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

1. STUDIES OF TOLERANCE OF MICE TO HEAT STRESSOR.

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INTRODUCTION

Living organisms are very influenced by their environment. The most important viable of the physico-chemical environment of living material is temperature. All animals and plants are able to live only within a very narrow range of temperature, with the exception of a few organisms as bacterial spores which can exist in a dried-up state. In general, organisms are killed at temperatures only a few degrees above at which they are accustomed to live. However, the high temperature lethal for an animal depends on duration of exposure to the elevated temperature, and high lethal temperatures are meaningful only if exposure times are given. There are very few records of heat death temperatures (1) (2). This might be due to the fact that many authors have neglected the time factor.

In a careful study of the heat death of any organisms, a curve might be plotted showing the length of time required for death at a whole series of temperatures. Such a curve would then give an accurate description of heat death in terms of temperature and time. At the present time, such a tabulation is scarcely possible (1). In determining the length of exposure necessary to produce death at a given temperature, the time 50 per cent of the organisms die is probably the best time to take according to Heilbrunn (1). There may be a great deal of variation in the time required to kill the most sensitive animals or in the time required to kill the least sensitive to heat.

Chung has made several works concerning mathematical relationship between intensity of stressor, duration of exposure and percentage of response, but many authors studied heat tolerance paid little attention to this point.

Chung difined stressors like heat and radiation as the "external stressors", which can be arbitrarily given to living bodies with a definite and constant intensity for a definite duration. He defined also stressors like drugs and toxic substances as the "internal stressors", a definite amount of which can be given into bodies at one time, for instance, intoxication and infection. He classified all stressors into the two categories of the external and internal stressors.

He has established general formulas (1) (3) (4) (5) expressing percentage (***P***) of probability of biological response in animals, exposed to stressor, in terms of intensity (i) of stressor and duration (t) of exposure. These formulas showed to be applicable to various biological phenomena concerning stress and response. (3) (6) (7) (8).

These formulas are as follows:

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

Where ***P*** is percentage of probability of response in animals exposed to stressor. P is 'probacent' (Chung's abbreviation of probability percentage), relative effective a-mount of stressor. i is intensity of stressor. t is duration of exposure. a, b, c, d, and n are constants depending on sorts of animals, stressors, responses, units and so on.

Chung and the author (6) presented formulas expressing percentage probability of response (cessation of ciliary movement) in paramecium caudatum exposed to heat stress in terms of air temperature of it's environment and duration of exposure. They also constructed formulas (9) approximately expressing human tolerance to temperature extremes, based on date (10) (11) (12) (13) (14) (15) reported in the literatures. Choi, Chung and Kim (7) established formulas expressing percentage probability of grade of burn on skin of the rats, subjected to hot air, in terms of air temperature and exposure time.

The purpose of this study is to establish formulas predicting heat tolerance in mice.

METHOD

In the experiments, 595 male and female mice were used, with weights ranging from 17 to 30 gm. They were exposed to dry hot environment of various air temperature for various durations until the minimum time was determined at which 100 % fatalities occurred. The criterion of death was absence of visible respiration. The time interval was then progressively decreased until 100% survival was attained at various temperatures.

A dry oven, 50x50x52.5 cm in size, and an incubator, 53x54x31 cm were used to get dry hot air cnditions of constant high temperature held within one per cent of error; with the oven from 60 to 200° c, with the incubator from 35 to

40°c. The inlet and outlet of air of both heat chambers were opened to protect animals from hypoia. No air currents are allowed in the heat chambers except for natural convection. Each five mice, confined in a wire cage with the floor of cork plate, were put into the heat chamber quickly. The experiments were done at the laboratory room temperature from 18°C to 24°C, and the relative humidity in the incubator was from 60 to 65% during experiment.

When the experiments were lasted longer than three hours, food and water were given to animals every 3 hours.

After exposure to heat stressor, the survival time of mice were recorded in the course of 30 day's observation. The reactions of the surviving animals were observed for one month. Body weight, food and water intake, and general liveliness were checked.

In other tests, changes of rectal, intra-abdominal and intracranial (brain) temperatures of surviving animals were measured with a micropyrometer and thermocouple immediately after exposure to heat stress. The body temperature of mice whose respiration ceased in the heat chamber in the condition of exposure in 100% of mortality was also measured.

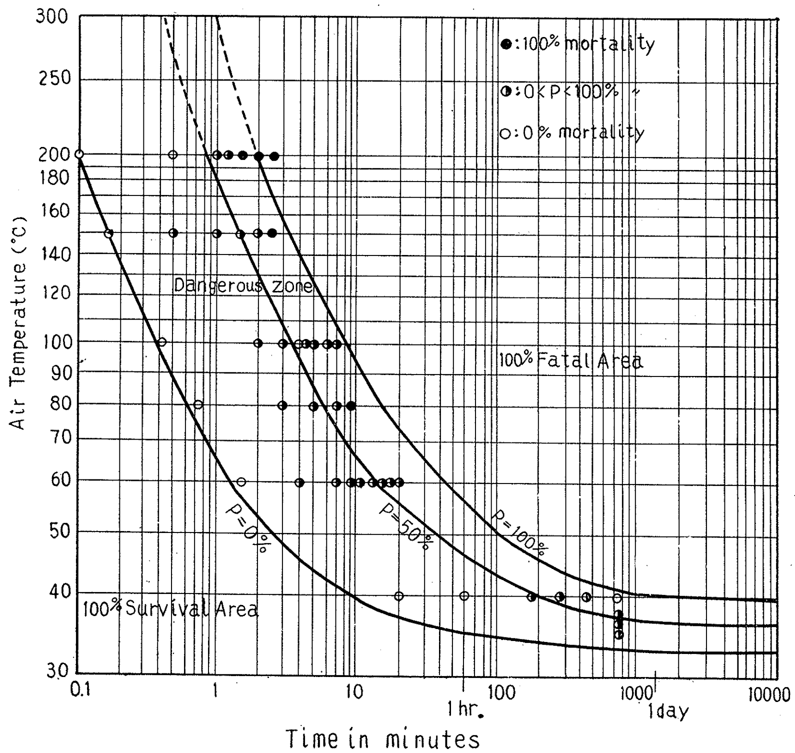

Fig. 1. Tolerance of mice to heat stress. Air temperature (°C) is plotted in ordinate and time of exposure in abscissa. Three curves of 0%, 50%, 100% 'probacent' of mortality are shown on a log-log scale. 100% survival area, dangerous zone and 100% fatal area are indicated.

RESULTS

MORTALITY. The mortality of mice exposed to dry hot air environment is shown in table 1. The results plotted on a log-log scale are presented in figure 1. Curve of mortality at each fixed temperature suggested a sigmoid form, but not accurately, when actual mortality was plotted in ordinate and time of exposure in abscissa in ordinary scale (Fig. 2, Fig. 3).

Table 1. Tolerance of mice to heat stressor. Relationship between temperature, duration of exposure and mortality of mice. (595 mice were exposed)

Temperaure Expos.	Duration or Expos. Min.	No. of Mice	No. Mortality Dead		% Probability of % Pro	
					Mortality Theoretical	bacent Theoretical
200°C	0.1	10	0	0	0	0
	0.5	10	4	40	29.1	44.49
	1.0	20	15	75	76.1	57.09
	1.17	20	19	95	92.9	64.72
	1.5	10	10	100	99.9	79.99
	2.0	10	10	100	100.0	99.38
	2.5	10	10	100	100.0	117.94
150°C	0.17	10	0	0	0.0	1.06
	0.5	10	1	10	0.1	19.39
	1.0	10	2	20	7.5	35.72
	1.5	10	6	60	76.5	57.32
	2.0	10	9	90	98.7	72.23
	2.5	10	10	100	100.0	83.46
98°C	0.42	10	0	0	0.0	0.79
	2.0	10	1	10	2.5	29.75
	3.0	10	4	40	27.5	44.05
	4.0	10	4	40	73.1	56.15
	4.5	20	13	65	88.5	62.12
	5.0	20	16	80	95.6	67.11
	5.5	20	19	95	98.7	72.15
	6.0	20	20	100	99.6	76.87
	7.0	10	10	100	100.0	82.48
80°C	0.75	10	0	0	0.0	2.21
	3.0	10	1	10	1.5	28.07
	5.0	10	5	50	30.5	44.83
	7.0	10	6	60	81.5	58.93
	9.0	10	10	100	98.2	71.11
60°C	1.5	10	0	0	0.0	1.06
	4.0	20	2	10	0.03	15.36
	7.0	20	4	20	1.5	28.34
	9.0	20	5	25	7.5	35.4
	11.0	20	10	50	20.5	41.72
	13.0	20	9	45	40.5	47.63
	15.0	10	8	80	59.0	52.27
	17.0	10	9	90	81.5	58.89
	18.0	10	9	90	85.5	60.42
	20.0	10	10	100	93.3	64.98
40°C	20.0	10	0	0	0.0	8.22
	60.0	10	0	0	0.6	25.11
	180.0	10	3	30	33.8	45.82
	270.0	10	8	80	62.5	53.21
	450.0	10	9	90	85.5	60.61
	720.0	10	10	100	97.1	68.97
38°C	720.0	20	8	40	45.5	48.85
37°C	720.0	20	4	20	12.5	38.45
35°C	720.0	15	0	0	0.05	17.23

SURVIVAL TLME. All death occured prior to removal of animals from the heat chamber in the condition of exposure causing 100% of mortality, for instance, to 200°C, for more than $1\frac{1}{2}$ minutes. In the conditions of exposure produced below 95% of mortality, some animals, however, once recovered from heat stroke when

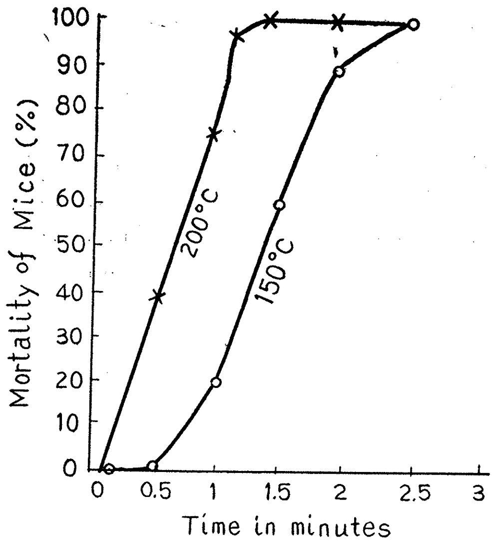

Fig. 2. Curves of mortality in mice exposed to dry hot environment of air temperature 150°C and 200°C. The curves suggest a sigmoid form, but not accurately.

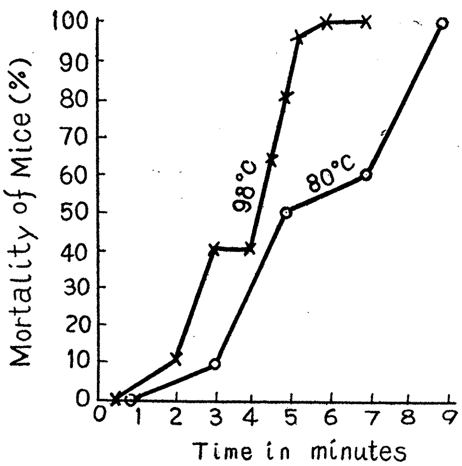

Fig. 3. Curves of mortality in mice exposed to dry hot environment of air temperature 80°C and 98°C. The curves suggest a sigmoid form, but not an accurate one.

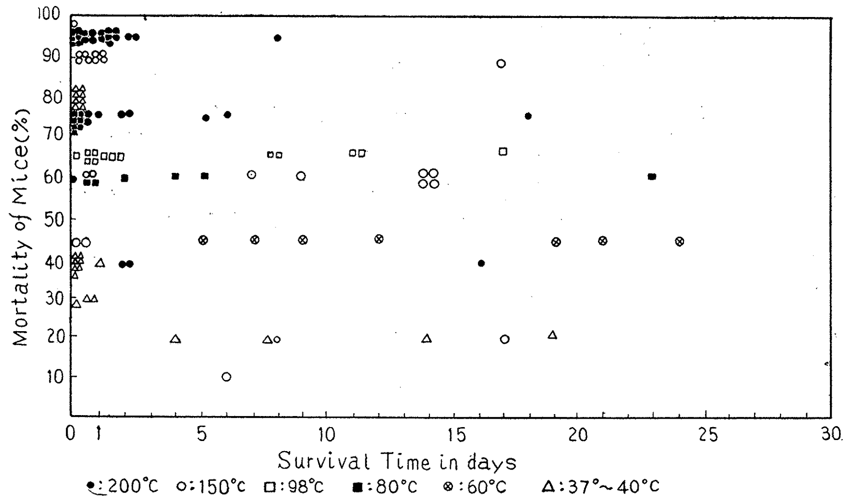

Fig. 4. Mortality and survival time of mice subjected to heat stress. Immediate deaths occured at conditions of exposure causing 100% of mortality. Below 95%, some animals died later between 24 hours and 30 days. Each point indicates one case of death resulting from heat exposure. Some parts of data at each temperature were plotted in the figure.

they were taken out from the chamber and some of them died within 24 hours or later between 24 hours and 30 days. Consequently, survival times of mice exposed to heat gradually increased as the percentage of mortality due to heat exposure decreased and it was shown in figure 4. Signs of heat pyrexia, heat exhaustion and heat stroke (16) (17) such as weakness, cyanosis, dullness, staggering, dyspnea, irregularity of breathing, Cheyne-Stokes type of respiration, convulsion, collapse, syncope and so on were observed in mice exposed to extreme heat. The body weight was found to be decreased in most fatal cases which died a few days after exposure to heat.

BODY TEMPERATURE. Changes of rectal, intra-abdominal and intracranial temperatures in the course of exposure to various hot air temperatures are shown in table 2, 3, 4 and graphically in figure 5. These body temperatures increased as the duration of exposure prolonged at each level of temperatures. They showed all

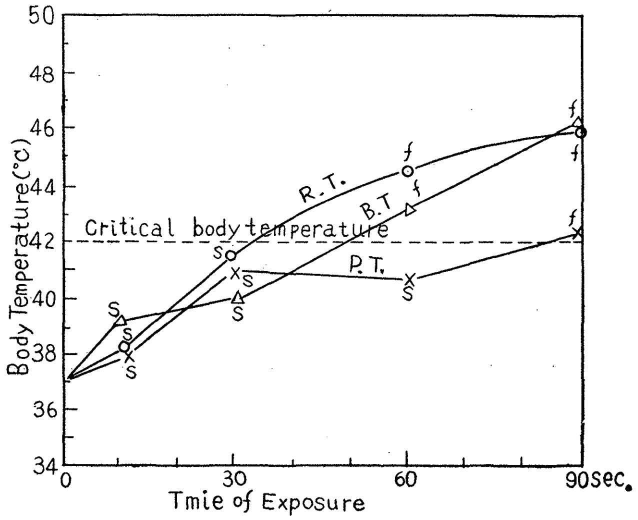

Fig. 5. Changes of body temperature of mice exposed to hot environment of 200°C air temperature for 10, 30, 60 and 90 sec. R.T. ○—○—× rectal temperature P.T. ×—×—× : intraabdominal temperature. B.T. △—△—△ intracranial temperature (brain). Symbols f and s indicate fatal and surviving case, respectively. The critical body temperature is considered to be 42°C.

Table 2. Changes of rectal temperature of mice exposed to hot environment of various air temperatures.
[(f): fatal cases, other: surviving cases]

Time / °C	10 sec	30 sec	1 min	1.5 min	5 min	15 min	17.5 min	20 min	1 hr	3 hr	4 hr
200	38.3	41.6	44.6 (f)	45.9 (f)	—	—	—	—	—	—	—
100	—	37.3	38.3	—	43.9 (f)	—	—	—	—	—	—
60	—	—	—	36.8	—	40	39.5	38.7	37.8	40.7	38.6
40	—	—	—	—	—	—	—	—	—	—	—

below 42°C in surviving animals and over 42°C in fatal cases, whent he body temperature was measured immediately after exposure to heat.

Table 3. Changes of intra-abdominal temperature of mice exposed to hot environment of various air temperatures.
[(f): fatal cases, others: surviving cases]

Time / °C	10 sec	30 sec	1 min	1.5 min	5 min	7.5 min	10 min	12 min	20 min	1 hr	3 hr	5 hr
200	38.1	41.1	40.7	42.3 (f)	—	—	—	—	—	—	—	—
100	—	38.2	38.0	—	43.1 (f)	—	—	—	—	—	—	—
60	—	—	—	37.7	—	42.0	40.2	41.5	—	—	—	—
40	—	—	—	—	—	—	—	—	38.9	38.9	40.9	38.1

Table 4. Changes of intracranial temperature of mice exposed to hot environment of various air temperatures.
[(f): fatal cases, others: surviving cases]

Time / °C	10 sec	30 sec	1 min	1.5 min	5 min	8 min	10 min	20 min	1 hr	3 hr	5 hr
200	39.4	40.5	43.5 (f)	46.0 (f)	—	—	—	—	—	—	—
100	—	36.0	38.0	—	40.5	—	—	—	—	—	—
60	—	—	—	36.8	—	37.8	37.0	—	—	—	—
40	—	—	—	—	—	—	—	37.4	38.2	40.8	37.7

DISCUSSION

Data are plotted on a log-log scale, where air temperature is plotted in ordinate and duration of exposure in abscissa. If points indicating specific percentage of mortality at each temperature were connected, they show almost a rectilinear line with a definite declination. Three lines indicating 0%, 50% and 100% of mortality seem to be parallel. Chung's formulas (1) concerning stress and biologic response are considered to be applicable to these data. According to his method of constructing the equations, the value of constant n are determined from the declination of the line connecting 50% mortality.

$$n = 0.7$$

Following four conditions are used to construct the formulas (5) expressing tolerance of mice to heat stress.

1) The constant 'a' in the formula (1 b) is determined from analysis of the data at 720 min of the longest exposure plotted in an ordinary graph as presented in figure 6.

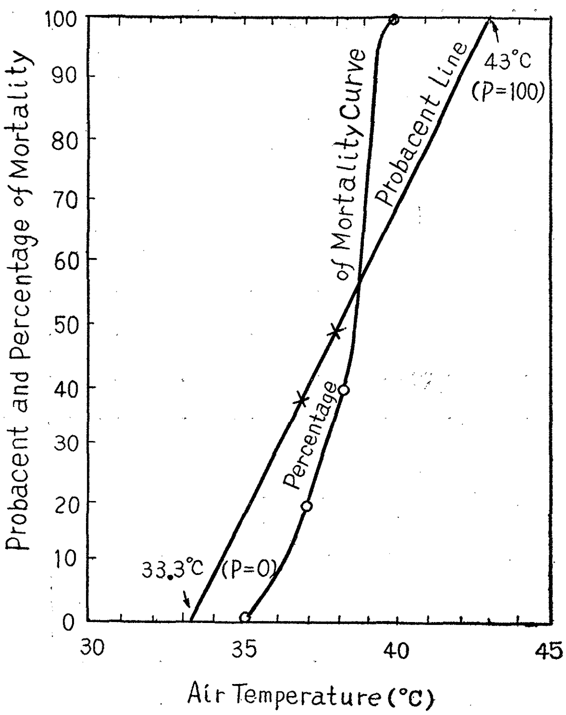

Fig. 6. Geometrical analysis of data to determine temperatures corresponding to P=O and P=100 at 720 minutes. the longêst time of exposure. Curve of actual percentages of mortality and probacent line corresponding them are plotted in an ordinary graph. Temperatures 33.3 and 43°C are obtained.

$$a = 33$$

2) The condition of exposure to 200°C for 0.1min is assumed as that of p=100

$$\frac{(200-33)\times 0.1^{0.7}-c}{0.7} = 0 \quad \cdots\cdots(2)$$

$$0.1 \qquad b+d$$

$$C = 33.4$$

3) The condition of exposure to 200°C for 2 min is assumed as that of $P=100$

$$\frac{(200-33)\times 2^{0.7}-33.4}{0.7} = 100 \quad \cdots\cdots(3)$$

$$2 \qquad b+d$$

4) The condition 43°C−720min, as P=100.
This is determined from graphical analysis as shown in figure 6.

$$\frac{(43-33)\times 720^{0.7}-33.4}{0.7} = 100 \quad \cdots\cdots(4)$$

$$720 \qquad b+d$$

values of the constants b and d are obtained from the equation (3) and (4): b= 0.074, d=2.266

Consequently, the following formulas (6) are constructed:

$$\left.\begin{aligned} \boldsymbol{P} &= \frac{10}{\sqrt{2\pi}}\int_{-\infty}^{P} e^{-(P-50)^2/200}\,dp \cdots\cdots\cdots\cdots (6a) \\ P &= \frac{(T-33)t^{0.7}-33.4}{0.074t^{0.7}+2.266} \cdots\cdots\cdots\cdots (6b) \end{aligned}\right\} \cdots\cdots\cdots (6)$$

where $\boldsymbol{P}$ is percentage of mortality in mice exposed to heat stress. P is probacent of mortality. T is air temperature (°C). t is duration of exposure in minutes.

These formulas (6) represent, for mice exposed to dry hot environment, an equation relating the mortality to the temperature T and the time of exposure t. Values predicted by these formulas (6) agree closely with observed data values as shown in Table 1. The constant 33 in the formula (6b) are the infinite survival temperature, unless other factors than heat stress begin to operate.

When the air temperature exceeds that of the skin, the body surface takes up in addition heat from its immediate surroundings; where evaporation is the only physiological way to dissipate excessive heat. Heatregulating function of rodent like mice is relatively poor, at least because of poorly developed sweat glands. (18) If the legs of rats are exposed to a temperature of 45°C in a water bath, the rats die in about 2½ hours. (19)

Heat death temperatures (1) (2) (20) reported for some mammals are as follows:

Animal	Lethal hot air temperature (°C)
Mouse	43°.3′
Rat	45° for 79 min
Guinea pig	48° for 100 min
Dog	44°–45°
Rabbit	44°–45°
Man	undetermined

Once the body temperature of mice rises above 42°C, a critical body temperature, the heat-regulating mechanisms may no longer dissipate the excessive heat being produced. Therefore the temperature of animal may then continue to rise until it causes death, unless this rise is checked artificially. This is suggested from the data as shown in table 2, 3, 4 and in figure 5.

According to Guyton, (15) the critical body temperature is 41.7°–43.3° C in man. When the body temperature rises to 43.3° to 45.6°C, the patient usually has only a few hours to live unless the body temperature is brought back within normal range very rapidly. Otherwise a viscious circle is thus established with ever-rising body temperature and consequently increasing metabolism and increasing heat productions. Numerous mechanisms of heat death are suggested, and no one mechanism operates for all animals. Heat may kill animals by enzyme inactivation, irreversible protein coagulation, toxic substances produced, change of the physical state of protoplasm or other factors.

SUMMARY AND CONCLUSIONS

1. Five hundred and ninety-five mice were subjected to dry hot environment of various air temperature of from 35 to 200°C, for periods of 6 seconds to 12 hours.

2. Data on mortality, survival time and change of body temperature are presented.

3. Critical body temperature for mice is 42°C.

4. Formulas are presented to express percentage probability of mortality in mice, exposed to heat stress, in terms of exposure time and magnitude of air temperature.

REFERENCES

1) Heilbrunn, L. V. : *An Outline of General Physiology, 3rd Edition.* W. B. Saunders Co., Philadelphia. 1955. P. 478–491.
2) Prosser, C. L. et al.: *Comparative Animal Physiology.* W. B. Saunders, Philadelphia. 19c2. P. 341–380.
3) Chung, S. J. : *Journal of the National Academy of Sciences, Republic of Korea* Vol. 2, 1960.
4) Cnung, S. J. : Studies of positive radial acceleration on mice. *J. Applied Physiology,* **14** : 52, 1959. *Republic of Korea Air Force J. Aviation Medicine,* **4**: 7, 1956.
5) Chung, S. J. : Studies of tolerance of paramecum to quinacrine hydrochloride. *ROKAF J. Aviat. Med.,* **6**:77, 1958.
6) Chung, S. J. and Cho, D. W. : Studies of tolerance of paramecium caudatum to heat stress. *Theses of Catholic Medical College,* 3:59, 1959.
7) Choi, J. H., Chung, K. C. and Kim, B. K. : Experimental studies of burn on rats. Report I. Mathematical formula expressing degress of burn on skin, fatality rate and resistance of skin to heat. *Theses of Catholic Medical College,* **3**:78, 1959.
8) Roh, B. L. : Studies of mathematical relationships between dose, time and percentage of response to drugs; and their application to determine lethal dose and to compare toxicity. *Korean Medicine,* **3**:10, 1960.
9) Chung, S. J. and Cho, D. W.: Formulas expressing human tolerance to temperature extremes. *Theses of Catholic Medical College,* **3**:72, 1959.
10) Taylor, C. L. : *Human tolerance for temperature extremes. Physics and Medicine of the Upper Atmosphere.* The University of New Mexico Press, Albuquerque. 1952. P. 548–561.
11) Buettner, K. : Effects of extreme heat in man. *J. A. M. A.,* **144**:732–738, 1950.
12) Mc Connell, N. J., Houghten, F. C. and Yaglou, C. P. : Air motion-high temperatures and humidities-reaction on human beings. *Traus. Am. Soc. Heating and Ventilating Engineers,* **30**: 167, 1924.
13) Blockley, W. V. and Taylor, C. L. : Human tolerance linits for extreme heat *J. Section Heat, Piping and Air Cond.,* May, 1949.
14) Skinner, J. B. and Pierce, W. M. : Control of excessive heat and humidity in industry: putting theory into practice. *J. Ind. Hyg. and Toxical.,* **27**:31–35, 1945
15) Guyton, A. C. : *Textbook of Medical Physiology.* W. B. Saunders, Philadelphia. 1956. P. 845–853.

16) Smillie, W. G. : *Preventive Medicine and Public Health,* The Maomillan Co,. New York. 1955. P. 153.
17) Stitt : Diagnosis, *Prevention and Treatment of Tropical Diseases,* 7 th Edition Blakiston Co., Philadelphia. 1945. P. 1082–1094.
18) Heilbrumn, Harris, LeFevre, Wilson and Woodward : *Physiol. Zool.,* **19**:404, 1946. cited from (1).
19) Gaalass : *J. Dairy Sci.,* **28**:555, 1945. cited from (1).
20) Kim K. S. : Studies on the tolerance of living organisms to changes of Osmctic pressure. *Journal of the Tokyo Women's Medical College,* **9**:1686, 1960.