Acta Medica Okayama

Volume 12, *Issue* 3 1958 *Article* 1

OCTOBER 1958

Studies on the megakaryocytes, platelets separation and degeneration

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Abstract

From these findings, we confirmed that the tongue-like process formations of the cells which are still believed as the platelets formation by many investigators, would be nothing but the presentation of the cell degeneration and platelets are separated only from the tips of tentacles.

Acta Med. Okayama. 12, 187—192 (1958)

STUDIES ON THE MEGAKARYOCYTES —PLATELETS SEPARATION AND DEGENERATION—

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Received for Publication, August 25, 1958

In our previous papers[1,2] we reported that megakaryocyte showed active pseudopodial movements and tentacle formation. We also proved that platelets were separated from the tips of tentacles. After that, we have investigated the motility, platelets separation and the course of degeneration of megakaryocytes of human beings and guinea pigs in vitro under various conditions of medium.

MATERIALS AND METHODS

The bone marrow fragments of normal adults, of nonthrombocytopenic patients and of guinea pigs have been used as materials. Applying our simple culture method devised in our laboratory, namely, using serum and vitamin B_{12} as media and adjusting temperature, osmotic pressure and pH, we studied the morphological changes of megakaryocytes under these conditions. Observation has been done under the phase-contrast microscope and the phenomena were caught on 16 mm movie film which speed was one frame per 2 or 4 seconds.

RESULTS

From the fragments of human bone marrow, cultured in the manner mentioned above and kept in an incubator at 37°C, some megakaryocytes have been found migrating to growth zone already 3 hours after the beginning of culture. They started to display deformative and pseudopodial movements at 8 or 10 hours afterward. Their active motility and tentacle formations reached maximum in 18 to 30 hours, and usually about 5% of megakaryocytes and at times over 20% of them were observed bearing tentacles. In general, it was difficult to distinguish the nucleus of megakaryocyte up to 30 or 36 hours of culture. The cell was diffusely and thickly full of fine or little coarse specific granules.

The enlarged tips of tentacles were filled with fine granules and were

always changing their forms very actively. The enlarged tips of tentacles relatively larger in size protruded out longer and longer, they even reached over on occasion 300 μ in length, and these slender tentacles began to possess small nodules similar to platelets every 15 to 30 μ apart, and sometimes platelets were separated from the tips of tentacles. Numerous platelets gathered around the main part of megakaryocyte displaying very active platelets formation and diminishing itself in volume. This fact shows that some megakaryocytes produce relatively quickly many platelets. Depending on the change of temperature, the strength of light, or medium in motion, the tentacles of megakaryocytes in this medium would rapidly retract their tentacles, showing only the pseudopodia, and subsequently the cells assumed the rest form.

Megakaryocytes grew inactive in their movements and revealed various signs of degeneration gradually around 48-hour culture. As the first sign of degeneration, the specific granules in cytoplasm assumed various sizes, or packed closely together, and increased their brilliancy.

On the other hand, we could recognize small vacuoles and secretion granules inside the cell, and thus the nucleus grew gradually clearer.

Following the first sign, vacuoles in some megakaryocytes increased in their size and occupied the major part of the cell body. Some megakaryocyte gradually got irregular structures and finally collapsed itself. Moreover, some lost the specific granules, got uniform brilliancy in the whole body and turned to hyalinization. While in a number of nuclei, the more brilliant they were the more pyknotic they themselves became, and had a tendency to fuse with several nuclear lobes.

Thus, even in the same medium the process of degenerative changes was not always the same in each megakaryocyte. A few nondegenerative megakaryocytes could be seen at the time of 72-hour culture, and hardly could be found after 92 hours.

After the standard medium, we applied various kinds of single medium, Ringer's solution, serum, and physiologic saline solution, but we found no megakaryocytes showing any tentacle formation. Their degeneration was most rapid in the physiologic saline solution, followed by serum and Ringer's solution in the order mentioned.

As for the influence of temperature, at 41°C and 30°C slight deformative movements were observed but above 43°C or below 20°C scarcely no such movements could be seen. Optimal temperature was between 35°C and 39°C.

In addition, with the purpose to see the effects of osmotic pressure and pH on megakaryocytes, we added saline solution, acids, or alkalis

at different concentrations and distilled water into the medium of simple culture method.

When the hypertonic saline solution had been added, the cell looked atrophic as a whole and on the surface many wrinkles appeared. Granules in the cytoplasm increased their brightness and size, and hyalinized finally. Previous to the hyalinization of cytoplasm sometimes fine hair-like processes appeared on the surface of the cell, which vacuolized themselves and fell out into the medium.

In the case where distilled water had been added, megakaryocytes rapidly inflated themselves and pushed out numerous small structureless spheroid or tongue-like processes which actively appeared and disappeared, and it seemed as if platelets having formed explosively from megakaryocytes. But then, these processes appearing like bubbles increased their own volume. When huge blebs covered the whole body, specific granules of cytoplasm moved into them. And when they ruptured, the cell itself collapsed at the same moment. On the other hand, the nucleus increased its volume and caused chromatolysis, then being led to pycnosis and necrosis, it disappeared by lysis in the end. In the case where relatively larger volume of distilled water had been added into the medium, the nucleus turned to hydrops and switched itself over to karyolysis.

N/50- to N/100-hydrochloric acid or caustic soda being added, the same phenomena as in the case of distilled water were observed.

These tongue-like processes were often seen on the megakaryocytes under pressure or in the medium in motion.

SUMMARY

From these findings, we confirmed that the tongue-like process formations of the cells which are still believed as the platelets formation by many investigators,[3,4,5] would be nothing but the presentation of the cell degeneration and platelets are separated only from the tips of tentacles.

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EXPLANATION FOR FIGURES

Fig. 1. Simple culture method devised in our laboratory.
Fig. 2. Schema of platelets separation.
Fig. 3. So-called platelets formation.
Fig. 4. Normal adult megakaryocyte showing tentacle formation (600×, DLL).
Fig. 5. Normal adult megakaryocyte showing pseudopodial movement (1000×, DLL).
Fig. 6. Resting non-degenerative megakaryocyte (1000×, DLL).
Fig. 7. Slightly degenerative megakaryocyte, showing specific granules in various sizes and with increased brilliancy (1000×, DLL).
Fig. 8. Degenerative megakaryocyte containing many small vacuoles (1000×, DLL).
Fig. 9. Vacuolar degeneration of megakaryocyte (1000×, DLL).
Fig. 10. Degenerative megakaryocyte showing small tongue-like processes (1000×, DLL).
Fig. 11. Degenerative megakaryocyte showing large blister formation (1000×, DLL).
Fig. 12. Megakaryocyte showing the beginning of collapse (1000×, DLL).

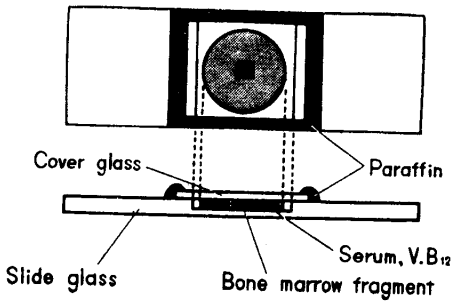

Fig. 1

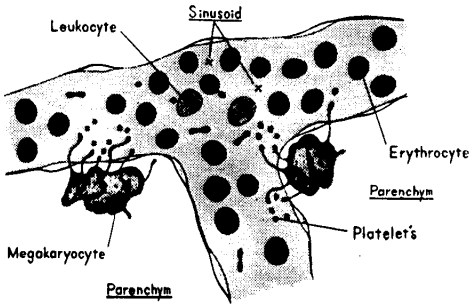

Fig. 2

Fig. 3

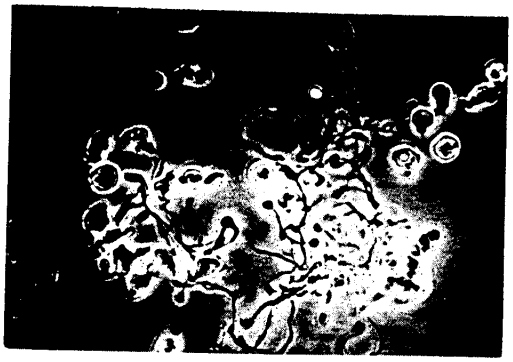

Fig. 4

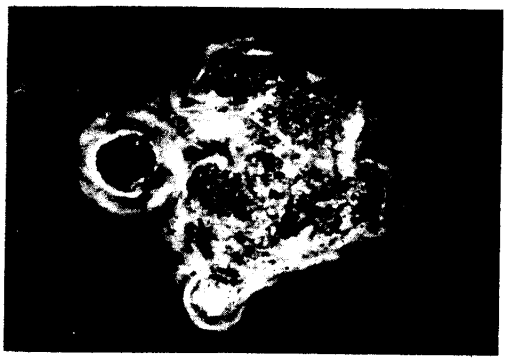

Fig. 5

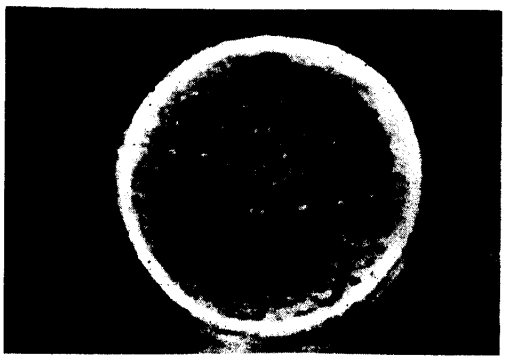

Fig. 6

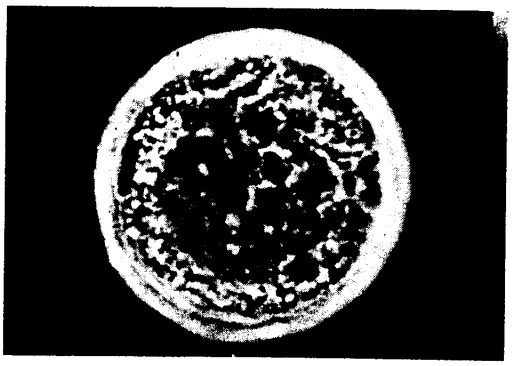

Fig. 7

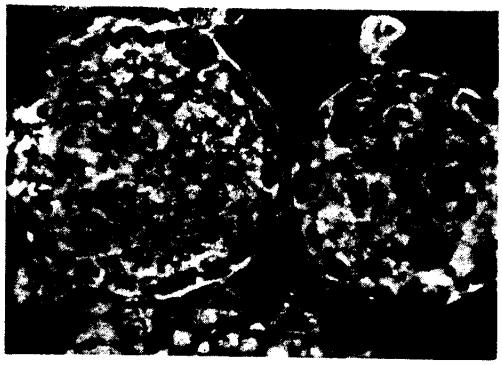

Fig. 8

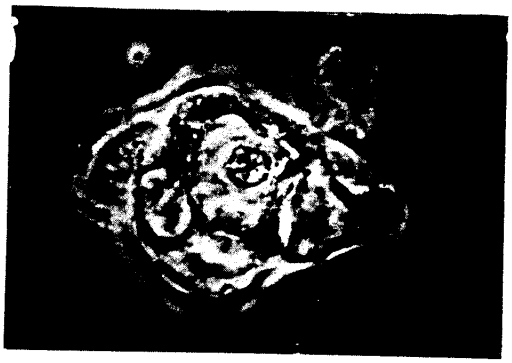

Fig. 9

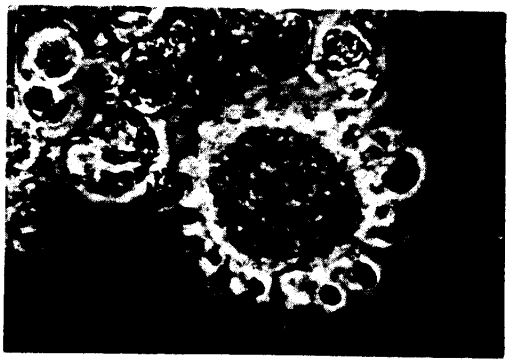

Fig. 10

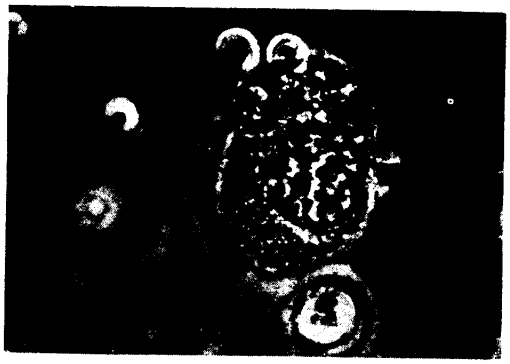

Fig. 11

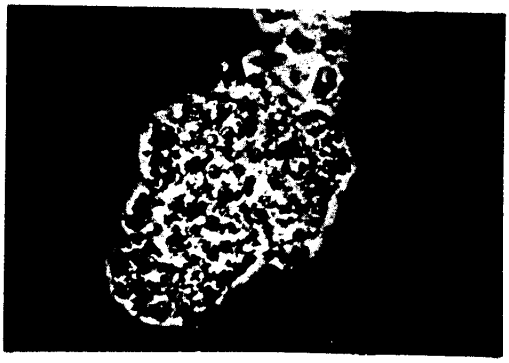

Fig. 12