INTERACTION BETWEEN ERYTHROCYTES DURING AGGREGATION STUDIED BY THE SCANNING ELECTRON MICROSCOPE

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KEY WORDS: scanning electron microscopy; aggregation of erythrocytes; dextran; rheopolyglucin.

Intravascular aggregation of erythrocytes develops in many diseases and pathological states, especially in myocardial infarction, shock, burns, operation stress, etc. [1].

It was shown previously by transmission electron microscopy that in the experimental sludging syndrome induced by high-molecular-weight dextran significant changes take place in the shape of the aggregating erythrocytes, with the formation of surface projections [1, 2]. By scanning electron microscopy it is possible to make a more adequate and broad study of this phenomenon, which may perhaps play an important role in the mechanisms of formation of erythrocyte aggregates.

The object of this investigation was to study the shape and interaction of erythrocytes in aggregates in rats with dextran sludging.

EXPERIMENTAL METHOD

Eight male Wistar rats weighing 180-200 g were anesthetized with pentobarbital (0.5 g/kg). By means of a biomicroscope the character of the blood flow in the mesenteric vessels could be observed visually and recorded photographically. To inject the substances and take blood samples a catheter was introduced into the carotid artery. The typical picture of dextran sludging was observed in the microvessels 5-7 min after injection of 2 ml of 20% dextran solution (mol. wt. 500,000). To restore the disturbed blood flow 2 ml rheopolyglucin was injected into the animal. Blood samples were taken through the same catheter before injection of dextran (control), after the injection, i.e., during the development of erythrocyte aggregation, and in the course of deaggregation induced by rheopolyglucin.

Blood samples were fixed for 1 h in 2.5% glutaraldehyde, pH 7.4, and centrifuged (for 5 min at 1000 rpm). The residue was washed twice with 0.1 M phosphate buffer, pH 7.4, postfixed for 1 h in 1% osmium tetroxide, pH 7.4, and dehydrated in alcohols of increasing strength. One drop of material prepared in this manner was applied to a clean grid and, after drying, was sprayed with gold. The preparation was studied in the scanning electron microscope with an accelerating voltage of 20 kV, using a Polaroid camera and materials for recording.

EXPERIMENTAL RESULTS

The investigations showed that the erythrocytes in control blood samples, taken through the catheter from the carotid artery before injection of high-molecular-weight dextran (D-500) into the rats, were of the usual shape and did not form distinct aggregates (Fig. 1). Only in some cases could formations of rouleaux type be observed, although they consisted of not more than three erythrocytes. It must be emphasized that in such cases the erythrocytes showed no visible changes and their contacts could be regarded as the simple resting of one cell on another.

Injection of the sludging agent D-500 into the animals led to marked aggregation of the erythrocytes as early as after 5 min. In blood samples taken at this time, distinct aggre-

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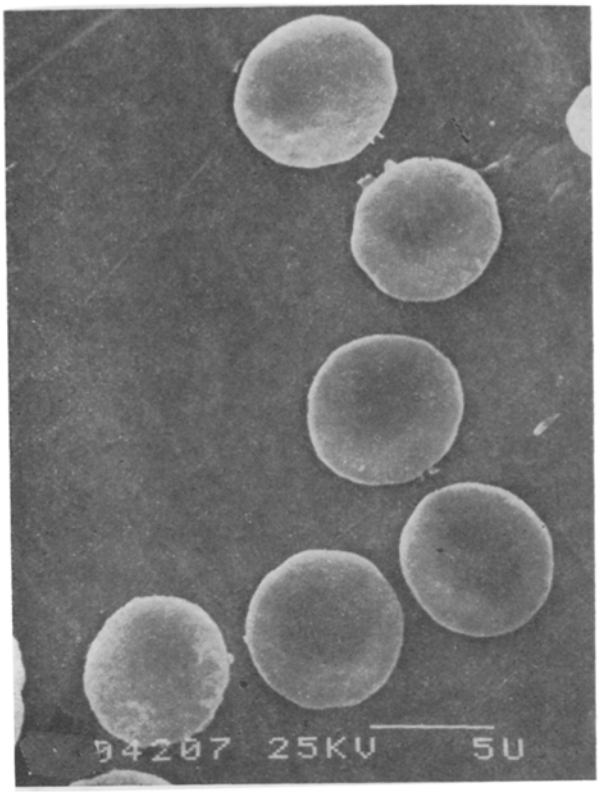


Fig. 1

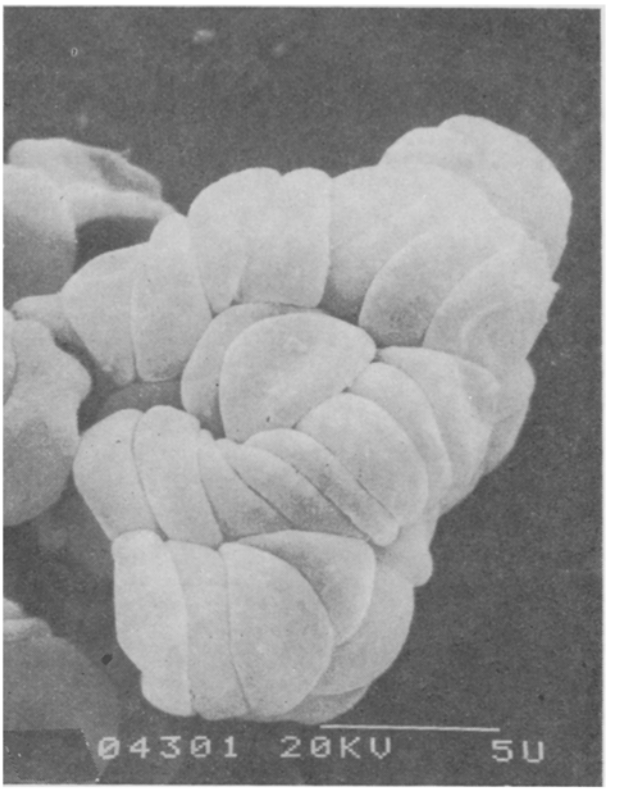
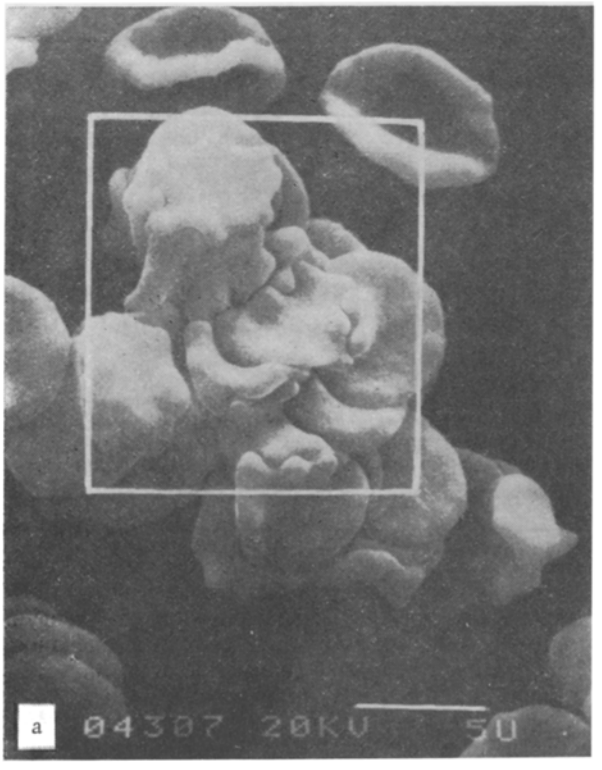


Fig. 2



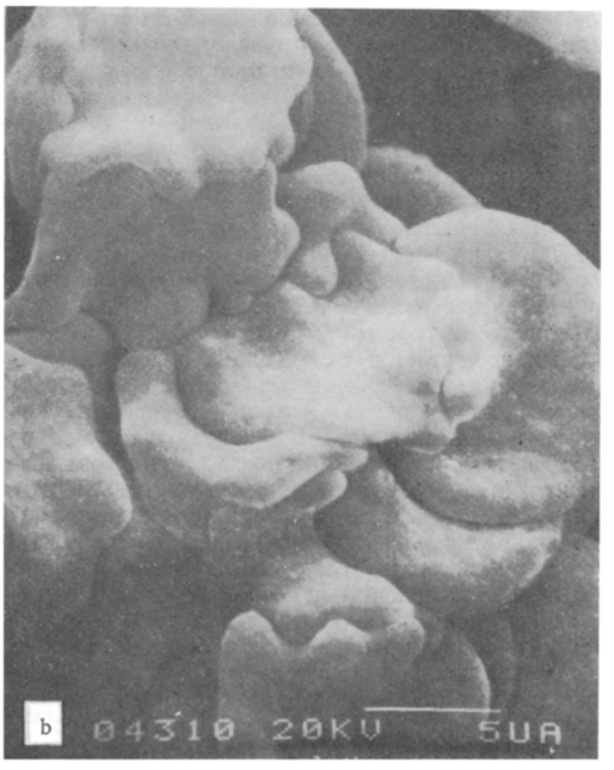


Fig. 3

gates, often circular in shape, could be seen in the scanning electron microscope. The smallest radius of these aggregates was numerically equal to the diameter of the erythrocyte. One such aggregate, consisting essentially of a comparatively long rouleau, transformed into a circular structure, can be seen in Fig. 2. It can be tentatively suggested that this transformation takes place, on the one hand, through the action of hemodynamic forces, twisting the rouleau in the flow of fluid, and on the other hand, through the increased binding strength of the erythrocyte in the rouleau, presenting fragmentation of the column in the rapid flow. It can be concluded from analysis of the type of union of the erythrocytes in these (circular) aggregates that in this case there were mainly simple contacts with no significant change in the shape of the adjoining surfaces. The general changes in shape of individual erythrocytes in these aggregates can easily be explained by the action of hemodynamic forces.

Together with the aggregates described above, with a more or less regular shape, larger aggregates with a more complex shape were frequently found (Fig. 3a). Analysis of the shape of these aggregates shows that it also is based on the formation of a rouleau, but this time it is branched and not linear (as in the first case). This shape is most easily explained by fusion of small rouleaux not only end-to-end, but also end-to-side, such as is observed, for example, during the formation of erythrocyte nets (reticulation of the flow). It will be clear that these aggregates remain intact in the rapid blood flow only on account of increased strength of binding of the aggregating cells together. This state of affairs is in harmony with the view that special contacts are formed between the erythrocytes in such aggregates. A characteristic feature of these aggregates is the presence of surface folds and projections in the region of contact between the erythrocytes, which significantly increases the total area of surfaces in contact, with a corresponding increase in the mechanical strength of the junctions (Fig. 3a, b). It is important to emphasize that the increased mechanical strength of these aggregates and their comparatively large size endow them with the properties of microemboli, formed when the balance is disturbed in the blood clotting system. However, by contrast with true emboli, all types of aggregates described above, including the strongest of them, underwent deaggregation as a result of the injection of rheopolyglucin in these experiments. Erythrocytes of the same animals as in Fig. 3, but after injection of rheopoly-

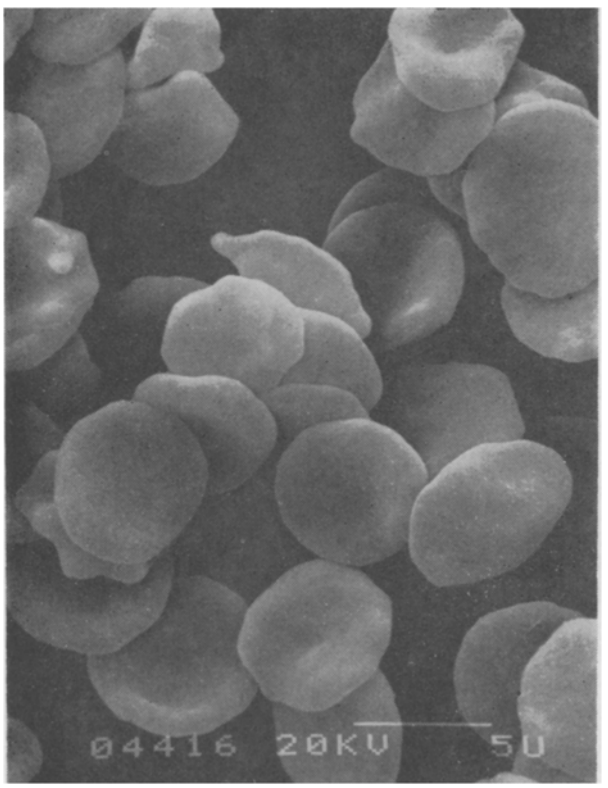


Fig. 4

glucin, are illustrated in Fig. 4. In this case distinct aggregates were not present, although the shape of the erythrocytes differs a little from the control (Fig. 1). The difference consists mainly in the presence of traces of the projections and folds seen in such a distinct form in Fig. 3b.

The results of this investigation, together with those of the writer's previous studies, using transmission electron microscopy [2] thus show that aggregation of erythrocytes is a unique process of intercellular interaction, characterized by a definite structural dynamics, especially in relation to intercellular contact. The most demonstrative feature here is the formation of surface folds and projections, increasing the total area of intererythrocytic contacts and thereby increasing the mechanical strength of the aggregates and, correspondingly, their "pathogenicity" in relation to maintenance of the normal blood microcirculation.

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HISTOCHEMICAL STUDIES OF MICROVASCULAR EFFECTORS REGULATING THE BLOOD SUPPLY TO THE CEREBRAL CORTEX

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KEY WORDS: pial arteries; adrenergic and cholinergic innervation; enzyme activity in vessel walls; regulation of the microcirculation.

Biomicroscopic studies have revealed active regions in the system of pial arteries which are effectors of regulation, whose function determines the intensity of the blood supply to microareas of the cerebral cortex and redistribution of the blood flow between them. These effectors were found to be sphincters in the branches of the pial arteries, precortical arteries, and pial arterial microanastomoses, located between terminal branches of the pial arteries [1, 2]. The object of this investigation was to study the adrenergic and cholinergic innervation and histochemical properties of smooth-muscle cells in the region of these microvascular effectors, and also in the initial segments of the radial intracerebral arteries.

EXPERIMENTAL METHOD

Experiments were carried out on 12 rabbits of both sexes weighing 2.5-3.5 kg. The animals were killed by air embolism and the brain was quickly removed from the skull. The pial vessels were studied in preparations of the pia mater which contained the whole branched system of pial arteries and veins and also fragments of intracerebral radial arteries 100-400 μ long.

Acetylcholinesterase activity was studied with the use of acetylcholine iodide as substrate and tetraisopropylphosphoramide as inhibitor of butyrylcholinesterase [4].

To obtain specific green fluorescence of catecholamines, 1.5% glyoxalic acid solution was dropped on the isolated pia mater [8]. The preparations were dried in a jet of air for 5-20 min, incubated for 5 min at 80°C, mounted in mineral oil, and examined in the Orthoflux fluorescent microscope (Leitz).

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