Brit. J. Pharmacol. (1951), **6**, 273.

THE DURATION OF COCAINE ANAESTHESIA IN THE RABBIT CORNEA. A STATISTICAL EXAMINATION

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(Received December 28, 1950)

For over half a century the rabbit cornea has been employed as a site for the testing of local anaesthetic activity. Sixteen years ago, however, Copeland (1924) recognized the relatively large variability in the duration of action of any one treatment when measured on different rabbits. This variability has been subsequently described by many other authors, among them Coles and Rose (1929), Rider (1930), and Sinha (1936, 1939a). The duration of action on the cornea of locally applied anaesthetics is of sufficient importance, however, to warrant its measurement in spite of the large variations involved, provided that the results obtained are treated statistically. Chance and Lobstein (1944) asserted that " the test . . . has not withstood statistical examination," yet statistical grounds for its rejection have so far not yet appeared. It is the purpose of this paper to show that the test gives values which are statistically valid, although considerable numbers of animals are needed for useful results.

METHOD

Albino rabbits of various breeds have been used, each animal being employed repeatedly with at least one week between tests. Right and left eyes were used alternately for cocaine treatment at successive tests, the other eye receiving the compounds of unknown potency. Thus, between two cocaine treatments on one eye, a minimum of two weeks was allowed to elapse, during which time the eye would have usually received a dose of another synthetic compound. On the average the rabbits remain in use for about twelve months. A total of about 150 animals provided the data quoted below.

In the test the conjunctival sac is flooded with test solution, 1–2 c.c. as necessary, and the lower lid held over the upper for one minute. The criterion of anaesthesia is taken to be the absence of a blink reflex when the cornea is stimulated with a bristle mounted on a glass rod. This bristle, about 15 mm. long, is mounted adjacent to another, roughly half a millimetre shorter. Sufficient pressure may thus be exerted to bend the longer until its partner just contacts the eye, thus effecting some degree of standardization of the stimulus. Animals which are nervous or blink excessively without stimulation are not used for the tests. Observations on treated animals are made at five-minute intervals after the instillation of the drug until the blink reflex returns. The arithmetical mean of the times of the last negative and the first positive response is taken as the duration of anaesthesia.

RESULTS

Since the tests involve the repetition of treatments with cocaine, extensive data have accumulated over four years of routine testing.

The distribution of duration times

Until the beginning of 1950, cocaine solutions were prepared in saline, the concentrations most commonly used being 1, 2.5, and 5 mg. per c.c. Frequency distributions of duration times corresponding to these three levels were obtained from the records. The three frequency polygons are shown in Fig. 1. These

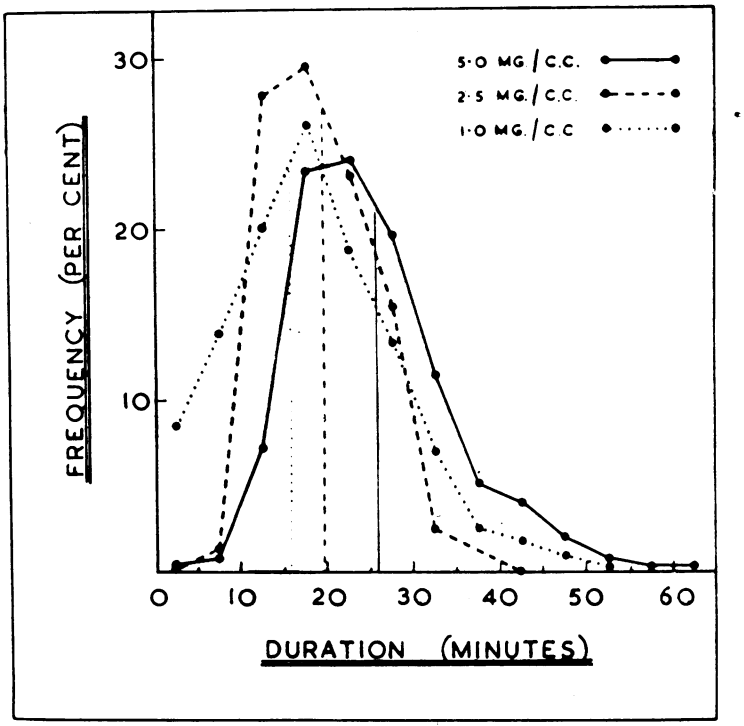

FIG. 1.—Frequency polygons of the durations of action of three concentrations of cocaine. The respective arithmetical means are indicated by the vertical lines.

values were not normally distributed with respect to time, and some simple normalizing transformation of the time values was sought. The best results were obtained with the logarithm of duration time, although frequencies at the lowest concentration were still not fitted by this means (see Table I). It was then observed that if the successive observations of duration were numbered 0, 1, 2, 3, etc., the corresponding frequencies for 1 mg./c.c. were distributed very nearly in a Poisson series. Although the total χ^2 measuring goodness of fit of this Poisson distribution showed significant deviation, this divergence was entirely contributed by the presence of thirteen values (2.3 per cent) which belonged to the upper tail of the distribution. If these are omitted, deviations from expectation have a probability of 0.96 (Table II). This relationship may, of course, be entirely spurious.

These findings suggest that above some threshold concentration (between 1 and 2.5 mg. per c.c.) duration times are log-normally distributed, while below the threshold the distribution is more skew. Since it is doubtful whether the threshold

TABLE I

Frequencies of observed (obs.) and expected (ex.) values of duration for three concentrations of cocaine in saline. Expected values derived on the basis of a log-normal distribution

Duration in minutes	Observed and expected frequencies for concentrations					
	1 mg./c.c.		2.5 mg./c.c.		5 mg./c.c.	
	Obs.	Ex.	Obs.	Ex.	Obs.	Ex.
2.5	48	46.8	0		2	
7.5	113	155.6	1	20.4	7	10.4
12.5	149	131.7	21		71	99.3
17.5	107	88.3	23	26.6	239	216.1
22.5	76	52.2	18	17.9	247	237.4
27.5	40	33.3	12		200	184.5
32.5	15	20.9	2	13.1	117	118.5
37.5	10	12.7	1		53	70.7
42.5	6	8.9			41	38.5
47.5	2				21	21.8
52.5	0				8	11.0
57.5	0	18.8			3	
62.5	1				4	12.8
67.5	2				8	
Total ..	569		78		1,021	
χ^2 (degr. freedom)	49.0 (9)		0.9 (3)		18.2 (10)	
p	<0.001		0.80		0.055	

TABLE II

Poisson distribution fitted to frequencies of duration times for 1 mg./c.c. cocaine in saline. o = observed; e = expected

Duration in minutes	All values			Omitting 13 highest values		
	o	e	$\frac{(o-e)^2}{e}$	o	e	$\frac{(o-e)^2}{e}$
2.5	48	46.7	0.036	48	45.6	0.126
7.5	113	116.8	0.124	113	114.1	0.011
12.5	149	145.9	0.066	149	142.6	0.287
17.5	107	121.7	1.776	107	118.9	1.191
22.5	76	76.0	0.000	76	74.3	0.039
27.5	40	38.0	0.105	40	37.1	0.227
32.5	15	15.8	0.041	15	15.5	0.016
37.5	10			8	7.9	0.013
42.5	6					
47.5	2					
52.5	0	8.1	20.544			
57.5	0					
62.5	1					
67.5	2					
Total ..	569			556		
χ^2 (deg. freedom)			22.692 (6)			1.910 (7)
p			<0.001			0.96

level is constant for all rabbits, we might expect to find some confusion of the two types of distribution at concentrations of about 1 to 2.5 mg. per c.c.

Some confirmation of these findings was available from results obtained since January, 1950. Since that date cocaine solutions were prepared in Sørensen buffer (*p*H 6), and a rather smaller series of results have been obtained with concentrations of 1 and 5 mg. per c.c. in this medium (Table III). An excellent fit is obtained by the log transformation at 5 mg. per c.c., and the Poisson series again gives a greater probability for the lower concentration.

TABLE III

Frequencies of observed and expected values for duration time for cocaine in buffer solution *p*H 6

Duration in minutes	Observed and expected frequencies for two concentrations				
	1 mg./c.c.			5 mg./c.c.	
	Obs.	Ex. (Poisson)	Ex. (log. normal)	Obs.	Ex. (log. normal)
2.5	16	12.6	17.5	1	10.3
7.5	29	27.8	34.5	7	
12.5	24	30.6	23.6	49	53 6
17.5	20	22.4	14.3	90	87.7
22.5	14	12.3	8.4	88	64 5
27.5	7			76	67.3
32 5	3	8.3	15.8	37	44.2
37.5	1			31	29.0
42.5				18	17.7
47.5				11	11.1
52.5				4	16.6
57.5				3	
62.5				1	
67.5				6	
Total ..	114	—	—	422	—
χ^2 (degr. freedom)		3.73 (5)	8 50 (5)		3.80 (9)
p		0.60	0.15		0.92

Further confirmation, although indirect, may be found in a paper by Rider (1930). Of results obtained with cocaine at 10 mg. per c.c. he wrote: " At least one-half of the rabbits tested have given a cocaine anaesthesia ten to fifteen minutes shorter than the value given " (i.e., the arithmetical mean of 27 minutes). This suggests that his results were distributed asymmetrically in the manner of log-normal distribution. More recently Sinha (1939a) gave histograms of duration times observed in groups of thirty to forty-five rabbits tested with three concentrations of cocaine. He observed that the coefficient of variation remained constant at about $\pm$ 20 per cent with mean values ranging between six and twelve minutes. This again indicates the applicability of a log-normal distribution.

Variation between rabbits

Rider, following the method of Coles and Rose (1929), adopted the practice of selecting for his experiments those animals which gave durations within a narrow

time range after the administration of 10 mg. cocaine per c.c. This procedure could have been partly justified if the variation between rabbits had been significantly greater than the experimental errors involved. In order to investigate the point, results on fifty rabbits, each of which was tested with cocaine at 5 mg. per c.c. four times in each eye, have been extracted and subjected to an analysis of variance (Table IV). The highly significant variance between rabbits confirms the earlier

TABLE IV

Analysis of variance of log duration times. Fifty rabbits tested four times in each eye with 5 mg. cocaine per c.c.

Source of variation	n	Mean square	F	p
Between rabbits	49	0.0793	5.55	<0.001
Between eyes	1	0.0181	1.27	>0.20
Between repetitions	3	0.1305	9.1	<0.001
Interactions:				
Rabbits × eyes	49	0.0224	1.56	Ca. 0.05
Rabbits × repetitions	147	0.0169	1.18	Ca. 0.20
Eyes × repetitions	3	0.0168	1.17	>0.20
Rabbits × eyes × repetitions (error) ..	147	0.0143		
Total variance	399	0.0252		

workers' grounds for eliminating those animals giving very high or low results. The question still arises, however, as to the justification of employing selected animals for comparative testing. While it probably would not affect the relative durations of different treatments, this would only be true in the absence of an interaction between the treatments and the rabbits. In a properly designed test the maximum of accuracy could be attained without selection of the experimental animals.

The analysis in Table IV also shows a highly significant mean square for " repetition." The four successive values of the geometric mean duration for fifty rabbits read once in each eye were : 1st 23.6, 2nd 21.8, 3rd 22.8, 4th 26.5 minutes, the grand mean being 23.6 minutes. Since no tendency was observed for values to continue to rise in rabbits used more than four times, it may be assumed that the variation observed is of a random nature, possibly influenced by the administration of other compounds, relatively more irritant, between successive trials with cocaine. It is to be concluded, therefore, that, within the limits of error indicated by the highest order interaction, absolute duration times are not repeatable. If consistent observations are to be obtained, the mean square for repetition must be considered as the error variance. This fact is discussed below at greater length.

As would be expected there is no over-all difference between the responses of right and left eyes, but the rather high mean square for the rabbits × eyes interaction indicates that there are slight differences between the results on the two eyes of individual animals. These differences would be minimized if the pairs of readings were made at the same time.

Since the log-normal distributions so far investigated (Tables I to III) consisted of a varying number of single observations on an undefined number of rabbits, it seemed desirable to check the distribution of the mean values of each rabbit in

the experiment analysed in Table IV. The number of animals was admittedly not large for the definition of a distribution, but reference to Table V shows a very satisfactory fit to be obtained by the log transformation.

TABLE V

Distribution of log-mean duration times for 50 rabbits (each value being the mean of four readings on each eye)

Log duration boundaries	Frequencies		
	Observed	Expected	
1.15–1.199	2	} 5.30	
1.20–1.249	5		
1.25–1.299	5	6.05	
1.30–1.349	9	8.70	$X^2 = 3.08$
1.35–1.399	11	9.90	6 d.f.
1.40–1.449	8	6.05	p = 0.80
1.50–1.549	4	}	
1.55–1.599	0	} 5.30	
1.60–1.649	1	}	

The relationship between concentration and duration

Since log duration is normally distributed for cocaine at concentrations above the threshold already described, we might expect to find a linear relationship between log duration and log dose. Sinha (1936) has shown that, while small quantities of cocaine solutions may give durations dependent on the volume administered, when the drug is applied in excess a maximum duration is obtained dependent only on concentration. In other words, with excess solution the log duration would be linearly related to log concentration. This was, in fact, Sinha's finding, giving the identity

$T = K.C^b$, which reduces to the more useful form of

$\text{Log } T = \text{Log } K + b \log C$,

Where T = duration time in minutes

C = concentration as percentage.

K is equivalent to the duration corresponding to Log $C = 0$, i.e., 1 per cent (w/v) or 10 mg. per c.c., and b the slope of the line.

In order to test this relationship with our own figures the results shown in Table VI were employed. These are the logarithmic means of all observations obtained at a variety of concentrations over a number of years. Since results with buffer solution were never significantly different from those with saline solution at the same concentration of cocaine, these have been pooled for this purpose. When the results

TABLE VI

Logarithmic means of duration times observed with varying concentration of cocaine

Concentration (mg./c.c.) ..	2.0	2.5	5.0	10.0	20.0	40.0	50.0	100.0
Mean duration (min.) ..	19.9	18.8	23.7	40.6	53.6	58.5	114.6	131.8
Number of observations ..	21	84	1,443	21	30	24	2	2

are weighted with their corresponding number of observations, analysis of the log values shows a highly significant linear regression mean square of 10.576, with a mean square for deviations from linearity of 0.137 (6 degrees of freedom). The latter is not likely to be significant, since the observations at different concentrations were made at different times. The regression coefficient b is equal to 0.467, standard error $\pm$ 0.053. The calculated value of K is 33.3 minutes (log K = 1.522).

In a subsequent publication Sinha (1939a) repeated his earlier observations, using rather larger groups of animals. He also tabulated some values of K and "n" (equivalent to our "b") derived from the work of other authors. These values, with some emendations and additions, are reproduced in Table VII with a list of

TABLE VII

DURATION (MINUTES) OF COCAINE ANAESTHESIA ON THE RABBIT CORNEA FROM VARIOUS AUTHORS

N.A. = No anaesthesia. I.A. = Incomplete anaesthesia. † = Values derived from best fitting regression lines. * = Omitted from computation of slope

Source	Duration (min.) for various concentrations (mg./c.c.) of cocaine						Slope (approx.)
	1.25	2.5	5.0	10.0	20.0	50.0	
				(*K*)			(*b*)
Young (general)†	13	17	24	33	45	70	0.47
Young (special experiment)..		23	30	38	47		0.33
Sollmann (1918)	N.A.	N.A.	10	20	30		0.79
Regnier (1923)†			9	15	23		0.66
Copeland (1924)	I.A.	2*	13	18	30	>40	0.60
Schmitz and Loevenhart(1924)					28		
Cohen (1925)	10	17	23	42	46†		0.47
Coles and Rose (1929) ..					60		
Rider (1930)				27			
Uhlmann (1930)†	39	49	62	78			0.34
Bovet (1931)			14	19	31	60	0.64
Sinha (1936)†	—	9	13	18	27	—	0.54
Sinha (1939a)†	—	4	6	8	12	—	0.47
Hulpieu *et al.* (1940) ..					47		
Gilman *et al.* (1942) ..					20		

their sources. Sinha did not appear to recognize the linearity of the regression of log duration time on log concentration implicit in the formula he had derived. Indeed, he wrote in 1936 "the log concentration plotted against time . . . gives an approximately linear relationship." This statement he repeated in a later paper on the "Anaesthetic Action of Pyrazoline Compounds" (Sinha, 1939b). Plotting duration against log concentration he found that the slopes (of the "approximate straight lines") for five compounds were dissimilar and inferred that the comparison of the concentrations producing anaesthesia of equal duration was of little significance. Such a sweeping conclusion might have been modified had he employed the log-duration/log-concentration relationship of his previous paper.

It will be seen from Table VII that there is a considerable variation in the duration quoted by different workers. The cause of the variation is discussed below. There is insufficient information to determine whether the slopes are more consistent between laboratories than are the duration times for any given concentration.

The method as a comparative assay

In an attempt to confirm the findings so far recorded and to assess the value of the technique for comparative testing, the following experiment was carried out. Sixteen rabbits were each tested four times at weekly intervals with four concentrations of cocaine in the right eyes and four concentrations of nupercaine in the left eyes. The animals were divided into four groups of four, and the order of treatments within the groups randomized in a latin square design. All drug solutions were in buffer (*p*H 6), and their identity was not known by the observer during the course of the experiments. The results obtained and their analysis are shown in Tables VIII and IX respectively.

TABLE VIII

THE DURATION-CONCENTRATION REGRESSION FOR COCAINE AND NUPERCAINE

The values quoted are expressed as the total number of stimulations producing no response at five-minute intervals after treatment. (Thus " 5 " corresponds to a duration of $5 \times 5 + 2.5 = 27.5$ minutes.) Each nupercaine group was made up from one rabbit from each cocaine group

Key to treatments:

C	Cocaine 2.5 mg./c.c.	F	Nupercaine 0.025 mg./c.c.
A	,, 5.0 ,,	E	,, 0.050 ,,
B	,, 10.0 ,,	G	,, 0.100 ,,
D	,, 20.0 ,,	H	,, 0.200 ,,

Group No.	Rabbit No.	Cocaine trials				Rabbit No.	Nupercaine trials			
		1	2	3	4		1	2	3	4
I	1	A7	C6	B6	D12	1	F7	H12	E6	G14
	5	B4	A1	D9	C1	2	E12	F2	G21	H25
	9	C6	D7	A4	B6	3	H22	G12	F0	E6
	13	D7	B7	C5	A7	4	G9	E7	H16	F0
II	2	A5	C4	B10	D12	5	F0	H6	E1	G6
	6	B14	A6	D11	C5	6	E14	F6	G13	H21
	10	C8	D13	A14	B13	7	H12	G10	F0	E3
	14	D9	B7	C9	A11	8	G5	E3	H14	F0
III	3	A4	C3	B5	D6	9	F0	H10	E0	G8
	7	B7	A8	D13	C3	10	E10	F5	G15	H24
	11	C7	D9	A4	B7	11	H20	G13	F4	E2
	15	D10	B14	C9	A13	12	G6	E2	H25	F2
IV	4	A5	C2	B4	D5	13	F0	H7	E2	G7
	8	B5	A3	D6	C1	14	E8	F0	G9	H13
	12	C6	D7	A5	B7	15	H14	G14	F9	E13
	16	D11	B8	C3	A6	16	G10	E8	H22	F1

The results confirm that the log-duration/log-concentration regression is linear for cocaine between 2.5 and 20 mg. per c.c. On the other hand, while nupercaine at strengths of 0.025 to 0.2 mg. per c.c. gave durations of the same order as the cocaine figures (see Fig. 2), there was a curvature of a low order of significance ($p = 0.05$–0.01). Moreover, the linear regression coefficients for the two drugs are significantly different, as are the log variances for error.

TABLE IX

THE DURATION-CONCENTRATION REGRESSION FOR COCAINE AND NUPERCAINE. ANALYSIS OF VARIANCE

Log values are taken throughout. Non-significant interactions have been pooled with the error sum of squares

Source of variation	d.f.	Cocaine			Nupercaine		
		Mean square	F	P	Mean square	F	P
Linear regression ..	1	0.8050	43.7	<0.001	9.5600	12.5	<0.001
Quadratic regression	1	0.0035	—	—	0.5059	6.5	0.05–0.01
Cubic regression ..	1	0.0000	—	—	0.0002	—	—
Groups	3	0.2133	11.6	<0.001	0.1266	1.65	0.20
"Rows"	3	0.1514	8.3	<0.001	0.5693	7.4	<0.001
Groups × rows ..	9	0.0597	3.3	0.01–0.001	0.2183	2.8	0.01
Trials (= "columns")	3	0.0212	1.2	>0.20	0.0140	—	—
Residual (ERROR)	42	0.0183			0.0767		
Linear regression coefficient (*b*)			0.333			1.148	
Standard error of *b*			±0.0503			±0.1029	
For the difference of these values of *b*			t = 7.12			p<0.001	
The ratio of nupercaine error variance to cocaine error variance			F = 4.19			p<0.001	

These three significant differences between the two drugs are sufficient to invalidate any single estimate of the relative potency of nupercaine in terms of cocaine which might otherwise have been obtained from the experiment. It would be reasonable to conclude that the two compounds differ qualitatively in their action on the cornea or in their interaction with it. Since this qualitative difference is due to the physico-chemical properties of the two compounds we might indeed find that the nature of the duration/concentration curve was a specific characteristic for every compound we could examine. It is likely, however, that unknown compounds could be classified as cocaine-like, nupercaine-like, and so on, the differences within each group not being detectable statistically by a reasonable number of experiments using this method.

Precision of the method

On the assumption that some compounds may have a qualitatively similar action to cocaine or nupercaine it is pertinent to consider the accuracy with which the activity of the unknown may be assessed relative to these standards. To demonstrate this we may compare the results obtained for 2.5 and 10 mg. per c.c. cocaine with those for 5 and 20 mg. per c.c., regarding them as pairs of dilutions of samples (*a*) and (*b*) respectively of cocaine. We find that the activity of the sample (*a*) is 99.6 per cent that of sample (*b*); to this deceptively accurate answer must be applied fiducial limits for p = 0.95 of 62–160 per cent. A similar examination of the nupercaine results gives us: sample (*a*) 0.025 and 0.1 mg. per c.c. = 100.7 per cent.

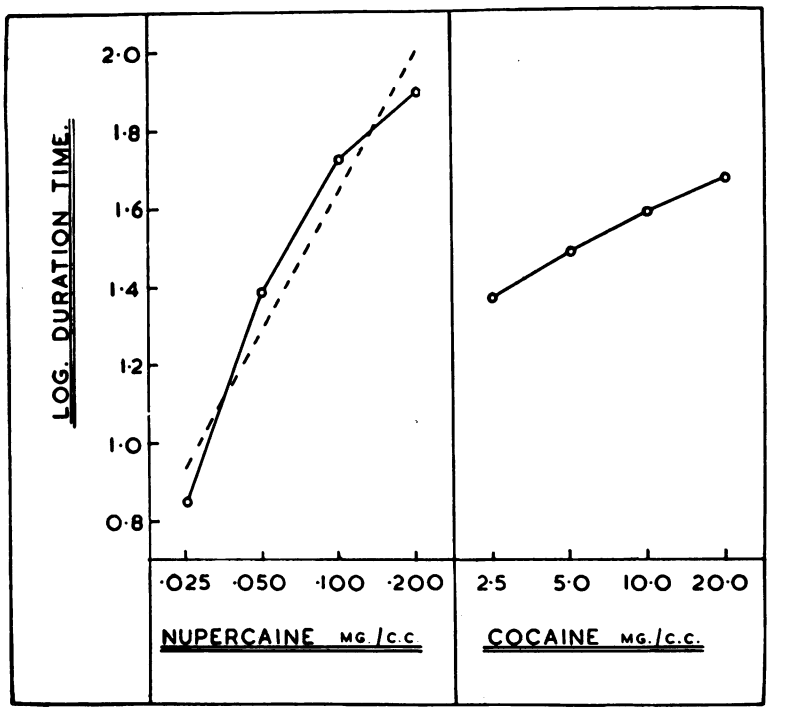

FIG. 2.—The regressions of log concentration and log duration for cocaine and nupercaine. The broken line for nupercaine is the fitted linear regression line.

as active as sample (*b*) 0.05 and 0.2 mg. per c.c., on the basis of linear regression. Fiducial limits are approximately 76–133 per cent. In neither example are the limits corrected for the variance of the regression coefficient.

It will be observed in Table IX that the mean square " trials " (i.e., the columns of Table VIII) is not significant against the error variance, where previously (Table IV) we obtained a significant repetition mean square. This discrepancy is probably accounted for by the fact that, although in the experiment " trials " are confounded with the rabbit × dose interaction, in the previously quoted results treatments with unknown compounds came between successive treatments with cocaine; thus, not only was there a possibility of the unknowns interfering with subsequent cocaine action, but the interval between trials with cocaine was at least twice as long in the earlier series as in the experiment.

By administering each treatment to each rabbit we are able to eliminate the rabbit to rabbit variation as " groups," " rows," and " groups × rows." The residual mean square may thus be used as an estimate of the error variance. It is interesting to note that for cocaine this value is of the same order as the final interaction mean square of Table IV.

The standard deviation of a single estimate of duration for each of the two drugs is shown in Table X. This is expressed both as percentage limits of the duration time observed and in terms of the corresponding percentages of concentration.

TABLE X

The error of a single estimate of duration time, as log increment and as percentage limits, in terms of time and concentration

	Cocaine		Nupercaine. Designed expt. (interaction error)
	General case (repetition error)	Designed expt. (interaction error)	
Standard deviation (% limits) ..	±0.3612 (44–230)	±0.1354 (73–137)	±0.2769 (53–189)
b	0.467	0.333	1.148
$\lambda = \frac{\text{s.d.}}{b}$ .. .. .. (% limits)	±0.7734 (17–593)	±0.4066 (39–255)	±0.2412 (57–175)

The latter values are obtained from the logarithmic value λ, which is given by the expression

$$\lambda = \frac{\text{standard deviation}}{\text{linear regression coefficient } (b)}$$

For comparison, similar values are listed derived from the general regression of Table VI, with a variance equivalent to the mean square for repetition found in Table IV. The advantage of a designed experiment will be seen by the great reduction in the value of λ compared with the general term.

Discussion

The greatest source of variation in results is undoubtedly inherent in the method, namely, the elicitation of the blink reflex. Many kinds of instrument have been employed as a stimulator. Leopold Ther (1949) quotes Kruchmann in 1895 and Schlüter in 1907 as using a bristle or Von Frey hair. This type was employed by Regnier (1923) and later by Bovet (1931), as well as by ourselves. Cohen (1925) used a toothpick tipped with cotton-wool. Most of the other authors quoted employed a rather more substantial stimulator following the lines of the blunt pencil point of Sollmann (1918). There is little evidence to suggest, however, that the choice in this respect has any consistent effect on the durations observed (see Table VII); nor can any conclusion be arrived at concerning its effect on the variability of results. Several workers have claimed an accuracy of the order of ± 20 per cent, but detailed analyses are not given.

It has commonly been observed that between the stages of no anaesthesia and complete blocking of the reflex there is a phase of partial anaesthesia which has two characteristics:

(1) the bristle applied to one point on the cornea may produce no response, but reapplied to another point may do so; and

(2) the response may be in the form of a slow closure of the eyelids, compared with the rapid movement of a normal untreated eye.

Regnier has claimed that the degree of anaesthesia may be estimated by the number of rapidly repeated stimuli required to produce a response. If this is

the case, since a bristle thus employed could not repeatedly fall on the same point of the cornea, we could not distinguish between an estimation of the degree of anaesthesia and the distribution of sensitive areas inferred in (1) above. Thus, while the bristle has the advantages of being less visible to the rabbits and of causing the minimum damage to the cornea, with a single stimulation we cannot guarantee to define the excitability of the whole area of the cornea. The response when produced may still be indistinguishable from a chance slow movement of the eyelids. The assessment of presence or absence of anaesthesia is then a highly subjective operation requiring considerable practice.

The relationship ($T = K.C^b$) discovered by Sinha appears to be true for cocaine in the concentration range 2.5 to 20 mg. per c.c., and may hold good up to concentrations of 50 mg. per c.c. or higher. It would seem unlikely that K should possess a universally valid value, but whether b varies significantly is a matter for conjecture. Nevertheless, under a given set of conditions, the log-duration/log-concentration relationship may validly be used for the comparison of anaesthetic compounds. That such comparisons may not always be resolved into a single estimate of relative potency has been demonstrated for cocaine and nupercaine, but it is not the purpose of this paper to investigate the mechanism by which the qualitative difference between these two drugs exerts itself; the nature of their difference is, however, of no small pharmacological importance. As Sinha rightly asserted, the fractional value of b for cocaine involves the use of relatively high concentrations of the drug for a given extension of its duration of action. Thus to double the duration we should require approximately four times the concentration. On the other hand, within the limits of the single experiment outlined above with nupercaine, the value of b is approximately unity. In other words to increase the duration by twofold would only require a twofold increase of concentration. It must be pointed out, however, that the curvature of the nupercaine regression line (Fig. 2) suggests that this property may not hold for very high values of duration and concentration. The nature of the regression clearly becomes of prime importance in assessing the relative merits of compounds to be employed for surface anaesthesia.

SUMMARY

The duration times for cocaine solutions at concentrations of 2.5 mg. per c.c. or greater are log-normally distributed. The absolute duration times for cocaine vary significantly both between rabbits and between treatments on the same rabbits when the animals are tested alternately with cocaine and compounds of unknown potency.

From 2.5 mg. per c.c. to about 50 mg. per c.c. the log duration is linearly related to log concentration for cocaine.

Nupercaine at 0.025 to 0.2 mg. per c.c. gave a different regression of log duration on log concentration from that of cocaine.

This difference and the precision of the method are discussed.

I should like to acknowledge my indebtedness to Dr. A. C. White and Mr. A. F. Green for advice and access to their accumulated data on corneal anaesthesia, to Miss H. Muller, who carried out the pharmacological tests, and to Mr. J. B. Attlesey for assistance in the computations.

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