because, in transforming percentage responses to logit, the observed means scatter about the logistic regression line within a reasonable limit. The variance due to deviation from regression is 78.8 which is far smaller than that in Table VIII. Such a result would be a special case and usually untransformed data also show linearity within responses from 85 to 25% according to our experiences. The significance of logistic transformation and method of calculation will be discussed in the next paper.

We wish to express our thanks to Prof. H. Kumagai and Prof. Y. Ito of University of Tokyo for guidance and help in the course of this work.

Summary

The usefulness and applicability of the small intenstines of mice for the assay of acetylcholine and atropine were examined and the results were treated statistically.

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84. **Keijiro Takagi and Masayasu Kimura** : Chemicopharmacological Studies on Antispasmodic Action. VI. On Statistical Treatment of Concentration-Action Relationship of Acetylcholine on Small Intestines of Mice.

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In order to study antispasmodic action, the mode of acetylcholine (ACh) action requipes to be clarified. This paper is concerned with the relation between ACh and acetylcholine receptor (R), and the theory of Clark[1] on concentration-action curve of ACh has now been recognized by using the statistical method shown in the preceding paper.[2]

Clark offered Langmiur's absorbtion formula to explain the action of ACh by the law of mass-action and then he has shown, though still very far from complete, that this formula could be applied on the concentration-action curve of ACh.

Recently, further observations with isolated guinea pig intestines by Matsumoto[3] supported this Clark's conception, but the tests of goodness of fit seemed to be nothing but graphic.

On the contrary, we have found that the method of logistic transformation was really and truely available for dealing with such observations. The formula (1) derived from the Clark's equation is identical with the formula (2) derived from logistic equation when the β and the K_A are respectively equal to n and $e^{-\alpha}$.

Clark's formula

$$A^n = K_A \frac{y}{y'-y} \longrightarrow e^{n w} = K_A \frac{y}{y'-y} \quad \cdots\cdots(1)$$

Logistic formula

$$P = \frac{1}{1+e^{-(\alpha+\beta X)}} \longrightarrow e^{\beta X} = e^{-\alpha} \frac{y}{H-y} \quad \cdots\cdots(2)$$

$$\longrightarrow \beta X = \log_e \frac{p}{1-p} - \alpha \longrightarrow Y = \beta X + \alpha \quad \cdots\cdots(3)$$

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1) A. J. Clark : J. Physiol., **61**, 530(1926).
2) K. Takagi, M. Kimura : This Bulletin, **4**, 444(1956).
3) S. Matsumoto : Medical Science, **6**, 113(1954).

A : Molar concentration of ACh
n : No. of ACh which combines with an ACh-receptor
K_A: Dissociation const. of ACh-receptor
y : Response of contraction
y' : Max. response of contraction

X : $\log_e A$ $\quad$ Y : $\log_e \frac{p}{1-p}$

H : Estimated max. contraction $\quad$ p : y/H

The concentration-action relationship can, therefor, be explained with the linear formula (3), derived in turn from the formula (2). From the β, we are finally able to expect the reaction order n on the combination between ACh and its receptor.

Results

An experiment for the determination of the ACh-concentration curve was made on 20 mice by

TABLE I. Contraction of Intestine for Acetylcholine (mm.)

$\times 10^{-8} M$		1.05	2.64	6.60	16.5	41.3	105	264	660
A_1	I_1	1.0	0.0	12.5	17.0	30.0	33.0	44.0	45.5
	I_2	0.0	1.0	18.5	23.0	41.0	49.5	49.0	43.5
A_2	I_1	1.0	2.0	12.0	16.5	37.0	44.0	52.5	56.5
	I_2	2.0	2.0	8.0	10.0	21.0	25.0	28.0	30.0
A_3	I_1	0.5	2.0	9.0	28.5	51.0	51.5	55.0	51.5
	I_2	1.5	5.0	14.0	16.0	47.0	63.0	64.0	57.5
A_4	I_1	4.0	3.5	11.0	24.0	38.0	38.0	53.0	56.0
	I_2	2.0	4.0	19.0	35.0	47.0	52.0	55.0	61.5
A_5	I_1	1.5	5.0	10.0	15.0	43.0	57.0	55.0	57.0
	I_2	4.0	6.0	10.0	22.0	36.5	41.0	46.0	40.5
A_6	I_1	3.0	5.0	13.0	33.0	43.0	71.0	71.5	80.5
	I_2	2.5	2.0	4.0	20.0	26.0	42.0	45.0	50.0
A_7	I_1	1.5	1.5	6.5	10.5	24.0	36.0	38.5	41.5
	I_2	0.0	5.0	18.0	36.5	51.0	64.0	78.0	76.0
A_8	I_1	4.0	5.0	7.0	10.0	37.0	61.0	55.0	53.0
	I_2	5.5	5.0	15.0	34.0	64.0	85.0	78.0	79.0
A_9	I_1	0.0	2.0	8.0	18.5	38.0	45.0	56.0	62.0
	I_2	3.5	1.5	11.0	16.0	35.0	61.0	53.0	61.5
A_{10}	I_1	1.0	1.5	10.0	12.5	45.0	43.0	53.0	46.0
	I_2	0.0	2.0	14.0	22.0	46.0	51.5	67.0	64.0
A_{11}	I_1	1.0	2.0	7.0	14.0	17.0	26.5	28.0	82.0
	I_2	1.0	2.0	8.0	12.0	22.0	33.0	38.0	39.0
A_{12}	I_1	0.0	4.0	17.0	22.0	39.0	41.0	44.5	48.0
	I_2	0.0	2.0	15.0	16.5	28.0	34.0	34.5	34.5
A_{13}	I_1	0.0	2.0	7.0	17.5	27.0	37.0	40.5	48.5
	I_2	0.0	4.0	12.5	32.0	45.0	58.0	61.5	63.0
A_{14}	I_1	2.0	5.0	12.5	37.0	53.0	59.0	73.0	83.0
	I_2	1.0	4.0	8.0	10.0	40.0	41.0	45.0	47.0
A_{15}	I_1	1.5	3.0	26.0	39.0	45.0	52.0	50.0	49.0
	I_2	4.0	10.0	21.5	42.0	49.0	55.0	49.0	57.0
A_{16}	I_1	1.5	2.0	8.0	19.0	33.0	46.0	49.0	52.5
	I_2	0.0	4.0	12.0	23.0	29.5	42.0	45.5	45.5
A_{17}	I_1	0.0	4.0	12.0	26.0	36.0	45.0	66.0	67.0
	I_2	2.5	4.0	9.0	23.0	31.0	49.0	45.0	55.0
A_{18}	I_1	1.5	4.0	11.0	16.0	45.0	51.0	51.5	73.0
	I_2	1.5	14.0	22.0	37.0	51.0	59.0	60.0	61.0
A_{19}	I_1	1.0	3.0	10.0	29.0	30.0	33.0	36.0	43.0
	I_2	0.0	2.5	20.0	32.0	37.0	55.0	56.0	72.0
A_{20}	I_1	1.5	2.0	9.0	15.0	26.0	32.0	34.0	39.0
	I_2	1.5	4.0	12.0	16.0	46.0	60.0	64.0	65.0
means		1.5	3.56	12.24	22.24	38.26	48.05	51.46	54.69

* I_1, I_2 were the two locations in the bath, to which two intestinal segments from an animal were attached.

the method described in the preceding paper.[2] Doses from $1.05\times10^{-8}M$ to $6.6\times10^{-6}M$ were divided into 8 levels and each dose was added with 0.8 cc. saline solution within 15 secs. The results shown in Table I are the contractive responses of small intestines of mice for the 8 ACh doses.

Concerning the date of Table I, from which lower 2 doses are omitted because of the significantly smaller variances in comparison with the others, the analysis of variance is shown in Table II.

Table II. Analysis of Variance for the Data of Table I

Adjustment for mean 343451.00

Nature of Variation	d. f.	Sum of Squares	Mean Square	
Animals	19	8029.65	422.61	
Doses	5	58805.28	11761.06	
Location	1	582.82	582.82	
$A\times L$	19	10407.18	547.75	
$A\times D$	95	5060.72	53.27	}
$D\times L$	5	131.30	26.26	} 47.08
$A\times D\times L$	95	3988.05	41.98	} (d. f. = 195)
Total	239	87005.00		

The interaction mean squares for $A\times D$ and $L\times D$ are smaller than the mean square for $A\times L\times D$, and the 3 components should be pooled to give the mean square of error $s^2=47.08$(d. f. = 195).

After the maximum response H has been assumed from the mean responses $\bar{u}$ as 55.1 mm., the mean response ratios $\bar{p}=\bar{u}/H$ are calculated, which in turn are transformed to logit by the logistic scale.[4] Two parameters α and β of $Y=\beta X+\alpha$ ……(3) and the maximum response H are then estimated with the method of maximum likelihood reported by us,[4] and shown in Table III and Figs. 1 and 2.

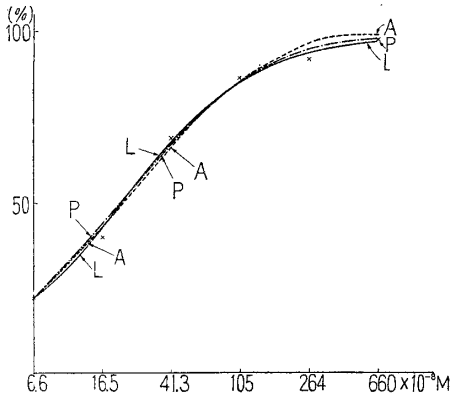

Fig. 1. Percentage Response (H=55.42) in comparison with Logistic(L), Probit(P), and Angular(A) Curves

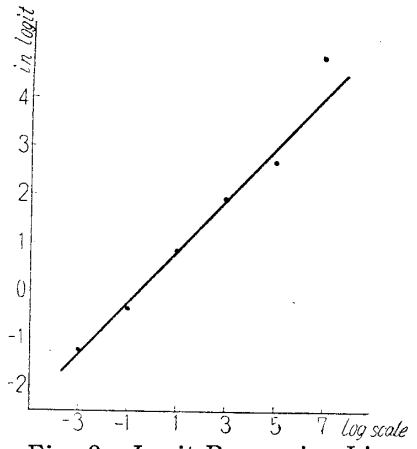

Fig. 2. Logit Regression Line for the Data of Table I

The estimates of α, β, and H are 0.2236, 0.5266, and 55.42, respectively.

For testing the deviation of observed values from the computed regression line, the expected logit Y_2 on the line was converted to P, multiplied by $H_2=55.42$ to give the expected response $\bar{u}$ on each dose. Then D_0^2 was calculated as the following and compared with s^2. $D_0^2=\sum\frac{n(\bar{u}-U)^2}{k-3}=\frac{40\times3.775}{6-3}=50.3$(d. f. = 3). $F_0=D_0^2/s^2$ showed no indication of serious deviations from the fitted sigmoid curve.

In order to return the modified dose metameter to the original log doses to base e, the observed slope $b=0.5266$ must be divided by $I(=2.303/2\times\log_{10}2.5)$ and given $b'=1.149$. Then the variance of b' is

$$V(b')=s^2V_{11}/H^2I^2=0.007298.$$

$$\text{Now}\quad t_0=\frac{1.149-1}{\sqrt{V(b')}}=1.74(\text{d. f.}=3).$$

The value of t_0 showed that b' does not deviate significantly from the theoretical value of 1. Finally, transforming the x of our linear formula in Table III into the X of concentration in the log scale, the following formula is given from

$$x=\frac{X-2.303\times\log_{10}2.89\times10^{-7}}{2.303/2\times\log_{10}2.5}.\qquad Y=1.149X+17.527.$$

4) K. Takagi, *et al.*: J. Pharm. Soc. Japan, **76**, 1186, 1191(1956).

Table III. Calculation for fitting a Logit Regression Line to the Data of Table I

$\bar{u}$ (mm.)	$\bar{p}=\bar{u}/H_1$	Exptl. Logit y_0	x	Expected Logit Y_1	P	$w=P^2Q^2$	nw	Working Logit y	$x'=1/Q$	nwx	nwy	nwx'
12.24	0.222	−1.25	−3	−1.35	0.206	0.02673	1.0692	−1.251	1.259	−3.208	−1.338	1.346
22.30	0.405	−0.38	−1	−0.25	0.438	0.06058	2.4232	−0.383	1.779	−2.423	−0.928	4.311
38.26	0.695	0.82	1	0.80	0.690	0.04576	1.9304	0.823	3.226	1.930	1.589	6.227
48.05	0.872	1.92	3	1.92	0.870	0.01281	0.5124	1.918	7.692	1.537	0.983	3.941
51.46	0.935	2.67	5	2.95	0.950	0.00223	0.0892	2.649	20.000	0.446	0.236	1.784
54.69	0.992	4.82	7	4.00	0.982	0.00031	0.0124	4.565	55.556	0.087	0.057	0.688
							6.0368			−1.631	0.599	18.297

$\bar{x} = S_{nwx}/S_{nw} = -0.2702$
$\bar{y} = S_{nwy}/S_{nw} = 0.0992$
$\bar{z} = S_{nwx'}/S_{nw} = 3.0309$

S_{nwx}^2	$S_{nwx'y}$	S_{nwxy}	$S_{nwx'x}$	$S_{nwx'^2}$
21.427	17.216	11.056	23.437	133.669
−) 0.441	−) 1.816	+) 0.162	+) 4.943	−)55.457
20.986	15.400	11.218	28.380	78.212

$$20.986\beta + 28.380\frac{dH_1}{H_1} = 11.218$$
$$28.380\beta + 73.212\frac{dH_1}{H_1} = 15.400$$

$$V_{11} = \frac{78.218}{78.212 \times 20.986 - 28.3804^2} = 0.0935$$
$V_{12} = -28.3804/836.204 = -0.03396$
$V_{22} = 20.987/836.204 = 0.02511$
$\beta = 11.2178 \times 0.0935 - 0.0396 \times 15.4005 = 0.5266$
$dH_1/H_1 = -0.0339 \times 11.218 + 0.0256 \times 15.4005 = 0.00588$
$dH_1 = 55.1 \times 0.00588 = 0.324$
$H_2 = dH_1 + H_1 = 55.424$
$\alpha = 0.0992 + 0.5266 \times 0.2702 - 0.0059 \times 3.0309 = 0.2236$
$Y = 0.2236 + 0.5266x$

Discussion

Usually for a set of data obtained with a single intestine a regression line is fitted and regression coefficients from many intestines are averaged, but a precise estimate of the maximum contraction can hardly be possible, because even in a higher concentration comparatively large variation is inevitable (see Table I). Acording to our experiences, number of responses obtainable from a single subject are limited and therefore it is difficult to draw a good fitted line for them. After recognizing in the data of Table I that there were no significant differences in sensitivity between each intestine, 40 responses in each dose level were averged. From these mean responses we can estimate with the method of maximum likelihood the fittest line as well as the maximum contraction height, which we thought now as the most reliable estimates.

In Table II, which shows the results of an experiment with 20 mice, the components for the animal (A), location (L), and the interaction between both are significantly larger than the triple interaction $A\times L\times D$, but the interactions $A\times D$ and $L\times D$ do not significantly deviate from the error variance $A\times L\times D$, so that the design of this experiment has a full appreciation of its validity. From the fact that $A\times D$ and $L\times D$ are small, we can pool the components for $A\times D$, $L\times D$, and $A\times L\times D$ as an error variance, and therefore the degrees of freedom for error can be increased. If $A\times D$ were significantly larger than $A\times L\times D$, we should consider the component for $A\times D$ as the error variance.

The above results that our data showed no serious deviation from a logistic sigmoid curve do not necessarily lead to the conclusion that the concentration-action relationship of ACh must obey the Clark's formula (1). If we accept Guddum's early conception that sigmoid curve is dependent upon different sensitivity in ACh receptors, a probit transformation must be applied rather than a logistic transformation, or else an angular transformation may also be used in such a case. Now, three regression lines through

each of these transformations with a graphic method[4] as $H=55.42$ are brought together for ready comparison in Fig. 1.

Each deviation from the regression line is logit $D_0^2=37.8\left(=50.3\times\frac{3}{4}\right)$, probit $D_0^2=81.0$, and angle $D_0^2=138.7$(d.f.$=4$), and then each value of F_0 compared with the error term$=47.08$ is 0.92, 1.97, and 3.36, respectively. As angle F_0 is more than $F^4_{195}\fallingdotseq 2.447$, the angular transformation is rejected significantly. To conclude, it is apparent that a logistic transformation might be the fittest. The final decision must be given after many experiments have been performed for a competitive inhibition by atropine or non-competitive one by papaverine-like substances.

We wish to expess our thanks to Prof. H. Kumagai and Prof. Y. Ito of University of Tokyo for guidance and help in the course of this work.

Summary

1) A concentration-action relationship of acetylcholine was demonstrated using isolated intestines of 20 mice.

2) For mean responses of six acetylcholine concentrations was fitted a logistic sigmoid curve by the method of maximum likelihood.

3) The maximum likelihood estimate of the mean slope was 1.146, which was proved not to be significantly different from the theoretical value of one.

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85. **Kiyoshi Futaki**: Circular Paper Chromatography. Studies on a Factor that Influences Rf and Determination of Rr Values of Photographic Developing Agents.

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Rutter[1,2] has described a modified chromatographic technique employing circular filter paper and called attention to its advantages such as speed and sharpness of separation, simplicity and compactness of apparatus, reproducibility, and control of rate of solvent flow.

Rosebeek[3] developed another technique of circular paper chromatography in which he employed a filter paper cone immersed in the eluant and just touching the center of horizontally supported filter paper.

Lüderitz and Westphal[4] extended Rutter's technique by applying discrete spots of material in a circle about the center of a paper rather than as a single spot at the origin, so that many different substances could be compared.

Rao and Giri,[5] employing Lüderitz and Westphal's technique, reported the factors that influence Rf significantly. According to them, these factors are the distance moved by the solvent and distance of the initial spot from the center of filter paper.

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1) L. Rutter: Analyst, **75**, 37(1950).
2) L. Rutter: Nature, **161**, 435(1948).
3) S. Rosebeek: Chem. Weekblad, **46**, 813(1950).
4) O. Lüderitz, O. Westphal: Z. Naturforsch., **7b**, 136(1952).
5) T. Rao, K.V. Giri: J. Indian. Inst. Sci., **35A**, 77(1953).