responsiveness of the one toe to the clip was not evident as the cycle proceeded.

Experiments with rats

Preliminary experiments showed that weanling white rats squeaked sharply when the clip was applied to the base of a toe. In one experiment, morphine was administered subcutaneously at different doses to 4 groups of animals and saline to a fifth group. The clip was applied in random sequence to the 18 toes of each animal before treatment and 30, 60 and 120 min after treatment. Morphine at 10 mg/kg completely, and at lower doses partially, suppressed the squeak-responses. The peak ED50 value at 30 min was estimated as 3.3 mg/kg, with a 95% fiducial interval of 2.8 to 3.9 mg/kg, and the information ratio was 5.5. After the lower doses of morphine, the toes on the forelimbs were on average less sensitive than those on the hindlimbs.

DISCUSSION

The term " analgesic " has two disadvantages. First, depression of pain may not be complete, so that the effect would be better described as " hypoalgesic." However, the term analgesic is so well established that we have not attempted to introduce a new one. The second disadvantage applies to animal tests, in which we do not know whether the subject actually feels pain and where, therefore, both terms analgesic and hypoalgesic may be inappropriate. Winder (1959) has applied the term " nociceptive " to reactions to noxious stimuli in animals and " antinociceptive " to drugs reducing such reactions. In the present work the compounds found active are known to relieve pain in man, and for these we have therefore continued to use the term analgesic drug.

Of the drugs examined, diethylaminoethylethoxybenzylnitrobenzimidazole, methadone, morphine, pethidine and codeine, in descending order of potency, were capable of suppressing the squeak-response completely. They suppressed not only the

squeak-response but other responses to the clip, and their order of potency corresponded with that in man. Substances of other types that relieve pain in man, such as antipyretic drugs, did not show appreciable activity in the multiple toe-pinch test in guinea-pigs. The failure of mephenesin, phenobarbitone and troxidone and of pentetrazole and strychnine to suppress the squeak-response showed that this response was insensitive to a number of non-analgesic drugs which are known to have central actions. The foregoing evidence suggests that the multiple toe-pinch test in guinea-pigs measures analgesic activity of the morphine type.

The method of testing one toe 14 times does not commend itself because, with morphine, the slope of the log dose-response curve and the information ratio were lower and the fiducial interval of the ED50 value was wider. However, as the potency of morphine was much greater, this variation of method might be useful for detecting the action of weak analgesic drugs.

The multiple toe-pinch test was successfully used in rats as well as guinea-pigs. Chicks also give a clear response to application of a clip to a toe (Schneider, 1961). There therefore seems good reason to think that this method of examining analgesic drugs can be used in a variety of toed species. We can envisage its extension to other sites or forms of stimulation. The principle of making correlated quantal observations so as to increase information from each experimental unit seems capable of wider application.

In the usual routine of testing, the posterior toe of the left forelimb was less responsive than the others. When the operations of the hands of the experimenter were exchanged, this lack of responsiveness disappeared, but was observed in the corresponding toe of the right forelimb. This bias arose from the right- or left-handedness of the operator.

The possibility that responsiveness to the clip may be greater in later cycles of testing suggests that experiments on the time-courses of analgesic drugs should be interpreted with caution. With morphine, peak potency was reached from 1 to 4 hr after treatment and after one or more cycles of testing. In this instance, at least, increase of responsiveness with testing has been more than compensated by increase in drug effect.

The lower responsiveness of one toe (no. 11) and the increasing responsiveness with temporal order of testing were seen with every active drug examined. These effects therefore seem unlikely to invalidate comparisons of potencies of drugs.

The increase in responsiveness during tests in which the clip was applied to all toes might be attributed to conditioning. Two facts, however, seem to contradict this explanation. First, responsiveness did not increase when only one toe was tested 14 times in a cycle. Secondly, the animals developed no signs of fear or distress in response to circumstances associated with application of the clip.

The information ratio is a novel feature of the statistical method. If all toes of an animal reacted alike, there would be no advantage in testing more than one toe and the information ratio would be theoretically unity. If the responses were not correlated, the information ratio would be equal to the number of toes tested. As the correlation between the responses decreased, the information ratio rose. For

example, in the experiments of Table 7, in which the average correlations between pairs of toes were 0.32 and 0.21, the information ratios were 3.5 and 4.7 respectively.

With guinea-pigs, it would be possible to test any number of toes up to 14 on each animal. Fig. 3 suggests that the information ratio would increase as the number of toes tested increased. For ethical reasons and for economy of animals, all the toes were tested in order to obtain the maximum information from each animal. When all toes are tested the information ratio exceeds unity and this expresses the main economic advantage of the test.

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