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Received July 2, 1956. P.S.E.B.M., 1957, v94.

Agglutination of Blood Cells in *Limulus polyphemus.** (22845)

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The horseshoe or king crab, *Limulus*, is as distinctive among the arthropods in its pattern of hemostasis as it is in other aspects(1). It appears to have neither fibrinogen nor the power of autotomy, relying on the toughness of its integument and a very large number of agglutinating cells to prevent blood loss. Not only does it possess many more of these cells than other arthropods(2), but the formation of the solid mass seems to differ qualitatively. Two cellular phases, agglutination and "gelation," have been distinguished(3), the latter of which is distinct from the process of gelation by polymerization of plasma fibrinogen which occurs in crustacea.

It has been difficult to study this phenomenon since procedures which stabilize vertebrate or even crustacean bloods—cooling, removal of calcium ions, addition of heparin, provision of hydrophobic surfaces, etc.—are largely ineffective in *Limulus*(4). By working quickly in the cold, both *Limulus* and crustacean cells can be separated before agglutination occurs but under these circumstances it is not possible to resuspend the centrifuged mass of cells. Agglutination is effectively prevented by formaldehyde fixation, a procedure which is used in counting blood cells or observing their structure(2). This drastic reagent would ordinarily be considered incompatible with any functional aspects but it was found to be effective at relatively low concentrations (0.05-0.10%). Higher concentrations (0.25-0.50%) resulted in some clumping and also precipitated some plasma protein. The cells separated from formaldehyde treated plasma could be readily resuspended in formaldehyde-free sea water or other salt solutions and maintained indefinitely without clumping.

However, these stable suspensions quickly agglutinated upon the addition of small quantities of *Limulus* plasma (0.01-0.05 volumes). In suspensions of 10-20 x 10^6 cells/cc this clumping began within 2 minutes and was complete in 10-15 minutes with large clumps of more than a hundred cells usually

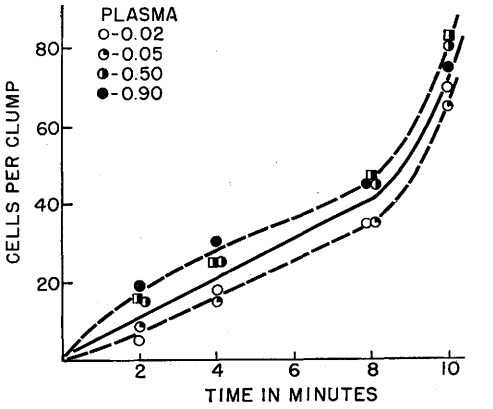

FIG. 1. The course of clumping of stabilized *Limulus* cells suspended in artificial sea water with various concentrations of added plasma; cell concentration, 14.8 × 10^6/ml; plasma concentrations as indicated (referred to whole plasma); temperature 22°; circles, fresh cells; squares, cells dried from the frozen state and resuspended. Ordinate represents avg No. of cells per clump.

* This is paper 23 of a series on "The formation of fibrin and the coagulation of blood" supported in part by research grant from The Public Health Service. We are indebted to Marion P. Meyer for assistance in the initial experiments which were conducted at Marine Biological Laboratory, Woods Hole, Massachusetts, with the support of the Wisconsin Alumni Research Foundation.

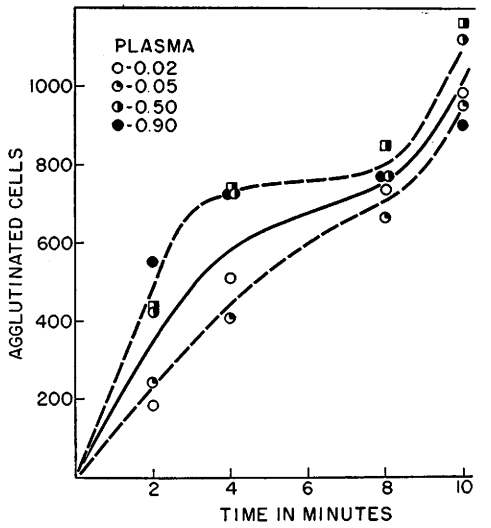

FIG. 2. The course of agglutination of *Limulus* cells at various concentrations of added plasma. Ordinate represents total No. of clumped cells in counting area. Conditions and symbols as in Fig. 1.

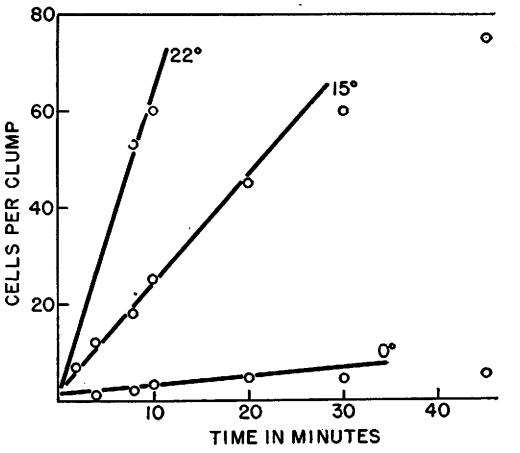

FIG. 3. The course of clumping of *Limulus* cells at 0, 15 and 22°C. Cell conc., 15.0 × 10^6/ml; plasma conc., 0.10.

settling out. The time course of this reaction is shown in Fig. 1. After an initial period, the increase in clump size is largely independent of the plasma concentration over the observed range of 0.02-0.90. A similar relation in terms of the total number of cells agglutinated is shown in Fig. 2. This suggests a 2-stage process and emphasizes the initial dependency on the plasma concentration. Heterologous plasma from the spider crab (*Libinia*) was ineffective in agglutinating *Limulus* cells.

Plasma-free cell suspensions could be frozen and dehydrated to yield a friable powder which, upon resuspension, was little different from the original material in microscopic appearance. It also appeared to have lost none of its functional attributes, insofar as these interests are concerned, since agglutination quickly occurred following the addition of plasma. Indeed, as is shown in Fig. 1 and 2, the course of the reaction was quantitatively identical to that before desiccation. Suspension of the cells in distilled water prior to drying or to concentrated salt solutions after drying produced the expected aberrations in appearance—crenation or rupture—but did not prevent the cells from clumping in the presence of plasma.

The very marked effect of temperature on cell clumping is shown in Fig. 3. The rate of clumping was reduced by a factor of 2.7 between 22° and 15°, and by a factor of 12 between 15° and 0°. The corresponding Q_{10} values (factor for a 10° temperature increase) were 4.1 and 5.2 respectively. Like calculations referred to the number of cells agglutinated gave very similar results ($Q_{10} = 4.6$ and 5.0).

The early stages of the reaction were definitely concentration dependent as is indicated in Fig. 4 which plots the initial rate against the log plasma concentration. A linear (semilogarithmic) relation is seen which suggests

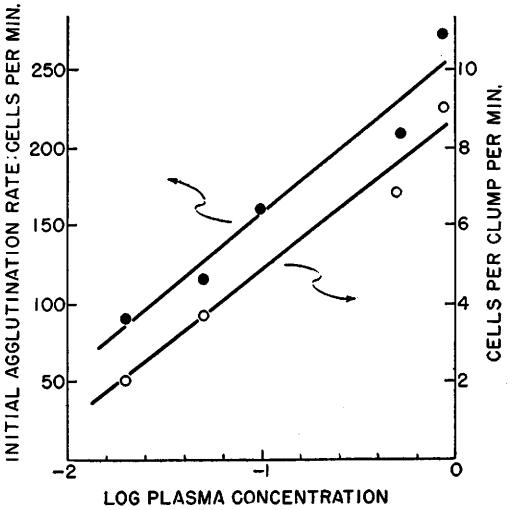

FIG. 4. Initial agglutination and clumping rates as a function of log plasma conc.

that at plasma concentrations below 0.002 no agglutination would occur.

Discussion. It is probable that formaldehyde modified the cell surfaces even though they could still be agglutinated once the free agent was removed since the second "gelation" stage was never observed in these stabilized systems. The effect on the plasma factor was more drastic since even after dialysis, the formaldehyde-treated plasma could not agglutinate the resuspended cells. Formaldehyde also very effectively prevents the interaction of fibrinogen to form fibrin (5). Inasmuch as heterologous plasma did not cause agglutination it would appear that a special "factor" was involved rather than a non-specific effect of, say, plasma protein.

The quantitative resistance of this cellular activity to drying is of interest but is in accord with the general durability of agglutinating systems. The very high temperature coefficient of the reaction is perhaps surprising. Much lower values have been observed for the polymerization of both vertebrate and invertebrate fibrinogens(6).

Summary. Stable cell suspensions were prepared from *Limulus* blood using 0.05-0.10% formaldehyde. Washed cells could be agglutinated by as little as 0.01 volume of fresh homologous plasma. The initial stages of this reaction showed moderate concentration dependence and very strong temperature dependence.

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Received July 27, 1956. P.S.E.B.M., 1957, v94.

Evidence for an Increased Intake of Sodium in Hypertension Based on Urinary Excretion of Sodium.*† (22846)

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In other papers, evidence has been presented which indicated that salt (*i.e.* sodium) intake played a primary role in the pathogenesis of human essential hypertension(1-2). Since this thesis was developed by semi-quantitative means, confirmation by quantitative technics seemed pertinent and forms the subject of the present report. Beginning in 1953, every member of the Brookhaven National Laboratory staff who reported to the staff clinic for annual physical examination by Dr. Robert A. Love was questioned as to his salt intake and classified into one of 3 groups as follows: 1) *Low intake*—this individual did not add, and had never added, salt to his food at table; 2) *Average intake*—this individual added salt to his food if *after* tasting, it was insufficiently salty; and 3) *High intake*—this subject routinely added salt to foods customarily salted, but did so *before* tasting them. The defects in this type of classification were recognized, but the author thought this was a method by which a large series could be studied readily and the implications of a low versus a high salt intake tested. The other papers may be consulted for further details but in summary the following results were obtained: Among the 1346 adults who were classified according to the categories listed above, there were 135, 630 and 581 in

* This work was carried out under auspices of Atomic Energy Commission.

† The author wishes to thank Phelps Crump, for his helpful criticisms of the statistical analyses.