

THE TEMPERATURE COEFFICIENT OF PHAGOCYTOSIS.

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Recently Madsen and Watabiki (1) have made some accurate measurements of the effect of temperature on the phagocytosis of bacteria. Fig. 1 is a reproduction of one of their figures showing the time curves of the number of bacteria ingested per leucocyte at different temperatures. In analyzing these results they endeavored to apply the familiar formula for a monomolecular reaction. In this formula, $K = \frac{1}{T} \text{Log} \frac{A}{A - x}$, they took A equal to the maximum number of bacteria ingested at the close of the experiment (instead of the maximum number of bacteria present, which, one is led to infer, was larger than A even at the higher temperatures) and x equal, as usual, to the number of bacteria ingested in time, T .

Considering their method of analysis it is not surprising that they found it impossible to calculate the temperature coefficient of phagocytosis from their figures. Inspection of Fig. 1 shows that the total number of bacteria ingested is smaller at the lower temperatures, *i.e.*, A is itself a function of temperature, increasing with rise of temperature. Now the accelerating effect of the higher temperatures is evidenced quite as much by the increase in A as by the increase in K , as they calculated it. The former factor they have completely discounted by their procedure, which is equivalent to "telescoping" the curves in Fig. 1 on the ordinate so that they all reach the maximum at the same point, and then comparing the times necessary for the different curves to reach the same ordinate. Moreover, the agreement of the experimental data with the formula for a monomolecular reaction was admittedly unsatisfactory at temperatures above 25°C. where the formula for a bimolecular reaction was usually found to give better results. But even if the results could be expressed

by the formula for a monomolecular reaction it could hardly be more than a meaningless coincidence. It could not indicate a rate proportional to the diminishing numbers of free bacteria because many bacteria were still present, at least at the lower temperatures, when phagocytosis had decreased to zero.¹ Nevertheless, the approximate empirical applicability of this formula to their results impressed the authors with the fact that phagocytosis obeys known physico-chemical

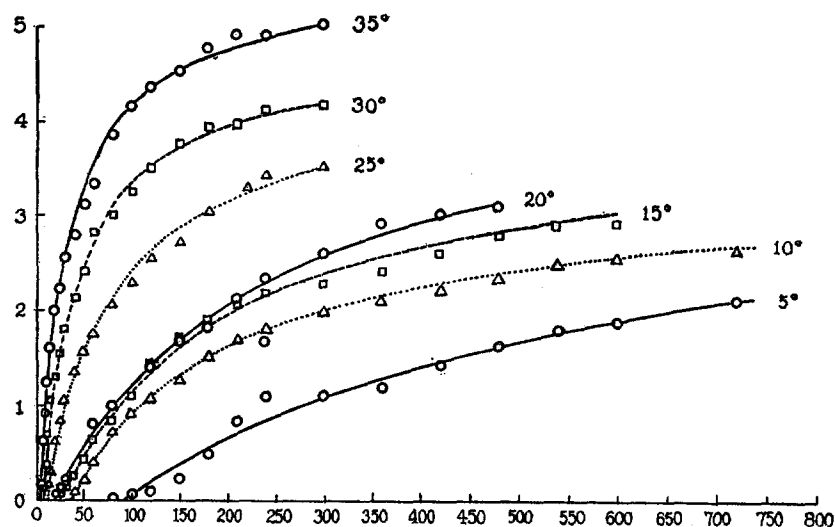


FIG. 1. Reproduction of curves from data of Madsen and Watabiki² showing the number of bacteria taken up per leucocyte (ordinates) as a function of time (abscissæ) at different temperatures. Note the shorter latent period, higher maximum and shorter duration of the experiment at higher temperatures.

laws. Actually, the attempt to force the experimental results to fit a known formula has merely beclouded the issue. It is worth while trying whether a more rational analysis of these curves will yield a more significant result.

Osterhout (3), in discussing the analysis of time curves, has pointed

¹ In a previous paper on phagocytosis (2) the writer found that the rate of ingestion of solid particles by leucocytes was proportional to the decreasing number of available particles, thus giving a constant K when calculated by the formula for a monomolecular reaction.

out that one should compare the times necessary for equal amounts of action rather than the amount of action at equal times. This is equivalent to saying that the rates of two reactions should be compared at *corresponding stages*. In the curves of Fig. 1, however, the corresponding stages are not points of equal amount of action because the maximum varies at different temperatures, but, rather, they are points of equal percentages of the total amount of action possible at that temperature. For comparative rates, therefore, we may take the *number of bacteria ingested per leucocyte per minute during the first half of the reaction; i.e.*, until one-half the maximum number of bacteria has been ingested. This criterion yields a value for the rate of the reaction which is far from being ideal but which seems to be the best approximation possible under the circumstances and certainly more rational than the original.

Following this procedure the rates of the reactions at different temperatures have been calculated from the data of Madsen and Watabiki.² In order to calculate from them the temperature coefficient, Q_{10} , of the reaction the logarithms of these rates have been plotted in Figs. 2 and 3 against the corresponding temperatures. The temperature coefficient for any interval of 10 degrees on the abscissæ is the antilog of the difference between the ordinates at the two temperatures; *i.e.*, the slope of the graph for that interval. The resulting graphs are practically straight lines which is rather an unusual result for biological processes. This means that the temperature coefficient is constant over the entire range from 5°–35°C. Q_{10} was found to be 2.05 ± 5 per cent and 2.0 ± 5 per cent in Figs. 2 and 3 respectively. For comparison with these curves the values of K , calculated by Madsen and Watabiki according to the formula for a monomolecular reaction, are also plotted in Figs. 2 and 3. Our improved analysis evidently gives a smoother curve, the probable error being only about one-half as large.

In a recent paper on phagocytosis (2) a formula was derived for calculating the chances of collision between leucocytes and particles of known size and density when stirred together in a common suspen-

² Madsen and Watabiki (1), Tables 1 and 2. The data on other tables could not be similarly treated because the experiments were not carried to completion.

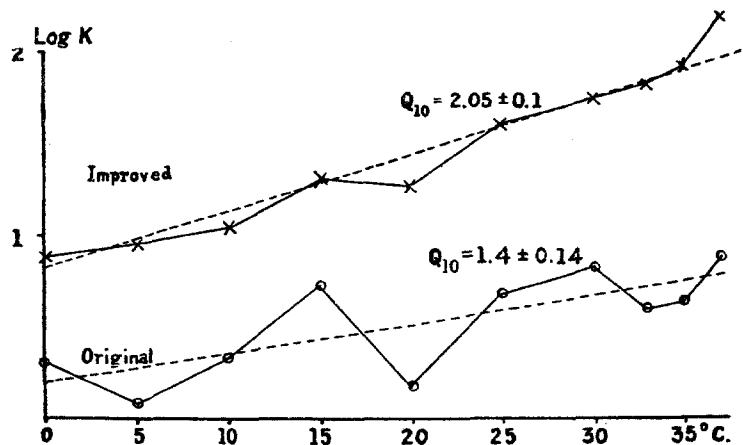


FIG. 2. Plots of the logarithms of K , the rate of phagocytosis (ordinates), against temperature (abscissæ). Data taken from Table 1 of Madsen and Watabiki. Lower curve from their own calculation of K by the formula of a monomolecular reaction. Upper curve shows the calculation of K by the improved method described in the text after correcting for viscosity of the medium. Slope of the graph (dotted line) represents the temperature coefficient of the process, Q_{10} , which is practically constant over this range of temperature. Calling the dotted line 100 per cent, the probable percentage deviation of the points from this line is only one-half as great by the new analysis. Before correcting for viscosity the new analysis gives higher values of Q_{10} than the original. Constants have been added to the values of $\text{Log } K$ in both experiments for convenience in plotting.

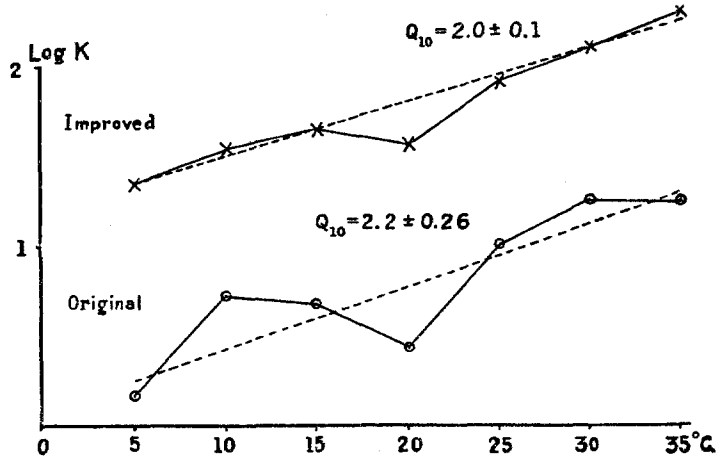


FIG. 3. Same as Fig. 1, but the data taken from another similar experiment of Madsen and Watabiki².

sion. Since this formula was based on Stokes's law for falling bodies, the chances of collision are inversely proportional to the viscosity of the medium. In the previous experiments the viscosity could be neglected because it did not vary. Where temperature is the variable, however, it is obvious that the viscosity becomes a factor of some significance because it decreased proportionally the number of meetings between leucocytes and bacteria. The rates of phagocytosis calculated by the improved method were, therefore, corrected by multiplying by the viscosity of water as given in Landolt and Bornstein's tables before plotting. If the same correction had been applied to the original values of K they would have given a temperature coefficient less than the new values. The correction for viscosity decreases the value of Q_{10} , as shown in Table I, but does not appreciably affect the form of the curve.

It is conceivable that the number of collisions between bacteria and leucocytes might be so great that this would not be a limiting factor. In experiments on the phagocytosis of solid particles this certainly was not the case since the number of particles ingested in a given time was always a constant percentage of the number present.

The last column in Table I gives values for Q_{10} obtained when the time is measured from the end of the latent period instead of the beginning, the correction for viscosity also being made. This procedure gives a slightly lower value for Q_{10} .

We may take, then, for the temperature coefficient of phagocytosis, as nearly as it may be obtained from these experiments, the value 2.0.³

This figure is not thoroughly satisfactory, however, because the curves in Figs. 1 and 2 are complicated by at least two reactions, each with its own temperature coefficient, besides the phagocytic reaction proper.

The first of these reactions is represented by the latent period. Madsen and Watabiki calculated its temperature coefficient from the

³ Ledingham, in studying the effect of temperature on phagocytosis of bacteria, concluded that it was due to the different rates of adsorption of opsonin by the bacteria. By previously incubating the bacteria with serum the accelerating effect of temperature could be nearly excluded. (Ledingham, J. C. G., *Proc. Roy. Soc. Biol., B.* 1907, lxxx, 188.)

length of the latent period by the formula of Arrhenius, and found $\mu = 15,000$ and $16,350$ respectively in their paper.² The data fit the simpler formula of van't Hoff equally well and gives Q_{10} as 2.35 and 2.6, respectively.

A second complicating reaction is evidenced in Fig. 1 by the fact that at higher temperatures phagocytosis ceases; *i.e.*, the maximum is reached sooner than at lower temperatures. This indicates that some secondary reaction is occurring which results in injury to the cells or otherwise makes phagocytosis impossible.⁴ The speed of this "lethal reaction," as we may call it, increases with the temperature. If the phagocytic reaction itself were accelerated by rise of temperature

TABLE I.

Temperature Coefficient of Phagocytosis.

	Uncorrected.	Corrected for viscosity.	Latent period deducted.
	Q_{10}	Q_{10}	Q_{10}
Fig. 2.....	2.7 ± 0.1	2.05	2.0
Fig. 3.....	2.5 ± 0.1	2.0	1.9

to the same degree as the lethal reaction, the maximum reached would be the same at all temperatures, for the more rapid phagocytosis at higher temperatures would just compensate for the shorter time available. Therefore, the fact that the maximum attained is higher at the higher temperatures, in spite of the shorter time, proves that the temperature coefficient of phagocytosis must be higher than the temperature coefficient of the lethal reaction.

In Fig. 4 are plotted graphs from which the Q_{10} of the lethal reaction can be approximated. Ordinates represent logarithms of the reciprocals of the time necessary for completion of the reaction, *i.e.*, time to reach the maximum; abscissæ represent temperature. These figures are, of course, highly inaccurate, probably particularly so at tem-

⁴ The maximum cannot be due entirely to any mechanical factor such as the exhaustion of the available bacteria or the filling up of the cells or it would be the same in all. The lethal reaction which determines the maximum might be referred to the toxic action of bacterial extracts.

peratures below 15° where the reactions were continued for 24 and 48 hours; and it has seemed justifiable, in calculating the value of Q_{10} , to discount these figures on account of secondary changes which might occur in such prolonged experiments. If this is done, the values for the temperature coefficient are 1.7 and 1.3 respectively. This bears out the qualitative conclusion arrived at from mere inspection of the curves in Fig. 1 that the temperature coefficient of the reaction resulting in injury to the cells was lower than that for the process of phagocytosis itself.

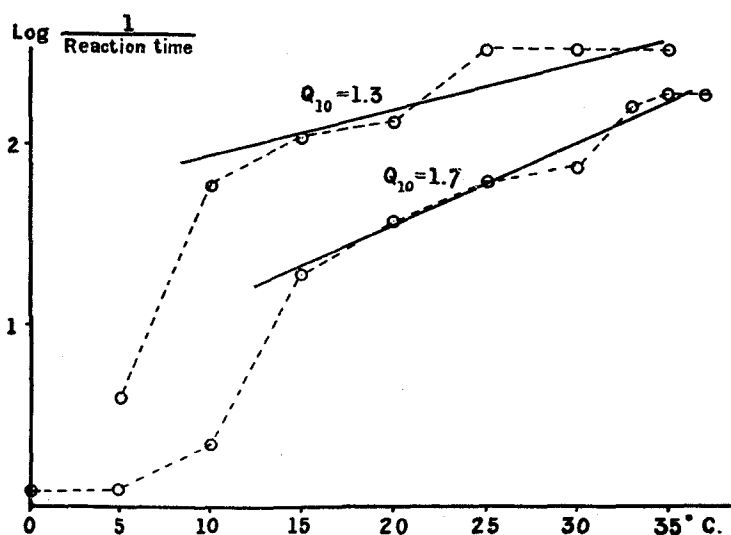
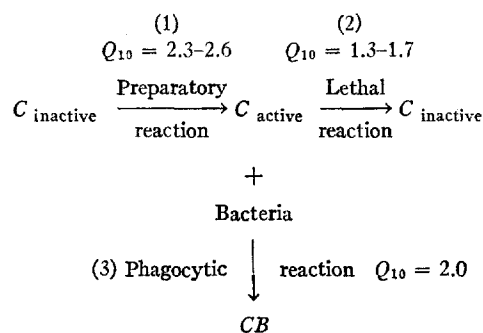


FIG. 4. Logarithms of the reciprocals of the times necessary to ingest the maximum number of bacteria (ordinates) plotted against temperature (abscissæ). Data taken from the same two experiments of Madsen and Watabiki as for the graphs of Figs. 2 (lower) and 3 (upper). Slope of the graph is proportional to the logarithm of the value of Q_{10} for that interval. Values calculated from the slopes of the solid lines are inserted. This is regarded as the temperature coefficient of the lethal reaction resulting in the death of the cell and cessation of phagocytosis. 3.75 and 2.5 have been added to the values for the upper and lower curves respectively for convenience in plotting.

Loeb (4) and Osterhout (5) have developed the conception of catenary reactions as applied to biological reactions, and the latter has recently discussed temperature coefficients from this point of view.

This general conception is obviously applicable to the experiments of Madsen and Watabiki, where three separate reactions can be clearly distinguished by mere inspection of their curves in Fig. 1. These reactions are: (1) a preparatory reaction represented by the latent period, during which it may be supposed that the leucocytes, originally inactive, are rendered active; (2) a lethal reaction which injures the leucocytes so that they are again inactive; and (3) the phagocytic reaction proper.

The relations between these three reactions may be diagrammed as follows:⁵



Here C stands for white blood corpuscles and CB for corpuscles containing bacteria. Now the rate of the phagocytic reaction (3) evidently depends upon the concentration of C_{active} ; and the accuracy of our analysis depends upon the assumption that this concentration is the same at different temperatures during the periods selected as corresponding periods in the reactions. The concentration of C_{active} depends in turn upon the relative rates of the preparatory and lethal reactions. Could we know the rates and dynamics of both these reactions as well as their temperature coefficients we could calculate the variation of C_{active} with time by Osterhout's equations (6) for the calculation of the concentration of M in the series $A \rightarrow M \rightarrow B$. In this way corresponding stages could be ac-

⁵ In this diagram no assumption is implied as to whether the effect of reactions (1) and (2) in "activating" and "inactivating" the cells is due to an action upon the cells themselves or upon the medium or bacteria. It merely states the fact that their phagocytic activity passes through a maximum during the experiment, due to two reactions.

curately selected. Unfortunately this is not possible. Moreover, the activity of the cells cannot be quite the same during the periods selected, since the higher temperature coefficient of the preparatory reaction compared to the lethal reaction would make the concentration of C_{active} pass through its maximum sooner at higher temperatures. An improvement might be made by taking as corresponding stages the times until the lethal reaction is one-half complete; *i.e.*, one-half the time necessary for cessation of phagocytosis instead of the time for the ingestion of one-half the total number of bacteria. The validity of this method depends upon the doubtful assumption that cessation of phagocytosis is caused entirely by the lethal reaction. It seems quite probable, however, particularly at the higher temperatures, that the filling up of the leucocytes with bacteria or the partial exhaustion of the free bacteria is another factor of importance. There is obviously a limit to the accuracy of interpretation which is possible. On the whole, the corresponding stages in the reaction probably would not differ so much from those which we have used that the *average* rates of ingestion in those periods would be seriously affected.

In conclusion, emphasis may be laid upon the central fact that the phagocytic curves of Madsen and Watabiki represent a complex of at least three reactions and consequently cannot be treated as a single monomolecular reaction without serious error. Osterhout's conception of catenary and (we may add) collateral reactions is not only applicable to the interpretation of these experiments, but obviously essential.

For comparison with the results of Madsen and Watabiki on the temperature coefficient of the phagocytosis of bacteria, it seemed of interest to determine the temperature coefficient of the phagocytosis of solid particles of carbon and quartz. For this purpose leucocytes obtained from a peritoneal exudate in the rat and particles of quartz or carbon of uniform sizes (2 to 4μ in diameter) were mixed in small glass-stoppered vials which were rotated slowly about their horizontal axes in water baths kept at the desired temperature. At frequent intervals small samples were removed and counts made of the number of particles not yet ingested. For the details of the procedure the reader is referred to a previous paper (2). The difficulty with the method is that both particles and leucocytes are likely to agglutinate

more or less during the experiment and that it is impossible to be sure whether the particles are actually inside the cells or merely stuck on the outside. Hence, if the particles stick on the outside more rapidly than they are ingested, it becomes impossible to measure the actual rate of ingestion or to determine the true temperature coefficient of ingestion. This is undoubtedly the explanation of the low temperature coefficient, 1.4, obtained by this method between 30° and 37°C.

The results of one experiment by the suspension method is shown in Fig. 5, where the percentage of particles *not* ingested is plotted as ordinates against time in minutes as abscissæ. Determinations were made at 37°, 27°, and 23°C. Inspection of these curves shows that the temperature coefficient is very low above 30°C. compared to that below 30°C. To obtain a quantitative comparison, the times, T , necessary for ingestion of 25, 50, and 75 per cent of the particles have been compared. Here, again, the rate of the reaction is conditioned in part by the viscosity of the medium, collisions taking place between cells and particles with proportionally less frequency in the more viscous medium at 23°C. The rate of the reaction, K , when corrected for viscosity is, then, the reciprocal of the time necessary for the ingestion of a given percentage of particles multiplied by the viscosity.

$$K = \frac{V}{T} \quad (1)$$

The temperature coefficient, Q_{10} , is now calculated according to the formula

$$\text{Log } Q_{10} = \frac{\text{Log } K_1 - \text{Log } K_2}{t_1 - t_2} \times 10 \quad (2)$$

where t is the temperature, as already explained. In this case the graph of $\log K$ against temperature is not a straight line as in the case of Madsen and Watabiki's results. In the experiment plotted in Fig. 5, Q_{10} is 1.26 between 23° and 27°C., and 5.6 between 27° and 37°C., when the figures for comparison are taken at the stage in the reaction when 50 per cent of the particles are ingested. The values obtained at other stages (25 and 75 per cent) in the reaction are given in Table II together with the results of seven other similar experiments. While the variations in the experimental figures are

large, the general tendency for Q_{10} to increase at lower temperatures is perfectly clean-cut.

The simplest interpretation of these results seems to be that at the lower temperatures the cells are actually too rigid to permit either the sticking on of the particles or the relatively greater changes in form which are necessary for ingestion. A slight increase in temperature now suffices to transform the protoplasm possibly from the *gel* to the

TABLE II.
Temperature Coefficient of Phagocytosis of Solid Particles.

Experiment No.	Particles		25 per cent ingested.	50 per cent ingested.	75 per cent ingested.	Remarks.
	Nature.	Size.				
		μ	Q_{10}	Q_{10}	Q_{10}	
1	Carbon.	4.7	1.1	1.3	1.0	Between 30°C. and 37°C. Grand average = 1.41 ± 0.06 .
2	Quartz.	6.0	1.9	1.6		
3	"	2.4	1.7	1.1	1.1	
4	Carbon.	4.7		1.2	1.4	
5	"	4.7	2.0	2.3	1.6	
6	"	4.7	1.4	1.3		
7	Quartz.	4.6	1.0	1.8	1.0	
8	"	2.4	1.3	1.2	1.3	
1	Carbon.	4.7	5.9	5.6	14.0	Between 23°C. and 27°C. Grand average = 18 ± 6 .
7	Quartz.	4.6	71.0	10.2	8.9	
9	Carbon.	4.7	10.7			

Comparisons were made at three different stages of the reaction; *i. e.*, when 25, 50, and 75 per cent of the particles had been ingested. In Experiments 1, 4, and 7, Q_{10} was measured from 27°C. to 37°C.

sol stage, thereby rapidly increasing the rate of phagocytosis; *i. e.*, Q_{10} is high. The low temperature coefficient above 30°C. might be taken to indicate that phagocytosis is merely a matter of surface tension changes. But this conclusion is not justified since many of the particles were merely stuck on the outside of the leucocytes singly or in clumps, making impossible any conclusion as to the temperature coefficient of the actual process of ingestion. The fact that no appreciable clumping occurs at lower temperatures shows that the

clumping is due to the increased stickiness or phagocytic activity of the cells.

In this connection the parallel fact is significant that the presence of solid particles has been found (2) to cause more rapid clumping of the leucocytes. Thus, at the optimum temperature for phagocytosis there is both an agglutination of cells due to particles and an agglutination of particles due to cells. The complete act of phagocytosis is

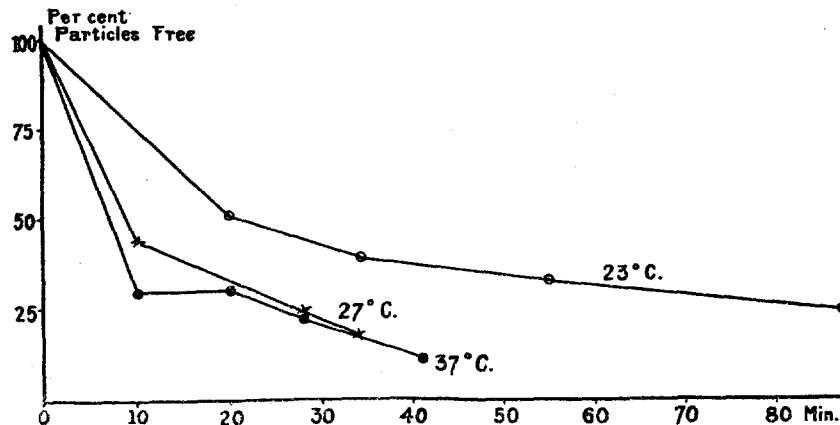


FIG. 5. Curves showing the number of particles of carbon still free (ordinates), *i.e.*, not ingested by leucocytes, as a function of time (abscissæ), at 23°, 27°, and 37°C. Inspection shows that the temperature coefficient is greater between 23° and 27°C. than between 27° and 37°C. See Table I for figures from this and other similar experiments.

evidently a complex of several reactions, and any figure which may be obtained for the temperature coefficient of the process must be interpreted from this point of view. The fact that Q_{10} is not constant must mean that new reactions become the limiting factors as the temperature changes. Below 30°C. the fluidity of the cell is the limiting factor. Above 30°C, it is the stickiness of the cells which would be expected to vary merely with the surface tension.

These results indicate then a marked change in the consistency of the protoplasm between 20° and 30°C. which did not appear in the

results of Madsen and Watabiki. Similar indications are found, however, in measurements of the temperature coefficient of the ameboid motion of leucocytes by Commandon (7) who took moving pictures of creeping leucocytes at 25°, 30°, and 35°C. and determined the rates of movement by subsequent measurements on the films. He gives the following figures:

Temperature.	Speed of leucocytes.	Q_{10}
°C.	μ per minute	
25	9.6	4.6
30	20.4	
30	20.4	1.5
35	25.2	

From these figures the Q_{10} has been calculated as described and was found to be 4.6 between 25° and 30°C., and 1.5 between 30° and 35°C.

Similar evidence of a rapid change in the temperature coefficient of ameboid movement below 30°C. was found in some preliminary experiments designed to measure the effect of temperature on phagocytosis by the "film method," previously described (2). In this method the leucocytes and particles are allowed to settle out between a slide and cover-slip in a thin film and the rate of phagocytosis is measured by counting the number of particles not yet ingested over equal areas. It is impossible to be sure, however, whether this rate is determined by the speed with which the leucocytes creep about from particle to particle or by the actual ease of ingestion. Demonstrations by this method, of the more rapid ingestion of carbon particles as compared with quartz, have shown that in this case at least the speed of the leucocytes is not the limiting factor. However that may be, the temperature coefficients of phagocytosis as measured by this method show the same marked increase at lower temperatures. This is shown in Table III.

TABLE III.

Temperature Coefficients of Phagocytosis by Film Method.

Experiment No.	Nature of particle.	Particles ingested.	27°-35°C.	20°-27°C.	10°-20°C.
		<i>per cent</i>	Q_{10}	Q_{10}	Q_{10}
10	Carbon.	25	4.2	7.9	6.8
		50	4.2	10.0	6.0
		75	7.1		
	Quartz.	25	1.7	20.0	7.6
		50	1.3		
		75	2.0		
11	Carbon.	25	3.2	9.3	
		50	4.0	8.7	
	Quartz.	25	2.0	25.0	
		50	1.6	18.6	
Averages.....			3.2 ± 0.4	14.2 ± 1.7	6.8

In each experiment a mixture of quartz particles 4.6μ in diameter and carbon particles 4.7μ was used. 0.3 cc. of this mixture in distilled water was added to 1 cc. of leucocyte suspension plus 0.4 cc. serum plus 0.2 cc. NaCl 2.25 per cent plus 0.2 cc. $\frac{M}{10}$ phosphate mixture of pH 7.5. Rates of phagocytosis, K , were taken equal to the reciprocal of the times, T , necessary for the ingestion of 25 per cent, 50 per cent, or 75 per cent of the particles. T was determined graphically. Q_{10} was calculated by equation (2). The dispersion of the average was calculated where the data were adequate.

SUMMARY.

1. The experiments of Madsen and Watabiki on the effect of temperature on the phagocytosis of bacteria are discussed and a new analysis of their curves is given, showing that the rate of phagocytosis is very nearly a logarithmic function of the temperature from 0° to 35°C .; *i.e.*, Q_{10} is constant over that range and is equal to 2.0.

2. New experiments are reported on the effect of temperature on the phagocytosis of quartz and carbon particles of uniform sizes, showing a marked increase in the temperature coefficient below 30°C .

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