HEART RATE AND LEUCOCYTE CIRCULATION IN CRASSOSTREA VIRGINICA (GMELIN)[1, 2, 3]

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It was noticed that leucocytes[4] in the circumpallial artery of the oyster at low temperatures tend to form aggregates which gradually become dispersed with elevation of ambient temperatures. On the basis of this observation, a hypothesis was formulated to interpret the fluctuation in the numbers of circulating leucocytes with changes in the ambient temperature in normal oysters. Assuming the number of circulating leucocytes is more or less constant in a normal oyster, to maintain the leucocytes in suspension, a certain amount of agitation must be present in the circulatory system or leucocytes will eventually settle out. Thus, the number of leucocytes in suspension is related to the intensity of agitation. The following series of experiments was designed to test the validity of the above hypothesis.

In studies of oyster experimental pathology, sampling of heart blood after intracardial injections of particulate or soluble materials is one of the established procedures. To understand the effect of this experimental manipulation on the heart rate and number of leucocytes in the blood is, therefore, of prime importance.

Materials and Methods

1. *Oysters, aquaria, and sea water*

The oysters used in this study were 8–15 cm. in length and were collected from the Navesink River near Red Bank, New Jersey. The shells were heavily infested with *Polydora* but, aside from weakening the structure of the shell, this appeared to have no effect on the functioning of the oyster. About 30–33% of the oysters collected from this area in the summer were parasitized by *Bucephalus cuculus*.

Groups of 4–8 oysters, depending on their sizes, were placed in each aquarium (Pyrex battery jar 25 × 25 cm.) with approximately 4 liters of sea water. Water temperatures in the aquaria for experiments performed in the winter usually approximated the ambient temperatures of the laboratory, *i.e.*, 15°–21° C., by placing the aquaria in a water bath, or were held at specific temperatures by using thermostatically controlled glass immersion heaters (±2° C.) in a constant temperature room.

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[4] In the oyster the terms *amebocyte, leucocyte,* and *phagocyte* are used interchangeably, as suggested by Stauber (1950).

The water used to hold oysters in the laboratory was collected from the Shrewsbury River at Atlantic Highlands, New Jersey, where the salinity varied from 20 to 27‰. Since ambient salinity change (Feng, 1960) has also been shown to influence the heart rate, all experiments listed below were conducted in the sea water of 20‰ which was obtained by diluting the stock water with appropriate amounts of aged tap water. The diluted sea water was stored in a constant temperature room (5° C.) before being used. Water was changed at least twice daily and vigorous aeration in each aquarium was maintained constantly.

2. *Preparation of oysters for injection, bleeding and heart rate studies*

Oysters were prepared for intracardial injection and bleeding by filing a window directly over the heart on the left valve of the shell with a power drill equipped with a circular grinding wheel. After a small hole was made in the nacreous layer, the opening was enlarged by subsequent fragmentation of the shell. For heart rate studies, however, the left valve anterior to the adductor muscles was completely removed, exposing the entire visceral mass, since according to Stauber (1940) valve closure also influences the heart rate. Oysters thus prepared were kept at 5° C. overnight to allow recovery from the shock of the treatment. Prior to the injection, the general physiological condition of each oyster was determined by noting (1) the strength of heart beat, (2) the response of adductor muscle to a mechanical stimulus such as a gentle tap on the aquarium wall, (3) the presence or absence of water pumping and (4) the ejection of fecal strings. Conditions (2) and (4) are not applicable to partially denuded oysters for heart rate studies, since their hinges are broken and consequently they are unable to snap their shells shut. Animals which did not meet the above standards and those which were infected with *Bucephalus* were discarded.

3. *Preparation of inocula*

Seitz-filtered sea water (20‰) was used as one of the inocula.

One pound of fresh spinach was ground in a solution of 0.40 *M* sucrose, 0.05 *M* Tris, pH 7.8, and 0.01 *M* NaCl for 30 minutes at 5° C. (Jagendorf and Avron, 1958). The spinach pulp was filtered through several layers of gauze to eliminate large coarse fibers. The filtrate was then centrifuged at 500 *g* for 30 minutes at 5° C. The supernatant was discarded and the sediment, consisting of almost pure chloroplasts, was reconstituted in 20‰ sea water to desired concentrations.

4. *Injection and sampling procedures*

Seitz-filtered sea water and spinach chloroplast suspension were injected *via* the ventricular route.

Injections were accomplished with a 1.0-ml. tuberculin syringe equipped with a 30-gauge (1½″) needle. Each oyster received 0.2 ml. of inoculum. There was some leakage immediately following the withdrawal of the needle, but in most cases the wound closed quickly. After injection the oysters were returned to the aquaria until the predetermined sampling intervals had passed.

At the appropriate interval, 0.05 ml. of blood was withdrawn from the ventricle by the same type of syringe and needle used for inoculation. The samples were used for estimating the number of leucocytes and chloroplasts.

5. *Enumeration procedures*

a. Heart rate

The heart was exposed for observation by the method described above. For temperatures above 15° C., the heart rates were calculated on the basis of the time required for 10 beats, while below 15° C., only 3 to 5 beats were used to calculate the number of beats per minute.

b. Unfixed oyster leucocytes

During each sampling 0.05 ml. of blood was withdrawn from the ventricle. Samples were not diluted in most cases; but where dilution was warranted, the sample was diluted with filtered sea water in the syringe and the appropriate dilution factor was noted in calculating the final number of unfixed leucocytes in a given volume of blood. The sample was shaken gently to keep the cells in suspension before the counting chamber was filled. The first two drops of blood expelled from the syringe were discarded. The remaining blood in the syringe was used to fill the improved Neubauer counting chamber by allowing the drops to flow under the cover glass.

c. Fixed oyster leucocytes

The procedure described above did not enumerate all the leucocytes in the sample, for in spite of the agitation of the blood sample, there was still a considerable number of leucocytes adhering to the wall of the syringe. An experiment was designed to compare the fixed and unfixed leucocyte counts from the same blood sample. From each oyster 0.25 ml. of blood was obtained by the method described above. The blood was first used to fill 5 Neubauer counting chambers and the remaining blood was fixed with a 5% acetic acid solution in 20‰ sea water (1 part of blood and 4 parts of fixative). The diluted fixed blood sample was shaken gently to dislodge the adhered leucocytes from the wall of the syringe, from which 5 replicates of the leucocytes were obtained as above.

d. Spinach chloroplasts

In estimation of the concentration of chloroplasts in an inoculum, the suspension was first diluted serially in filtered sea water as follows: 1:10, 1:100, 1:1000 and 1:10,000. The counting chambers were filled with aliquots from the last three dilutions. Counting procedures similar to those for enumeration of oyster leucocytes were used to determine the total number of chloroplasts per ml. of inoculum. For estimating the removal of chloroplasts from the blood stream, the free chloroplasts present were counted simultaneously with the leucocytes in the sample.

e. Statistical treatment of the data

A graphic representation of heart rates and leucocyte counts was derived by calculating the symmetrical confidence limits of these numerical characteristics at the 99% level of probability. Such confidence limits were calculated for a group of control figures by substituting the available information in the following expressions:

$\bar{x} - ts/n$,

where $\bar{x}$ = the arithmetic mean,

t = "Student's" distribution value at the 99% level of significance,

s = standard deviation and

n = the number of observations.

The 99% confidence limits of the control group are plotted. Any point of the experimental groups which falls outside the control limits is considered to be significantly different from the $\bar{x}$ at the 99% level of probability. This method of analysis is the standard procedure used in the study of fluctuations of oyster leucocyte counts, heart rates and chloroplast counts; hence it obviated the requirement of performing many tests of significance between groups of variables.

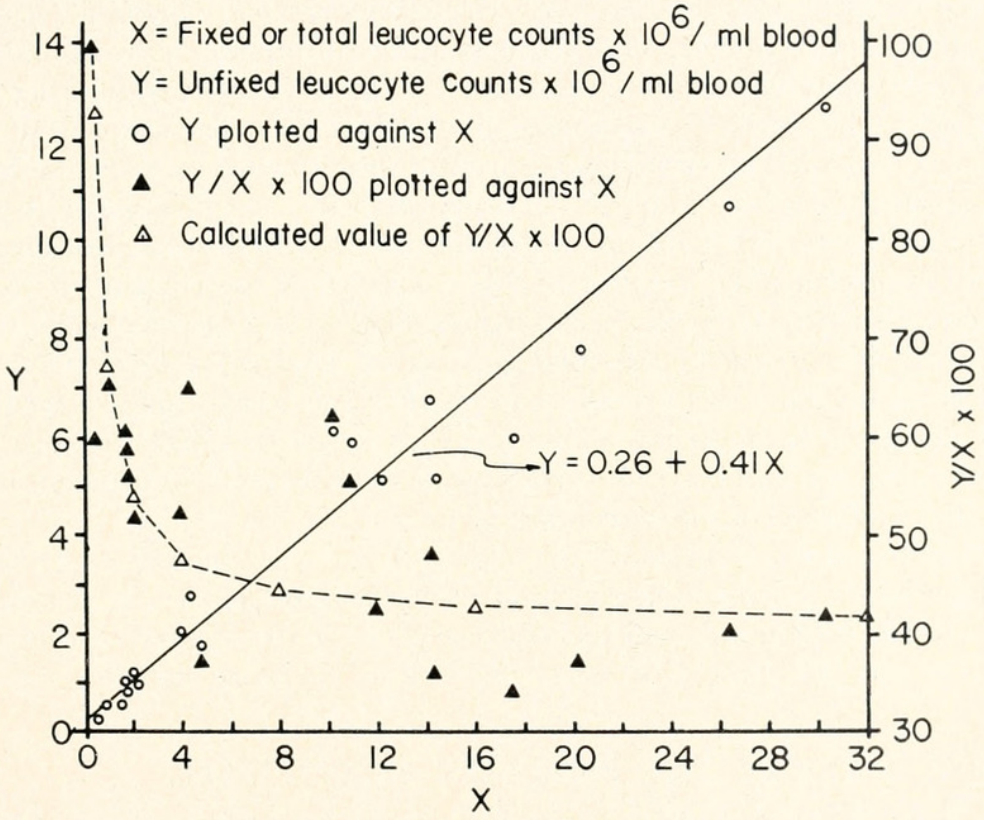

FIGURE 1. Relationship between unfixed and fixed leucocyte counts.

RESULTS

1. *Unfixed leucocyte counts vs. fixed leucocyte counts*

When 20 pairs of unfixed and fixed leucocyte counts are plotted, the points exhibit a linear relationship (Fig. 1). The diagram suggests that an increase in the unfixed leucocyte counts is accompanied by an increase in the fixed leucocyte counts (or the total number of leucocytes). The formula for this regression line is $Y = 0.26 + 0.41X$, where Y is the unfixed leucocyte counts, X is the fixed (or total) leucocyte counts, 0.26 is the intercept of the line with the Y axis, and 0.41 is the slope of the line.

The number of unfixed leucocytes may be expressed as a percentage of the total number of leucocytes ($Y/X \times 100$). When these percentages are plotted against

TABLE I

Variability of leucocyte counts in a group of 88 apparently normal oysters at 18° C.

Leucocyte $\times 10^6$/ml. of heart blood	0.5	2.0	3.5	5.0	6.5	8.0	9.5	11.0	12.5	14.0	15.5	17.0
Frequency	15	20	24	14	3	4	1	2	1	3	0	1
Mean	4.10											
$s(x - x)^2$	1027.83											
Standard Deviation	3.43											
Standard Error	0.37											
n	88											

the total number of leucocytes (X), an exponential curve is obtained (dash line in Fig. 1). The curve suggests that there is little difference between unfixed and fixed leucocyte counts when the total number of leucocytes is in the order of 0.2×10^6/ml. blood. Presumably this is due to the fact that the chances of collision between leucocytes and the syringe wall are not great, hence most of the leucocytes remain in suspension. The greatest decrease of the $Y/X \times 100$ term occurs at X values between 0.5 to 4.0×10^6/ml. of blood. The adhesion of leucocytes to the syringe wall is probably the greatest in this range, since the chances of contact are enhanced and also the space on the syringe wall does not appear to be restricted. However, further increase in the total number of leucocytes from 4.0 to 30.0×10^6/ml. of blood does not seem to affect the percentage term; this is reflected in the flatness of the curve between the above range of leucocyte concentrations. It further suggests that the space of the syringe wall is probably a limiting factor in determining the magnitude of the percentage term.

Based on the above analysis, it is concluded that the unfixed oyster leucocyte count, although not representing the total number of leucocytes is, nevertheless, a valid index of the total number of leucocytes. The data presented in this study are all in terms of unfixed number of leucocytes unless otherwise stated.

2. *Variability of leucocyte counts in apparently normal oysters*

Eighty-eight oysters of assorted sizes were prepared for sampling of heart blood as described above. Prior to the counting of leucocytes, the oysters were removed from the cold room (5° C.) and were placed in sea water of 18° C. as shown in Table I. The range of counts varies from 0.5×10^6 to 17.0×10^6 per ml. of blood with a mean of $(4.10 \pm 0.37) \times 10^6$ per ml. of blood. Neither sex (Table II) nor wet weight of oysters (3.5–30.1 gm., Fig. 2) is correlated with the number of leucocytes.

TABLE II

Test for the significance of mean leucocyte counts obtained from 33 male and 22 female oysters

Sex	Mean	$s(x - x)^2$	n	SE_D	$x_2 - x_1$	$\pm t$	d.f.	*P*
Male	3.23	317.05	33	1.02	0.27	0.26	53	0.5
Female	3.50	225.00	22					

3. *Temperature-heart rate relationship*

Ten partially denuded oysters were placed in sea water at room temperature (23.5° C.), followed by chilling in the cold room. While the water was being chilled, the heart rates were recorded for 23.5°, 21.0°, 18.5°, 14.5°, 11.5° and 10.0° C., respectively. After the observation was completed, the oysters were placed in fresh sea water and kept in the cold room overnight. The next morning, they were allowed to warm up to room temperature in fresh sea water. During the

FIGURE 2. A scatter graph of oyster body weights vs. leucocyte concentrations. The plot is based on observations made on 59 oysters.

warming process, heart rates were recorded at the above temperature gradient. Three sets of such data were obtained, which were combined to construct the temperature-heart rate curve of Figure 3. The result indicates that the heart rate changes from approximately 5 beats per minute at 10° C. to 29 beats at 23.5° C. and confirms the general conclusion reached by earlier workers (Roughley, 1926; Takatsuki, 1927; Federighi, 1929; and Stauber, 1940).

4. *Temperature-leucocyte number relationship*

Twenty-one oysters were used in this experiment. At 6°, 12°, 18° and 22° C, blood samples were taken from each oyster for the counting of leucocytes. Oysters

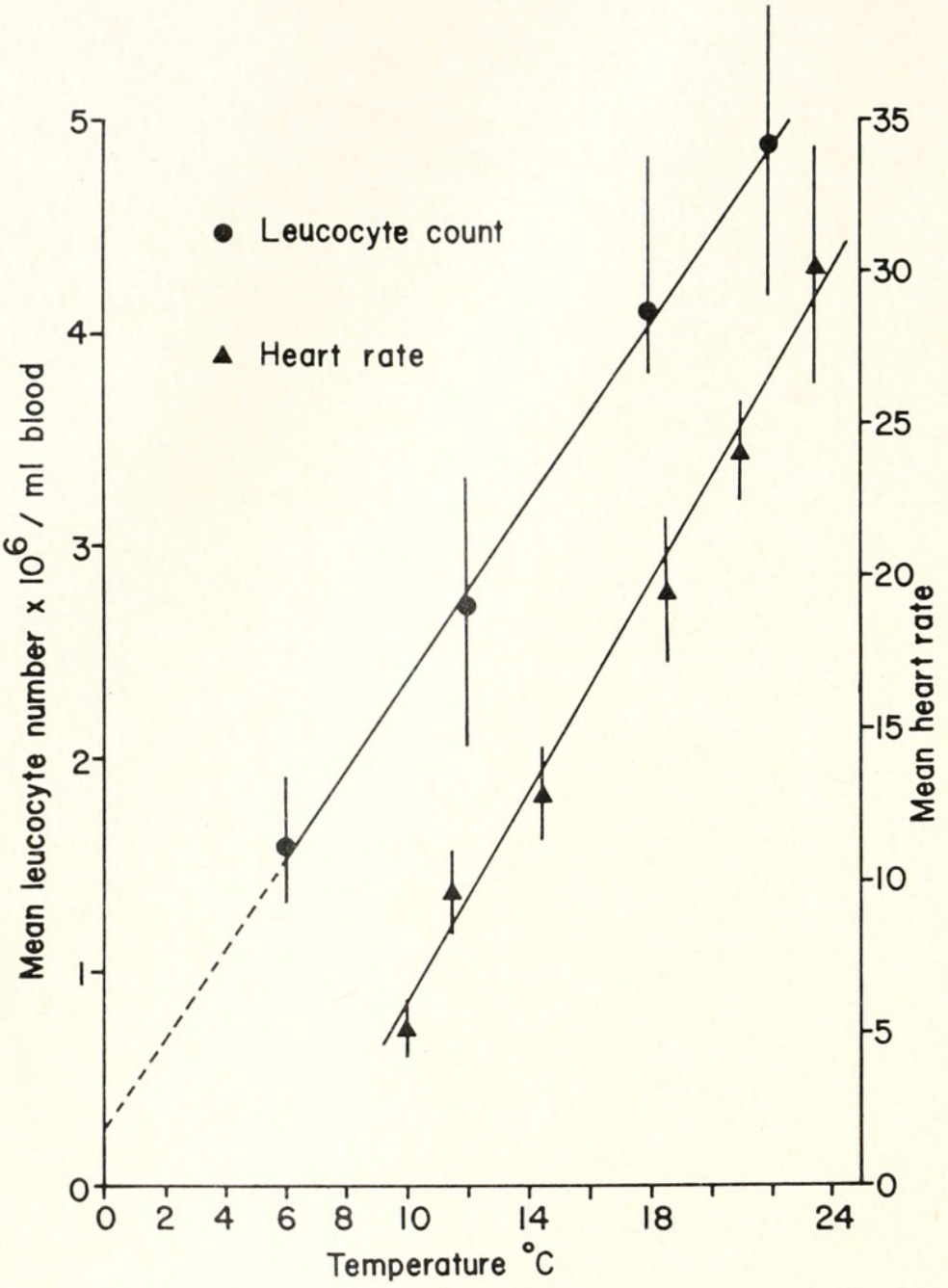

FIGURE 3. The effect of temperature on the leucocyte number and heart rate of oysters. Each point represents average heart rate of three determinations on 10 oysters. The mean leucocyte count in heart blood at 6°, 12°, 18° and 22° C. is obtained from a group of 21 oysters. The vertical lines are ranges of the means.

were allowed to stabilize themselves at each temperature for at least 12 hours prior to sampling.

When the mean number of leucocytes at 6° C. is compared with that at 18° and 22° C., the means behave as if they were drawn from different populations, in spite of the fact that all figures were derived from the same 21 oysters (Table III, P less than 0.01). The linearity of the curve (Fig. 3) suggests that the relation between the number of leucocytes in suspension and the temperature change is a simple one. The extrapolated estimate below 6° C. is probably a close approximation to the true event, *i.e.*, leucocytes are nearly 100% settled out at 0° C., since the

TABLE III

Test for significance of mean leucocyte counts obtain from 21 oysters at 6°, 12°, 18° and 22° C.

Temperature ° C.	6°	12°	18°	22°	Remarks
Mean $\times 10^6$	1.06	2.67	4.08	4.86	
$s(x \times \bar{x})^2$	36.31	179.20	217.10	192.57	
Standard Deviation	1.31	2.92	3.22	3.28	
Standard Error	0.29	0.65	0.72	0.68	
n	21	21	21	21	
d.f. $= (n_1 + n_2 - 2)$	—	40	40	40	6° C. *vs.* 12° C.
$\pm t$	—	1.49	3.17	4.40	6° C. *vs.* 18° C.
P	—	>0.10	<0.01	<0.01	6° C. *vs.* 22° C.
d.f.	—	—	40	40	
$\pm t$	—	—	1.44	2.33	12° C. *vs.* 18° C.
P	—	—	>0.10	>0.02	12° C. *vs.* 22° C.
d.f.	—	—	—	40	
$\pm t$	—	—	—	0.80	
P	—	—	—	>0.50	18° C. *vs.* 22° C.

heart is probably quiescent at this temperature. It is possibly true (depending on the length of time) that *in vivo* at 0° C. leucocytes might not be expected to settle out completely due to the increased viscosity of "plasma." However, no leucocytes were found in the supernatant of a blood sample contained in a test tube which was allowed to stand overnight at 25° C. Extrapolation of the temperature-leucocyte relation beyond 22° C. is uncertain; the curve may become asymptotic if the mean of 4.85×10^6 leucocytes per ml. blood at 22° C. is the maximum for oysters, or it may rise further if all leucocytic clumping is not yet dispersed or if small foci of leucocytic accumulation in the tissues are resolved.

5. *Effects of mechanical stimuli (repeated bleedings) and of injection of sea water*

This experiment was attempted in order to discover the effects of repeated bleedings in small amounts of 0.05 ml. per bleeding on the number of leucocytes, and to

TABLE IV

Changes in the level of oyster leucocytes during a 20-hour period at 16° C.

Time (hr.)	0	$\frac{1}{4}$	$\frac{1}{2}$	$\frac{3}{4}$	1	3	5	10	20	Grand* total
Mean	3.20	4.15	4.00	3.15	3.45	3.65	3.10	2.55	3.40	3.57
$s(x - \bar{x})^2$	29.10	42.03	62.00	73.03	59.73	38.53	24.90	64.23	61.40	463.10
σ	1.71	2.05	2.49	2.70	2.44	1.96	1.58	2.53	2.48	2.27
σ_m	0.57	0.69	0.83	0.90	0.82	0.65	0.53	0.84	0.83	0.24
n	10	10	10	10	10	10	10	10	10	90
d.f.	98	98	98	98	98	98	98	98	98	
$\pm t$	0.49	0.76	0.56	0.41	0.16	0.11	0.64	1.32	0.22	
*P**	>0.50	>0.40	>0.50	>0.50	>0.50	>0.50	>0.50	0.20	>0.50	

* Test for significance of the grand total mean and the means obtained at various time intervals.

establish a base line as the control for certain of the studies cited below. Ten oysters held at 16° C. were bled nine times: 0, $\frac{1}{4}$, $\frac{3}{4}$, 1, 3, 5, 10 and 20 hours after the start of the experiment. The grand total mean of leucocytes per ml. of blood based on 90 observations was 3.57×10^6 with a standard error of 0.24 and $s(x - \bar{x}) = 463.1$. The statistical constants of leucocytes obtained at each time interval are summarized in Table IV. Each mean is compared with the grand total mean to ascertain whether the difference between them is significant. The large P value, in each case, suggests that the mean number of leucocytes at any time interval during the 20-hour period does not differ significantly from the population mean. The data are also presented in graphic form (Fig. 4).

Concurrently with the above experiment, five oysters were each injected with 0.2 ml. of sea water. Heart blood samples were obtained at the following intervals: $\frac{1}{4}$, $\frac{1}{2}$, $\frac{3}{4}$, 2, 4, 8 and 96 hours after the injection. Significant changes in leucocyte numbers during the first $1\frac{1}{2}$ hours are observed (Fig. 4). Immediately after the injection, the number of circulating leucocytes drops abruptly; it reaches the lowest point in $\frac{1}{2}$ hour and slowly regains its normal number in about $1\frac{1}{2}$ hours. The sudden drop in leucocyte numbers after the injection of sea water is ascribed to the displacement of oyster blood in the ventricle by the inoculum or to dilution or to disturbance of heart rate or all three together. The gradual mixing of the inoculum with the influx of oyster blood containing many leucocytes results in the subsequent rise of leucocyte numbers. After 2 hours the number of leucocytes in this group

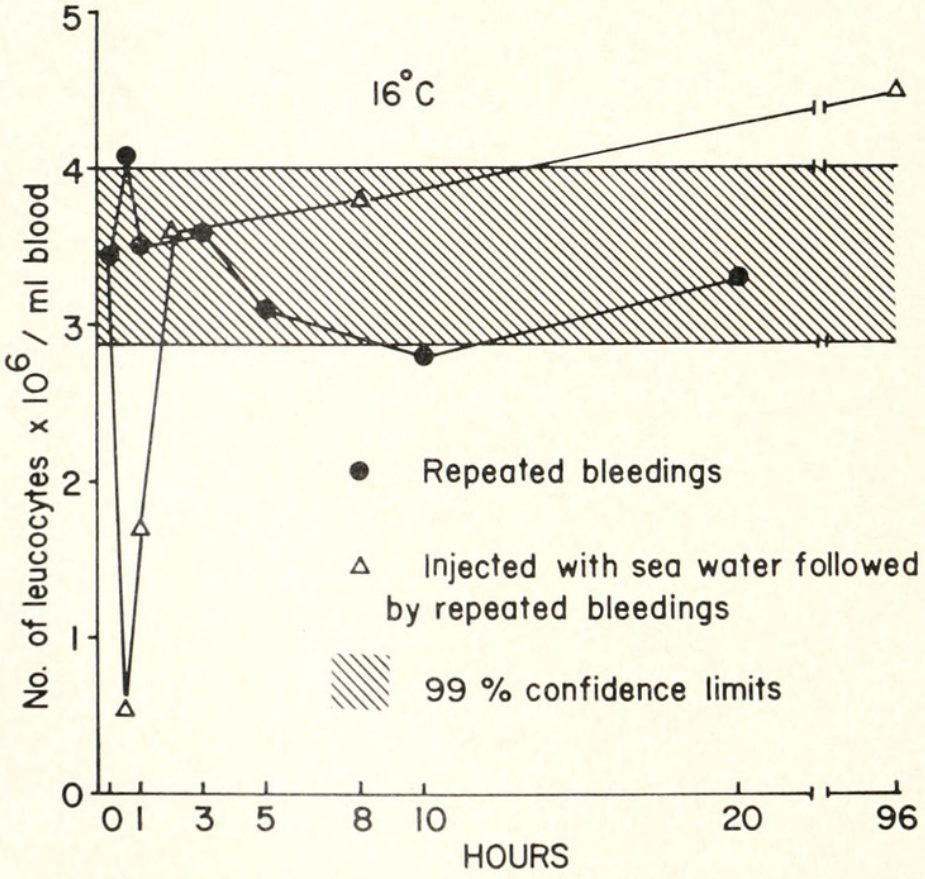

FIGURE 4. The effect of injection of sea water and repeated bleedings on the number of leucocytes in samples of oyster heart blood at 16° C. The cross-hatched area represents the 99% confidence limits of normal leucocyte number in control animals.

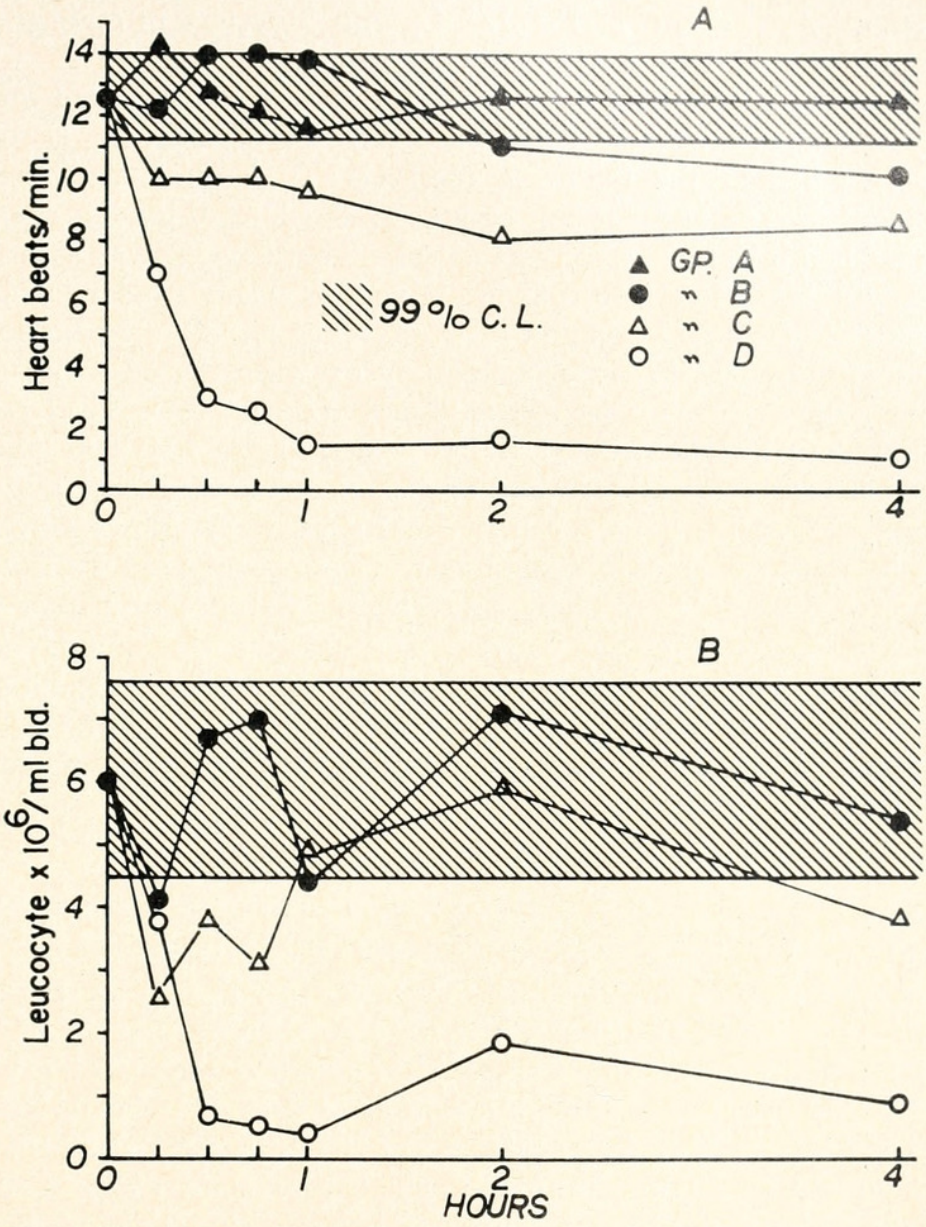

FIGURE 5. The effect of various experimental manipulations on the heart rate (a) and corresponding leucocyte number (b) of oysters. Group A is the control while Groups B, C, and D represent (1) repeated bleedings only, (2) injection of spinach chloroplasts, followed by repeated bleedings, and (3) injection of sea water, followed by repeated bleedings, respectively. The cross-hatched areas represent the 99% confidence limits of heart rates and leucocyte numbers presumed to be present in unhandled oysters and in oysters subject only to repeated bleedings, respectively.

is no longer considered to be significantly different from the control group (Fig. 4), even though one point does fall slightly outside the limits.

6. *Effects of injection of spinach chloroplasts*

Nineteen oysters with hearts exposed were divided into four groups: A, B, C and D. Group A served as control. The remaining three groups were designed to determine the effects of repeated bleedings alone (Group B), injection of sea water followed by bleedings (Group C) and injection of spinach chloroplasts followed by bleedings (Group D) on the heart rate and number of leucocytes. Counts were

made on Groups A, B and C at 15°–17° C. with 5 oysters in each group, and on Group D at 14°–16° C. with 4 oysters in the group.

All experiments lasted for 4 hours. Heart rates were recorded for all groups but leucocyte numbers were sampled regularly for Groups B, C and D only. Samples were taken at the following intervals: 0, $\frac{1}{4}$, $\frac{1}{2}$, $\frac{3}{4}$, 1, 2 and 4 hours. Leucocyte counts were not made for Groups C and D at 0-hour, since 0-hour was also the time when injections were performed. Therefore, it was assumed that the 0-hour leucocyte counts for Groups C and D were probably similar to that of Group B. Each oyster in Group D was given 0.2 ml. of an inoculum containing 6.0×10^9 chloroplasts per ml., while in Group C each oyster was injected with 0.2 ml. of sea water. Ninety-nine per cent confidence limits of heart rates for Group A were calculated and plotted in the manner similar to that for the leucocyte counts.

Repeated bleedings apparently have little effect on the heart rate during the 4-hour period (Fig. 5). Even when the mean heart rate slows down to 8 beats per minute in Group C, which is a significant drop as compared with that of Group A, the leucocyte counts do not show appreciable difference from that of Group B one hour post-injection (Fig. 5). Injections of chloroplasts definitely suppress the heart rates which in turn probably contribute to the delay of the mixing process, among other things. However, the leucopenia immediately following the injection of chloroplasts could be due to incomplete mixing or other unknown factors which are independent of the heart rate.

Discussion

Leucopenia and leucocystosis which are associated with infections in mammals are frequently used as indices of host response; the securing of such information becomes essential in clinical diagnosis. Two parameters may be employed as indices of host responses: (1) changes in ratio of various cells, and (2) quantitative changes in the total number of leucocytes. Although changes in the various types of blood cells after injection of foreign materials or even after hemorrhage were observed by Cameron (1934) for the larvae of the wax moth caterpillar, and also presumably normal leucocyte counts are available for some fishes, amphibians and reptiles (Gemeroy, 1938), the possibility of using this method in detecting host response in poikilothermic vertebrates and invertebrates has yet to be explored. It is probably true that one of the most serious hindrances to the progress is the lack of detailed leucocyte histology with respect to these animals. As a result of the present study, however, it is evident that even the quantitative aspect of this problem is complicated by the fact that the numbers of circulating leucocytes are strongly influenced by the heart rate, which is in turn dictated by temperature and/or mechanical stimuli, *e.g.*, injections of particulate and soluble materials. Thus, the mobilization of leucocytes to the site of invasion in oysters at lower temperatures may be greatly handicapped by the settling out of leucocytes at these low temperatures. The role of the two accessory hearts in circulation has been well established (Hopkins, 1934; Eble, 1963). A comparison of the two accessory hearts and systemic heart with respect to their position, size and mechanical output suggests that the contribution of the former in this context is relatively minor (Eble, 1963; Stauber, personal communication). The available evidence also indicates that they are as temperature-dependent as the systemic heart (Stauber, personal communication). Since leucocytes are also known to participate in the nutritive process, it

is possible that the number of circulating leucocytes may fluctuate with stages of feeding, although experimental evidence is still lacking on this point. The transient disappearance of circulating granulocytes following intravenous injection of staphylococci and pneumococci in small mammals (Rogers, 1958) is attributed to adhesion of circulating granulocytes to the capillary endothelium (Wood, Smith, Perry, and Berry, 1951). A similar phenomenon in the oyster, which occurs immediately following intracardial injection of sea water and chloroplasts, is thought to be associated with the incomplete mixing of the inoculum and the influx of blood from the auricles.

Bang (1961) describes the development of "intravascular clotting or thrombosis" in the circumpallial artery of the oyster following intracardiac injection of tissue extracts. He further reports that the "clotting" disappeared spontaneously within two hours after the injection. Based on the findings of the present study, an alternative interpretation of Bang's observations might be offered. It is clearly demonstrated in the present study that the formation and dispersal of leucocyte aggregates or "clotting" can be manipulated by placing oysters at 5° C. followed by gradual warming to 23° C. This observation is further correlated with circulating leucocyte numbers, temperature and heart rate (Fig. 3). The spontaneous disappearance of "clots" within 2 hours after the injection, as reported by Bang, could, therefore, be ascribed to restoration of normal heart rate following recovery of the heart from trauma, since it is the pulsation of the heart which keeps the leucocytes in suspension. It was also not surprising to find that "clotting" did not occur in his control oysters injected with sea water, since in the studies reported here the reduction in heart rate from 13 to 9 beats per minute following injection of sea water (Fig. 5a) did not sufficiently reduce the velocity of blood flow to affect sludging of circulating leucocytes (Fig. 5b). Therefore, what Bang observed in his experimental animals could be a transient sludging of leucocytes induced by temporary heart failure rather than "clotting," which implies the involvement of biochemical processes. Such settling or sludging of blood corpuscles has also been observed in traumatized or sick experimental animals, sick domestic animals and in human patients (Knisely and Bloch, 1942; Bloch, 1956; Harding and Knisely, 1958; Knisely, Warner and Harding, 1960). These authors have clearly demonstrated that the cause of sludging is largely physical, namely, reduction in the velocity of blood flow, created by experimental manipulations, pathological conditions or other factors. The physical aspect of this problem is reflected by the use, in their analysis of the phenomenon, of formulae originally designed for civil engineers in studying the sedimentation rates of various particles in a fluid medium and the transportation of silt by moving water.

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Summary

It was demonstrated that the number of circulating leucocytes from the heart blood samples of 21 oysters and the heart rate of oysters increase linearly with the rising ambient temperature at which the oysters were held. At ambient temperatures of 6°, 12°, 18° and 22° C., the mean leucocyte number per ml. heart blood

was found to be 1.6, 2.7, 4.1 and 4.9 million, respectively. The corresponding heart rates were 5 (10° C.), 9, 19 and 26 beats per minute. These findings indicate that the fluctuation of leucocyte counts in the blood of experimental oysters is probably associated with the intensity of agitation exerted by the heart beat, which is in turn influenced by the temperature. Effects of repeated bleedings and of injections of sea water and spinach chloroplasts on heart rate and number of leucocytes were also studied. Repeated bleedings do not affect the heart rate and the number of leucocytes, while injection of sea water and spinach chloroplasts does reduce the heart rate and the leucocyte number significantly for a period of two to at least four hours, depending on the type of inoculum used. These effects, therefore, do influence (1) the settling or sludging of leucocytes, (2) the mixing time of the inoculum and (3) the probable accumulation of leucocytes to the site of invasion, especially in oysters at lower temperatures.

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