STUDIES OF TOLERANCE OF SEVERAL KINDS OF ANIMALS TO HEAT, ELECTROSHOCK, HYDROGEN AND HYDROXYL IONS.

IV. STUDIES OF TOLERANCE OF PARAMECIUM CAUDATUM TO HYDROGEN AND HYDROXYL IONS.

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INTRODUCTION

Living matter is typically surrounded by water solutions. Wherever water exists, there must of course be hydrogen and hydroxyl ions, and concentration of these ions is a factor of some importance. (1) Animals maintain a relatively constant hydrogen ion concentration. Sea water is alkaline (PH 8.0 to 8.1), where the blood and body fluids of marine animals have a PH usually between 7.2 and 7.8. The reaction of the natural fresh water environment may vary from a PH of 3.2 to one of 10.6. (1) The relation of various organisms e. g. bacteria (2) and protozoa to the PH of their environment has often been studied. (3) Land vertebrate blood is slightly alkaline, and have usually a PH near 7.4 (4). In birds and mammals, a change in the hydrogen ion concentration of the blood is believed to have marked effects on various parts of the body.

The effect of acidity and alkalinity has been studied on various biological systems, e. g. heart, smooth muscle, blood vessel, red blood cells and brain etc. There has, however, been little information presented to date concerning mathematical relationship between PH and time of exposure in tolerance of living cells. The author considered that Chung's formulas (5) (6) (7) (8) (9) (10) might be applicable to concentration time relationship in PH tolerance of unicellular animal like Paramecium Caudatum.

Paramecium is supposed to be a convenient experimental animal in such a research because of a complex organism having ciliary motion, besides being a single, isolated cell.

$$\left.\begin{aligned} \boldsymbol{P} &= \frac{10}{\sqrt{2\pi}}\int_{-\infty}^{P} e^{-(P-50)^2/200}\, dp \cdots\cdots\cdots\cdots (1a) \\ P &= \frac{(i-\partial)t^n - c}{bt^n + d} \cdots\cdots\cdots\cdots\cdots\cdots\cdots (1b) \end{aligned}\right\} \cdots\cdots\cdots\cdots (1)$$

Where $\boldsymbol{P}$ is per cent probability of response in animals exposed to a kind of stressor. P is 'probacent' (Chung's abreviation of probability percentage). It shows relative effective amount of stressor. i is intensity of stressor. t is duration of exposure. a, b, c, d and n are constants, which are determined by sorts of animal, stressor, response and units and so on.

The purpose of this study is to examine tolerance of Paramecium Caudatum to hydrogen and hydroxyl ions in the environmental fluid and to establish formulas expressing per cent probability of response of paramecia in terms of PH and time of exposure.

METHOD

Reaction of Paramecium Caudatum to changes of acidity or alkalinity in culture fluid was studied by means of microscopic observations. Criterion of tolerance of paramecia was the absence of visible ciliary motion and locomotion. Fluid of straw infusion was used as culture media, which was made by boiling 1.000 ml redistilled water containing four grams of straw for about twenty minutes. 61 ml of M/15 Na_2HOP_4 and 39 ml of M/15 KH_2PO_4 solutions were added to the 900 ml fluid of straw infusion above described. The PH of the culture fluid was so adjusted to be 7.2. The experimental studies were done in room temperature from 29°C to 31°C in July and August.

Animals were exposed to solution of various hydrogen and hydroxyl ion concentrations in the test tubes. The PH range of acidity was from 4 to 6.5. The PH range of alkalinity was from 8 to 10.5. The PH of fluid was determined by a Beckman H_2 type PH meter. Buffer solutions of various PH values were made by several buffer systems (10) as indicated in the Table 1.

In the experiments on tolerance of paramecia to acidity, animals of which ciliary movement ceased were microscopically surveyed and counted at various time of exposure. In this work on tolerance to alkalinity, cell membrane of paramecia were destructed rapidly and lysis of cell occurred relatively soon. Consequently, surviving and locomoting paramecia were counted at various time of exposure. Experiments of relatively longer period than two hours were carried out in test tubes containing the same number of paramecia with the control tubes. After well shaking to make the paramecium suspension homogeneous, two drops were put on a slide glass with a small pipet. There after, number of animals seem in the visual field of the centor of the drop were microscopically surveyed and counted. Simultaneously, number of animals of the control drop was counted. The results in average were

Table 1. Method of producing buffer solutions of various PH values in this experiment.

PH	Buffer system	Volume ratio	Degree of dilution (Ratio)		Remarks PH changes during experiment
			Buffer solution	Culture fluid of paramecia	
4.0	$\frac{M}{5}$ CH_3COOH $\frac{M}{5}$ CH_3COONa	18.5 1.5	1	6	none
5.0	$\frac{M}{5}$ CH_3COOH $\frac{M}{5}$ CH_3COONa	8.0 12.0	1	6	none
5.5	$\frac{M}{5}$ CH_3COOH $\frac{M}{5}$ CH_3COONa	5.9 14.1	1	9	none
5.75	$\frac{M}{5}$ CH_3COOH $\frac{M}{5}$ CH_3COONa	4.2 15.8	1	6	from 5.7 to 5.8 during the experiment lasting for 80 min.
5.95	$\frac{M}{5}$ CH_3COOH $\frac{M}{5}$ CH_3COONa	2.9 17.1	1	12	from 5.95 to 6.1 after 24 hours.
6.5	$\frac{M}{5}$ Na_2HPO_4 $\frac{M}{15}$ KH_2PO_4	0 10	1	7	none
7.2	$\frac{M}{15}$ Na_2HPO_4 $\frac{M}{15}$ KH_2PO_4	6.1 3.9	1	9	(contol culture fluid) none
8.0	$\frac{M}{10}$ Na_2HCO_3 $\frac{M}{10}$ $NaHCO_3$	1.0 9.0	1	7	none
8.7	$\frac{M}{10}$ Na_2CO_3 $\frac{M}{10}$ $NaHCO_3$	2.5 7.5	1	7	none
9.0	$\frac{M}{10}$ Na_2CO_3 $\frac{M}{10}$ Na_2HCO_3	4.0 6.0	1	7	none
9.55	$\frac{M}{10}$ Na_2CO_3 $\frac{M}{10}$ $NaHCO_3$	9.0 1.0	1	7	from 9.5 to 9.6 during experiment for 60 minutes
10.0	$\frac{M}{10}$ Na_2CO_3 $\frac{M}{10}$ $NaHCO_3$	9.0 1.0	1	5	none
10.5	$\frac{M}{5}$ $Na_2B_4O_7$ $\frac{M}{10}$ $NaOH$	2.0 8.0	1	2	none

compared each other. Paramecia were treated by culture fluid at various values of PH for various durations until the minimum time was determined at which 100% cessation of ciliary motion occurred. The time interval was then progressively decreased until 0% cessation of ciliary motion was attained at various values of concentration. The longest period was for twenty four hours.

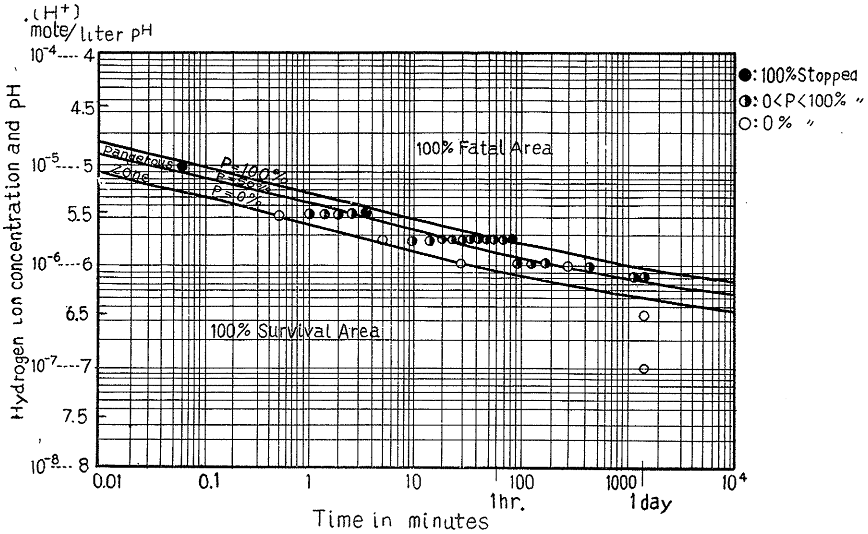

Fig. 1. Tolerance of Paramecium Caudatum to hydrogen ions in the environmental fluid. Abscissa: time of exposure, ordinate: hydrogen ion concentration and PH. Three curves of 100%, 50% and 0% 'probacents' of cessation response of ciliary motion are shown on a log-log scale.

RESULTS

The results of the experiments on tolerance of Paramecium Caudatum to acidity and alkalinity of the environmented fluid are shown in Table 2 and 3, respectively.

The results plotted on a log-log scale are presented in Fig. 1 and 2. Even slight changes of PH values gave more marked influence to the time until cessation of ciliary movement of paramecia in the acid range (PH 7) than in the alkaline range (PH 7) as indicated with difference of the declination of curves in the Fig. 2 and 3.

DISCUSSION

Obtained data are plotted on a log-log scale, where intensity of stimulus i. e. concentration of hydrogen and hydroxyl ions are taken along the ordinate and duration of exposure along the abscissa. If points indicating specific percentage of response at each level of the above ions, i. e. different PH values, are connected, they reveal

Table 2. Tolerance of Paramecium Coudatum to Hydrogen Ions (PH>7)

PH	Time of Expos. min.	No. of Animals	No. of Stopped	% Stopped	% Probability Theoretical	% Probacent Theoretical
5.0	0.06	270	270	100	100	93.56
5.5	0.5	279	0	0	0	
	1.0	380	35	9.2	1	8.78
	1.5	320	65	20.3	14.5	38.82
	2.0	260	114	43.8	42.5	48.20
	2.5	327	255	77.9	72.5	56.02
	3.5	292	292	100.0	96.7	68.33
5.75	5	623	0	0	0	6.05
	10	598	28	4.6	0.4	23.44
	15	574	72	12.1	6.5	35.03
	20	570	132	23.1	27.5	44.04
	25	670	416	62.0	55.5	51.44
	30	636	488	76.7	77.9	57.70
	35	587	504	85.5	90.7	63.24
	40	614	546	88.9	96.6	68.29
	45	674	623	92.4	97.3	69.31
	50	538	506	93.8	99.7	77.31
	55	621	590	95.3	99.9	81.16
	65	549	542	98.7	99.9	87.66
	80	549	549	100.0	100.0	97.01
5.95	30	764	0	0	0	0.76
	90	324	32	9.9	1.5	27.65
	120	628	101	16.1	6.5	34.67
	180	328	190	57.9	44.5	48.57
6.0	300	263	179	68.0	41.0	47.71
	420	240	200	83.3	82.5	59.18
6.1	1200	409	365	89.2	74.0	56.41
	1440	422	382	90.2	90.1	62.87
6.5	1440	1028	0	0	0	– 43.83
7.0	1440	Control	0	0	0	– 80.28

a rectilinear line with a definite declination. Three lines indicating 0%, 50% and 100% seem to be parallel. Chung's formulas (1) relating to stress and biologic response are considered to be applicable here. According to his method, (7) the values of n in the formula (1) are obtained from the declination (θ) of the lines connecting points indicating 50% responses as follows:

n=tan 14°20′=0.256..............................in acid range.

n=tan 30′=0,5 ....in alkaline range.

Exposure times t_1, t_2 and t_3 corresponding to P=O, P=50, P=100 at PH 5.5 are determined from calculation and geometrical analysis in a graph with values of probacent as shown in Fig. 3, where a normal frequency curve for $t^{0.256}$ is considered.

Table 3. Tolerance of Paramecium Cadudatum to Hydroxyl Ions (PH>7)

PH	Time of Expos. Min.	No. of Animals	No. of Stopped	% Stopped	% Probability Theoretical	% Probacent Theoretical
10.5	0.5	1028	1028	100	92.1	64.02
10.0	1	476	0	0	0.02	14.69
	2	459	50	10.9	2.97	31.14
	3	476	147	30.8	26.1	43.59
	4	476	290	58.8	66.2	54.19
	5	476	432	90.7	91.2	63.58
	6	476	474	99.7	98.6	72.0
	7	476	476	100.0	99.9	79.82
9.55	10	284	0	0	0.1	19.14
9.55	10	284	0	0	0.1	19.14
(9.5–9.6)	20	284	97	34.1	10.5	37.30
	30	284	202	71.1	54.9	51.23
	40	284	254	89.4	89.9	62.76
	50	284	276	97.1	98.9	73.05
	60	284	284	100.0	100.0	82.36
9.0	360	1060	411	39.7	36.8	46.61
	1440	1060	1060	100.0	100.0	112.66
8.7	1440	1110	262	23.6	23.5	42.71
8.0	1440	1208	0	0	0	− 13.56
7.2	1440	Control	0	0	0	− 25236

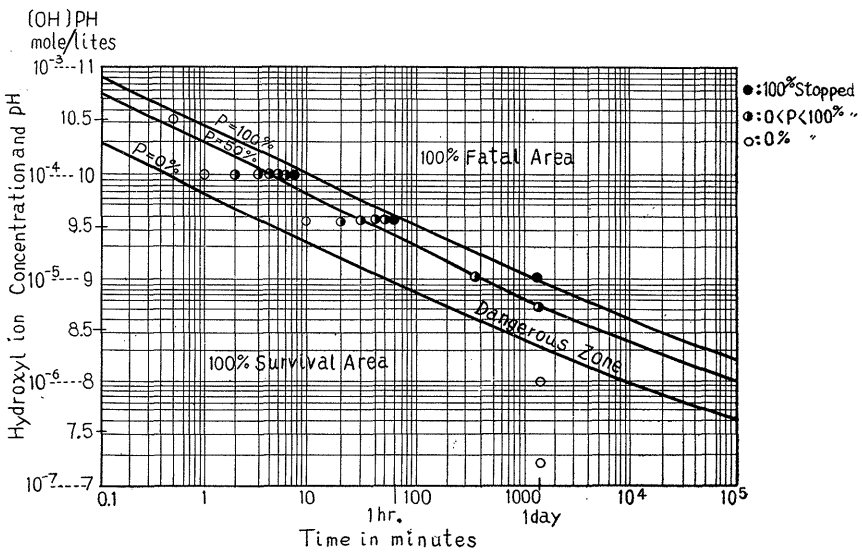

Fig. 2. Tolerance of Paramecium Caudatum to hydrooxyl ions in the environmental fluid. Abscissa: time of exposure, ordinate: hydroxyl ion concentration and PH. Three curves of 100%, 50% and 0% 'Probacents' of cessation response of ciliary motion are shown on a log-log scale.

$t=0.34$ min, $(\therefore 0.34^{0.256}=0.76)$ $t_2=2.1$ min, $(\therefore 2.1^{0.256}=1.21)$ $t_3=6.9$ min. $(\therefore 6.9^{0.256}=1.64)$

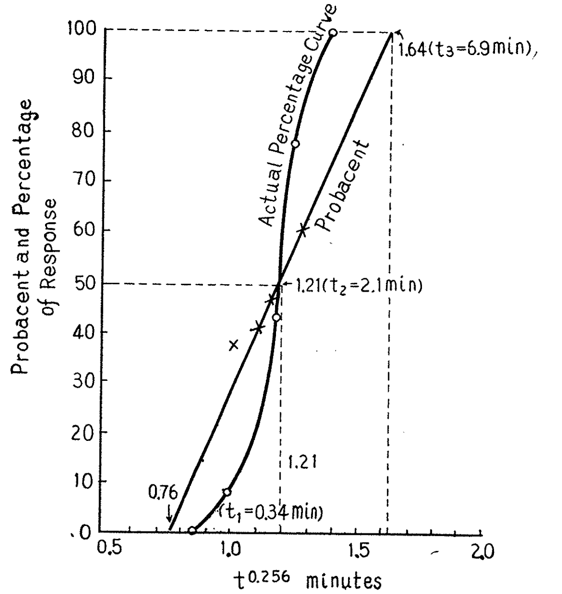

Fig. 3. Geometrical analysis of time corresponding to probacent 0, 50 and 100% at PH 5.5. The abscissa indicates values of t 0.256 and the ordinate values of probacent and percentage of response. Probacent points (x) corresponding to actual percentage of data (o) are plotted.

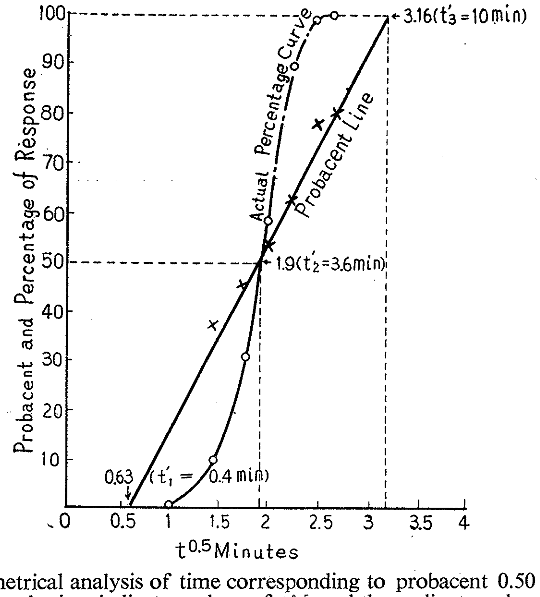

Fig. 4. Geometrical analysis of time corresponding to probacent 0.50 and 100% at PH 10. The abscissa indicates values of $t^{0.5}$ and the ordinate values of probacent and percentage of response. Probacent points (x) corresponding to acttual percentage of response of data (o) are plotted.

Exposure times t'_1, t'_2 and t'_3 corresponding to P=0, P=50, P=100 at PH 10 are likewise determined as shown in Fig. 4.

$t'_1=0.4$ min, $t'_2=3.6$ min, $t'_3=10$ min.
$(0.4^{0.5}=0.63)$ $(3.6^{0.5}=1.9)$ $(10^{0.5}=3.16)$

Four conditions as below described are used to construct the formulas (6) expressing tolerance of paramecia to hydrogen ions.

1) $a=10^{-6.8}$............(2) The constant 'a' in the formula (1b) is determined from the data plotted on a log-log scale.

2) The condition of exposure to concentration $10^{-5.5}$ of hydrogen ions (PH 5.5) for 0.34 min is assumed as that of P=P.

3) Similarly, the condition of $10^{-5.5}$............2.1 min, as P=50.

4) The condition of $10^{-6.1}$............1440 min, as P=62.93 Corresponding to actual percentage of response 90.2.

$$\frac{(10^{-5.5}-10^{-6.8})\times 0.34^{0.256}-C}{0.34^{0.256}b+d}=0 \quad \text{.................(3)}$$

$$\therefore C=2.28\times 10^{-6}$$

$$\frac{(10^{-5.5}-10^{-6.8})\times 2.1^{0.256}-2.28\times 10^{-6}}{2.1^{0.256}b+d}=50 \quad \text{..............(4)}$$

$$\frac{(10^{-6.1}-10^{-6.8})\times 1440^{0.256}-2.28\times 10^{-6}}{1440^{0.256}b+d}=62.93 \quad \text{......(5)}$$

Values of b and d are obtained from the formulas (4) and (5).

$b=3.35\times 10^{-10}$ $d=2.67\times 10^{-8}$

Consequently, the following formulas (6) are constructed:

$$\left.\begin{aligned} \boldsymbol{P} &= \frac{10}{\sqrt{2\pi}}\int_{-\infty}^{P} e^{-(p-50)^2/200}\,dp \quad \text{.................(6a)} \\ P &= \frac{(10^{-PH}-10^{-6.8})t^{0.256}-2.28\times 10^{-6}}{3.35\times 10^{-10}t^{0.256}+2.67\times 10^{-8}} \quad \text{......(6b)} \end{aligned}\right\} \text{.........(6)}$$

where $\boldsymbol{P}$ is per cent probability of cessation response of ciliary motion of paramecia exposed to acid range of PH. p is probacent of response. PH is the negative logarithm of the hydrogen ion concentration. t is time of exposure in minutes. These formulas (6) represent, for percentage ($\boldsymbol{P}$) of cessation response of ciliary motion to the concentration of hydrogen ion i. e. PH and the time of exposure (t).

Following four conditions are used to construct the formula (7) expressing tolerance of paramecia to hydroxyl ions i. e. alkalinity of culture fiuid.

5) $a=10^{-6.5}$............(7). The constant 'a' in the formula (1b) is determined from the data plotted on a log-log scale. Hydroxyl ion concentration $[OH]^-$ is represented by 10^{-14+PH}, therefore if PH is 7.5, then $[OH]^-$ is $10^{6.5}$.

6) $[OH]^- = 10^{-4}$ (PH=10)............0.4 min, as P=0

7) $[OH]^- = 10^{-4}$ (PH=10)............10 min, as P=100

8) $[OH]^- = 10^{-5.3}$ (PH=8.7)............360 min as P = 42.8 corresponding to actual percentage of response 23.6.

Values of b, c and d are similarly obtained from the above equations.

$b = 4.82 \times 10^{-9}$ $\quad c = 6.28 \times 10^{-5}$ $\quad d = 2.51 \times 10^{-6}$

The formulas (7) is constructed.

$$\left.\begin{aligned} \boldsymbol{P} &= \frac{10}{\sqrt{2\pi}} \int_{-\infty}^{P} e^{-(p-50)^2/200} dp \cdots\cdots (7a) \\ P &= \frac{([OH^-] - 10^{-6.5})t^{0.5} - 6.28 \times 10^{-5}}{4.82 \times 10^{-9} t^{0.5} + 2.51 \times 10^{-6}} \cdots\cdots (7b) \\ P &= \frac{(10^{PH} - 10^{7.5})t^{0.5} - 6.28 \times 10^{9}}{4.82 \times 10^{5} t^{0.5} + 2.51 \times 10^{8}} \cdots\cdots (7c) \end{aligned}\right\} \cdots\cdots (7)$$

Where $\boldsymbol{P}$, p, t and PH indicate the similar meanings to those of the formula (6). $[OH]^-$ is concentration of hydroxyl ions in moles/liter.

The above formulas (7) express the tolerance of paramecia to alkalinity of culture fluid. Values predicted by these formulas (6) and (7) agree closely with observed data values as shown in Table 2 and 3. The constant 6.8 and 7.5 in the both formulas are the tolerable PH value of infinite survival and ciliary movement in acidity and alkalinity, unless other factors than the chemical stressors of hydrogen and hydroxyl ions begin to operate.

According to Yanagihu (12), optimum range of PH for paramecia is from PH 7 to 7.2, and they are able to grow between PH 6 and 8.

Alexander (1) described, the protozoan, Euglena gracilis, can live from PH 2.3 to PH 11, and Euglena mutabilis can tolerate PH 1.4 to 7.9 and grow from PH 2.1 to 7.7. Most protozoa have more restricted PH range. (13). Fisher (1) reported that chick heart fibroblasts can grow between PH 5.5 and 8.5, and the mammalian cells studied by Barta were able to grow between PH 7.4 and 9.5. It is found from this study that there is a definite mathematical relationship between PH and duration of exposure for tolerance limits in paramaecia, as expressed in Formulas (6) and (7).

SUMMARY AND CONCLUSIONS

1. Tolerance of Paramecium Caudatumto hydrogen and hydroxyl ions i. e. PH change of environmental culture fluid was examined by means of microscopic observation.

2. Formulas are presented to express approximtely the tolerance (per cent probability of cessation response of ciliary motion) of paramecia in terms of time of exposure and concentration of hydrogen and hydroxyl ions.

3. The above formulas were able to be constructed by applying Chung's general formulas concerning stress and response.

REFERENCES

1) Heilbrunn, L. V. : *An Outline of General Physiology*. W. B. Saunders Co., Philadelphia. 1955. P. 541-545.
2) Fusita, A. : *Theories and Practice of PH Determination*. Mankodo, Tokyo, 1954. P. 165.
3) Buchanan and Fulmer : *Physiology and Biochemistry of Bacteria*, Vol. 2, Baltimore, 1930.
4) Prosser, C. L. et al : *Comparative Animal Physiology*. W. B. Saunders. Philadelphia. 1952. P. 80.
5) Chung, S. J. : Studies of positive radial acceleration on mice. *J. Applied Physiology*, **14**:52, 1959. *ROKAF J. Aviation Medicine*, **4**:7, 1956.
6) Chung, S. J. : Studies of tolerance of paramecium to quinacrine hydrochloride. *ROKAF J. Aviat. Med.*, **6**:77, 1958.
7) Chung, S. J. : *J. of the National Academy of Sciences, Republic of Korea*, Vol. 2, 1960.
8) Chung, S. J. and Cho, D. W. : Studies of tolerance of paramecium caudatum to heat stress. *Theses of Catholic Medical College*, **3**:59, 1959.
9) Cho, D. W. : *Studies of tolerance of mice to heat stress.*
10) Roh, B. L. : Studies of mathematical relationship between dose, time and percentage of response, and their application to determine lethal dose and to compare toxicity. *Korean Medicine*, **3**:10. 1960.
11) Yoshikawa, S. : *Clinical Biochemistry, Experimental.* Kyodo-Isho Publishing Co., Tokyo. 1955. P. 438.
12) Yanagihu, R. : *Paramecium*. Nakayama-Shoten, Tokyo, 1955. P. 15.
13) See Von Dach : *Ohio Jour. Sci.*, **43**:47, 1943. Loefer: *Arch. f, Protistenk.*, **85**:209, 1935. *Physiol. Zool.*, **15**:333, 1942. quoted from (1).