Biochimica et Biophysica Acta, 556 (1979) 278—291

BBA 78520

BIOCHEMICAL AND ULTRASTRUCTURAL STUDY OF THE DISRUPTION OF BLOOD PLATELETS BY STREPTOLYSIN O

JEAN-MARIE LAUNAY [a] and JOSEPH E. ALOUF [b,*]

[a] *Laboratoire de Biochimie, Hôpital Saint-Louis, 75010 Paris and* [b] *Unité des Antigènes Bactériens (ERA CNRS 794), Institut Pasteur, 28 rue du Docteur-Roux, 75724 Paris, Cedex 15 (France)*

(Received March 21st, 1979)

Key words: Streptolysin O; Cytolysin; Dense Body; Ultrastructure; (Platelet)

Summary

The membrane-damaging protein toxin, streptolysin O, proved highly lytic on human, guinea-pig and rabbit platelets. About 15 molecules of toxin were sufficient to lyse one cell. Platelet disruption was assessed by electron microscopy, clearing of cell suspensions and assay of lactate dehydrogenase, serotonin, monoamine oxidase and glutathione peroxidase released in the extracellular fluid. This egress reflected the damage of both plasmic and organelle membranes. A quantitative study of lactate dehydrogenase and serotonin liberation taken as respective markers of the cytosol and dense bodies was undertaken as a function of toxin concentration. No platelet aggregation or shape change was elicited by streptolysin O. The ghosts resulting from platelet lysis retained properties of the native membrane such as aggregability and serotonin uptake. Dense bodies were easily separated after gentle disruption of the plasmic membrane by small amounts of toxin. Platelet lysis by streptolysin O proved a useful procedure for the determination of protein content, enzyme activities and serotonin assay on the same lysate in contrast to usual methods.

Introduction

Streptolysin O, a cytolytic streptococcal toxin purified to homogeneity is a protein of about 60 000 daltons [1]. It is the prototype of the group of bacterial thiol-activated cytolysins (for reviews see Refs. 2 and 3). These toxins are immunologically related and possess a number of common properties: they lyse

* To whom all correspondence should be addressed.

eukaryotic but not prokaryotic cells, they are activated by thiols and their biological activity is specifically inhibited by cholesterol. The cytolytic process is reflected by morphological damage of cell membranes and the leakage of cytoplasmic or organelle-bound constituents [3—5]. There is indirect evidence that membrane cholesterol is both the receptor and target site for SH-activated toxins [4].

Similarly to other cytolytic proteins [5,6], streptolysin O and related toxins are potential molecular probes of membrane organization by virtue of their specific sterol affinity [2—5] as illustrated for cereolysin binding on various membranes [4]. Most studies of streptolysin O action have been undertaken on mammalian erythrocytes which are a convenient model system for assay (hemolysis) in addition to their relatively well-known membrane structure. In contrast, our knowledge on toxin interaction with platelets is still limited. Partially purified streptolysin O [7,8] and tetanolysin [9] have been shown to induce lysis of these cells. As a preliminary approach to the isolation of platelet membrane for ultimate structural investigations, we report here a quantitative and ultrastructural study of the cytolytic effect of purified streptolysin O on human, guinea-pig and rabbit platelets. Cell damage has been monitored by the assay of serotonin and some cytosol or organelle-bound enzymes released after interaction with toxin. The terms liberation or release used here will refer to the egress of serotonin or any other platelet component from toxin-damaged platelets. 'Platelet release reaction' is used to indicate the secretion of internal metabolites without concomitant lysis [10].

Materials and Methods

Glassware and vessels. Whole blood and platelet preparations were exposed only to plastic surfaces or siliconized vessels.

Chemicals. [^{14}C]Serotonin binoxalate (56 Ci/mol), [^{14}C]tryptamine bisuccinate (49.7 Ci/mol) and β-[^{14}C]phenylethylamine, HCl (53 Ci/mol) were purchased from New England Nuclear (Boston, MA). Bovine serum albumin was from Serva, formaldehyde solution from British Drug Houses, glutaraldehyde, L-cysteine and ADP from Sigma. All other chemicals were analytical grade.

Preparation of [^{14}C]serotonin-labeled human platelet suspensions. Blood from healthy adult donors was collected into 1/9 vol. citric acid/citrate/dextrose mixture. Platelet rich plasma was isolated by centrifugation for 15 min at 220 $\times g$ (room temperature) and incubated with [^{14}C]serotonin (1 mCi/l) in 0.2 M Tris buffer, pH 7.4, [11] by shaking for 20 min at 37°C. Labeled platelets were separated from plasma and unbound serotonin by gel filtration at room temperature on a Sepharose 2B column [11].

Platelet treatment with formaldehyde. The fixation of gel-filtered platelets with 1.5% (v/v) formaldehyde solution was carried out as described by Costa and Murphy [12].

Guinea pig and rabbit washed platelets. These platelets were separated according to Pletscher et al. [13] and suspended (10^9 cells/ml) in Tyrode buffer (pH 7.4).

Preparation of rabbit platelet dense bodies. Platelet-rich plasma was treated

either by sonication [14] or by incubation with 130 units of streptolysin O (20 s, 37°C, pH 6.5). The action of the toxin was then stopped by adding 100 combining units of antitoxin. Sonicated or toxin-treated preparations were then applied on a Urographin gradient and spun down (Fig. 1). The dense bodies were separated and suspended in 500 µl water to induce osmotic shock.

Toxin preparation. Highly purified streptolysin O ($4 \cdot 10^5$ hemolytic units/mg protein in the reduced state) [1] was used to avoid interference of other streptococcal exoproteins. Samples of stock toxin solution (32 µg protein/ml) kept at −80°C were thawed and activated prior to use by adding 100 µl of 0.25 M cysteine per ml. Toxin titer was 200 units/ml before reduction and 15 000 units/ml after reduction. One hemolytic unit (2.5 ng of reduced protein equivalent to $4 \cdot 10^{-8}$ µmol) is the greatest dilution of toxin that produces 50% lysis of 0.5 ml of rabbit erythrocyte suspension ($3 \cdot 10^8$ cells/ml) [15]. Platelet buffer was used for toxin dilutions.

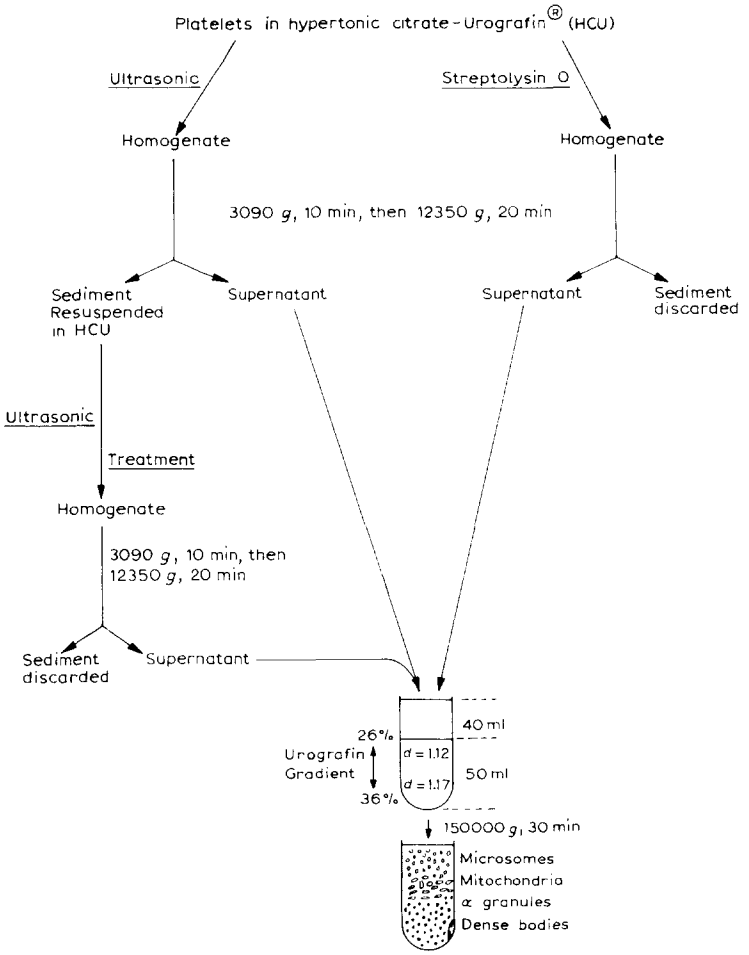

Fig. 1. Flow sheet of the steps of fractionation of subcellular organelles of rabbit blood platelets using a continuous density gradient of Urographin ® (Schering, Berlin). Adapted from Da Prada et al. [14]. See text for further details.

Antistreptolysin O antibodies. Human γ-globulin preparation having an antistreptolysin O titer of 600 combining units/ml [15] was used to inhibit the cytolytic activity of the toxin (one combining unit neutralizes 50 hemolytic units of streptolysin O).

Inactive toxin preparations. Three hemolytically inactive preparations were used as controls in parallel with active streptolysin O: (a) unreduced stock solution diluted 300 times, (b) reduced toxin incubated (20 min, 22°C) with 20 ng of cholesterol/unit [15] and (c) reduced toxin incubated (20 min, 22°C) with antistreptolysin O γ-globulins.

Assay system with [^{14}C]serotonin-labeled gel-filtered platelets. Unless otherwise stated, 200 μl of platelets ($3 \cdot 10^7$ cells) were preheated at 37°C for 10 min before adding 60 μl of test substance or buffer (controls). After appropriate incubation times the tubes (set in quadruplicate for each dose of agent) were ice-cooled for 2—5 min and centrifuged at 2200 × *g* for 10 min at 4°C. Two 50-μl aliquots of each supernatant and untreated platelet suspension were added to 10 ml of scintillation fluid and counted. The percentage of serotonin liberation was expressed as:

$$\frac{\text{cpm toxin-treated supernatant} - \text{cpm buffer-treated supernatant}}{\text{total cpm untreated platelets} - \text{cpm buffer-treated supernatant}} \times 100$$

Turbidity measurements. The turbidity of platelet suspensions was recorded continuously in a Unicam spectrometer at 520 nm in cuvettes maintained at 37°C against either Tris buffer (pH 6.5) or platelet-poor plasma separated from platelet-rich plasma by centrifugation at 2200 × *g* (10 min, 4°C).

Platelet aggregation and shape change. These determinations were performed on platelet-rich plasma according to Born [16] in a Born-Michal type IV aggregometer. Each test contained 250 μl to 1 ml of human platelet-rich plasma ($4 \cdot 10^8$ cells/ml) and 10 μl of challenging agent. The mixture was stirred at 1000 rev./min (37°C).

Electron microscopy. Washed guinea-pig or human gel-filtered platelet suspensions (1 ml) were introduced into polystyrene tubes and incubated at 37°C for 10 min and then 300 μl of reduced streptolysin O (150 units/$1.5 \cdot 10^8$ platelets) were added. Control tubes contained 300 μl of Tyrode buffer instead of toxin. The mixtures were incubated at 37°C.

After 5, 10, 15 and 45 min, the tubes were ice-cooled for 2 min and centrifuged at 2200 × *g* for 15 min at 4°C. The pellets were recovered and treated for electron microscopy.

Control and toxin-treated human platelets were fixed for 1 h in 2.5% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.2, washed twice with the same buffer without glutaraldehyde, postfixed for 10 h in 1% OsO_4, dehydrated and embedded. Ultrathin sections (50 nm) were examined with a Siemens-101 electron microscope at a beam acceleration of 80 kV. Washed guinea-pig platelets were prepared for electron microscopy as described by Tranzer and Richards [17].

Chemical and physical disruption of human gel-filtered platelets. Platelet disruption by $Zn(OH)_2$ was performed by adding 0.2 ml 10% (v/v) $ZnSO_4$ aqueous solution and 0.1 ml of N NaOH to 1 ml of platelet suspension. The mixture was shaken and centrifuged at 1200 × *g* (10 min, 4°C).

Disruption by $HClO_4$ was carried out by mixing 1 ml of N $HClO_4$ solution and 1 ml of platelet suspension. The mixture was then processed as described for $Zn(OH)_2$ treatment. Ultrasonic treatment was carried out on platelet suspension for two 30-s periods in ice using a Branson sonifier operated at 50 kHz. The platelets were also disrupted by three successive freezing and thawing cycles (—20°C, 37°C).

Assay of enzyme activities and serotonin. Monoamine oxidase (EC 1.4.3.4) activity was determined radiometrically using [^{14}C]tryptamine ($5 \cdot 10^{-6}$ M) and β-[^{14}C]phenylethylamine ($5 \cdot 10^{-6}$ M) as substrates for the types A and B of the enzyme [18]. Glutathione peroxidase (EC 1.11.1.9) [19] and serotonin [20] were assayed fluorimetrically. Lactate dehydrogenase (EC 1.1.1.27) was determined spectrophotometrically [21].

Results

Effect of streptolysin O on turbidity of human platelet suspensions. A continuous decrease of turbidity with almost no lag was observed upon incubation (37°C) of 0.1 unit of reduced toxin with platelet-rich plasma or gel-filtered platelets (Fig. 2). Optimal clearing was obtained after 30 min and corresponded with a 50% decrease of initial turbidity. No change in turbidity was observed with any of the three types of inactive toxin preparations. The change in light transmissibility of platelet suspensions was not due to either settling or aggregation since at the end of the experiment the absorbance of cuvette content after stirring was very close to that recorded after 30 min. Platelet clearing was due to platelet lysis as confirmed by the findings reported in the following paragraphs and by platelet count.

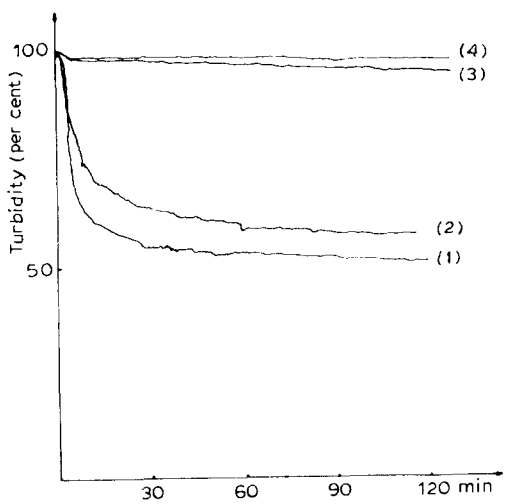

Fig. 2. Effect of streptolysin O on turbidity of human platelet suspensions. Curves (1) and (2), 1 ml of platelet-rich plasma ($\sim 4 \cdot 10^8$ cells) and gel-filtered platelets ($3 \cdot 10^7$ cells) incubated at 37°C with 300 µl of reduced toxin (0.1 unit) under the conditions described in Materials and Methods. Curve (3), 1 ml of either type of platelets incubated with 300 µl of reduced toxin (0.1 unit) previously neutralized with antitoxin. Curve (4), control platelets incubated with buffer. The records are not at the same scale, the sensitivity of the apparatus was adjusted differently for the two different platelet suspensions.

Effect of streptolysin O on aggregation and shape change. Unreduced and reduced streptolysin O even at high lytic concentration (50 units) failed to induce aggregation and shape change of plasma-rich platelet (Fig. 3). The possible inhibitory effect of plasma cholesterol was excluded, since no modification of the hemolytic titer of toxin preparation was observed after incubation with plasma. It is well known that plasma cholesterol is bound to phospholipids and proteins and hence unavailable for combination with streptolysin O [2].

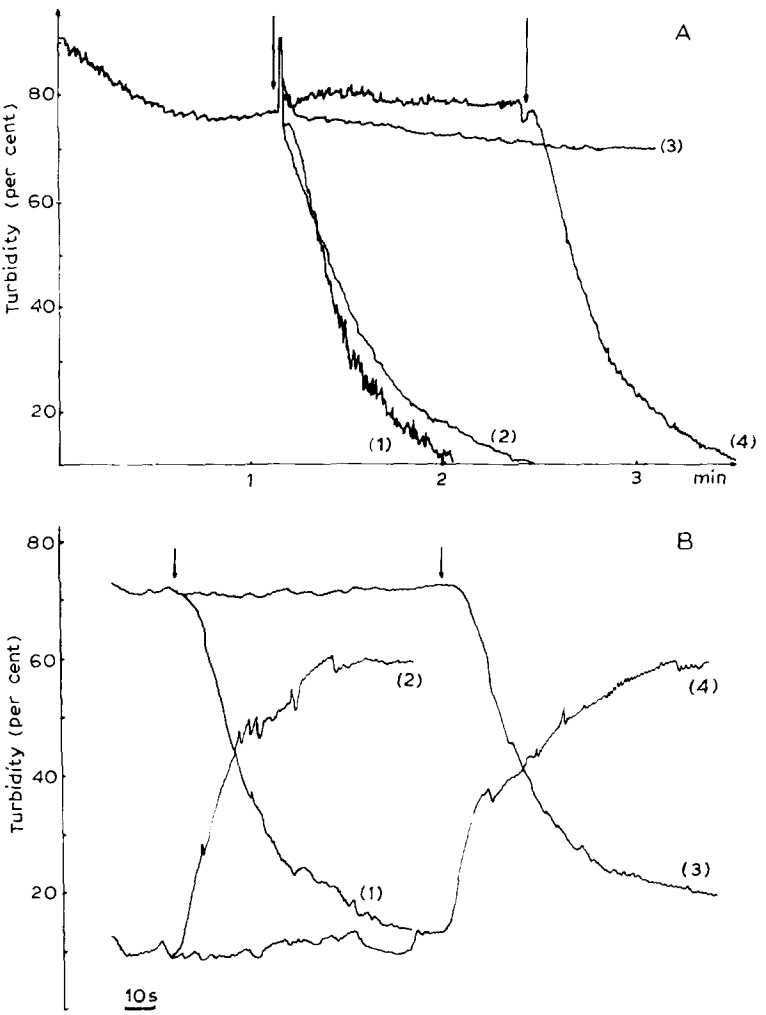

Fig. 3. Effect of streptolysin O on aggregation and shape change of human platelet-rich plasma ($4 \cdot 10^8$ cells/ml). The arrows indicate the addition of test products. Experimental conditions: (A) 250 µl of platelet sample + 10 µl of test product; curve (1), $4 \cdot 10^{-6}$ M ADP; curve (2), 50 units of toxin + $4 \cdot 10^{-6}$ M ADP added simultaneously; curve (3), unreduced (inactive) toxin; curve (4), 0.1 unit of toxin + $4 \cdot 10^{-6}$ M ADP added after 1.5 min. (B) 1 ml of platelet sample + 10 µl of test product. Curve (1) (aggregation); curve (2) (shape change) after addition of 10^{-4} M ADP; curves (3) (aggregation) and (4) (shape change), 50 units of toxin and addition of 10^{-4} AMP after 1.5 min.

Toxin-treated platelet ghosts remained aggregatable by ADP with concomitant shape change. However, aggregation velocity was reduced by about 30% (Fig. 3a).

Ultrastructural study of toxin-treated platelets. This study was undertaken on guinea-pig (Fig. 4) and human platelets (Fig. 5) challenged with four units of streptolysin O per 10^6 cells. This is the amount required for optimal serotonin liberation. After 5 min of incubation the cytoplasmic membrane appeared disrupted with almost complete leakage of the cytoplasmic material. However most intracellular organelles (dense bodies, α-granules) were still apparently intact and scattered inside the ghost. After 10 min they appeared as concentric rosettes near the inner face of the fragmented membrane. Organelle lysis became evident after 15 min and total disruption was observed within the last 30 min of incubation.

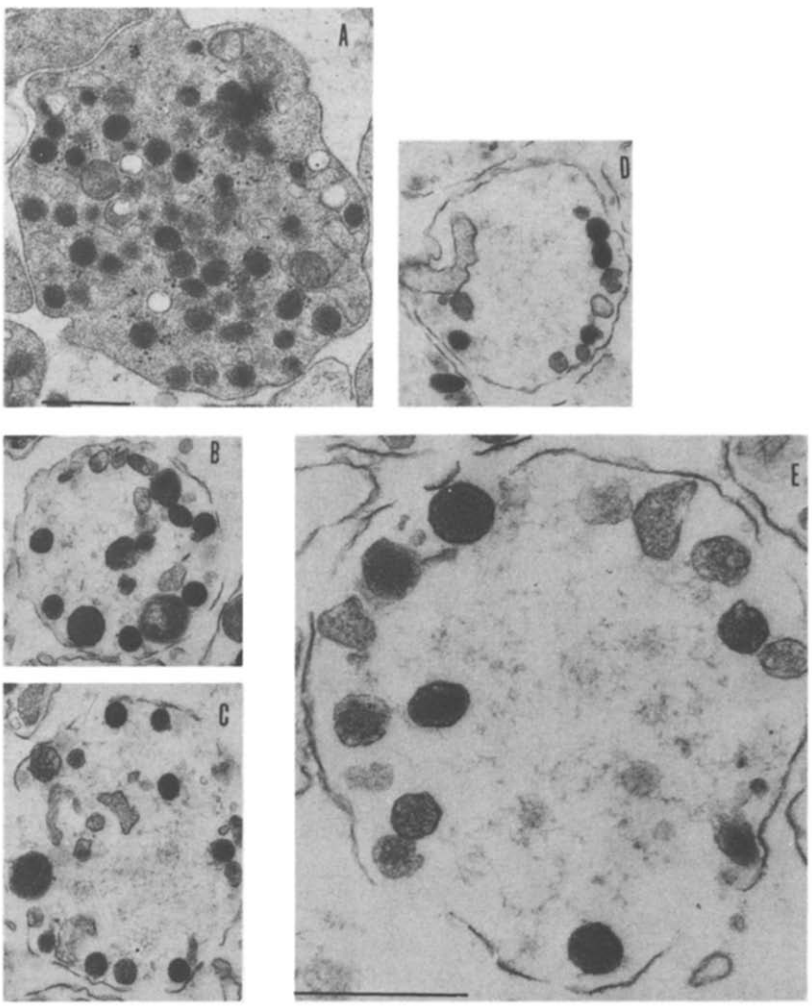

Fig. 4. Electron micrographs of ultrathin sections of guinea-pig platelets (10^8 cells). (A) controls; (B, C, D) after 5, 10 and 15 min incubation at 37°C with 400 units (~1 μg) of streptolysin O (magnification ×22 000), bar 0.5 μm; (E) after 20 min (magnification ×45 000), bar 0.5 μm.

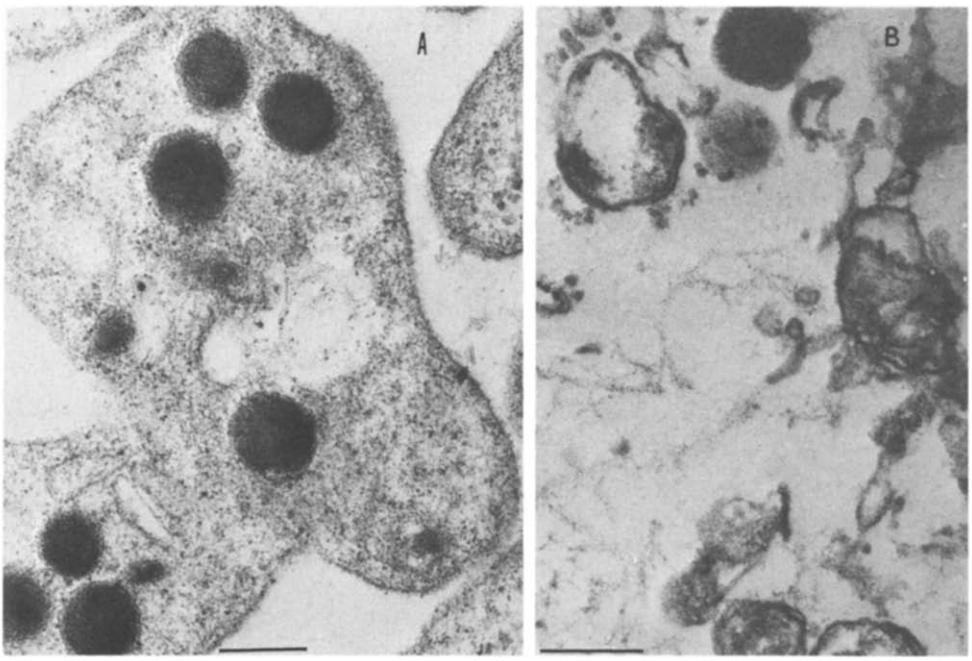

Fig. 5. Electron micrographs of ultrathin sections of human gel-filtered platelets (10^8 cells) (A) controls; (B) after 45 min incubation at 37° C with 400 units of streptolysin O (magnification ×60 000), bar 0.2 μm.

Liberation of lactate dehydrogenase and serotonin as a function of toxin concentration. All experiments were performed on [^{14}C]serotonin-labeled human gel-filtered platelets ($3 \cdot 10^7$ cells), 4 h after blood withdrawal. It is well established that serotonin taken up from surrounding medium is incorporated into dense bodies and behaves as autochtonous platelet serotonin.

The platelets were incubated for 45 min with varying concentrations of streptolysin O. Platelet damage was followed by the quantitative determination of lactate dehydrogenase activity and [^{14}C]serotonin in the supernatants of incubation mixtures. Lactate dehydrogenase is a cytoplasmic component. Its liberation is a good marker of plasmic membrane damage whereas serotonin is essentially located in dense bodies. The liberation of both substances was optimal at pH 6.5 and 37°C, as found for hemoglobin release from erythrocytes [15].

The dose-dependent liberation of lactate dehydrogenase and serotonin was sigmoidal but markedly different as reflected by the magnitude of the concentration range over which the release of these two markers took place. The data were plotted on a log-prob graph which linearizes the sigmoid curves (Fig. 6). Enzyme liberation (slope ~3) was initiated at a level of 10^{-4} unit of toxin per $3 \cdot 10^7$ cells, and was total for $6 \cdot 10^{-2}$ unit of toxin (on the basis of enzyme activity released by freezing and thawing). The dose-response curve for serotonin escape was quite different depending on whether native or formaldehyde-treated platelets were used. It was almost identical to that of lactate dehydrogenase (same slope) for formaldehyde-treated platelets. As no release reaction takes place in this case [12], liberated serotonin is attributable to

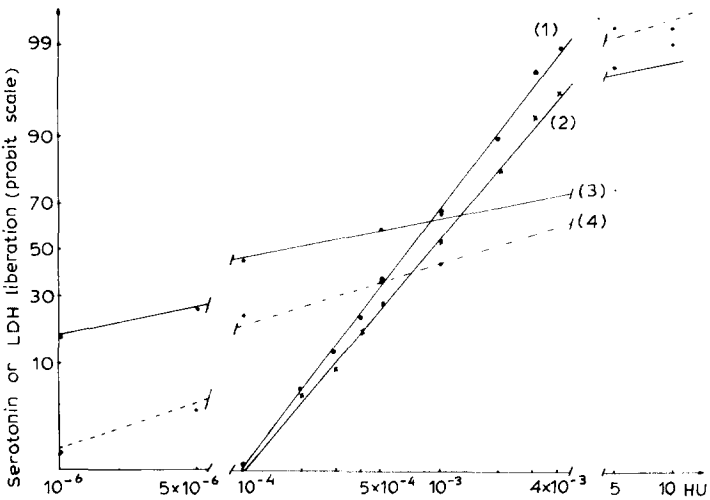

Fig. 6. Quantitative liberation of human gel-filtered platelets serotonin and lactate dehydrogenase (LDH) as a function of streptolysin O concentration (Log-prob scale). Curve (1), lactate dehydrogenase liberation at pH 6.5; curve (2), serotonin liberation at pH 6.5 from formaldehyde-treated platelets, curves (3) and (4), serotonin liberation from native platelets at pH 6.5 and 7.4. The symbol HU is for hemolytic unit.

dense body disruption. A small fraction may, however, originate from the extragranular compartment.

Serotonin escape began as low as 10^{-6} unit of toxin per $3 \cdot 10^7$ cells; a dose two orders of magnitude lower than that found to initiate enzyme release. Nevertheless, 50 units of toxin were apparently necessary for the liberation of all platelet serotonin. This behavior is reflected by the flat slope (0.5) of the dose-response curve.

No significant escape of [^{14}C]serotonin was noticed with untreated platelets. This escape was inhibited when the platelets were challenged with reduced toxin previously incubated with antitoxin or cholesterol. For a concentration of 20 ng of sterol per unit of toxin the inhibition was total. The platelets appeared microscopically undamaged at this dose.

Kinetics of serotonin egress from toxin-damaged platelets. [^{14}C]Serotonin-labeled, gel-filtered platelets were treated with 130 units of toxin per $3 \cdot 10^7$ cells under the conditions described in the previous paragraph. Marker liberation was detectable 2 min after incubation with toxin and was linear for up to 15 min. At this time about 80% of the total platelet [^{14}C]serotonin had already escaped. The release of the remaining fraction took place more slowly between 15 and 45 min. These findings correlate with ultrastructural studies.

Comparative study of serotonin content and enzyme activities from platelets disrupted by streptolysin O and other agents. The determination of serotonin, monoamine oxidase and glutathione peroxidase in the supernatant of human gel-filtered platelets disrupted by a high amount of streptolysin O (Table I) has been carried out as a possible method for the estimation of total platelet content in these components. This approach was compared to four other usual disruptive methods used for the same aim: deproteinization with $Zn(OH)_2$ or $HClO_4$, sonication, freezing and thawing. The first two methods are suitable for serotonin assay but not for enzyme determination, wheras the last two proce-

TABLE I

COMPARATIVE EVALUATION OF SOME PLATELET CONSTITUENTS DETERMINED AFTER DISRUPTION BY STREPTOLYSIN O AND USUAL DISRUPTIVE PROCEDURES

Each values is the mean of 20 experiments ± S.D. for serotonin and glutathione peroxidase and three experiments ± S.D. for monoamine oxidase.

	Streptolysin O	$Zn(OH)_2$	$HClO_4$	Sonication	Freezing-thawing
Human gel-filtered platelets					
Serotonin [a]	2.40 ± 0.08	1.25 ± 0.41	2.27 ± 0.41	—	—
Monoamine oxidase [b]	0.28 ± 0.03 0.030 ± 0.003	— —	— —	0.21 ± 0.03 0.021 ± 0.001	0.18 ± 0.01 0.019 ± 0.002
Glutathione peroxidase [c]	459.0 ± 22.5	—	—	270.0 ± 22.5	450.0 ± 80.0
Rabbit platelet dense bodies [d]					
Serotonin	18.4 ± 1.6	—	—	21.0 ± 0.17 [e]	—

[a] Expressed as nM/mg platelet protein.

[b] Expressed as m I.U./mg platelet protein using as substrates β-phenylehtylamine (upper figure) or tryptamine (lower figure).

[c] Expressed as m I.U./mg platelet protein.

[d] Expressed as μM/mg protein. Dense bodies were isolated as described in Materials and Methods and lysed by osmotic shock.

[e] According to Pletscher and Da Prada [25]. Total protein was determined by the method of Lowry et al. [40] on platelet or dense body pellets dissolved in N NaOH.

dures are appropriate for enzyme determination but not for serotonin.

Platelet disruption by streptolysin O gave the following results: (a) monoamine oxidase activity was significantly higher ($P < 0.05$) than that determined after sonication or freezing and thawing, (b) glutathione peroxidase activity was also higher ($P < 0.05$) as compared to sonication but did not differ from freezing and thawing treatment, (c) serotonin was twice as high as that assayed on $Zn(OH)_2$-treated platelets. Serotonin found on $HClO_4$-treated platelets was of the same order but the data were widely dispersed ($P < 0.05$).

Isolation of platelets ghosts and dense bodies. The platelet ghosts resulting from the disruption of human gel-filtered platelets by streptolysin O (50 units/$3 \cdot 10^7$ cells) were collected by centrifugation at 105 000 $\times g$ for 1 h. The pellet was washed and suspended in buffer, pH 6.8. Preliminary experiments indicated that the sedimented material retained functional activity of the native membrane as regards aggregation by ADP (Fig. 3) and serotonin uptake (competition for uptake with native platelets).

Dense bodies were readily separated from platelets treated by moderate amounts of toxin sufficient to disrupt the cytoplasmic membrane without significant damage to the organelles. This procedure was much easier and less time-consuming than sonication technique (Fig. 1). The amount of serotonin released by osmotic shock of dense bodies isolated by either method was of the same order (Table I).

Discussion

In this article we report an ultrastructural and biochemical study of platelet disruption by streptolysin O as a preliminary step for investigations on platelet membrane structure. This toxin and the related thiol-activated cytolysins [2,3,5], which lyse eukaryotic cells by binding to membrane cholesterol, are interesting tools in membranology [22]. Other cytolysins or enzymes [5,6] have been employed for probing membrane structure. This has been recently shown with phospholipases in the study of phospholipid asymmetry in platelets [23]. SH-activated toxins as well as polyene antibiotics which share similar properties proved useful probes of cholesterol localization and distribution in erythrocyte and *Acholeplasma* membranes [4,22,24].

As shown by electron microscopy (Figs. 4 and 5), platelet cytoplasmic membrane as well as organelle membrane were the target of Streptolysin O. The disruption of the cytoplasmic membrane was reflected by the clearing of cell suspensions and the release of cytosol material such as lactate dehydrogenase. Evidence for organelle lysis was given by the liberation of serotonin, monoamine oxidase and glutathione peroxidase (Table I) which are respective markers of dense bodies [25], mitochondrial external membrane [26] and dense tubular system [27]. Lactate dehydrogenase escaped for toxin concentrations less than $6 \cdot 10^{-2}$ unit ($\sim 1.2 \cdot 10^{-10}$ μmol) per $3 \cdot 10^7$ cells. From the amount of toxin ($\sim 10^{-2}$ unit) that released 50% of platelet enzyme (Fig. 6) one may claculate that about 16 molecules were sufficient to lyse one platelet. This cell is therefore as sensitive as a human or a rabbit erythrocyte which requires about 50—100 molecules for lysis [28] if one takes into account the smaller surface area (7-fold) of platelets [29]. The similar slope of the dose-response

line of lactate dehydrogense escape from platelets (Fig. 6) and that of hemoglobin release from the same number of erythrocytes under identical conditions [28] also suggests a similar sensitivity of the plasmic membrane of both cells. One may also infer from these data about 10^{-2} unit of toxin were sufficient to create heavy membrane changes to allow the escape of a macromolecule such as lactate dehydrogenase. Such lesions have been termed 'functional holes' in studies of toxin effects on fibroblasts [3,30]. Rb^+ and hemoglobin release from toxin-treated erythrocytes [31] as well as electron microscopy [32] suggest lesions rather large in diameter.

More than 40% of the serotonin escaped at toxin concentrations below those required to initiate enzyme egress indicating that this small molecule was able to diffuse through very slightly damaged membranes. A minor fraction of serotonin may also originate from the extragranular compartment [33]. Release reaction appeared unlikely since the pH optimal (6.5) for lysis is unsuitable for this reaction [34]. Drastic disruption of platelets by 50 units of toxin or more was necessary for total liberation of serotonin as also shown for staphylococcal α-toxin [35]. This behavior may be due to the adsorption of serotonin on ghosts and possible reuptake. Indeed after platelets were treated with formaldehyde which inhibits the release reaction and reuptake [12] serotonin escape corresponded to that of lactate dehydrogenase (Fig. 6).

Platelet lysis by streptolysin O neither induced aggregation nor shape change in contrast to other cytolysins such as clostridial α-toxin, staphylococcal β-hemolysin (alone or associated to other phospholipases [22,36]) and staphylococcal α-toxin [37]. Platelet ghosts kept important functional properties of the native membrane such as aggregability by ADP and serotonin uptake. ADP-induced aggregation has also been reported for other isolated platelet membranes [38]. These findings show that platelet aggregability does not necessarily depend on the metabolism of the intact cell.

The streptolysin O-ghosts appear as a clean system very suitable for membrane studies. Only nanogram amounts of purified toxin are required, several orders of magnitude lower than detergents, chaotropic molecules and other usual lytic agents. Such agents (as well as physical treatments) drastically disrupt the membranes and provoke heavy membrane modifications which are still poorly defined, in contrast to the well-known molecular target (cholesterol) of streptolysin O.

Platelet lysis by appropriate amounts of toxin proved a powerful, rapid and simple procedure for the separation of dense bodies the structure of which remains almost unknown. These organelles were separated in a single step prior to ultracentrifugation in contrast to the multi-step sonication technique (Fig. 1).

Platelet lysis by streptolysin O allowed for the determination of total protein, serotonin and enzyme activities on the same platelet lysate. The toxin appears therefore to be a useful reagent for analytical purposes as compared to the customary techniques, which have well-known limitations.

It remains to be determined whether the in vitro effects of streptolysin O have an in vivo counterpart on platelets in streptococcal infections in man during which the toxin is known to be elaborated and released into the organism [39]. A possible disrutpion of platelets leading to the liberation of intra-

cellular components (vasoactive amines and factors of blood coagulation, inflammation and platelet aggregation) may be of pathological significance. A thrombocytolytic activity elicited experimentally in rabbits by a partially purified preparation of streptolysin O has been reported [8].

Acknowledgements

This work was supported by the Centre National de la Recherche Scientifique (ER 794). The advice, helpful criticism and encouragment of Drs. Da Prada and J.G. Richards (Hoffman-La Roche, Basel) who also prepared dense bodies and performed electron micrographs of guinea pig platelets are gratefully acknowledged. We thank Dr. L. Chevance and Mr. M. Adrian for performing the electron micrographs on human platelets, Mrs. M. Mester for carrying out some aggregation experiments and Miss M. Buhler for technical assistance. We express our thanks to Professor R.Y. Stanier and Dr. S. Iscaki for reviewing the manuscript.

References

1 Prigent, D., Geoffroy, C. and Alouf, J.E. (1978) C.R. Acad. Sci., Ser. D 287, 951—954
2 Bernheimer, A.W. (1976) in Mechanisms in Bacterial Toxinology (Bernheimer, A.W., ed.), pp. 86—97, Wiley, New York, NY
3 Smyth, C.J. and Duncan, J.L. (1978) in Bacterial Toxins and Cell Membranes (Jeljaszewicz, J. and Wadström, T., eds.), pp. 129—183, Academic Press, New York, NY
4 Cowell, J.W. and Bernheimer, A.W. (1978) Arch. Biochem. Biophys. 190, 603—610
5 Alouf, J.E. (1977) in The Specificity and Action of Animal, Bacterial and Plant Toxins, Receptors and Recognition, (Cuatrecasas, P., ed.), Ser. B, Vol. 1, pp. 221—270, Chapman and Hall, London
6 Freer, J.H. and Arbuthnott, J.P. (1976) in Mechanisms in Bacterial Toxinology (Bernheimer, A.W., ed.), pp. 170—193, Wiley, New York, NY
7 Bernheimer, A.W. and Schwartz, L.L. (1965) J. Pathol. Bacteriol. 89, 209—223
8 Merucci, P., Vella, L. and Cacciapuoti, B. (1964) Prog. Immunobiol. Stand. 1, 127—129
9 Cox, C.B., Hardegree, C. and Fornwald, R. (1974) Infect. Immunol. 9, 696—701
10 Holmsen, H. and Day, H.J. (1970) J. Lab. Clin. Med. 75, 840—855
11 Lévy-Tolédano, S., Rendu, F., Besson, P. and Caen, J. (1972) Rev. Eur. Etud. Clin. Biol. 17, 513—518
12 Costa, J.L. and Murphy, D.L. (1975) Nature 255, 407—408
13 Pletscher, A., Bartholini, G. and Da Prada, M. (1966) in Mechanisms of Release of Biogenic Amines (Von Euler, U.S., Rossel, S. and Uvnas, B., eds.), pp. 165—175, Pergamon Press, Oxford
14 Da Prada, M., Richards, J.G. and Lorez, H.P. (1978) in Platelets, a Multidisciplinary Approach (De Gaetano, G. and Garattini, S., eds.), pp. 331—353, Raven Press, New York, NY
15 Prigent, D. and Alouf, J.E. (1976) Biochim. Biophys. Acta 443, 288—300
16 Born, G.V.R. (1962) Nature 194, 927—929
17 Tranzer, J.P. and Richards, J.G. (1976) J. Histochem. Cytochem. 24, 1178—1193
18 Wiberg, A. and Oreland, L. (1976) Med. Biol. 54, 137—141
19 Ramos-Martinez, J.I., Launay, J.M. and Dreux, C. (1979) Anal. Biochem., in the press
20 Bogdanski, D.F., Plestcher, A., Brodie, B.B. and Udenfriend, S. (1956) J. Pharmacol. Exp. Ther. 117, 82—88
21 Ginsberg, M.H., Kozin, F., O'Malley, M. and McCarty, D.J. (1977) J. Clin. Invest. 60, 999—1007
22 Bernheimer, A.W. (1974) Biochim. Biophys. Acta 344, 27—50
23 Chap, H.J., Zwaal, R.F.A. and Van Deenen, L.L.M. (1977) Biochim. Biophys. Acta 467, 146—164
24 Blau, L. and Bittman, R. (1978) J. Biol. Chem. 253, 8366—8368
25 Pletscher, A. and Da Prada, M. (1975) in Biochemistry and Pharmacology of Platelets, Ciba Found. Symp. 35, New Ser., pp. 261—279, Excerpta Medica, Basel
26 Tipton, K.F., Houslay, M.D. and Mantle, T.J. (1976) in MAO and its Inhibition, Ciba Found. Symp. 39, New Ser., pp. 5—31, Excerpta Medica, Basel
27 Breton-Gorius, J. and Guichard, J. (1976) Nouv. Rev. Fr. Hematol. 16, 381—389
28 Alouf, J.E. and Raynaud, M. (1968) Ann. Inst. Pasteur, Paris 114, 812—827
29 Frojmovic, M.M. and Panjwani, R. (1976) Biophys. J. 16, 1071—1089

30 Thelestam, M. and Möllby, R. (1975) Infect. Immunol. 12, 225—232
31 Duncan, J.L. (1974) Infect. Immunol. 9, 1022—1027
32 Dourmaskin, R.R. and Rosse, W.F. (1966) Am. J. Med. 41, 699—710
33 Costa, J.L., Murphy, D.L. and Kafka, M.S. (1977) Biochem. Pharmacol. 26, 617—621
34 Pollard, H.B., Teck-Goldman, K., Pazoles, C.J., Creutz, C.E. and Shulman, N.R. (1977) Proc. Natl. Acad. Sci. U.S.A. 74, 5295—5299
35 Nosál, R., Rasková, M. and Masek, K. (1969) Toxicon 7, 255—262
36 Chap, H. and Douste-Blazy, L. (1974) Eur. J. Biochem. 48, 351—355
37 Jeljaszewicz, J., Niewiarowski, S., Poplawski, A. and Blawat, L. (1966) Thromb. Diath. Haemorrh. 15, 69—83
38 Steiner, M. (1974) in Platelets Production, Function, Transfusion and Storage (Baldini, M.G. and Elbe, S., eds.), pp. 197—206, Grune and Stratton, New York, NY
39 Wannamaker, L.W. (1973) Circulation 49, 9—18
40 Lowry, O.H., Rosebrough, R.J., Farr, A.L. and Randall, N.J. (1951) J. Biol. Chem. 193, 265—275