

## THE RATE OF KILLING OF CLADOCERANS AT HIGHER TEMPERATURES.

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### I.

At temperatures definitely supranormal duration of life becomes very rapidly less as the temperature is raised. The high values of the temperature coefficients obtained for such phenomena<sup>1</sup> have been compared with those found for "thermal destruction" of organic compounds and for the heat denaturing and coagulation of proteins.<sup>2</sup> These comparisons do not especially explain why the temperature coefficients should be so large. Nor have the majority of the published experiments considered very closely the precise form of the curve for rate of killing at higher temperatures, and the way in which this curve makes transition to that for duration of life at inframaximal temperatures. Information of this sort was desired for the location of "upper critical temperatures" for diverse forms of Cladocera, thus facilitating their physiological characterization. Such "upper critical temperatures" were expected to give material for comparison with critical temperatures (Crozier, 1924; 1925-26) occurring within the zone of completely reversible thermal effects. The data were obtained by determining death curves for cladocerans, clonally uniform, at each of a number of "lethal temperatures." The results permit fairly exact comparisons of the "velocities of killing," and the calculation of temperature characteristics ( $\mu$ ) for certain phases of this

<sup>1</sup>Loeb (1908); Moore (1910); Lepeschkin (1910; 1923); Goodspeed (1911); Ayres (1916); Groves (1917); Bigelow (1921); Smith (1923); Heilbrunn (1924, *a*); Klopp (1924-25); Collander (1924); Crozier and Stier (1924-25, p. 440).

<sup>2</sup>Arrhenius (1907; 1913); Chick and Martin (1910; 1912, *a, b*); Hartridge (1912); Hecht (1918-19); Lewis (1926, *a, b, c*).

process which may be compared with the results of some earlier experiments.

## II.

Two species of Cladocera were used. *Daphnia pulex* was selected from a small rain-filled pond near Cambridge, Massachusetts. Large parthenogenetic females were the only kind present at that time in the pond. The animals were allowed to remain at laboratory temperature for 1 to 3 days before they were employed in the experiments. *Moina macrocopa* was secured from a clone originating from material supplied by Dr. A. M. Banta. It occurs near Cold Spring Harbor, Long Island, during the summer. The animals used were derived from one parthenogenetic female and were raised in mass cultures. Only adult females were used.

Temperature control was obtained by a water bath heated by an immersion heater with relay and mercury thermoregulator. Aggitation of the bath was by a motor-driven stirring rod. The temperature in the bath was constant to  $\pm 0.05^{\circ}\text{C}$ . during the course of an experiment. A series of test-tubes, each containing 40 cc. of culture water, was suspended in the bath and allowed to become adjusted to the temperature of the bath. A dense culture of animals of the desired age was previously prepared so that from 15 to more than 100 animals could be drawn up into a pipette with about 2 cc. of culture water. Such a group was introduced into the first tube, then after a proper interval a similar group was introduced into the second tube, and so on until each of the tubes in the series contained animals. A proper interval was allowed to elapse after the introduction of the last group, and then the rack containing all the tubes was quickly lowered into a large jar containing cold water. The temperature inside the experimental tubes fell within the first few seconds below the temperature lethal for that species of cladoceran. The culture water and animals were then transferred to a small wide mouthed bottle and allowed to stand until the animals showed a sharp division between those dead and those alive. For *D. pulex* this time is about 12 hours, but for *M. macrocopa* 4 or 5 hours sufficed for a sharp separation. A greater delay before reexamining the animals was avoided because some of the dead animals might start to disintegrate. The individuals were tabulated as "dead" and "alive," and as many experiments were made at a given temperature and time as would provide a total of from 60 to 200 animals upon which to base one point on the mortality curve.

Experiments at different temperatures were run on the same day, so that unknown but possible irregularities in the stock would be discounted. Also, at the higher temperatures, when the time was short (5 to 50 seconds), the initial temperature in the tubes was slightly above the point desired, so that the addition of

the 2 cc. of culture water along with the animals served to bring the temperature to the right point.

In Fig. 1 the plotted points represent, for each of the several temperatures, the percentage of *D. pulex* that died after the given number of second's exposure. The temperature range is from 32° to 37°C. The curves fitted to the plotted points represent the distribution of resistances at each temperature. For 34° three points for 100 per cent dead, at 3, 4 and 5 minutes, are not shown for the sake of simplicity. One point, 36 per cent for 34°, is based on but 25 individuals; another, of 56 per cent for 32°, is based on but 16 animals. These points consequently are not given much weight. Fig. 2 shows similar curves for *M. macrocopa*. In this species the lethal temperatures are higher, ranging from 40° to 47°C. This form is more easily handled than the other and consequently the upper and lower temperature limits could be approached more exactly. No attempt was made to determine accurately the shape of the ends of the curves, but attention was chiefly directed to that portion representing from 20 to 80 per cent dead. These methods therefore differ considerably from those used by von Transehe (1913), and seem to have avoided certain sources of irregularity.

### III.

Results gotten by this procedure are given in Fig. 1 (*D. pulex*), and in Fig. 2 (*M. macrocopa*). From the best fitting mortality curves, which were first adjusted in final form before any use was made of them, it was possible by interpolation to obtain the time at each temperature required to give a certain percentage of survivors. In this way figures are secured which permit comparison of the rates of the processes underlying killing at the different temperatures. Since full time was allowed for recovery, the figures used represent irreversible destruction. We have chosen to compare the times required to produce death (*a*) in 40 per cent, and (*b*) in 70 per cent of the individuals. The reciprocals of these times are plotted logarithmically, in Figs. 3 and 4, against  $1/T^\circ$  abs. The graphs are almost or quite rectilinear between 32° and 37° for *D. pulex*, and between 42° and 46° for *M. macrocopa*. The critical thermal increments for 40 per cent and for 70 per cent killing are sensibly identical for *D. pulex*, being  $\mu = 119,400$ . The values of  $\mu$  for *M. macrocopa* are not quite the same at 40 per cent and at 70 per cent, but the difference is in all likelihood within the probable

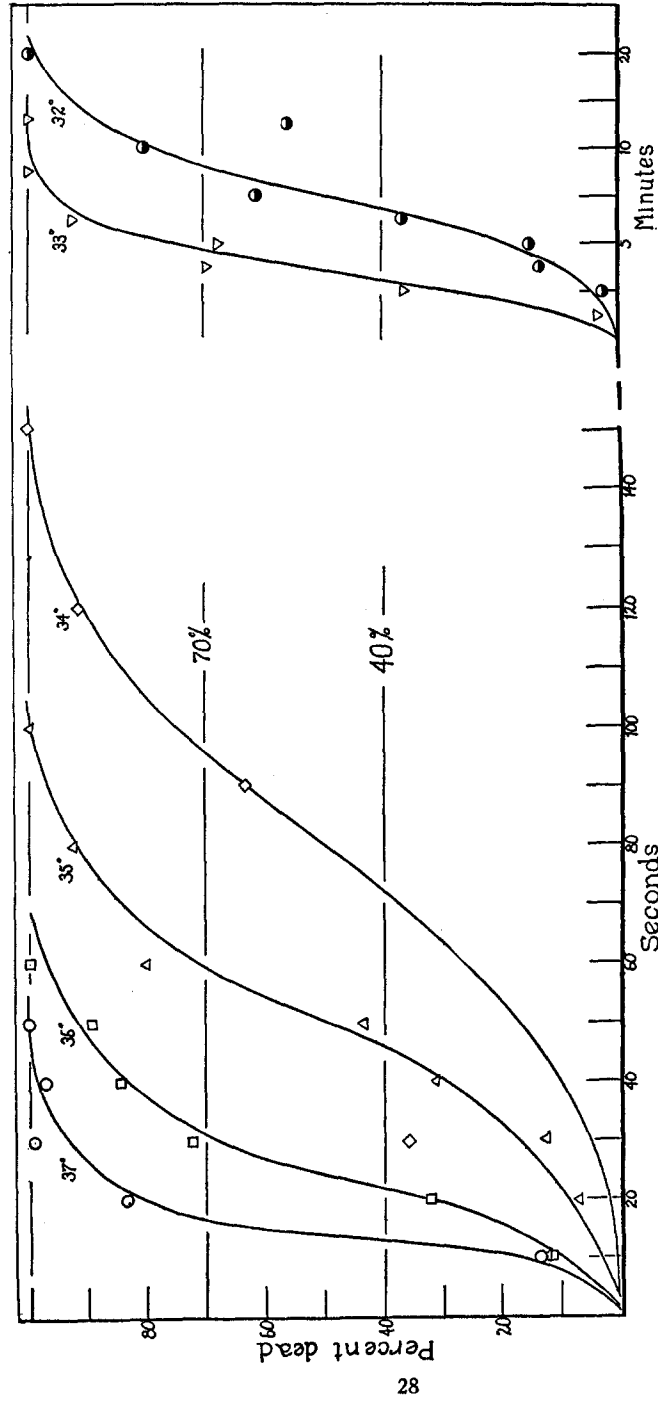


FIG. 1. Curves showing velocities of killing of females of *D. pulex* at temperatures from 32° to 37°C. Each point is based on from 25 to almost 200 animals and the curves are drawn through the observed points with proper regard to the relative weights of the points. The time scale for 32° and 33° is shortened. The animals were collected from a pond near Cambridge, Massachusetts, during October and November, 1926. Horizontal lines showing 40 per cent and 70 per cent dead are drawn and the intersections of these lines with the curves give the times used in obtaining the points plotted in the graphs in Fig. 3.

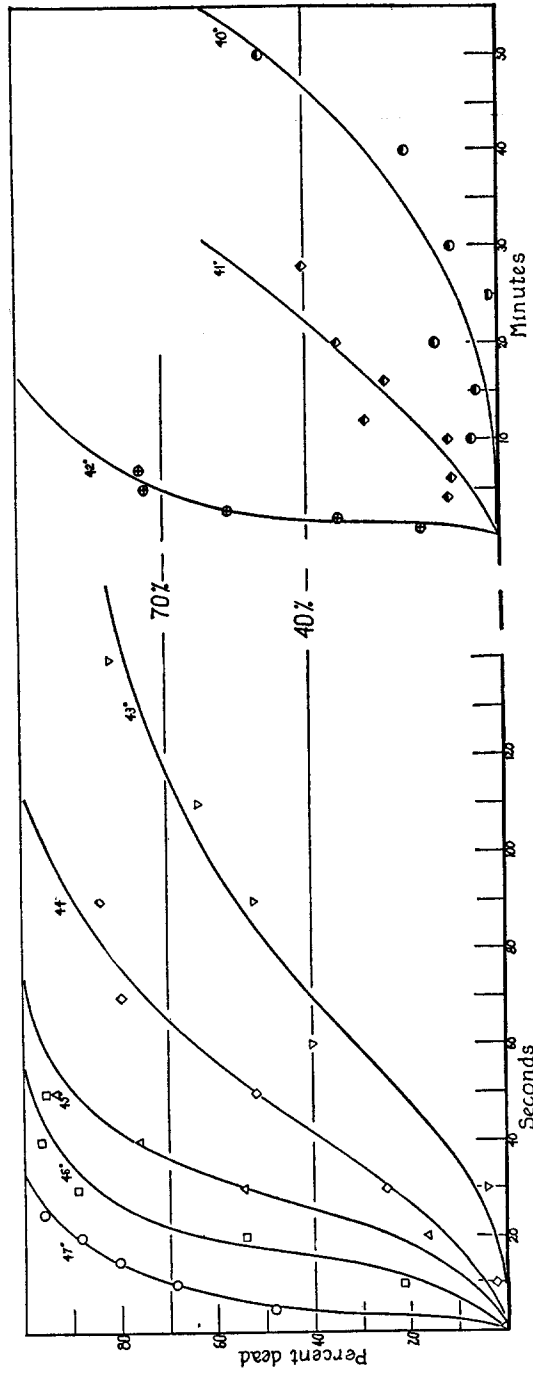


FIG. 2. Curves showing the velocities of killing of females of *M. macrocephala* at temperatures from 40° to 47°C. The females used in these experiments were all from one clone raised in the laboratory. The treatment of the data is similar to that for *D. pulex* (Fig. 1).

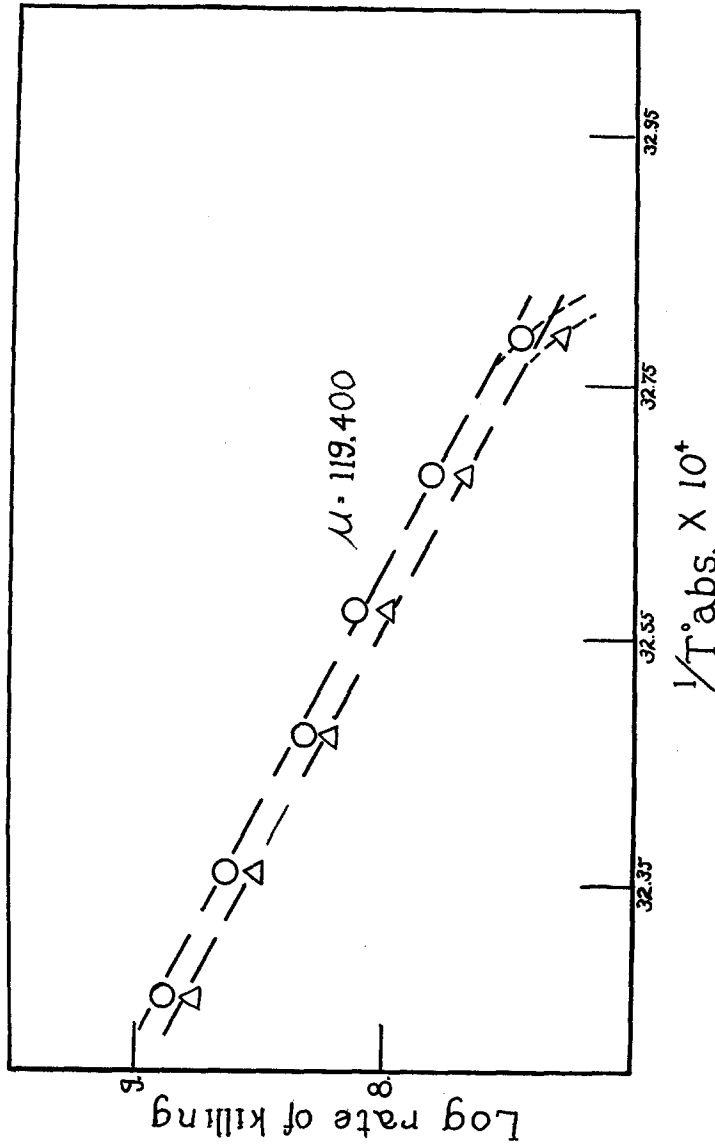


FIG. 3. *D. pulex*. Logarithms of the reciprocals of the times necessary to kill 40 per cent (circles) and 70 per cent (triangles) plotted against the reciprocals of the absolute temperatures. The slopes of the two lines are sensibly identical and yield  $\mu = 119,400$ .

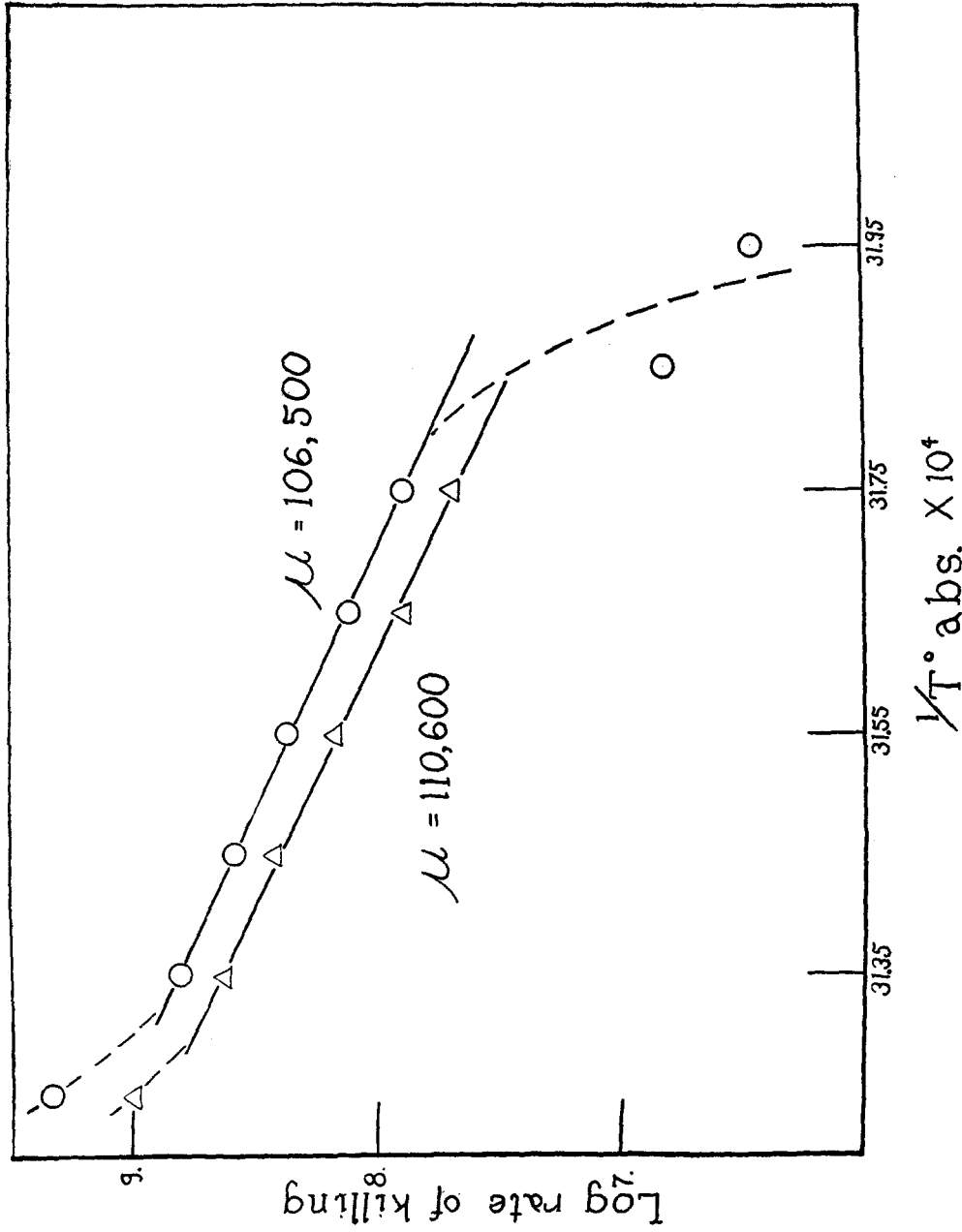


FIG. 4. *M. macrocephala*. Treatment as for *D. pulex* in Fig. 3. The slope of the lines between 42° and 46° yields  $\mu = 106,500$  for 40 per cent dead and  $\mu = 110,600$  for 70 per cent dead. The points for 40 per cent dead at 40° and 41° show an abrupt transition to the curve of life duration at sublethal temperatures.

TABLE I.

Estimations of temperature characteristics from data of various observers upon processes involving thermal destruction. As has long been known, the values of  $\mu$  are quite high. In such series of data, however, it is necessary to recognize that "breaks" of several kinds occur at the boundaries of temperature zones within which a fairly constant fit to the Arrhenius equation is obtained. In view of the nature of such cases it is not surprising to find that, with increasing temperature, at these breaks change may occur to a higher or to a lower value of  $\mu$  (or to a zone in which the equation no longer holds). It is to be noted that in certain cases objection might perhaps be raised to the type of effect which has been used as the basis of measurement. For this reason we have not tabulated the result of von Transehe's (1913) measurements, which for *D. magna* give a very high apparent  $\mu$  (234,000, above 38°C.).

Material	Source	Value of $\mu$	Temperature range °C.
<i>Drosophila</i>	Loeb and Northrop, 1917	107,900	31-35
Starfish, larvæ	Jacobs, 1919	160,000	32-36
<i>Tubularia</i>	Moore, 1910	116,200	29-36
		"	25-28
Sea urchin, larvæ	Loeb, 1908	93,600	27-32
		131,400	24-27
<i>Botrytis</i>	Smith, 1923	85,700	31-50
Sea urchin	Moore, 1910	72,800	36-42
" "		101,900	36-40
Bacteria	Bigelow, 1921	63,600	100-140
<i>Ceramium</i>	Ayres, 1916	27,700	28-38
Sea urchin, eggs ("viscosity")	Heilbrunn, 1924, <sup>b</sup>	114,800	32-37
<i>Cumingia</i> , eggs ("viscosity")	" "	64,000	32-43
<i>Paramecium</i>	Jacobs, 1919	34,600	36-40
		217,000	40-43
		106,000	36-40
Barley grains	Goodspeed, 1911	41,500	56-68
Wheat "	Groves, 1917	73,500	60-75
		20,300	75-87
" "	" "	53,000	70-92
" "	" "	48,400	60-80
		73,500	80-91
<i>Pisum</i>	Collander, 1924	94,500	35-55
<i>Draparnaldia</i>	" "	63,600	35-55
<i>Elodea</i>	" "	73,800	35-55
<i>Beta</i>	" "	94,500	40-60
<i>Brassica</i>	" "	94,500	40-60
<i>Tradescantia</i>	" "	73,800	40-65



error; the value of  $\mu$  for 40 per cent dead is 106,500 while that for 70 per cent dead is 110,600 calories. The points for *M. macrocopa* give a slightly better fit than do those for *D. pulex*. This undoubtedly is due, first, to the fact that the death curves were based on larger numbers of individuals, and second, that all the individuals of *M. macrocopa* were from the same clone.

It is clear that within a certain range of temperatures the Arrhenius or Marcelin-Rice equation applies with some exactness. But it is also apparent that in the vicinity of a certain temperature there is fairly abrupt transition to the ordinary curve of life duration. We believe it desirable that such transition points be established, where possible, with all attainable exactness. For *D. pulex* this transition occurs near 31°C., as the animals will live and reproduce at 30° but die at 32°C. It will also be noted that the two points for 32°C. (Fig. 3) show a tendency to "fall off" in the same manner as is shown so strikingly in the graph for *M. macrocopa* (Fig. 4). The critical temperature for continued growth of *M. macrocopa* is much higher. Some females will live for days at a temperature of 40°C., and will reproduce normally at 39°C. The critical temperature for the continued life of this species must be in the neighborhood of 39.5° or 40°C.; at least, this holds when the animal is subjected to abrupt changes in temperature. At the uppermost temperatures used the "velocity of killing" shows abnormal acceleration. These features occur in similar data with other organisms.

#### IV.

The  $\mu$  values here obtained may be compared with those computed for some similar cases. Several values of  $\mu$ , and the sources of the data, are given in Table I. There is possibly a tendency for the values to be grouped around 70,000 and around 90,000 to 100,000; but the cases are too few, and the methods employed insufficiently uniform, to permit decision at present.

The killing of protoplasm by exposure to higher temperatures is accompanied not only by visible coagulative processes, roughly measurable as increased "viscosity" (Heilbrunn, 1922; Heilbrunn, 1924, *b*), but, before these are well under way, by the structural alteration of the chondriome constituents (Policard and Mangelot,

1922; Fauré-Fremiet, 1925). Without the production of extreme effects, it is known that brief exposure at high temperatures may produce injury followed by hysteresis or by more or less incomplete recovery (*cf.* Wurmser and Jacquot, 1923; Klopp, 1924-25). It has been quite generally assumed that such disturbances are traceable to modifications of the colloidal state of protoplasm, and this interpretation permits certain views as to the process of thermal injury, as well as of the mechanism of adaptation to higher temperatures (*cf.* Jacobs, 1919). The coagulative process evident in connection with thermal killing involves proteins. The heat coagulation of protein follows the course of a first order process (Chick and Martin, 1910; 1912, *a, b*; Lewis, 1926, *a, b, c*) only when the pH is constant. During the coagulation of protoplasm this condition is pretty certainly not fulfilled, as a general thing.

The curve relating velocity of killing to temperature should therefore be expected to show a number of discontinuities, and it must be regarded as surprising that (over certain ranges of temperature) the agreement with the Arrhenius equation is in fact as good as it is. The velocity of heat coagulation of protein is governed by the velocity of the denaturing process, which Lewis (1926) shows to be hydrolytic. The typically high values of the temperature characteristic Lewis (1926, *a, b, c*) interprets as due to the additive nature of heats of activation, the hydrolysis of the protein which is preliminary to its flocculation being catalyzed at several linkages simultaneously (unless the possibility also be recognized that intramolecular changes may be responsible for quantitatively different cleavage mechanisms on either side of one or more transition temperatures). It might then be supposed that the observed critical increments should be integral multiples of that for a single cleavage. With comparable organisms it should be tempting to apply this notion to the temperature characteristics for thermal destruction. In the present case, the difference between the mean temperature characteristics pertaining to *Daphnia pulex* and to *Moina macrocopa* is  $119,400 - 108,600 = 10,800$ , which may safely be rounded off to 11,000. If one ventures to regard this as perhaps signifying hydroxyl ion catalysis, the mean number of simultaneous linkage cleavages in the former case is 11, in the latter 10. There is no reason to suppose identical cleavage mechanisms in all

organisms, nor dependence of killing upon any one sort of protein even in a single cell. Hence a certain suggestive tendency of the perhaps best ascertained among known values of  $\mu$  (Table I) to *approach* a series with a common divisor of about 11,000 is probably illusory.

## SUMMARY.

In spite of obvious possible sources of disturbance, the "velocity of killing" of organisms at supranormal temperatures, properly determined, tends to adhere to the Arrhenius equation for relation to temperature. Over certain ranges of temperature the relationship between *log velocity of killing* and  $1/T^\circ$  *abs.* is linear. Interpreted as due to the thermal denaturing of protein, it is possible that differences between the temperature characteristics for the killing process in closely related forms may be suggestive in regard to the mechanism of the denaturing. The temperature limits within which the linear relationships appear may be classed among those temperature levels which are critical temperatures for protoplasmic organization.

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