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A COMPARISON OF HEAT AND PRESSURE ANALGESIOMETRIC METHODS IN RATS

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In most of the methods which are in common laboratory use for assessing analgesic activities the pain threshold is measured from the response to superficial heat stimulation. The effect of analgesics on pain involving the deeper structures is equally if not more important. Methods for this purpose have been described by Eddy (1928), Haffner (1929), Molitor and Latven (1937), and Friend and Harris (1948). We describe here a simple pressure method, which does not suffer from certain of the disadvantages of these earlier techniques, and compare the results with those obtained by radiant heat stimulation.

METHODS

Pressure method.—The apparatus shown in Fig. 1 was designed to give a uniformly increasing pressure on the tail of the rat. The vertical syringe (A) is connected by transparent "Portex" tubing to the horizontal syringe (B) and to the mercury manometer (C). All air in the system is displaced by a mixture of equal volumes of liquid paraffin and kerosene. At rest, the distance between the head of the plunger of syringe A and the surface of the base of the stand D should be approximately equal to the mean diameter of the tails of the rats. The manometer scale is then adjusted to give zero reading.

With the tail of the rat under syringe A the pressure on it is increased by a uniform depression of syringe B. Readings which are estimates of pain threshold are taken from the manometer when the rat responds—first by struggling, then by squeaking. The pressure is then immediately released.

The nature of the syringe head will have its effect on the observed threshold. In the experiments to be described it was 25 mm. in diameter, slightly concave at the centre, and so rounded that only about 20 mm. of tail was actually in contact with it.

For convenience and standardization we have incorporated a mechanical drive for the horizontal syringe. For the design and construction of this device, which is described in the appendix, we are indebted to Mr. E. I. Godfrey, of these Laboratories. It provides a uniform increase of pressure of 2 cm. mercury per second and an instantaneous release.

Heat method.—The apparatus described by Thorp (1946) for thermal irradiation of the tip of the rat's tail was used; but instead of obtaining thresholds in terms of minimal heat intensity required for a response, we have measured reaction times to a

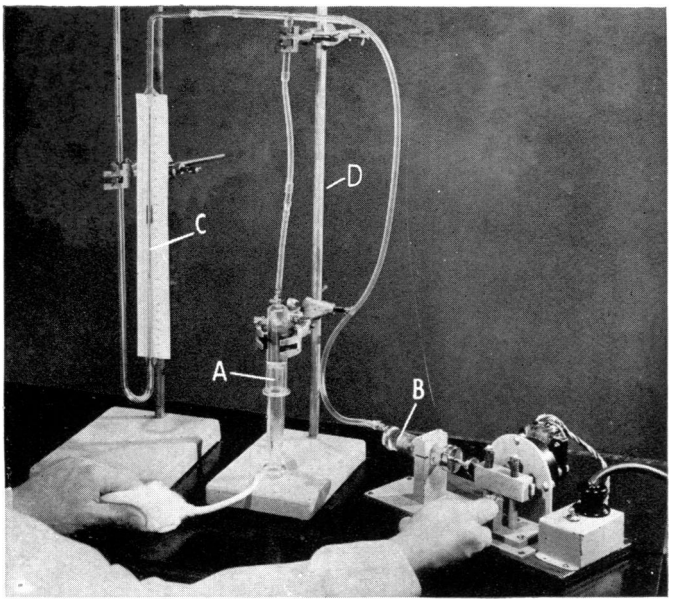

FIG. 1.—The pressure apparatus in use. A and B are syringes connected to the mercury manometer C.

single exposure of constant intensity (100 volts applied to the lamp) as used by D'Amour and Smith (1941).

All experiments were carried out on an inbred strain of Wistar rats.

RESULTS OBTAINED WITH THE PRESSURE METHOD

The normal threshold

The distribution of thresholds.—The normal pressure thresholds in cm. of mercury were examined in groups of 180 rats at several ages. The values were found to be distributed log-normally at each age. Consequently all the quantitative analyses of threshold levels reported below have been carried out on the logarithmic values, although antilogs are quoted for several of the examples for ease of interpretation.

Site of stimulation.—The thresholds determined above were measured at the tip of the tail. It was then found that if pressure be applied at the middle or the base of the tail the thresholds rose in that order. Geometric mean values for struggle and squeak responses of 40 rats of varying age were:

	Struggle	Squeak
Tip	7.3 cm.	8.5 cm.
Middle	10.5 cm.	12.1 cm.
Base	13.2 cm.	14.8 cm.

On practical grounds it was decided to continue observations at the tip of the tail.

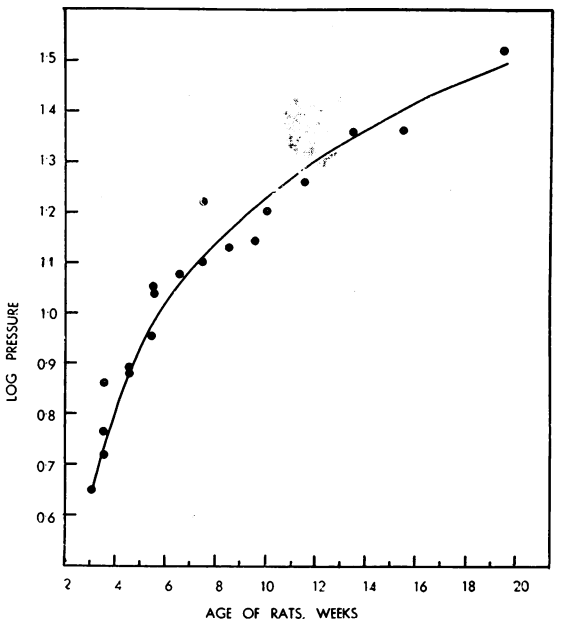

FIG. 2.—The relationship between pressure threshold and age of the rats.

The effect of age.—Three-week-old, newly weaned rats are very sensitive to pressure stimulation, with thresholds of 4–5 cm. mercury. This sensitivity decreases rapidly with age, as reference to Fig. 2 will show ; there, mean thresholds for groups of rats of different ages estimated over several months are plotted as log values. The number of rats per group varied between 20 and 180. Some representative data in this respect are shown in Table I. The ratio of the mean squeak to the mean struggle threshold remains fairly constant at 1.2 for ages of $3\frac{1}{2}$ to $8\frac{1}{2}$ weeks. Thereafter it is subject to rather wider variation, but the overall mean for 3 to $19\frac{1}{2}$ weeks remained at 1.2.

TABLE I

THE PRESSURE THRESHOLDS OF NORMAL RATS AT DIFFERENT AGES

Age, weeks	Number of rats	Mean struggle threshold, cm. Hg	Log. variance (struggle)	Mean squeak threshold, cm. Hg	Log. variance (squeak)	Ratio of means, squeak/struggle
$3\frac{1}{2}$	180	6.53	0.0117	7.87	0.0109	1.20
$4\frac{1}{2}$	144	7.05	0.0051	8.54	0.0084	1.21
$5\frac{1}{2}$	96	9.82	0.0297	12.25	0.0103	1.25
$7\frac{1}{2}$	180	15.39	0.0148	17.78	0.0104	1.16
$8\frac{1}{2}$	100	11.99	0.0204	14.66	0.0133	1.22

The observed values for the rat-to-rat variance differ considerably, but there appears to be a minimum variation between animals of about 4 to 5 weeks old. For general purposes we prefer to use rats between the ages of 3 and 6 weeks with as narrow a range of age as possible in each experiment.

Sex of rats.—No significant difference between the sexes has been observed in rats up to 8 weeks. The males then tend to show rather higher thresholds than females of the same age. It is advisable, therefore, to use equal numbers of each sex per treatment group, particularly with older rats.

Reproducibility of the pain threshold.—Groups of 20 normal rats, at each of three ages, were tested five times at 20-minute intervals. Analysis of variance showed that the variance between trials was never significantly different from the variance between rats, although both tended to be significant against the residual (error) mean square. Similar findings were made when the rats were tested once each morning and afternoon for three consecutive days. The absence of systematic variation among repeated readings showed that with normal threshold pressures no damage was sustained by the tail.

In view of the equal variability between trials and between rats, we might well expect to find no correlation between the individual readings for each rat before and after treatment with an analgesic. Therefore to assess the rise in pain threshold separately for each rat would merely increase the error of the final observations (see below).

The threshold under analgesics

Damage to the tail.—No obvious physical damage to the tail has been observed in rats treated with analgesics after the application of a pressure of four times the control value, our general "cut-off" pressure, but in an experiment using 3-week-old rats injected with 8 mg./kg. morphine sulphate a pressure of eight times the normal threshold caused bruising. (These animals were not allowed to recover.) Stimulation repeated every 15 minutes after treatment with morphine appears both to shorten the apparent duration of the drug action and to depress the threshold upon recovery. Such hypersensitivity may sometimes be detected as long as 24 hours after injection by a decrease in the normal threshold of approximately 15 per cent.

Correlation with the normal threshold.—In a test on 20 $3\frac{1}{2}$-week-old rats the mean "squeak" threshold was initially 7.4 cm. and after 2.5 mg./kg. of morphine subcutaneously was 13.8 cm. The coefficient of correlation for the 20 pairs of individual observations was estimated as:

$$r = +0.116, \text{ from which } p = 0.65$$

Hence there was no significant correlation between the initial values and corresponding thresholds after treatment.

Since there is no statistical advantage to be gained from the measurement of individual elevation of threshold, only post-injection readings need be made on each rat, and the design of quantitative tests is simplified.

The standard response curve.—A typical dose-response curve for morphine sulphate injected subcutaneously into 7-week-old rats is shown in Fig. 3. The thresholds were measured 30 minutes after injection, and each point represents the mean of the log thresholds for struggle and squeak of 10 rats. None of the rats injected with 16 mg./kg. responded at or below the cut-off pressure of 50 cm. Hg. The curve shows significant flattening between 1 and 2 mg./kg. Between 2 and 8 mg./kg.

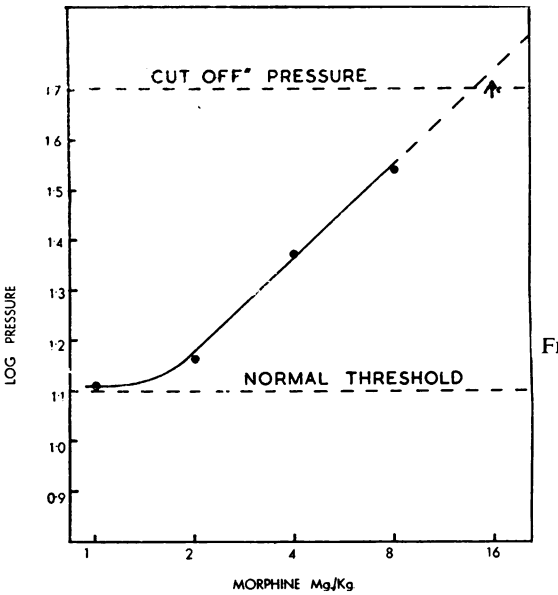

FIG. 3.—The elevation of the pressure threshold by graded doses of morphine sulphate. Mean thresholds for groups of ten rats at 30 minutes after subcutaneous injection.

the regression is linear with a regression coefficient of 0.625, S.E. ± 0.052. The standard deviation of a single observation in this experiment was ± 0.070 (equivalent to approximately ± 16 per cent).

Comparative tests with the pressure method

Quantitative technique.—It is possible to conduct comparative tests of unknown compounds against morphine as a standard. The results of such a test, and their analysis, are presented in Tables II and III. In this experiment the compound

TABLE II

RESULTS OF A COMPARATIVE TEST OF MORPHINE AND 191C49

Values are the means of struggle and squeak thresholds for each rat measured 30 minutes after injection

	Morphine sulphate (mg./kg.)		191C49 (mg./kg.)	
	2.0	3.0	2.0	3.0
Pain thresholds, cm. Hg	10	20	8	11
	9	12	4	12
	12	10	12	16
	6	20	6	8
	9	16	7	13
	12	14	10	6
	14	14	8	18
	9	17	8	15
	11	20	12	19
	16	12	14	14
Logarithmic means, cm. Hg	10.4	15.1	8.4	12.5

TABLE III

ANALYSIS OF VARIANCE OF LOG THRESHOLDS OBSERVED IN THE COMPARATIVE TEST OF MORPHINE AND 191C49

Source of variation	n	Mean square	F	p
Between drugs	1	0.07744	4.04	0.05
Between doses	1	0.27855	14.53	<0.001
Departure from parallelism ..	1	0.00046	—	—
Residual (error)	36	0.01916		

Common slope, b = 0.916, standard error ±0.249

191C49, 3-diethylamino-1 : 1-di(2-thienyl)but-1-ene hydrochloride (Adamson and Green, 1950), was compared with morphine sulphate in 3½-week-old rats. The mean threshold before treatment for the 40 rats was 5.73 cm. Hg ; the values 30 minutes after injection are shown in Table II. While the log-threshold/log-dose lines for the two drugs are shown to have significant regressions and to be parallel, the standard error of the combined slope is rather large. Taking this into account, the ratio of activity is found to be 0.80 × morphine with fiducial limits for p=0.95 of 0.53 – 1.00 × morphine sulphate.

Greater precision could be attained by increasing the number of rats tested or by increasing the dose interval to reduce the standard error of the slope. The latter alternative is not always practical for the reason given below.

Quantal technique.—As other workers have found in using different methods of stimulation, it has been our experience that the quantitative technique is frequently invalidated by some animals in a group treated with an analgesic failing to respond to pressures of about four times the control value. Any statistical device of substituting positive values for "insensitive" rats such as devised by Ipsen (1949) is unreal, and we prefer to use a quantal interpretation of results. Arbitrarily, an animal is considered to show positive "analgesia" when its threshold is at least twice the mean value of a control group. A standard probit analysis is possible on such results, an example of which is illustrated in Fig. 4 and Table IV. At the foot of the latter are quoted the fiducial limits of the relative potencies of the several compounds in terms of morphine sulphate 30 minutes after injection.

While the ratio quoted for phenadoxone is in agreement with the value found by Basil, Edge, and Somers (1950) in the rat, the amidone value appears somewhat diversely in the literature. Thus Scott, Kohlstaedt, and Chen (1947)—see also Chen (1948)—found that the potency of amidone was about twice that of morphine sulphate in nullifying the pain from pinching the rat's tail, whereas the majority of experimenters who have used radiant heat stimulation have reported that amidone is 1 to 1.3 times morphine (Thorp, Walton, and Ofner, 1947 ; Cahen, Epstein, and Krementz, 1948 ; Thorp, 1949 ; Hougs-Olsen, 1949 ; Bonnycastle and Leonard, 1950). A notable deviation from the heat ratios is the value of 3.4 : 1 found by Tainter and Buchanan (1949). Because at the time it seemed that the relative activity of amidone and morphine might be related to the type of stimulus used, an extensive comparison of these compounds by the heat and pressure methods was begun here in 1948.

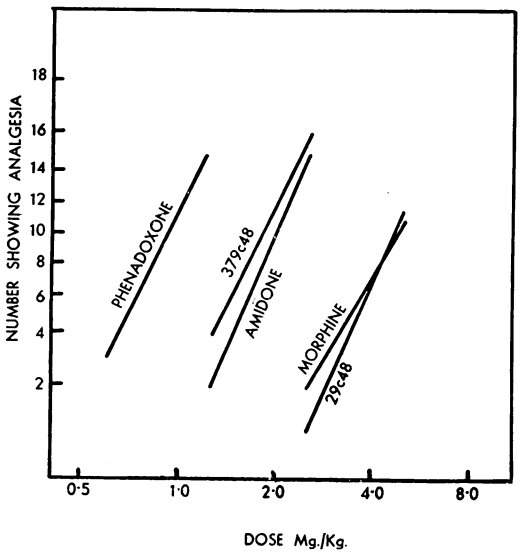

FIG. 4.—Probit response lines of five drugs by the pressure method. Groups of twenty rats were used; details of the analysis of this experiment are given in Table IV.

TABLE IV

SOME ITEMS IN THE QUANTAL ANALYSIS OF A COMPARATIVE TEST BY THE PRESSURE METHOD USING A GROUP OF 20 RATS AT EACH DOSE

Drug	Morphine sulphate	Phenadoxone	29C48*	379C48†	Amidone
Dose, mg./kg.	2.5 5.0	0.6 1.2	2.5 5.0	1.25 2.5	1.25 2.5
Rats showing "analgesia"	2 11	3 15	1 12	4 16	2 15
Weighted mean log dose ..	0.4965	−0.1514	0.5206	0.1505	0.1847
Weighted mean probit response	4.63	4.92	4.74	5.00	4.92
Slope	4.65	5.65	6.31	5.32	6.64
Std. error of slope ..	±1.38	±1.52	±1.82	±1.50	±1.63
Log potency	0	0.699	$\bar{1}$.995	0.411	0.363
Variance of log potency ..	—	0.00321	0.00344	0.00320	0.00338
Ratio to morphine ..	1	5.00	0.99	2.58	2.31
P.95 limits of ratio ..	—	3.87–6.46	0.76–1.29	2.00–3.33	1.77–3.00

χ^2 for homogeneity of slopes = 0.952, 4 d.f., p = 0.91. Common slope = 5.66, std. error ±0.715.

* 6-Piperidino-4: 4-diphenyl-5-methyl-3-hexanone hydrobromide. † 6-Piperidino-4: 4-diphenyl-3-heptanone hydrochloride. (Ofner, Thorp, and Walton, 1949; Ofner, Walton, Green, and White, 1950.)

THE COMPARISON OF PRESSURE AND HEAT RESULTS

The normal thresholds

We have found the reaction times to heat stimulation, like the pressure thresholds, to be log-normally distributed in our experiments. Confirmation in regard to heat reaction times may be found in the data published by Ercoli and Lewis (1945). A similar frequency table given by D'Amour and Smith (1941) does not conform to either the normal or log-normal type, but this may be due to the mixed population of rats which these workers used. The logs of the heat reaction times have been used in the analyses described in this paper.

Experiments on normal rats showed that when equal areas of the tail were irradiated the sensitivity to heat was greatest at the tip. In contrast to the pressure thresholds, heat reaction times showed no definite change with age, although they frequently tended to be shorter in the older rats.

A comparison of the variances by the two methods showed that the heat reaction times were more variable on different occasions in groups of the same age than were the pressure thesholds, but that the internal variances of each were of the same order. Further, the variance of the heat reaction times like the variance of pressure thresholds was approximately the same between observations made at 20-minute intervals as between rats.

In order to justify our later comparative tests with analgesics, an experiment was conducted on 40 rats in which both pressure thresholds and heat reaction times were observed on each rat with the minimum delay between the two measurements (approximately 15 seconds). Alternate animals were subjected to pressure first and heat first. Analysis of variance showed that neither measurement was affected by the order in which they were determined.

The relative activity of morphine and amidone

All the tests in this section have been carried out in the following manner. Four doses of each drug have been injected subcutaneously into groups of 10 or 20 rats, while a control group received injections of saline. A twofold dose interval was used throughout. Pain thresholds to heat and then to pressure were determined before and 30 minutes after treatment. The mean threshold for the saline group was never significantly different before and after treatment; the latter estimate was used as the control value in assessing quantal responses. Probit analyses showed that within every test the regressions for the two drugs were parallel, although the slopes varied from test to test. It was possible to compute for each experiment

(*a*) the ED50, being the dose required to produce " analgesia " in 50 per cent of the animals, and

(*b*) the ratio of the ED50 value for amidone compared with that for morphine.

Tests were performed on two series of rats. The first series were tested at $3\frac{1}{2}$ weeks of age, and then fortnightly until $19\frac{1}{2}$ weeks old, the rats being randomized between successive occasions. The second series consisted of groups of previously unused animals at various ages from $4\frac{1}{2}$ to 14 weeks.

It should be mentioned that at ages above two months rats become difficult to handle and their responses poorly defined, particularly by the pressure method. This has been reflected to some extent by the variation of the results. The estimates of ED50 shown in Table V varied for both drugs and in both series. In the series of previously unused rats the ED50 increased between the ages of $3\frac{1}{2}$ and 10 weeks, which has been our general experience in many other tests. A similar trend was seen in the rats which were repeatedly injected, but even after the first treatment the sensitivity to the analgesics seems to have decreased, suggesting the development of some degree of tolerance. A similar comment was made by Ercoli and Lewis (1945). The divergence between the two series did not, however, increase with the infrequent injection given. The overall mean estimate for the ED50 in the previously used rats is twice that in the fresh animals, as shown in Table VI.

TABLE V

THE ED50s (MG./KG.) FOR MORPHINE AND AMIDONE IN RATS OF DIFFERENT AGES ESTIMATED 30 MINUTES AFTER SUBCUTANEOUS INJECTION

Age in weeks		Amidone			Morphine sulphate		
Series I	Series II	Pressure-struggle	Pressure-squeak	Heat	Pressure-struggle	Pressure-squeak	Heat
3½		1.3	1.3	1.1	2.9	2.7	3.3
	4½	1.6	1.5	1.0	3.6	3.8	2.1
5½		2.0	3.2	2.0	5.1	5.6	4.6
7½		3.1	2.8	2.4	7.2	7.4	4.7
	8½	1.7	1.7	1.5	3.5	3.6	3.6
9½		2.7	3.7	2.5	8.1	6.9	7.1
	10	2.0	1.9	1.1	5.5	4.3	2.7
11½		2.6	2.6	3.0	6.7	5.7	8.2
13½		2.1	2.2	4.8	4.9	4.8	4.8
	14	1.0	1.0	1.6	1.8	2.0	2.3
	14	2.0	2.2	3.3	3.1	2.8	7.6
15½		7.0	4.1	6.0	24.0	10.6	10.2
19½*		2.3	2.4	2.8	8.2	8.8	7.1

* Tests on Series I were not carried out at 17½ weeks of age.

TABLE VI

UNWEIGHTED LOG MEAN ED50s FOR MORPHINE AND AMIDONE. "UNUSED" RATS REFER TO SERIES II OF TABLE V AND THE 3½-WEEK OBSERVATIONS ON SERIES I. "USED" RATS REFER TO THE REMAINDER OF THE FIRST SERIES

Rats	Amidone			Morphine sulphate		
	Pressure-struggle	Pressure-squeak	Heat	Pressure-struggle	Pressure-squeak	Heat
Unused	1.6	1.6	1.5	3.2	3.1	3.2
Used	2.8	2.9	3.1	7.9	6.9	7.5

There it will also be seen that the ED50s for struggle and squeak responses are identical. It follows that the correlation observed between these two thresholds in normal rats is maintained after treatment with these analgesics. For this reason only the squeak estimates have been used in calculating activities. While the mean ED50 for all groups was the same for pressure and for heat responses, the values differed significantly between tests. The ratios of activity of amidone and morphine found are listed in Table VII.

It was possible to show statistically that these ratios were completely homogeneous throughout all the tests. The age of the rats, their previous usage, and the method of stimulation produced no significant variation of the values. In particular the mean log ratios for pressure (squeak) and heat were:

Pressure 0.3396, variance 0.0004037
Heat 0.3385, variance 0.0004796

From these we obtain for the difference of the two estimates,

$$t=0.64,\ p=0.65$$

TABLE VII

RATIOS OF ACTIVITY OF AMIDONE AND MORPHINE SULPHATE, MEASURED BY PRESSURE AND HEAT STIMULATION. THE WEIGHTS ARE THE RECIPROCALS OF THE VARIANCES OF THE LOG RATIOS

Age in weeks		Pressure				Heat			
Series I	Series II	Ratio		Weight		Ratio		Weight	
3½		2.1		221		3.0		144	
	4½		2.5		340		2.0		139
5½		1.8		185		2.3		104	
7½		2.7		260		2.0		537	
	8½		2.1		289		2.2		173
9½		1.9		224		2.8		240	
	10		2.3		213		2.3		118
11½		2.2		215		2.7		146	
13½		2.2		219		2.5		167	
	14		2.0		55		1.4		96
	14		1.3		117		2.3		43
15½		2.6		103		2.0		77	
19½		3.1		60		2.6		101	

There being no significant difference, the grand mean ratio may be quoted as amidone$\equiv$2.23 $\times$ morphine sulphate, with fiducial limits for p=0.95 of 2.08 - 2.39. Before this particular comparison of amidone and morphine, several other comparative tests had given a mean activity ratio of 2.38 with a log variance of 0.0003390. Combining the two values gives an estimate for the ratio of 2.29 with fiducial limits for p=0.95 of 2.17 - 2.41.

The activity of pethidine

When tested by various heat methods the analgesic activity of pethidine in the rat seems to vary under different conditions. For example, the following estimates of the pethidine/morphine ratio have been reported: 0.05 to 0.1 (Bonnycastle and Leonard, 1950), 0.14 (Cahen, Epstein, and Krementz, 1948), 0.15 (Thorp, Walton, and Ofner, 1947), and 0.2 (Tainter and Buchanan, 1949). The last authors named also quote Bliss and Sevringhaus (1947) as having analysed the results of a collaborative study in which several laboratories participated and found that the relative potency of pethidine varied between 0.05 and 0.45 times that of morphine. One possible explanation of this variation is to be found in the observation made by Davies, Raventos, and Walpole (1946) that the slope of the pethidine regression line was only half that of morphine. Because of the variation in the relative activity of pethidine by the heat method, a comparison of the heat and pressure ratios may only be made when the two effects are measured in parallel.

We have compared pethidine hydrochloride and morphine sulphate in a similar manner to that already described for the comparison of amidone and morphine. Six experiments were carried out, and in each about 120 rats were used and two or more doses of each analgesic were injected. The estimated slopes of the regression lines and activity ratios are shown in Tables VIII and IX respectively.

By the heat method the slopes of pethidine and morphine were significantly different in only one of the six tests, while by the pressure method no significant

TABLE VIII

THE SLOPES OF THE REGRESSION OF PETHIDINE HYDROCHLORIDE AND MORPHINE SULPHATE BY THE QUANTAL TECHNIQUE

"p" = probability of differences occurring by chance ("t" test)

Exp. No.	Heat method			Pressure method		
	Slopes		"p"	Slopes		"p"
	Pethidine	Morphine		Pethidine	Morphine	
1	2.79	2.58	0.85	3.36	3.61	0.85
2	1.79	3.85	0.20	5.34	5.00	0.85
3	0.86	4.97	0.015	2.72	4.02	0.40
4	2.99	2.33	0.75	3.65	4.65	0.65
5	2.99	6.31	0.25	6.64	3.99	0.35
6	7.06	13.08	0.15	4.44	7.91	0.075
χ^2 between experiments ..	7.79	10.06	—	5.17	7.62	—
p	0.15	0.075	—	0.40	0.20	—
Weighted means	2.43	3.88	0.10–0.05	3.75	4.94	0.10
Variance of means ..	0.240	0.368	—	0.204	0.321	—

TABLE IX

THE ACTIVITY RATIO OF PETHIDINE HYDROCHLORIDE : MORPHINE SULPHATE BY THE HEAT AND PRESSURE METHODS

"p" = probability of difference of log ratios occurring by chance ("t" test)

Exp. No.	Heat method ratio	Pressure method ratio	"p"
1	0.11	0.15	0.30
2	0.12	0.20	0.05–0.02
4	0.18	0.44	<0.001
5	0.24	0.46	0.01
6	0.25	0.29	0.35
χ^2 between experiments	19.7	41.8	—
p	<0.001	<0.001	—
Weighted geometric means ..	0.20	0.27	0.001

differences were found. The slopes were homogeneous between tests for each drug by each method, and the four means showed that the pethidine slope was more shallow than the morphine slope by both methods. The probability of the observed differences between the means occurring by chance was low, although not significant at the 5 per cent level.

By using the respective weighted mean slopes in the five tests not showing internal significant differences, the pethidine/morphine activity ratios shown in Table IX were calculated. By neither method were these ratios homogeneous, and in each experiment pethidine was more potent by the pressure than by the heat method. The five differences between the (log) ratios were homogeneous ($p=0.10$), and the mean of these indicated that the pressure ratio was about 1.5 times the heat ratio with probability limits of 1.25–1.85 ($p=0.95$).

From the foregoing evidence it would appear unlikely that the pethidine and morphine regressions are in fact parallel, and no single estimate of relative potency would therefore be valid, either for heat or pressure. The mean ratios quoted in Table IX represent only the order of potency under the conditions required to elevate the pain threshold by 100 per cent in 50 per cent of rats.

Discussion

The heat and pressure methods are similar in that the intensity of stimulus may be assumed to increase nearly linearly with time. Whereas in the heat method it is largely the superficial layers of the tail which are stimulated, in the pressure method the deformation of the deeper structures is presumably the chief cause of excitation.

Both thresholds were found to be log-normally distributed, and a study in normal animals showed that, whereas heat reaction times tended to decrease with increasing age, the pressure thresholds very markedly increased. This may be explained largely by physical considerations in so far as the area exposed to heat irradiation increases with the diameter of the tail, whereas the pressure per unit area decreases. The greatest increase in the normal pressure thresholds with age was concurrent with the period of maximal rate of anatomical development of the tail.

A point of some theoretical interest arises from the observation that the normal thresholds for heat reaction time and " struggle " have a greater variance than that for " squeak." This may be correlated with the behaviour pattern of a rat when a painful stimulus is applied to the tail, since the animal endeavours to remove its tail from the source of stimulation, and only when this is unsuccessful does it squeak " in protest." We may draw a parallel with some unpublished experiments carried out in man at these laboratories in collaboration with Dr. A. C. White, where thresholds for five degrees of pain ranging from " discomfort " to " intolerable " were determined by applying pressure to the base of the finger-nail with a modification of the analgesic apparatus described in this paper. The analysis of 90 observations involving 8 subjects showed that the variances decreased as the degree of pain increased. The variance between rats was of the same order as that for the lower degrees of pain in man.

It has been demonstrated by Miller (1948) and by Ipsen (1949) that there is little or no correlation between individual thresholds to heat stimulation before and after an analgesic. We have confirmed this observation with pressure stimulation, and have shown that no correlation should be expected, since the variance between repeated readings is as great as that between rats. This would indicate a high degree of homogeneity among the rats studied ; under such conditions the procedure of determining individual increases in pain threshold cannot therefore be recommended, as it only serves to increase the error of the test.

Miller (1948) in his *Critique of Analgesic Testing Methods* pointed out that no method had been evolved for combining into a single parameter measurements of the intensity of effect and duration of action of analgesics. While duration of action should be considered in assessing the value of new analgesic compounds, the combination of it with degree might result in important differences between rates of onset and duration of effect being overlooked. Estimates of the degree of analgesia are frequently invalidated by some animals in a group failing to respond to stimulation,

and in addition some practical considerations also make it undesirable to include duration of action among the routine observations. First, the time taken for several readings at intervals after injection reduces the number of animals which may be tested on any one occasion. Second, repeated subjection to high levels of pressure or long exposure to heat reduces the apparent duration of action of the drug. This may be overcome by the use of fresh animals for each time reading, but this is uneconomic. Further, from some small experiments we have carried out measuring the proportion of animals whose thresholds significantly exceeded the normal range for $p=0.99$, it would appear that the measurement of duration of action of analgesics is subject to wider experimental errors than the simple measurement of degree ; it is most likely therefore that a combination of the two would give less accurate results than degree alone. On these grounds we prefer to treat intensity of effects at an arbitrary time interval and duration as separate entities both in the design of the tests for determining them and in their interpretation.

When tested on rats by either the radiant heat or the pressure method the potency of amidone is constant in many age groups at about 2.3 times that of morphine. In contrast pethidine shows a more shallow regression than morphine and the relative activities of the two compounds vary with the level of analgesic effect. The difference in regression is probably largely responsible for the variability in the pethidine/morphine ratio observed in these laboratories and elsewhere. A more important difference is inherent in the observation that, relative to morphine, pethidine is about 1.5 times more active by the pressure method than by the radiant heat method. It seems that pethidine may have either a greater analgesic effect on pain caused by pressure than pain due to heat or a greater influence on the flight reaction to pain (struggle and squeak) than on the reflex of removing an appendage from a source of stimulus.

The example of pethidine emphasizes the necessity for considering the method of test when comparing analgesic ratios in the same species. It also shows that even within one species and with one method the relative activities of analgesics obtained may depend on the response level chosen and on the sensitivity of the animals used. The interval between injection and the measurement of thresholds may also be of importance ; in the comparative tests described above observations were made 30 minutes after subcutaneous injection.

SUMMARY

1. A method is described for determining the pain threshold of rats to a gradually increasing pressure on the tail. These estimates and heat reaction times are log-normally distributed. The effect of age, sex, and site of stimulation were investigated. By both methods the variance between repeated readings is as great as between rats ; the importance of this in the design of analgesiometric tests is discussed.

2. The sensitivity of rats to morphine and amidone decreases up to a certain age. In an extensive comparison in rats of many age groups the ratio of the activity of amidone to morphine sulphate was constant at about 2.29 : 1 whether tested by the radiant heat or the pressure method. The fiducial limits of the ratio, for $p=0.95$, were 2.17 to 2.41.

3. The increase in the analgesic effect for a given increment in dose was less with pethidine than with morphine. For this reason the relative activity of the two compounds by any one method is dependent on the response level chosen and the sensitivity of the rats used. A comparison by the heat and pressure methods showed that pethidine had a relatively greater effect than morphine on the " flight reaction " in response to pressure on the tail of the rat than on the reflex of removing the tail from a source of radiant heat stimulation.

4. A small degree of " tolerance " to amidone and morphine occurred even after a single injection in the rat.

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APPENDIX

THE MECHANICAL DRIVE FOR THE PRESSURE APPARATUS

BY

E. I. GODFREY

The driving mechanism photographed in use in Fig. 1 is shown in more detail in Fig. 5. It consists of three main components mounted on a common base (D).

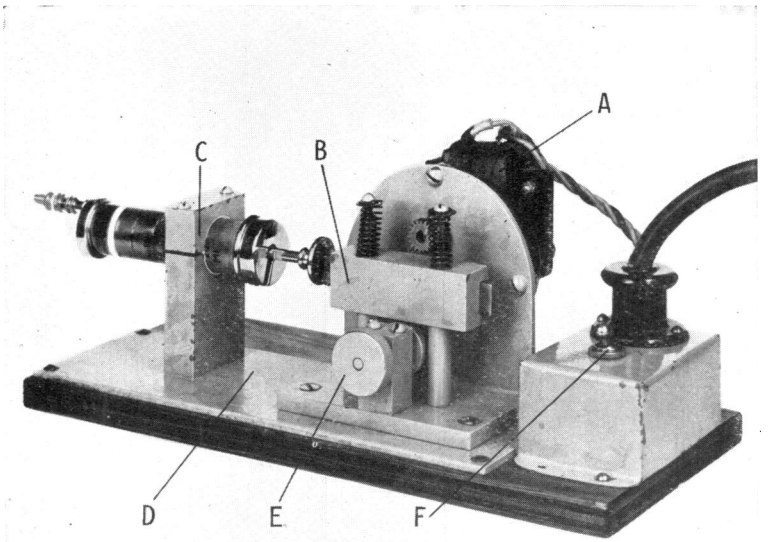

FIG. 5.—The mechanical drive of the pressure apparatus (for key see text).

These are the driving motor A, a "Synclock" electric motor (1 r.p.m.), suitably geared to a rack carried by the rack-casing B, and the syringe housing C in which is clamped the horizontal syringe of the pressure apparatus. E indicates a knob operating the quick-release mechanism described below, and F a switch controlling the motor.

The rack and its casing are removed for the photograph in Fig. 6. The gear G is mounted on the motor driving shaft, and operates on the coaxial gears H and I. The latter meshes with the rack when in operation. The knob E, operating the cam K, causes the rack casing to be raised or lowered on its supporting pillars J. In Fig. 7 the rack L may be seen in its casing B. The springs shown are to prevent the rack from lifting during the driving operation. When the rack is disengaged it is free to slide in its case, and the pressure in the syringe system is automatically released.

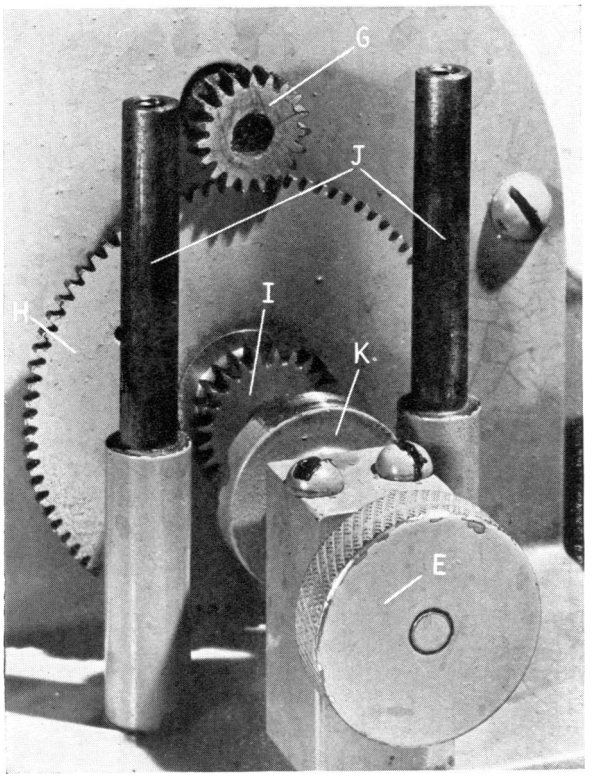

FIG. 6.—Details of the driving gears and quick-release device (rack and casing removed).

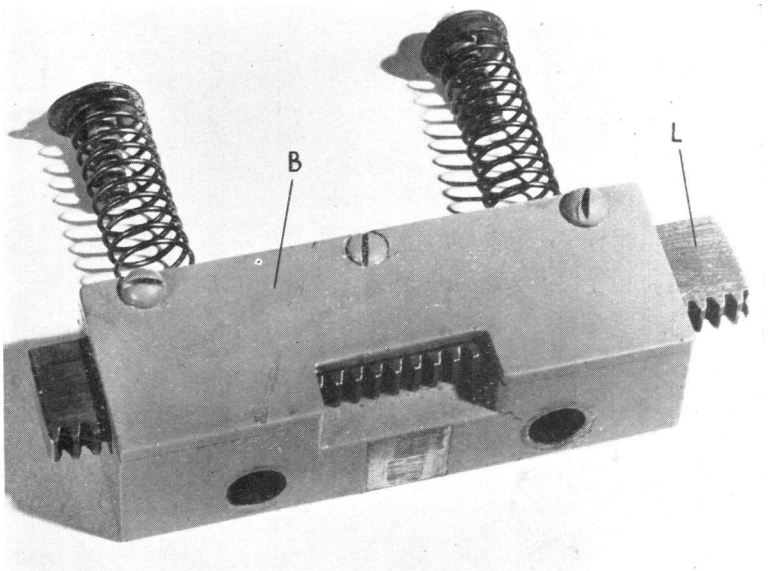

FIG. 7.—The rack and its casing.