enzymes from phagocytosing leukocytes by cyclic AMP and agents such as prostaglandins that elevate tissue levels of cyclic AMP has been reported (*13*). This action might be explained, at least partially, by our findings that catecholamines and cyclic AMP strengthen the integrity of lysosomes. The apparent alteration of physical properties of the lysosome membrane, perhaps induced by cyclic AMP, might influence certain intracellular events, such as the peripheral migration of lysosomes and subsequent fusion with heterophagic vacuoles, that lead ultimately to the extracellular release of lysosomal contents. Moreover, this action of the catecholamines might be responsible for their anti-inflammatory effects. If this hypothesis is valid, then our finding that cholinergic agents elicit an opposing action to that of catecholamines on lysosomes suggests that the autonomic nervous system might play an important role in regulating or controlling the inflammatory process by virtue of the capacity of neurohormones to modulate the structural integrity of lysosomes.

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Complement-Induced Platelet Protein Alterations

Abstract. *Polypeptides of high molecular weight are deleted from the sedimentable fraction of platelets subjected, while intact, to complement action. These polypeptides, distinct from the previously described thrombin-sensitive protein of this fraction, also diminish after platelets are exposed to thrombin. They may be of importance in the molecular events underlying complement-triggered changes in platelet function.*

The capacity of complement to generate platelet coagulant activity has been demonstrated (*1*). It is likely that failure of complement-platelet interaction is responsible for the coagulation abnormality in the blood of rabbits congenitally deficient in the sixth component of complement (C6) (*1, 2*). Activation of complement can trigger aggregation and release of vasoactive amines (*3*) and has been implicated in the precipitation of intravascular coagulation (*4*). We now report complement-induced alterations in high-molecular-weight polypeptides residing in the sedimentable portion of human and rabbit platelets. Similar alterations can be induced by thrombin. These changes may reflect molecular events underlying complement-triggered changes in platelet function.

Citrate was added to human and rabbit blood to prevent coagulation. Platelets were washed and isolated by modifications of methods already described (*5*). The cellular constituents of the blood were separated from plasma by centrifugation and washed twice in Tyrode buffer containing 2 percent bovine serum albumin; calcium was omitted, and 0.03*M* adenosine was added to prevent aggregation. These procedures were carried out at room temperature. The cells were then resuspended in the buffer, and the erythrocytes and leukocytes were removed by centrifugation at 800*g* for 2 minutes. Platelet preparations contained less than three erythrocytes or leukocytes per 10,000 platelets. Platelets were gently sedimented by centrifugation at 800*g* for 5 minutes and resuspended in plasma, serum, or buffer to a concentration ranging from 200,000 to 700,000 per cubic millimeter.

Antiserum to human platelets was produced in C6-deficient rabbits. Disrupted platelets were subjected to molecular sieve chromatography in a column of Sepharose 2B (2.5 by 34 cm), and the void volume fraction was used for immunization. Citrated plasma was prepared from the blood of the immunized animals and heated to 56°C for 1 hour; the heat precipitable material was removed by centrifugation.

Inulin was prepared as described (*6*). Human thrombin (1000 U.S.P. unit/mg) was provided by D. L. Aronson and prepared as described (*7*). Citrated human or rabbit plasma was freed of platelets by centrifugation at 51,000*g* (average) for 30 minutes in a swinging bucket rotor. Rabbit serum was prepared by allowing blood to clot in a glass for 2 hours at 37°C and was twice absorbed with tribasic calcium phosphate (10 mg/ml) to remove residual prothrombin.

The effect of complement on human platelets was determined in the following manner. Washed platelets were resuspended in normal human plasma, normal rabbit plasma, or normal rabbit serum. As a control, platelets were suspended in C6-deficient plasma or C6-deficient serum. Complement was then activated by the addition of 1/20 volume of undiluted heated antiserum to platelets, and the mixture was incubated for 30 minutes at 37°C. The mixture was then sonicated at setting number 1 on a Bronson sonifer with a microtip for two 15-second periods. After separation by centrifugation at 51,000*g* (average) for 30 minutes, the sedimentable fraction was then solubilized and reduced in a mixture of 6 percent sodium dodecyl sulfate (SDS), 0.04*M* dithiothreitol, 0.1*M* EDTA, 0.1*M* tris, *p*H 8.0; this reaction mixture was kept for 30 minutes at 37°C and then for 18 hours at room temperature. It was then alkylated with 0.33*M* iodoacetamide (15 minutes at 37°C). Samples (250 μg) of this material—measured as protein (*8*) with purified C3 as a standard—were then subjected to electrophoresis at 5 Ma for 12 hours in columns (6 by 200 mm) of 5 percent polyacrylamide gel containing 1 percent SDS. The gels were stained with Coomassie blue.

The changes induced in rabbit platelets by complement were determined in a similar manner, except that colloidal inulin (1 mg/ml) was used to activate complement. The effect of thrombin on human platelets was assessed by adding 10 unit/ml to a suspension of platelets in Tyrode albumin buffer (without calcium) and incubating at

37°C for 30 minutes. Sonication and isolation of the sedimentable fraction was then carried out as above. Proteins of known molecular weight were subjected to electrophoresis in parallel with platelet proteins for estimation of their molecular weights.

The effect of complement on human and rabbit platelet polypeptides is shown in Figs. 1 and 2. Activation of complement with intact human platelets and antibody to human platelets produced consistent deletion of high-molecular-weight polypeptides numbers 1 and 3 from the sedimentable fraction of human platelets (Fig. 1, gel C; Fig. 2, gel F). Similar results were seen with 17 preparations from nine individuals. Diminution of bands 1, 2, 3, and 5 from rabbit platelet preparations was seen after activation of complement by inulin (Fig. 2, gel G). Plasma or absorbed serum served equally well as a complement source for these experiments. The effect of complement on these polypeptides could only be demonstrated if the platelets were intact at the time complement was activated. Disruption of platelets by freeze-thawing prior to activation of complement (Fig. 1, gel B; Fig. 2, gel E) prevented these changes from occurring.

The thrombin-sensitive protein (190,000 daltons) (band 5 in the human preparations) of Baenziger *et al.* (*9, 10*), did not appear to be altered by complement. Quantities of thrombin (1 unit or less) which cause deletion of the 190,000-dalton band had no observable effect on the complement-deleted polypeptides. However, larger concentrations of thrombin (10 unit/ml or more) did cause diminution of these polypeptides in human platelets (Fig. 2, gel C).

As has been reported (*9, 10*) disruption of platelets by sonication prevented the release of the thrombin-sensitive, 190,000-dalton protein by thrombin (Fig. 2, gel B). Similarly, only a minimal change in band 1 and no decrease in band 3 could be detected if thrombin was added to disrupted platelets. A relative decrease in the intensity of band 1 with a reciprocal increase in the intensity of band 3 was sometimes seen when disrupted human platelets were exposed to thrombin or plasma (Fig. 2, gels A and B). The relative intensity of these two bands also varied from preparation to preparation.

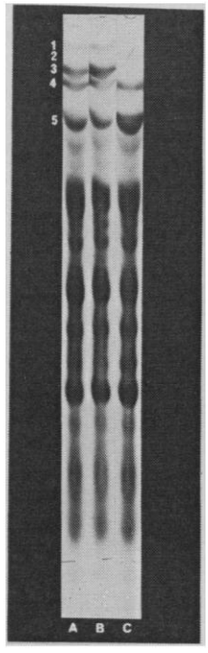
Complement components C3 and C4 are known to be the most abundant in serum. However, addition of 20 μg of these proteins per gel produced no appreciable change in the high-molecular-weight region of the electrophoresis pattern. The subunits of these proteins moved considerably faster in SDS gels as compared to the platelet polypeptides (Fig. 2). It is therefore unlikely that the changes noted represent alterations in platelet-associated C3 or C4.

The band deleted from human platelets after complement action may represent aggregates or altered forms of the same polypeptides. This is suggested by the reciprocal variations in intensity of these bands which was sometimes noted. At this time, their identification with previously described platelet proteins cannot be made. Platelet myosin contains a major subunit whose molecular weight in SDS is approximately 200,000 (*11*). Thrombin has been shown to alter platelet myosin in intact equine platelets in a manner analogous to the polypetide alterations we describe (*12*). However, the identification of platelet myosin with one or more of these polypeptides cannot be made at present.

The methods used do not distinguish between constituents of the plasma membrane and other sedimentable portions of the platelet. This approach was chosen to maximize the chance of detecting alterations in platelet structure by complement, and at the same time to exclude changes that are simply the result of release of soluble material secondary to lysis.

The effect of complement on human and rabbit platelets is similar, suggesting that the affected peptides are analogous. Inulin is known to activate complement by the alternate pathway, a property shared by the biologically important polysaccharides endotoxin and zymosan (*13*). Although we have not been able to demonstrate a clear-cut effect of inulin on human platelets, the potent alternate pathway activator, zymosan, has been used to provoke serotonin release from human platelets (*14*).

Platelets must be intact at the time of complement activation for the polypeptide deletions to occur. This suggests that these changes are not the immediate result of interaction between complement and platelet membrane, but rather are secondary to that initial event. A variety of changes in platelet proteins can be induced by enzymes (*10, 15*). However, there is no evidence at present for the association of enzyme activity with the terminal com-



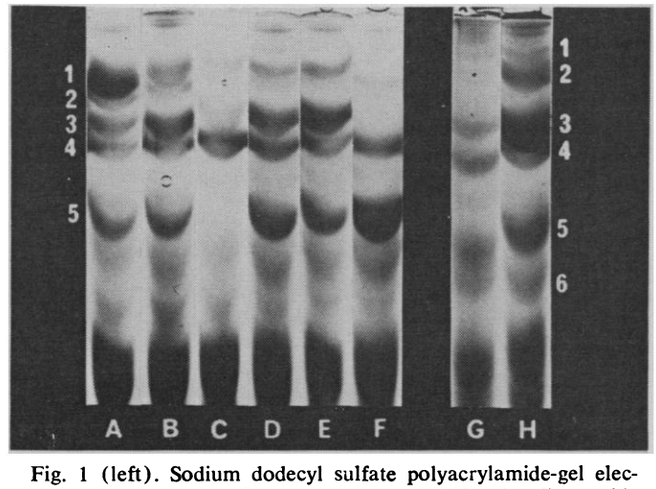


Fig. 1 (left). Sodium dodecyl sulfate polyacrylamide-gel electrophoresis patterns of the reduced and alkylated sedimentable fraction of complement-treated human platelets. (A) Platelets were suspended in C6-deficient rabbit plasma as a control and antibody to platelets was added. (B) Platelets were suspended in normal rabbit plasma, but were disrupted by freeze-thawing before the addition of antibody to platelets. (C) Platelets were suspended in normal rabbit plasma and antibody to platelets was added. Note deletion of bands 1 and 3 in this gel only. Fig. 2 (right). Cathodal (high-molecular-weight portion) of SDS polyacrylamide gels. The effects of thrombin and complement are compared. (A) Human platelets were suspended in buffer as a control. (B) Human platelets were disrupted by sonication before the addition of thrombin. (C) Thrombin was added to intact platelets. There is a marked diminution of bands 1 and 3 and a total loss of band 5. (D to F) These gels are the same as those in Fig. 1 and represent the effect of complement on human platelets. (G) Rabbit platelets were suspended in normal rabbit plasma and inulin was added; there is a diminution of bands 2, 3, and 5. (H) Rabbit platelets were suspended in C6-deficient plasma as a control and inulin was added.

plement components (C5–C9). Complement may act by inducing specific alterations in membrane structure, making these polypeptides susceptible to enzyme action. However, simple rupture of membranes by mechanical means does not cause these changes to occur.

Protein alterations induced by complement may vary in different cells. In sheep erythrocyte membranes as noted by Knüfermann *et al.* (*16*) the slight diminution in high-molecular-weight polypeptides that occurs after complement action is in contrast to the total deletion we have reported here. Similar studies of other cell types known to be functionally altered by complement may also reveal characteristic changes.

Complement and thrombin trigger platelet aggregation, release reactions, and the development of coagulant activity. Both induce similar changes in platelet polypeptides. Thus, these deletions may reflect occurrences common to different mechanisms of platelet activation. Their description here provides an initial step in delineation of the molecular events underlying complement platelet reactions.

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Development of Rabbit Visual Cortex: Late Appearance of a Class of Receptive Fields

Abstract. *In young rabbits before the age at which the eyes open, only three of the seven receptive field types described in the adult visual cortex are detectable. The remaining four receptive field types—which share the property of having radially asymmetric fields—appear later, coincident with a decline in the percentage of cells that are visually responsive but not classifiable as to receptive field type.*

Increasing attention has been paid to the receptive field organization of central neurons in neonatal and perinatal organisms. Cases in which the development of adult receptive field organization continues postnatally are of special interest for the following reasons: (i) Analysis of a developmental sequence may be expected to provide clues as to the neuronal circuitry underlying receptive field properties. (ii) Such analyses should provide information about the mechanisms by which this circuitry is established. (iii) Additionally, normative data on the ontogeny of receptive field organization may be used as a basis for study of developmental alterations produced by environmental manipulation.

All of the visual receptive field types found in the superior colliculus of the adult rabbit are present in the superior colliculus of the neonatal rabbit with apparently adult-like organization before the time at which the eyes open (eye opening) (*1*). The same appears to be true for the cat visual cortex (*2*, *3*) although the responses of the cells to stimulus orientation are not as selective as in the adult. Superior colliculus cells of the cat, on the other hand, do not develop motion and directional selectivity until eye opening (*4*). Of the seven receptive field types found in the striate cortex of the adult rabbit (*5*), three—those with more or less radially symmetric fields (*6*)—are present before eye opening, which occurs at about 10 days postnatal. The other four receptive field types—those with radially asymmetric fields—do not appear until 2 to 8 days after eye opening. The appearance of these latter receptive field types is correlated with a decline in the percentage of cells classified as indefinite—cells which, while clearly responsive to light, are so unpredictable in their behavior that they cannot be identified as one of the seven adult receptive field types.

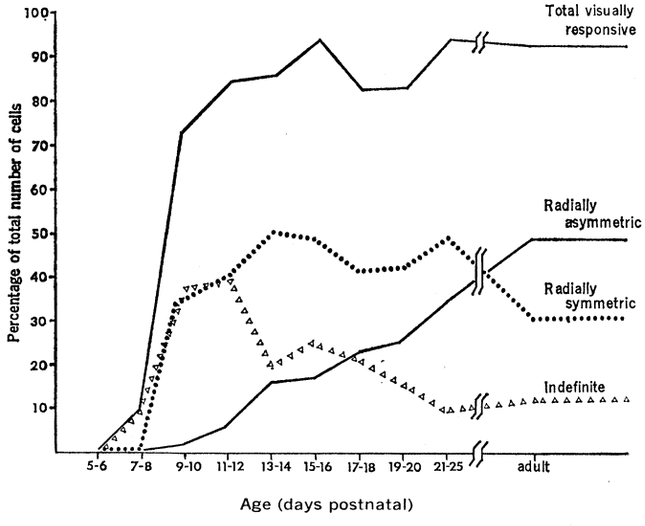

Fig. 1. Time course of percentage of units encountered that had radially symmetric and radially asymmetric receptive fields in rabbit striate cortex. Also shown are the percentages of units encountered that were responsive to visual stimuli and the percentage of units that could not be classified as to receptive field type. Eyes usually opened on day 10 or day 11.