TEMPERATURE RELATIONS IN PHAGOCYTOSIS[1]

DORALEA R. HARMON, CHRISTINE ZARAFONETIS, AND PAUL F. CLARK

Department of Bacteriology, University of Wisconsin Medical School, Madison, Wisconsin

The relation of fever and of subnormal temperatures to resistance and to well-being has been a matter of debate and varying practice for centuries. Since the dawn of history hot and cold topical applications and baths have had wide popularity, and antipyretic drugs have been used to a greater or less extent ever since their discovery.

In 1878 Pasteur, Joubert, and Chamberland noted that susceptibility to anthrax could be induced in fowl, animals normally resistant to this infection, simply by immersing them up to the thighs in a bath held at 25 C. The animals so treated suffered a reduction in temperature to 37 to 38 C and died of anthrax within 24 to 30 hours following inoculation with *Bacillus anthracis*, whereas the controls remained alive. Subsequently, Wagner (1890) confirmed their report, indicating further that "hypothermy diminishes the mobility and phagocytic functions of the leucocytes." When the animal's temperature was lowered, the tide of battle was turned against the leucocyte and the animal succumbed. Similar experiments with antipyretics were not so striking.

Modern interest in the therapeutic use of the febrile state was awakened by the report of Wagner von Jauregg (1918–19) that cases of tabes dorsalis and general paresis were improved following artificial infection with malaria. Although many of the physiological responses to fever have been clearly described, specific studies on the influence of fever on defense mechanisms have been few.

One of the most frequently cited reports is that of Rolly and Meltzer (1908), who studied in rabbits the effect of artificially increased temperature on resistance to infection, on phagocytosis, and on antibody production. Their figures, however, showed no greater differences than might be due to individual variation; in some of their experiments only two or three animals were used for a given procedure. Continuing the studies on phagocytosis, they reported that ingestion of several species of bacteria by human leucocytes *in vitro* was increased with temperature increases from 6 C to 39.5–40 C; beyond that point a decreasing activity occurred. The experiments with guinea pigs and guinea pig leucocytes gave irregular results.

Ellingson and Clark (1942), in a more complete study, disagreed with the findings of Rolly and Meltzer, especially in regard to the persistence and the production of antibodies. In rabbits already actively immunized against *Eberthella typhosa*, the induction of fever of 106 to 107 F (corresponding to about 41 C) was followed by rapid reduction of antibody titers as compared with controls with normal temperatures. In rabbits passively immunized with homologous antityphosus serum, fever of this degree accelerated the decline of antibody

titers. Specific antibody responses were impaired when animals were subjected repeatedly to severe fever during the period of immunization. Their phagocytosis studies (*in vitro*) were few, but both with staphylococci and guinea pig leucocytes and with staphylococci and human leucocytes, an increase in the number of bacteria ingested was observed up to a temperature of 104 F or 40 C. Admittedly, this phase of their work was incomplete, and need for further study along this line was stressed by the authors.

Two other papers—one by Ledingham (1908), the other by Madsen and Wulff (1919)—are noteworthy in the field of phagocytosis-temperature relations. Ledingham observed enhanced phagocytosis with elevations in temperature from 18 to 42 C. He considered that this increased reaction was due to more adequate sensitization (i. e., sensitization in a much shorter time) at the higher temperature levels. Testing his hypothesis, he found that both opsonization and phagocytosis were favorably influenced, but opsonization to a more marked degree. He likewise noted that the rate of the reaction was involved rather than the end point.

Madsen and Wulff claimed that the optimum temperature for phagocytosis *in vitro* is that of the animal's body at the time the cells are withdrawn. If the temperature of the individual is normal, then maximum phagocytosis by that individual's cells should be at the normal body temperature for that particular animal species (around 37 C for humans, 39 C for guinea pigs and rabbits, and 41 C for fowls). Should the individual develop a febrile temperature, then optimum phagocytosis, according to these authors, would take place at that febrile temperature.

In attempting to analyze earlier phagocytic studies, it should be borne in mind that the lack of mechanical devices to ensure uniform mixing of phagocytic systems was characteristic of that period, so that chances for contact between the phagocytes and the bacteria were less and probably therefore did not so closely approximate those within the animal body.

A recent paper concerning phagocytosis-temperature relations is that of Cottingham and Mills (1943), who report experiments dealing with the effects upon phagocytic functions of polymorphonuclear leucocytes of vitamin-deficient white rats subjected to environmental temperatures of 68 F and 90–91 F. They concluded that phagocytic powers of deficient animals (a number of deficiencies were observed) were lessened regardless of environmental temperature. In their work, however, only 40 cells on each of two smears per animal per temperature were counted. In our experience, a count of 400 cells per animal per temperature is essential for a quantitative estimation of the extent of phagocytosis. These investigators went to the opposite extreme of that of the earlier workers and used extremely rapid shaking (560 reversals of direction per minute).

In view of the limitations of the earlier work and with the desire to pursue the observations of Ellingson and Clark, we undertook these studies on phagocytosis-temperature relations.

METHODS

The experiments reported here are divided into two parts: A, those concerned with phagocytosis over the range of 22 to 42 C, and B, those dealing with phagocytosis in the range of 37 to 45 C (with comparisons at 25 and 50 C).

A. Polymorphonuclear leucocytic exudation was induced in the peritoneal cavities of ten normal, mature guinea pigs, five normal, mature rabbits, and five normal, mature mice by the injection of starch aleuronat paste (3 per cent potato starch boiled in distilled water with 5 per cent aleuronat added) mixed with an equal volume of tryptose broth. The amounts injected were 10 to 20 ml in guinea pigs, 30 to 40 ml in rabbits, and 3 ml in mice.

After 18 to 24 hours the animals were exsanguinated by bleeding from the heart, and the serum so recovered was used in the phagocytic system. Various time intervals after injection of the irritant into mice were tested (4 to 24 hours) in an attempt to increase the yield of leucocytes, but consistent results were not obtained. Leucocytes were withdrawn from the peritoneal cavity after bleeding by washing out the area with a suitable amount of sterile physiological salt solution. The suspension was then centrifuged gently and standardized by means of the Neubauer counting chamber to 25,000 to 30,000 cells per mm³. Leucocytes in all experiments were used within 4 hours after recovery from the test animal; the cells were retained at refrigerator temperature until ready for use, usually within an hour of their recovery.

The bacterial suspension employed was a laboratory strain of *Staphylococcus aureus* (hemolytic, mannitol-fermenting, and coagulase-positive) grown on tryptose agar for 18 to 24 hours, washed off with saline, shaken with sterile glass beads to break up the bacterial clumps, and standardized to McFarland $BaSO_4$ standard no. 1. In practice this suspension was diluted with an equal volume of saline just before its incorporation in the phagocytic system. The ratio of leucocyte to bacteria varied from 1:40 to 1:60. The standard bacterial suspension was discarded after a 6 weeks' period and a fresh emulsion prepared.

The leucocytes and serum were first pipetted into cleaned, sterile pyrex tubes (73 by 8 mm); the bacteria were added just before the mixture was incubated. One-tenth ml of each reagent was added; the tubes were sealed with paraffined corks and attached to the wheel of an electrically driven rotating device so constructed as to make 2.5 revolutions per minute about an axis 20 degrees from the long axis of the tube. This machine was also used by Ellingson and Clark (1942), but was modified so that the tubes containing the phagocytic system were rotated end over end. This modification was considered of greater value in the attainment of optimum contact between the leucocytes and the staphylococci. Five temperatures were included in each test: 22, 27, 32, 37, and 42 C. The temperatures of the water bath were carefully checked, and variations of more than 0.25 to 0.50 C were prevented.

After incubation for 10 minutes, the tubes were removed and 0.02 ml of the mixture placed on a slide and smeared over the surface with cigaret paper. After drying, the slides were stained in a dilute solution of Loeffler's methylene

blue, the extent of dilution being determined separately for each experiment since the staining properties of leucocytes vary considerably from animal to animal. The use of this dilute stain permitted differentiation of the bacteria from the leucocytic nuclear material, which under these conditions stains less intensely.

On each of two smears for each temperature 200 leucocytes were examined for the presence of phagocytosis, making a total of 400 leucocytes observed at each temperature. The chief method of evaluation of the phagocytic reaction was that of Hamburger (1912), noting the percentage of active leucocytes; but a rough phagocytic index was also obtained according to the method of Leishman (1902), by recording the number of cells ingesting "few" (five or less) organisms and those phagocytizing "many" (more than five) bacteria. The labels of the slides were covered with tape and arbitrarily assigned a number by another person in order to rule out personal bias.

The body temperatures of the rabbits were recorded, and all showed normal temperatures. The temperatures of a representative sample of guinea pigs were taken and were found to be in the normal range. No attempt was made to obtain body temperatures of the mice (see figures 1, 2, and 3).

B. The procedure for the tests in the range of 37 to 45 C at 2-degree intervals (with experiments at 25 and 50 C for comparison) was the same as in the 22 to 42 C experiments with a few changes. The separate components of the phagocytic systems (serum, staphylococci, and leucocytes) were incubated for 1.5 minutes before being mixed at the temperatures at which they were to be rotated. Another variation was rotation of the tubes in the water bath for 5 minutes instead of 10. The staphylococci of this series only were maintained in a lyophilized state after preliminary tests had indicated little or no difference in the avidity with which these were ingested when compared with freshly prepared suspensions in systems containing the same leucocytes and serum. The body temperatures of the five guinea pigs from which cells and serum were obtained for this series varied from 39 to 40 C, a range considered normal (see figure 4).

PRESENTATION OF RESULTS

A. The results of representative phagocytic experiments in the guinea pig and rabbit groups are shown in figures 1 and 2. Actual counts are given: separate counts made by two individuals for the guinea pig group, those of a single observer for all other experiments. Variation in the evaluations by the investigators at a single temperature were 5 per cent or less except in a few instances. When great variation occurred, careful recounting was the practice and the discrepancies were ruled out mathematically.[2]

Figure 3 is a summary of the data from the guinea pig, rabbit, and mouse

[2] Method for ruling out: adding together all the figures obtained at a given temperature except the one in question; averaging; finding the deviation of each figure from the average; averaging the deviations; multiplying the average deviation by the total number of determinations, including the questionable figure. If the figure obtained thus is less than the deviation of the doubtful figure from the average, then that determination may be discarded.
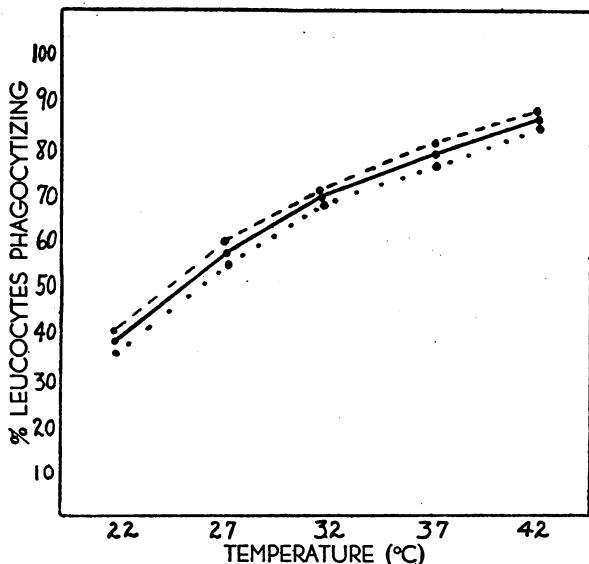
FIG. 1. REPRESENTATIVE GUINEA PIG EXPERIMENT

Top and bottom curves: results obtained by two individuals (D. H. and C. Z., respectively).
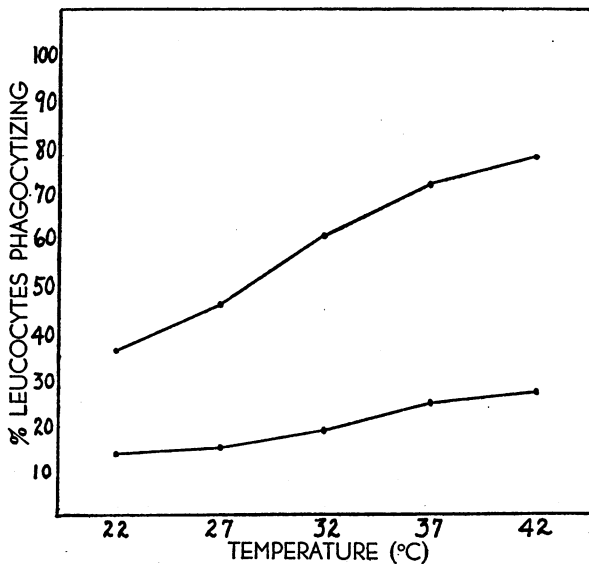Middle curve: average of the two counts

FIG. 2. REPRESENTATIVE RABBIT EXPERIMENT

Top curve: percentage of leucocytes phagocytizing.   Lower curve: percentage of leucocytes
ingesting "many" (i.e., more than five) bacteria

groups. The figures were obtained by designating the findings in each test at
22 C as unity (1.00) and comparing those at the four higher temperatures with
this base. This is done for reasons of convenience and is intended merely to
picture the average phagocytic ranges in each of the three groups.

Increases in phagocytic values with each rise in temperature (numerator = number of tests showing increase, denominator = number of tests in series) are shown below:

Guinea pig group:
>   22 to 27 C—9/10 (exactly the same value at the two temperatures was obtained in the other test)
>   27 to 32 C—9/10 (a significant decrease of 11 per cent occurred in the tenth test)
>   32 to 37 C—8/10 (insignificant decreases of 1 per cent and 3 per cent were observed in the other two tests)
>   37 to 42 C—8/10 (an insignificant decrease of 2 per cent appeared in one of the remaining two tests; there was a much greater decrease in the other, 12 per cent)
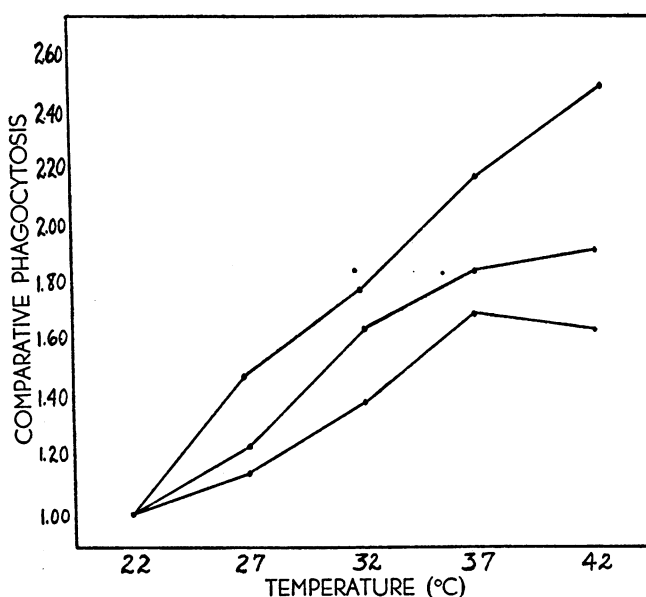

FIG. 3. PERCENTAGE OF LEUCOCYTES PHAGOCYTIZING IN EACH GROUP
Top curve: guinea pig group (average of ten). Middle curve: rabbit group (average of five). Bottom curve: mouse group (average of five)

Rabbit group:
>   22 to 27 C—5/5
>   27 to 32 C—5/5
>   32 to 37 C—4/5 (an insignificant decrease of 2 per cent occurred in the other)
>   37 to 42 C—4/5 (there was a significant decrease of 13 per cent in the fifth test)

Mouse group:
>   22 to 27 C—4/5 (a small decrease of 3 per cent was observed in the fifth test)
>   27 to 32 C—3/5 (the decreases in the remaining two tests were 1 per cent and 2 per cent, respectively)
>   32 to 37 C—5/5
>   37 to 42 C—2/5 (two tests gave small decreases of 3 per cent and 1 per cent, respectively, while in the other test the decrease was significant, 11 per cent)

B. The average comparative phagocytosis in the higher temperature range is shown in figure 4. Results were obtained by averaging the comparative phag-

ocytosis values in the leucocyte-serum systems from the five guinea pigs at each temperature. Here, it will be noted, the comparative base (1.00) is placed at 25 C.

Increases in phagocytic values with each rise in temperature were as follows:

25 to 37 C—5/5

37 to 39 C—3/5 (of the remaining tests, one showed a decrease of 4 per cent at the higher temperature, but the other showed exactly the same value at both temperatures; two of the tests showing increases did so by only 1 per cent and are to be considered as the same as the lower temperature values)
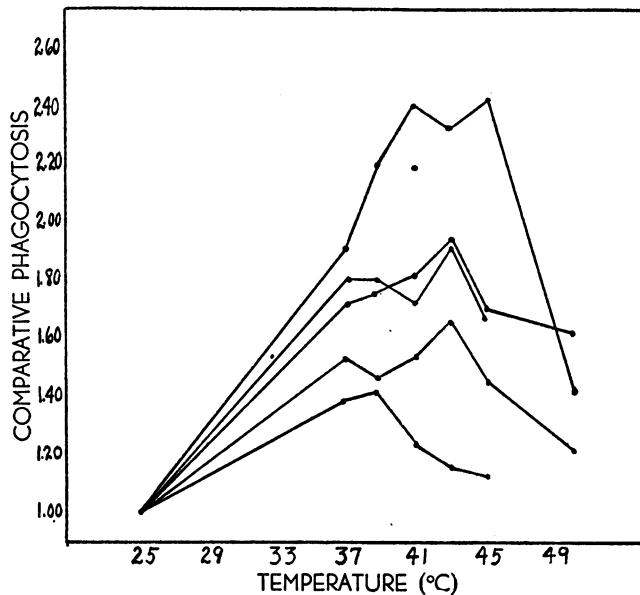

FIG. 4. PHAGOCYTOSIS AT 37 TO 45 C WITH COMPARISONS AT 25 AND 50 C
Each curve represents one complete experiment (guinea pig leucocytes and serum vs. staphylococci)

39 to 41 C—3/5 (one of the other tests gave a decrease of 3 per cent at 41 and the second, a decrease of 6 per cent; in one of the tests at which an increase occurred the amount of enhancement was only 1 per cent and is to be considered the same as at the lower temperature)

41 to 43 C—3/5 (decreases were of 3 per cent in each of the other two cases; increase in one experiment was an insignificant 2 per cent)

43 to 45 C—1/5 (decreases were all over 5 per cent except in one test where the drop was only 2 per cent; the one experiment giving an increase did so by 4 per cent)

45 to 50 C—0/5 (in two tests the leucocytes were too disintegrated to be examined. In two of the other 3 tests the drops were 28 per cent and 13 per cent, respectively; in the third the decrease was only 2 per cent)

DISCUSSION

It is readily observed that the over-all picture of phagocytosis *in vitro* by the exudative polymorphonuclear leucocyte in the guinea pig and rabbit groups is one of increase with each successive 5-degree rise in temperature over the range of

22 to 42 C.   In the mouse group there was less phagocytosis at 42 as compared with 37 C, but this series of tests was unsatisfactory because of our inability to obtain a leucocytic exudation comparable both in quality and in quantity with those of the other two species.   The irritant proved either too damaging, producing a sloughing of tissue cells, or too mild for a reaction of any kind.   Four of the five experiments finally completed with any degree of technical parallelism with those of the other two animal groups displayed a range of phagocytosis well below that of the other species.   The fifth mouse test, however, was technically comparable to the guinea pig and rabbit tests.   Nevertheless, the results at each temperature in all five tests were averaged together to obtain the mouse group curve presented in figure 3.   Because of the inadequacy of the mouse experiments our discussion is confined to the results obtained with guinea pigs and rabbits.

Although the lower temperatures are unphysiological for the animals employed in these tests, we considered it of importance to include them for the establishment of a curve.   There is no doubt that phagocytosis increases, and at a consistent but somewhat decreasing rate, from 22 to 37 C.   The next question obviously concerns the reaction between 37 and 42 C.

Two preliminary experiments were run with guinea pig phagocytic systems at the temperatures 37, 39, and 41 C.   The first gave results varying only 1 per cent at the three temperatures; the second showed a decrease of 11 per cent and 10 per cent, respectively, at 39 and 41 compared with 37 C.   For a temperature range containing 2-degree increments, an even more rigid technique than the one described must be employed.   These increased precautions should include separate incubation of each component of the phagocytic system at the temperature at which the whole is to be tested, in order that incubation of the entire system may begin at the designated temperature instead of going through a brief period of adjustment.   This condition was strictly adhered to in the five subsequent tests shown in figure 4.   The arbitrary time of this preincubation was set at 1.5 minutes to avoid possible injury to the leucocytes.   Here, it will be recalled, lyophilized organisms were used, and the period of rotation was shortened from 10 minutes to 5 minutes.

In figure 4 are the data, in comparative form, from the five experiments (guinea pig leucocytes and sera) within the range of 37 to 45 C with controls at 25 and 50 C.   An increase appears in the percentage of actively phagocytic leucocytes beyond 37 C to about 43 C, followed by a decrease after this temperature.   In only one test was there an enhancement at 45 C, and this rose insignificantly above the value obtained at 41 C.   In this test (the topmost curve in figure 4), are observed a significant increase in phagocytosis from 37 to 41 C, a leveling off at 41, 43, and 45 C, and a decided drop at 50 C.   In the other tests the fall occurred after 43 C, except in the experiment represented in the lowest curve in which 39 C was the high point.   The decreases shown on the graph between 37 and 41 C, except possibly in the lowest curve, are considered of little, if any, significance (i.e., less than 5 per cent decreases in percentage of active leucocytes).   The results at 50 C did not fall so low as those at 25 C in the three

tests in which values could be obtained at that high temperature (in two experiments the white cells were too disintegrated to be counted). These results are interesting, for it appears that white cells can be subjected to temperatures higher than one observes even in patients with the severest febrile reactions and still give a good account of themselves—at least, *in vitro*. The white cells in the three experiments in which they could be counted at 50 C appeared to be morphologically as sound as those at the lower temperatures.

These results contradict the claims of Madsen and Wulff that optimum phagocytosis *in vitro* occurs at the temperature of the animal body at the time of withdrawal of the leucocytes, the only exception being shown in the lowest curve of figure 4 in which phagocytosis dropped sharply after 39 C, the approximate body temperature of the guinea pig contributing leucocytes and serum.

Fenn (1921–22) recalculated the results of Madsen and Watabiki (1919), who failed to determine a temperature coefficient in their studies. By calculation of the number of bacteria ingested per leucocyte per minute during the first half of the reaction, Fenn found that the rate of phagocytosis was almost a logarithmic function of temperature from 0 to 35 C and that Q_{10} was constant over that range and was equal to 2.0. His own experiments did not show comparable results, for he obtained an increase in temperature coefficient below 30 C and a lowered coefficient above 30 C. He considered that this increased coefficient at lower temperatures might be due to change of the leucocytic protoplasm from the *gel* to the *sol* state, a phenomenon easily effected by slight increases in temperatures in the lower range. The lower coefficient at higher temperatures, might, he thought, indicate that at higher temperatures, phagocytosis is dependent upon surface tension alterations.

By comparing the phagocytosis at each temperature with that at the next level, we observed a steady, progressive increase over the range of 22 to 42 C, the rate of this increase diminishing slightly at higher temperatures. Although our first tests (figure 3) did not establish a point of decline in the percentage of actively phagocytic cells, it seemed reasonable to assume it would not be far above 42 C, judging from the decrease in the rate of the reaction at the higher temperatures. This assumption was borne out in the tests pictured graphically in figure 4, in which the rate continued to decrease to the point of decline.

How the increase in the amount of phagocytosis can be correlated with the impairment of production and maintenance of antibodies at fever temperatures is not clear. It must be remembered, however, that the experiments of Ellingson and Clark on the effect of temperature on phagocytosis, as well as those presented here, were carried out *in vitro*, whereas their tests concerning antibodies were made *in vivo*. In the actual infectious process the leucocyte is subjected to elevated temperatures for longer periods than the arbitrary interval selected for these *in vitro* studies. It is entirely possible that the white cell becomes "weakened" in the febrile body before it comes in contact with the bacterial cell and so is less effective in combating invasion. Some information regarding this phase could be obtained by placing the polymorphonuclear cell at varying temperatures for different periods of time before running phagocytic tests *in vitro*

with them.   Repeated attempts on our part to develop a satisfactory technique
in vivo for phagocytosis paralleling the tests *in vitro* were unfruitful because of
difficulty in obtaining suitable leucocytic exudation to balance the bacterial sus-
pension injected.

What, then, are the mechanisms involved in these phenomena of increase, on
the one hand, and decline, on the other?   What is the role of complement in
these reactions?   What are the physicochemical changes concerned?   What part
does mobility of the leucocytes play?   What is the effect of the particular organ-
ism, *Staphylococcus aureus*, employed in these tests?   Specifically, what is the
influence of temperature on coagulase activator (Smith and Hale, 1944; Hale and
Smith, 1945)?   Are there changes in the metabolism of the leucocyte, and if so,
are such changes a cause or an effect of increased ingestion?   Is digestion of the
organisms at higher temperatures likewise increased, or is it decreased, or does it
remain fairly stable?   It may be that the white cells are actually under adverse
conditions at elevated temperatures and are artificially stimulated to an enhanced
activity which lasts only during the short periods of these tests.   During such
stimulation the leucocyte may be unable to cope with the engulfed organisms,
and when the initial stage has passed, the cells may begin to disintegrate, thus
freeing organisms which have been damaged little or perhaps not at all.   The
accumulation of various metabolic products and the presence of leucocidin should
likewise be kept in mind as possible participants in these involved processes.
Obvious suggestions for future research, in addition to points already indicated,
include investigation of different time limits, the effects of immune serum, the
behavior of various types of organisms, and the activity of other types of phago-
cytes, particularly human cells.   One must be aware also of possible physiological
differences between blood leucocytes and exudative cells (Fleischmann, 1939).
The crucial test regarding the practical value of increased temperature can be
made only by use of *in vivo* procedures, and only then can parallels be accurately
drawn.   It is still unclear whether fever is advantageous or detrimental to the
patient.

SUMMARY AND CONCLUSIONS

In a series of studies of phagocytosis *in vitro*, exudative guinea pig and rabbit
polymorphonuclear leucocytes in a system with fresh homologous normal serum
and *Staphylococcus aureus* showed enhanced phagocytic powers with 5 C incre-
ments within a temperature range of 22 to 42 C.   These results are based on 10-
minute incubation of the complete phagocytic system in a controlled water bath.
Data here included are from the 15 tests (10 guinea pig and 5 rabbit) of which the
techniques were the most satisfactorily standardized.   In all, over 30 experiments
were performed, and, regardless of variations in technique, the reaction showed
the same trend.

Fifteen experiments with mice did not lead to clear-cut results; this was due to
failure to secure an adequate leucocytic response.   The results of the five tests
in which there was a moderate amount of exudation are presented; they indicate
an increased ingestion up to 37 C followed by a decline at 42 C.

In five tests with guinea pig leucocytes and fresh homologous normal serum (vs. *Staphylococcus aureus*) in which the temperatures ranged from 37 to 45 C with 2 C increments and with comparisons at 25 and 50 C, phagocytosis increased to a point approximating 43 C and declined rapidly beyond that point.

The increase in phagocytosis proceeded at a slightly decreased rate as elevated temperatures were reached.

REFERENCES

COTTINGHAM, E., AND MILLS, C. A. 1943 Influence of environmental temperature and vitamin deficiency upon phagocytic functions. J. Immunol., 47, 493–502.

ELLINGSON, H. V., AND CLARK, P. F. 1942 The influence of artificial fever on mechanisms of resistance. J. Immunol., 43, 65–83.

FENN, W. O. 1921–22 The temperature coefficient of phagocytosis. J. Gen. Physiol., 4, 331–345.

FLEISCHMANN, W. 1939 The metabolism of damaged cells and tissues. Cold Spring Harbor Symposia Quant. Biol., 7, 290–300.

HALE, J. H., AND SMITH, W. 1945 The influence of coagulase on the phagocytosis of staphylococci. Brit. J. Exptl. Path., 26, 209–216.

HAMBURGER, H. J. 1912 Physikalisch-chemische Untersuchungen über Phagozyten. Wiesbaden.

LEDINGHAM, J. C. G. 1908 The influence of temperature on phagocytosis. Proc. Roy. Soc. (London), B, 80, 188–195.

LEISHMAN, W. B. 1902 Note on a method of quantitatively estimating the phagocytic power of the leukocytes of the blood. Brit. Med. J., 1, 73–75.

MADSEN, T., AND WATABIKI, T. 1919 Meddelanden svenska k. Vetenskopsak, Nobelin-stitut, 5. (*Cited by* Fenn, 1921–22.)

MADSEN, T., AND WULFF, O. 1919 Influence de la température sur la phagocytose. Ann. inst. Pasteur, 33, 437–447.

PASTEUR, L., JOUBERT, AND CHAMBERLAND. 1878 La théorie des germes et ses applications à la médicine et à la chirurgie. Bull. acad. méd., 2d Series, 7, 432–453.

ROLLY, F., AND MELTZER. 1908 Experimentelle Untersuchungen über die Bedeutung der Hyperthermie. Deut. Arch. klin. Med., 94, 335–364.

SMITH, W., AND HALE, J. H. 1944 The nature and mode of action of staphylococcus coagulase. Brit. J. Exptl. Path., 25, 101–110.

WAGNER, K. E. 1890 Contribution à l'étude de l'immunité. Le charbon des poules. Ann. inst. Pasteur, 4, 570–602.

WAGNER VON JAUREGG, J. 1918–19 Über die Einwirkung der Malaria auf die progressive Paralyze. Psychiat.-Neurol. Wochschr., 20, 132–134, 251–255.