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Clottable Protein in Limulus: Its Localization and Kinetics of Its Coagulation by Endotoxin*

From the Marine Biological Laboratory, Woods Hole, Massachusetts, The Department of Medicine, The Johns Hopkins University School of Medicine and Hospital, and the Department of Pathobiology, School of Hygiene and Public Health, The Johns Hopkins University, Baltimore, Maryland

J. LEVIN and F. B. BANG

The mammalian platelet is intimately involved in coagulation and hemostasis (1). Mammalian platelets accelerate coagulation (2), but are not necessary for it to occur (3); during the coagulation process they aggregate (4), and lose their granules (5). Lysates of mammalian platelets contain a thrombin-clottable protein (platelet fibrinogen) (6). The blood of Limulus, the horseshoe crab, contains only one type of cell, the amebocyte (Fig. 1). When whole blood is withdrawn a cellular clot composed of aggregated amebocytes quickly forms. Thereafter, the aggregated amebocytes shrink, the cells degranulate, and a liquid phase appears (7, 8). The liquid material undergoes gelation[1]) when it is exposed to bacterial endotoxin, and has been designated as pre-gel (8). The production of pre-gel is enhanced by disruption of amebocytes, and the rate of gelation is directly related to the concentration of endotoxin (8). Cell free Limulus plasma is incoagulable (8). A schematic representation of the proposed coagulation mechanism in Limulus is shown in Fig. 2. These observations suggest that pre-gel may be present in the amebocytes and is released during clotting; or a factor liberated from amebocytes during clotting either reacts with a substance already present in plasma to form pre-gel, or constitutes a pro-coagulant which is activated by endotoxin.

Endotoxin of a Vibrio species, pathogenic for Limulus, is capable of causing massive intravascular coagulation, amebocytopenia, incoagulability of the blood, and death (9). The resemblance of this reaction to the Shwartzman phenomenon in rabbits (10), the occasional occurrence of intravascular coagulation during gram negative endotoxemia in humans (11), the role of platelets in the acceleration of mammalian coagulation by

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1) Gelation is defined as the coagulative process which occurs when Limulus pre-gel reacts with endotoxin to produce a gel.

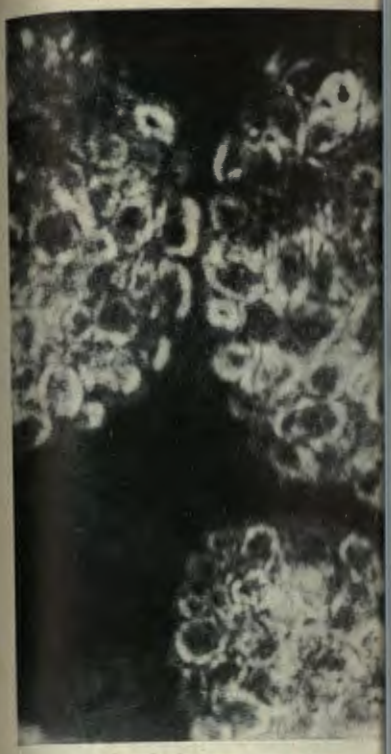

Fig. 1. *Photomicrograph of Limulus a*
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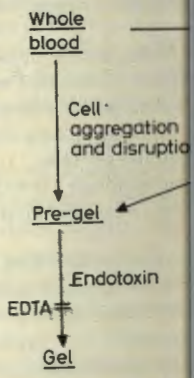

Fig. 2. *Schematic representation of pr*
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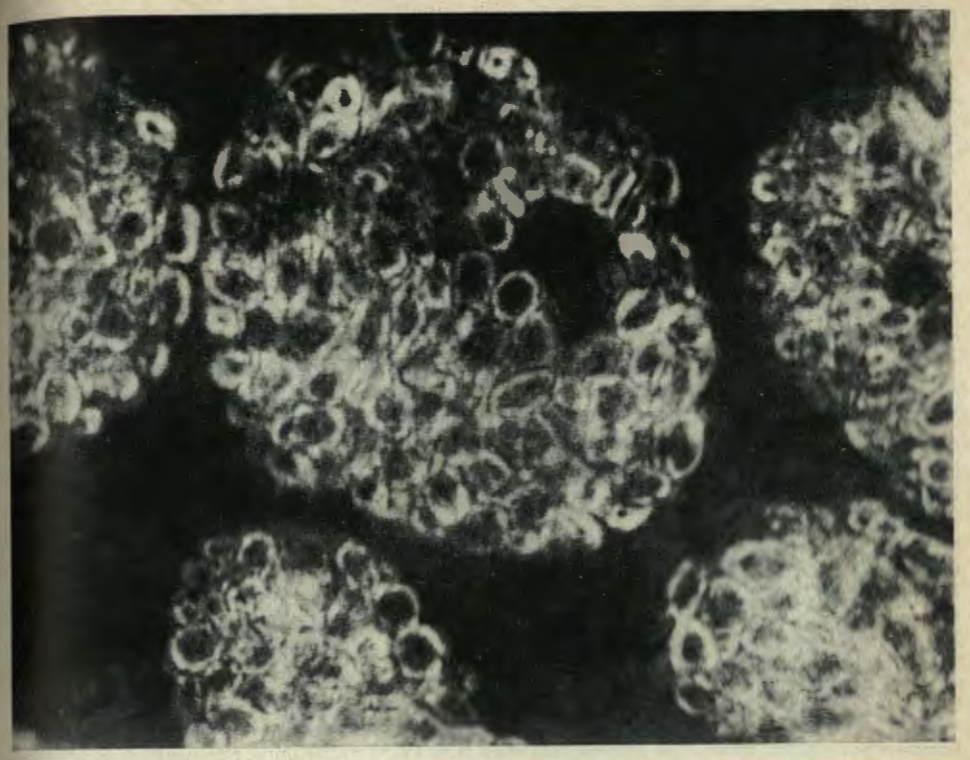

Fig. 1. *Photomicrograph of Limulus amebocytes.* Original magnification 3500× using a polarizing microscope. One amebocyte and portions of 4 others are shown. Densely packed granules, which largely obscure the nucleus, are present in the cytoplasm of each amebocyte.

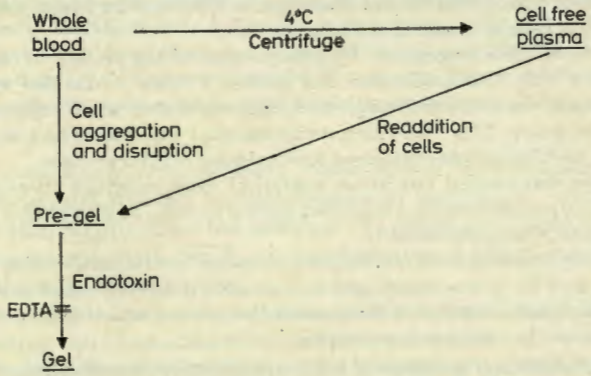

Fig. 2. *Schematic representation of proposed coagulation mechanism in Limulus.* Cell free plasma is incoagulable. EDTA blocks the gelation of pre-gel by endotoxin.

endotoxin *in vitro* (12), and the morphological and functional similarity between mammalian platelets and amebocytes prompted further investigation of coagulation in Limulus.

Our data indicate that amebocytes are the only source of clottable protein in Limulus blood, and the non-cellular elements do not appear to contribute to the coagulation process. The rate of gelation of cell lysate prepared from washed amebocytes is related to the concentration of endotoxin but not to the concentration of protein, suggesting that the reaction between endotoxin and clottable protein is enzymatic.

Methods

Limuli were obtained from the Marine Biological Laboratory Supply Department, and were kept in running sea water. Their sizes ranged from 8–12 inches across the cephalothorax. Blood was obtained by cardiac puncture by inserting a 20 gauge needle at the joint between the thoracic and abdominal segments, after the area had been cleansed with 70% ethyl alcohol. Sterile and pyrogen free glassware and syringes, prepared by steam sterilization at 16 lbs pressure for 45 min followed by 2 hrs at 170° C in a drying oven, were used throughout.

Tests were carried out in *silicone-free 13 × 100 mm glass test tubes* which were immediately capped after reaction mixtures were added to prevent contamination with air-borne moisture containing endotoxin. Experiments were carried out at room temperature, which ranged from 20° to 25° C.

Amebocytes were obtained from *blood* which was drawn directly into sterile, pyrogen free, siliconized (SC-87 Dri-Film, General Electric Company, Waterford, New York) syringes, preheated to 40° C and containing tris-buffered (Sigma 7–9 [Tris (hydroxymethyl) aminomethane], Sigma Chemical Company, St. Louis, Missouri) N-ethyl maleimide (NEM) (Mann Research Laboratories, New York, New York) at pH 7.28 and 40° C. The ratio of whole blood to NEM was 1:1, producing a final concentration of NEM of 5×10^{-3} M. NEM was used to prevent amebocyte aggregation (13). Blood samples were centrifuged at 600 rpm for 1 min, the supernatant material poured off, and the sedimented cells resuspended in 10 ml of buffered NEM, 1×10^{-2} M at 40° C. This procedure was repeated twice. The amebocytes were then twice washed and resuspended in artificial buffered sea water (Marine Biological Laboratory Supply Department, Woods Hole, Massachusetts). Cells were examined by phase microscopy and normal morphological appearance ascertained prior to use. Experiments were carried out immediately following the preparation of washed cells. For some experiments, the washed cells were lysed by freezing and thawing 4 times in dry ice in acetone. The cell lysate was cleared by centrifugation, at 2,000 rpm for 5 min, yielding a clear colorless liquid composed of cellular lysate in artificial sea water.

Protein concentrations were determined by precipitation of the protein in cell lysate with 1 M trichloroacetic acid (TCA). The precipitate was washed 3 times in distilled water at 4° C. The remaining supernatant was extracted with ether, and the water phase examined for remaining protein by U.V. absorption. The TCA precipitable material was lyophilized, dried, and weighed; and for purposes of calculation was considered to equal total cellular protein.

Ultracentrifugation was carried out in an analytical ultracentrifuge (Beckman Instruments, Fullerton, California), equipped with a Schlieren optical system, at 59,780 rpm and 23.5° C. Sedimentation coefficients (S) are uncorrected.

Cell free plasma was prepared from native blood obtained from animals which were precooled at 4° C for 24 hrs. The shed blood was centrifuged immediately in a refrigerated centrifuge at 20,000 G for 10 min. Cell free plasma prepared in this manner, from blood which did not contain NEM, was incoagulable, even after the addition of endotoxin.

Increase in optical density was measured with a spectrophotometer (Beckman DU, Beckman Instruments, Fullerton, California). *Increase in turbidity* as measured by light scattering was carried out with a photofluorometer (Model A, Farand Optical Company, New York, New York) equipped with blue filters and used as a nephelometer. Instantaneous recording was accomplished with a direct writer (Nesco Instruments, Costa Mesa, California).

The *endotoxin* used was either E. coli endotoxin (Lyophilized lipopolysaccharide of Escherichia coli, Difco Laboratories, Inc., Detroit, Michigan) or endotoxin obtained from the originally isolated Vibrio species, by a method previously described (8). The endotoxin was diluted with pyrogen free,

distilled water. Except where indicated, final concentration of E. coli endotoxin in incubation mixtures was 5 μg/ml, an amount far in excess of that necessary to produce complete gelation (8). The changes noted after the addition of endotoxin to cell lysate or pre-gel were graded as follows: Gel-formation of a solid gel; Viscid-marked increase in viscosity but no solid gelation; Flocculant-macroscopically visible flocculation but no marked change in viscosity; Liquid-no change.

Disodium ethylenediamine tetraacetate *(EDTA)* was used as a 0.162 M solution in buffered sea water.

Results

Localization of Clottable Protein

Effect of varying amebocyte concentration in cell free plasma. Native, cell free plasma did not gel in the presence of endotoxin. Washed amebocytes were added to incoagulable cell free plasma to produce various concentrations of amebocytes as shown in Table 1. These mixtures were incubated for 24 hrs at room temperature, during which time the cells aggregated and disrupted. The fluid phase, which appeared following cellular disruption, did not gel in the absence of endotoxin. However, following the addition of endotoxin, gelation occurred, and the final degree of gelation was clearly related to the initial concentration of packed amebocytes.

Table 1. Relationship between Concentration of Amebocytes and the Degree of Gelation.

Volume of packed Amebocytes ml	Volume of cell free plasma ml	24 hr pre-gel +endotoxin[1])
3	2	Gel
1	4	Viscid
0.5	4.5	Flocculent
0.2	4.8	Liquid

1) Packed amebocytes and cell free plasma were mixed in the ratios indicated. Twenty-four hours later, the supernatant (pre-gel) was removed. E. Coli endotoxin (0.1 ml containing 50 μg/ml) was added to 0.9 ml of the liquid pre-gel. The degree of gelation 30 min after the addition of endotoxin was noted; there was no additional change after 24 hrs. Grading of degree of gelation: Gel-formation of a solid gel; Viscid-marked increase in viscosity but no solid gelation; Flocculent-macroscopically visible flocculation but no marked change in viscosity; Liquid-no change.

Clottability of cell lysate from washed, frozen-thawed cells. Lysates prepared from washed amebocytes did not gel in the absence of endotoxin. However, cell lysate gelled solidly within 30 min after the addition of endotoxin (Table 2). EDTA blocked this effect of endotoxin, but NEM, at concentrations which inhibited amebocyte aggregation, did not prevent gelation.

No acceleration of gelation was noted when cell free plasma was added to incubation mixtures containing cell lysate diluted in artificial sea water.

To confirm the presence of clottable protein in the amebocytes and to demonstrate that plasma factors are not required for coagulation, cell lysate was mixed in various proportions with artificial buffered sea water. As shown in Fig. 3, the increase of opacity following the addition of endotoxin was related to the original concentration of the cell lysate. As little as 10% cell lysate (by volume) was sufficient to cause visible change in the incubation tube after the addition of endotoxin. The only source of protein in these solutions was the cell lysate.

Table 2. Coagulation of Cell Lysate by Endotoxin[1]).

Volume of cell lysate ml	Distilled H_2O ml	0.162 M EDTA ml	5×10^{-2} M NEM ml	Endotoxin ml	Appearance at 30 min
0.9	0.1			0.1	Gel
0.9			0.1	0.1	Gel
0.9		0.1		0.1	Liquid
0.9	0.1				Liquid
0.9					Liquid

1) The indicated substances were added to cell lysate and the degree of gelation 30 min after the addition of endotoxin was noted; there was no additional change after 24 hrs. Endotoxins of both E. coli and Vibrio produced similar results.

Fig. 3. *Demonstration of clottable protein in lysate of amebocytes.* One-tenth ml of endotoxin was added to 0.9 ml of various dilutions of cell lysate in artificial sea water. The percent values indicate per cent cell lysate (by volume) in artificial sea water. Following the addition of endotoxin, a solid gel was present in the tubes containing greater than 10% cell lysate. The only source of protein in these solutions was the cell lysate.

Protein concentration in amebocytes. The concentration of protein in cell lysate and the relation to original cell concentration was studied in two pools of Limulus blood, each obtained from four animals (Table 3). Approximately 13.9×10^6 amebocytes contained 1 mg protein.

Table 3. Concentration of Protein in Limulus Amebocytes.

	Experiment 1[3])	Experiment 2[3])
Amebocyte count/100 ml original pooled blood	20×10^8	55.7×10^8
Amebocytes/ml washed cell suspension	16×10^6	53×10^6
Protein (mg/ml) in cell lysate	1.1	4.0
Amebocytes/mg protein in cell lysate[1])	14.5×10^6	13.3×10^6
"Clottable" protein (mg/100 ml)[2])	137	418

1) Amebocytes/mg protein $= \frac{\text{line 2}}{\text{line 3}}$

2) "Clottable" protein for purposes of calculation was considered equal to total protein and $= \frac{\text{line 1}}{\text{line 4}}$

3) In each experiment, whole blood from 4 animals was pooled and the studies performed.

Characteristics of Clotta

UV absorption pattern. The spe obtained from amebocytes showed (Fig. 4). This peak was not present removed.

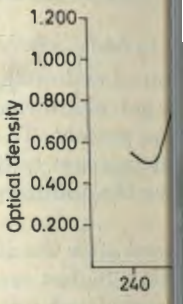

Fig. 4. *Ultraviolet absorption pattern of ly* sea water at pH 7.28.

Ultracentrifugation studies. Ana lysate had two peaks; the slower had a sedimentation coefficient (S ficient (S) of the faster peak, co material in the slower moving peak

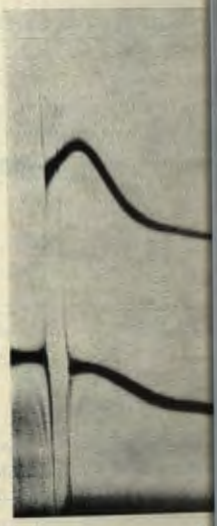

Fig. 5. *Ultracentrifugation pattern of ly* were noted, as demonstrated in this p been reached. The sedimentation coeff the slower peak was 1.75. The upper c contained material remaining after a s

Characteristics of Clottable Protein Obtained from Amebocytes

UV absorption pattern. The spectral absorption pattern of the clear cell lysate obtained from amebocytes showed a peak of maximal absorption at 270–275 mμ (Fig. 4). This peak was not present after gelation had occurred, and the gel had been removed.

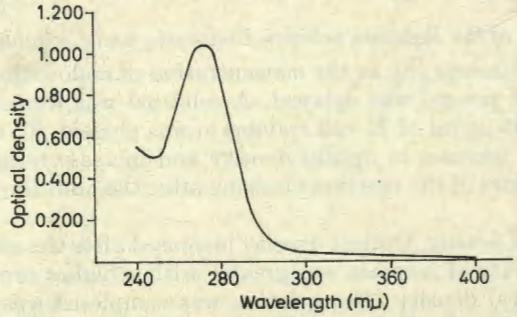

Fig. 4. *Ultraviolet absorption pattern of lysate of amebocytes.* The cell lysate was in buffered artificial sea water at pH 7.28. Maximal absorption was at 270–275 mμ.

Ultracentrifugation studies. Analytical ultracentrifugation revealed that the cell lysate had two peaks; the slower moving peak contained most of the material, and had a sedimentation coefficient (S) of approximately 1.75. The sedimentation coefficient (S) of the faster peak, containing less material, was 11.3. The quantity of material in the slower moving peak was markedly decreased following gelation (Fig. 5).

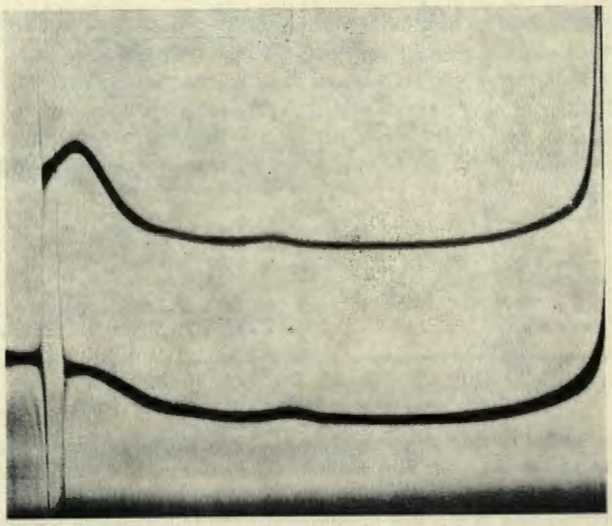

Fig. 5. *Ultracentrifugation pattern of lysate of amebocytes* (representative experiment). Two peaks were noted, as demonstrated in this picture taken 24 min after the full speed of 59,780 rpm had been reached. The sedimentation coefficient (S) of the material in the faster peak was 11.3 and in the slower peak was 1.75. The upper cell contained untreated lysate of amebocytes. The lower cell contained material remaining after a sample of the same lysate had been gelled by endotoxin, and the gel removed.

Temperature stability. The clottable protein was stable at 4° C for 72 hrs, and when frozen for 1 week. Heating the cell lysate for 30 min at 56° C caused some precipitation of protein, and rendered the lysate incoagulable. To rule out the possibility that only a critical enzyme had been destroyed, making impossible the detection of intact clottable protein, 0.1 ml of unheated cell lysate was added to 1.0 ml of heated lysate, and the mixture incubated with endotoxin. Visible gelation did not occur.

Kinetics of the Reaction between Endotoxin and Clottable Protein

In previous experiments (8), as the concentration of endotoxin was decreased, the onset of gelation of pre-gel was delayed. A solid gel was formed when as little as approximately 0.005 μg/ml of E. coli endotoxin was present. To confirm and extend these observations, increase in optical density and increase in light scattering were measured as indicators of the reaction occuring after the addition of endotoxin to cell lysate.

Increase in optical density. Optical density increased after the addition of endotoxin to cell lysate. The rate of increase was greater with a higher concentration of endotoxin, but the optical density after gelation was completed was approximately the same after 120 min (Fig. 6). The rate of the reaction decreased as the concentration of endotoxin in identical samples of cell lysate decreased (Fig. 6, panel on the right). Furthermore, the addition of EDTA to the incubation mixture inhibited the gelation of cell lysate by endotoxin and there was no significant increase in optical density.

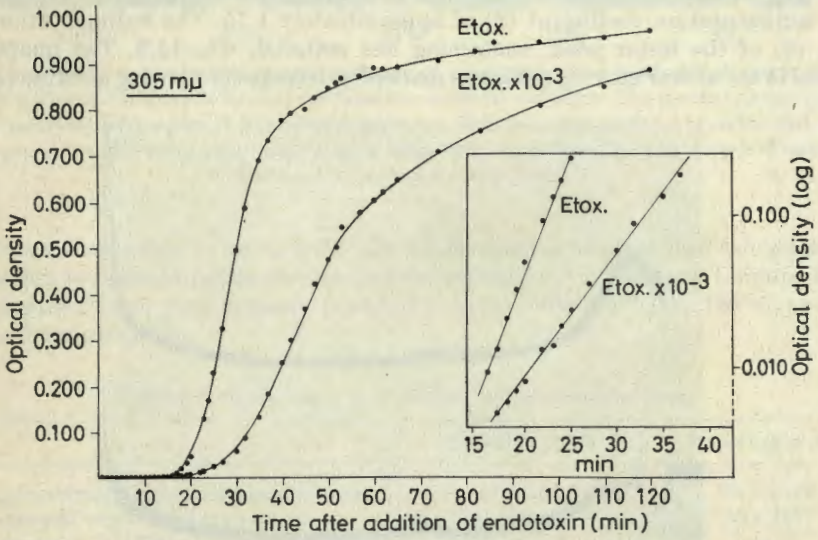

Fig. 6. *Increase in optical density following the addition of different concentrations of endotoxin to lysate of amebocytes.* One-tenth ml of Vibrio endotoxin was added to 0.9 ml samples of the same cell lysate. The relative concentrations of endotoxin (Etox.) are indicated. Protein concentration was constant. The maximal rate of increase in optical density following the addition of endotoxin to cell lysate was greater with a higher concentration of endotoxin (panel on the right).

In contrast, the maximal rates of increase in optical density when the concentration of endotoxin was constant and the concentration of clottable protein varied, were similar (Fig. 7), indicating that the rate of gelation was independent of the concen-

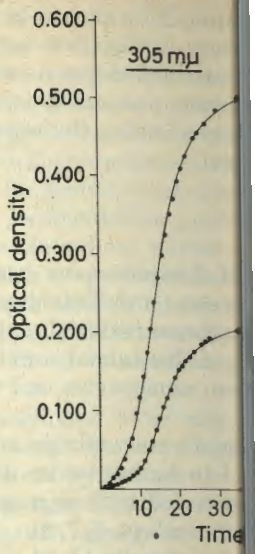

Fig. 7. *Increase in optical density follo lysate of amebocytes.* One-tenth ml of E lysate. The concentration of protein i that in B. The final optical density of The maximal rates of increase in optic and the concentration of clott

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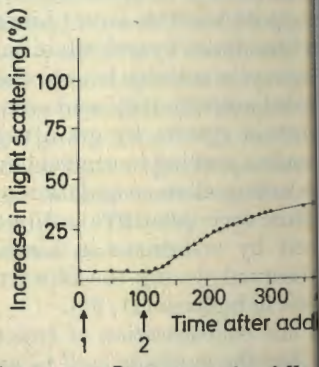

Fig. 8. *Increase in light scattering follo lysate of amebocytes.* One-tenth ml of di point 1 to produce a concentration of was increased to 0.00

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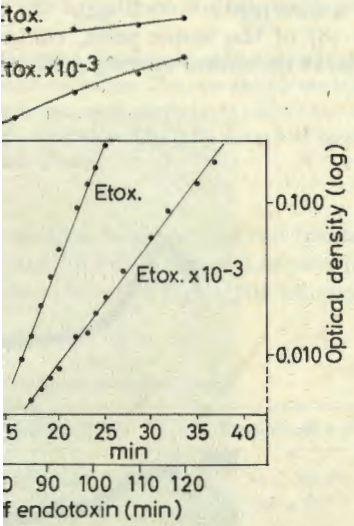

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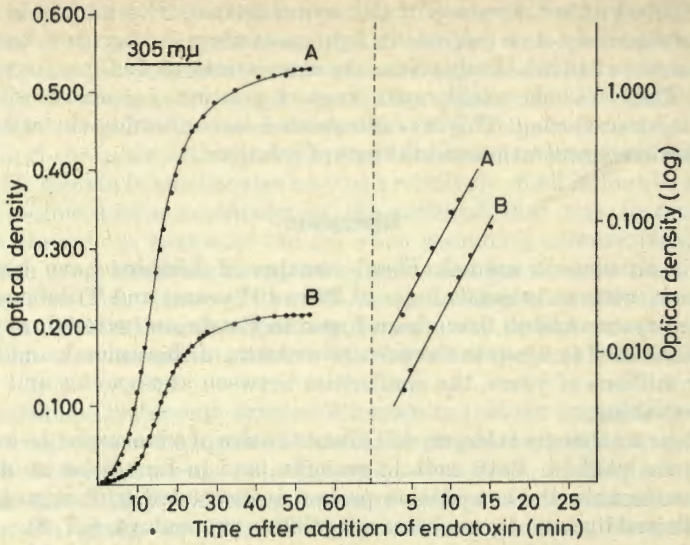

Fig. 7. *Increase in optical density following the addition of endotoxin to different concentrations of lysate of amebocytes.* One-tenth ml of E. coli endotoxin (50 μg/ml) was added to 0.9 ml samples of cell lysate. The concentration of protein in the sample represented by A was 2½ times greater than that in B. The final optical density of line A was 2½ times greater than line B (panel on the left). The maximal rates of increase in optical density when the concentration of endotoxin was constant and the concentration of clottable protein varied were similar (panel on the right).

tration of coagulable protein in the range tested. The final optical densities differed by a factor of 2½, as would be predicted by the fact that the protein concentration in sample A was 2½ times greater than that in sample B (Fig. 7, panel on the left).

Increase in light scattering. Increase in light scattering was measured following the addition of increasing concentrations of endotoxin to the same sample of cell lysate (Fig. 8). No increase in light scattering was observed when the concentration of endo-

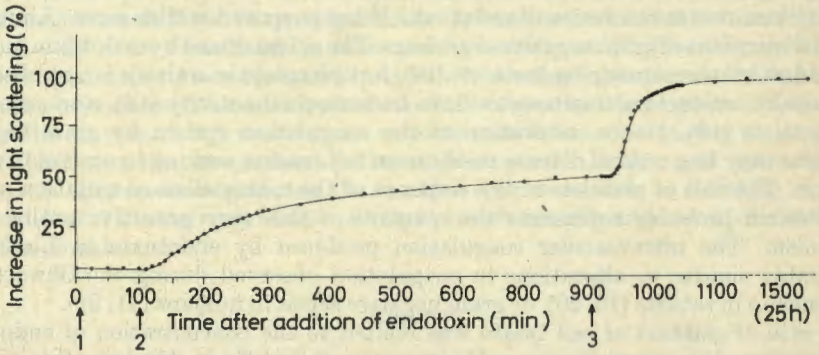

Fig. 8. *Increase in light scattering following the addition of increasing concentrations of endotoxin to lysate of amebocytes.* One-tenth ml of diluted E. coli endotoxin was added to 0.9 ml of cell lysate at point 1 to produce a concentration of 0.00004 μg of endotoxin/ml. The concentration of endotoxin was increased to 0.004 μg/ml at point 2, and to 4 μg/ml at point 3.

toxin was 0.00004 μg/ml. Increase of the concentration of endotoxin to 0.004 μg/ml resulted in a relatively slow increase in light scattering. A very slow but continuous increase persisted at 15 hrs. At this time, the concentration of endotoxin was increased to 4 μg/ml. There was the usual rapid onset of gelation, associated with a marked increase in light scattering. This was interpreted as confirming the relationship between endotoxin concentration and the rate of gelation.

Discussion

Limulus is an ancient animal. Fossil remains of Limulus have been found in Devonian rock, with an estimated age of 290×10^6 years; and Trilobites, which are similar to embryonic Limuli, have been found in Cambrian material, approximately 500×10^6 years old (14). Despite the primitive nature of this animal, and its apparent stability for millions of years, the similarities between amebocytes and mammalian platelets are striking.

Although the amebocyte is larger, and about the size of a macrophage, in many ways it resembles the platelet. Both contain granules, and in Limuli (as in other invertebrates) and mammals, the coagulation process is associated with aggregation of the cells (15), followed by loss of granules as coagulation proceeds (4, 5, 7, 8).

Both amebocytes and platelets aggregate at the site of injury to blood vessels (1, 16). As evolution has occurred, and the coagulation system has become more complex, plasma factors have become increasingly important in coagulation. We have shown that the Limulus amebocyte is the *sine qua non* of coagulation in that animal, and is the only source of clottable protein in the blood. Amebocytes are essentially packets of coagulation potential which can function without accessory plasma factors. It is not known whether there is a plasma activator in Limulus blood. Some activating mechanism presumably accounts for the rapid aggregation and alteration of amebocytes in shed blood, but the mechanism of endogenous activation of coagulation has not yet been explained.

Platelets appear to have fibrinogen on their surface and may actually contain fibrinogen (17). Whether platelet fibrinogen is distinct from plasma fibrinogen is unclear. The finding that amebocytes contain clottable protein provides a biological precedent for the concept that mammalian platelets contain fibrinogen.

The coagulation system of Limulus, through the mediation of amebocytes, is exquisitely sensitive to the action of endotoxin. What purpose does this serve? Limuli live in an environment of gram negative organisms. The gel produced by endotoxin immobilizes endotoxin-containing bacteria (9, 18), but phagocytic activity is not observed. Additionally, cultures of amebocytes have bacteriocidal activity (18), as do mammalian platelets (19). Hence, activation of the coagulation system by gram negative organisms may be a critical defense mechanism in Limulus, serving to control bacterial infection. The role of platelets in the response of the mammalian coagulation system to endotoxin probably represents the remnant of this very primitive antibacterial mechanism. The intravascular coagulation produced by endotoxin in Limulus is remarkably similar to alterations in coagulation observed during the Shwartzman phenomenon in rabbits (10, 20), or gram negative sepsis in humans (11, 21).

The rate of gelation of cell lysate was related to the concentration of endotoxin, which appeared to be rate limiting. It is recognized that the methods used to prepare endotoxins would be expected to destroy enzymatic activity. Nevertheless, our data are consistent with the concept that an enzymatic system mediates the conversion of the cellular protein into a gel by endotoxin. Endotoxin may activate an enzyme which,

in turn, directly reacts with the cellular protein. The mechanism by which endotoxin causes gelation of clottable protein may provide insight, on a molecular level, into at least one of the many biological activities of endotoxins. Gel inactivates endotoxin or at least interferes with its ability to produce further gelation (8). Fibrin absorbs thrombin and has been described as an antithrombin (22). There is perhaps a parallelism between the anti-thrombin activity of fibrin and the inactivation of endotoxin by gel.

The clottable protein in amebocytes may be a relatively small molecule, as suggested by the low sedimentation coefficient of the material that was consumed during gelation, the amorphous nature of the gel when examined microscopically, and the ease with which the gel could be disrupted by mild mechanical agitation.

Heparin and sodium citrate do not block the action of endotoxin on Limulus coagulation, but EDTA does (8). Similarly, heparin and sodium citrate do not block the aggregation of mammalian platelets by endotoxin *in vitro*, but EDTA is inhibitory (19).

N-ethyl maleimide is the only substance known to prevent aggregation of Limulus amebocytes, and it has been suggested that NEM prevents amebocyte aggregation by reacting with sulfhydryl groups (13). However, this action of NEM may be unique since two other sulfhydryl inhibitors, iodoacetamide and mercaptoethanol, did not block amebocyte aggregation (23). NEM does not act as an anticoagulant; i. e., it does not block the action of endotoxin on the clottable protein present in cell lysate. The use of NEM to prevent cell aggregation may provide a means of distinguishing between the roles of cellular and plasma coagulation factors in other invertebrates.

Summary

When blood is withdrawn from Limulus, the horseshoe crab, a cellular clot composed of amebocytes quickly forms. Amebocytes are the only type of cell in Limulus blood. During coagulation, Limulus amebocytes undergo morphological changes that are strikingly similar to those seen when mammalian platelets undergo aggregation and viscous metamorphosis. The clottable protein in Limulus blood is derived entirely from amebocytes, and gelation does not require extracellular factors. Cell free Limulus plasma is incoagulable. The cellular localization of clottable protein in Limulus blood provides a precedent for the presence of fibrinogen in mammalian platelets.

The clottable protein in lysates of amebocytes has a spectral absorption pattern with a maximum at 270–275 mμ, appears to have a low sedimentation coefficient, is stable at −20° C for 1 week, and is destroyed by heating at 56° C for 30 min. The protein gels upon exposure to endotoxin; and the rate of gelation is related to the concentration of endotoxin but is independent of the concentration of protein in the range tested. The kinetics of this reaction are consistent with the concept that an enzymatic system mediates the conversion of the cellular protein into a gel by endotoxin. Increase in light scattering during the reaction detects as little as 0.004 μg of E. coli endotoxin/ml. This conversion will perhaps provide insight into one of the biological activities of endotoxins.

Résumé

Le sang du Limulus, crabe en fer à cheval, forme rapidement après collection un caillot composé d'amoebocytes. Ces amoebocytes sont les seules cellules sanguines du Limulus. Pendant la coagulation, les amoebocytes du Limulus subissent des altérations morphologiques qui sont très semblables à celles des plaquettes de mammifère

au cours de l'agrégation et de la métamorphose visqueuse. La protéine coagulable du sang de Limulus provient entièrement des amoebocytes et la formation d'un gel ne requière pas de facteur extracellulaire. Le plasma de Limulus, sans cellules, est incoagulable. La localisation dans la cellule, chez le Limulus, d'une protéine coagulable crée un précédent pour la présence du fibrinogène dans les plaquettes de mammifères.

La protéine coagulable dans les lysats d'amoebocytes à un maximum d'absorption entre 270 et 275 mμ, a un faible coéfficient de sédimentation, est stable à —20° C pendant 1 semaine et est détruit par la chaleur à 56° C, 30 min. La protéine exposée à l'action d'une endotoxine forme un gel; la vitesse de gélification est proportionnelle à la concentration d'endotoxine mais est indépendante de la concentration de la protéine dans les limites étudiées. La cinétique de la réaction indique qu'un système enzymatique provoque la conversion de la protéine cellulaire en un gel, en présence d'endotoxine.

L'augmentation de la dispersion de la lumière pendant la réaction permet de détecter aussi peu que 0.004 μg d'endotoxine d'E. coli par ml. Cette réaction pourra peut-être expliquer l'une des actions biologiques de l'endotoxine.

Zusammenfassung

Wenn Blut von der Hufeisenkrabbe Limulus gewonnen wird, so entsteht schnell ein zelluläres Gerinnsel, das aus Amoebozyten besteht. Die Amoebozyten sind die einzigen Zellen, die das Limulusblut enthält. Die morphologischen Veränderungen der Limulus Amöbozyten während der Gerinnung sind jenen der Säugetierplättchen während Aggregation und visköser Metamorphose außerordentlich ähnlich. Das gerinnungsfähige Eiweiß des Limulusblutes stammt zur Gänze aus den Amöbozyten, die Gelierung erfordert nicht die Gegenwart irgendwelcher extrazellulärer Faktoren. Zellfreies Limulusplasma ist ungerinnbar. Die zelluläre Lokalisation des gerinnungsfähigen Eiweißes im Limulusblut stellt einen wichtigen Hinweis für die Gegenwart von Fibrinogen in Säugetierplättchen dar.

Das gerinnungsfähige Protein aus Lysaten der Amöbozyten hat ein Maximum der Absorption bei 270–275 mμ, es hat einen niedrigen Sedimentationskoeffizienten, es ist bei —20° eine Woche lang stabil und wird durch Erhitzen auf 56° in 30 Min. zerstört. Das Eiweiß geliert, wenn es Endotoxin ausgesetzt wird. Die Schnelligkeit der Gelierung hängt von der Konzentration des Endotoxins ab, ist aber von der Konzentration des Eiweißes im untersuchten Bereich unabhängig. Die Kinetik der Reaktion entspricht dem Konzept, daß ein enzymatisches System die Umwandlung des zellulären Eiweißes in ein Gel durch Endotoxin vermittelt. Die Steigerung der Lichtbrechung während der Reaktion läßt die geringe Menge von 0,004 μg von Escheria coli – Endotoxin per ml erkennen. Diese Umwandlung gibt vielleicht die Möglichkeit, einen Einblick in eine der biologischen Aktivitäten des Endotoxins zu erhalten.

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