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Abstract

In 1955 SANO found mitochondria by the supravital stain with Janus green B in the basophilic stippled cells from the circulating blood of the lead intoxicated rabbitsl , and in 1956 by means of electronmicroscope VALLEJO-FREIRE, BRUNNER et al. found mitochondria in the reticulocytes2,3, and later at the end of 1956 BRAUNSTEINER et al. also succeeded in revealing mitochondria and the vesicular structure by electron microscope in the ultra thin section of young red cells4. We also have found the mitochondria and the endoplasmic reticulum in young red cells. It has been discussed long whether the reticulum of reticulocytes is a preexistent structure or an artifact. The fact that the mitochondria exist in the reticulocyte seems to support strongly the preexistence theory of the reticulum, substantia reticulo filamentosa. However, the fact that the reticulum has several characteristics different from the general mitochondria5,6 can not be ignored. In this paper we should like to demonstrate the photos of mitochondria and the endoplasmic reticulum in the denucleated red cells revealed by electron microscope comparing to the picture of reticuluocyte appeared by supravital stain.

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MITOCHONDRIA AND ENDOPLASMIC RETICULUM IN THE DENUCLEATED RED CELLS, WITH SPECIAL REFERENCE TO THE RETICULUM OF RETICULOCYTE

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In 1955 SANO found mitochondria by the supravital stain with Janus green B in the basophilic stippled cells from the circulating blood of the lead intoxicated rabbits[1], and in 1956 by means of electronmicroscope VALLEJO-FREIRE, BRUNNER et al. found mitochondria in the reticulocytes[2, 3], and later at the end of 1956 BRAUNSTEINER et al. also succeeded in revealing mitochondria and the vesicular structure by electron microscope in the ultra thin section of young red cells[4]. We also have found the mitochondria and the endoplasmic reticulum in young red cells. It has been discussed long whether the reticulum of reticulocytes is a preexistent structure or an artifact. The fact that the mitochondria exist in the reticulocyte seems to support strongly the preexistence theory of the reticulum, substantia reticulo filamentosa. However, the fact that the reticulum has several characteristics different from the general mitochondria[5, 6] can not be ignored. In this paper we should like to demonstrate the photos of mitochondria and the endoplasmic reticulum in the denucleated red cells revealed by electron microscope comparing to the picture of reticuluocyte appeared by supravital stain.

Materials and Methods

Cells : — Young red cells of the bone marrow and the circulating blood from the anemic rabbits served as materials. Anemia was caused by the repeated blood drawing by the heart punction, about 20 cc. once a day for 3 to 4 days, and the materials were obtained at the reticulocyte crisis appearing a few days after the last depletion.

Specimen for the electron microscope and the optic microscope : — A small piece of bone marrow aspirated from femoral marrow or a drop of

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venous blood were fixed with 1% buffered osmic acid, embedded in the mixture of methyl- and n-buthyl-methacrylate conventionally and sectioned by using ultrathin section apparatus of Shimazu Co. The thin section of about 150 to 300 Å were observed on collodion film under the microscope of Hidachi Co. For the observation of reticulum by the optic microscope the blood was smeared after the supravital stain on the dye film of Nile blue, neutral red or brilliant cresyl blue, fixed with methanol and stained with Giemsa. Or the smear without supravital stain was dried in ether gas, fixed with methanol and stained with Giemsa. Another sample of blood was stained supravitally mixing with 0.5% Nile blue saline solution and hemolyzed, washing with distilled water by the repeated centrifugation. The cell suspension obtained by decanting the superntant was mounted on the collodion film, dried and removed the dye washing with methanol and observed under the electron microscope.

Observations and Results

As is demonstrated in Fig. 1, in the cytoplasm of erythroblast there are seen cut surfaces of several mitochondria appearing as gross oval or elongated vesicules having doubly layered cristae. Besides these a number of small vesicular structures of scanty in contents are also recognizable. As is generally accepted these scanty vesicular structures are the cytoplasmic basophilia or the endoplasmic reticulum (PORTER). These organellae, both mitochondria and endoplasmic reticulum, are also seen in some of the denucleated red cells (Figs. 1, 2 and 3). The mitochondria in the denucleated red cells appear oval or round in shape and some of them are scanty in contents which will be the picture of decomposition as pointed out by BRAUNSTEINER et al.[4] The round or oval shape should be the cut surface of elongated mitochondria as has been demonstrated by BRUNNER et al. in the cells fixed with formol. The small vesicular endoplasmic reticulum should be also the cut surface of the elongated structure of the endoplasmic reticulum. These cells containing the mitochondria and the endoplasmic reticulum are large in shape and less in hemoglobin contents comparing to the matured red cells. Therefore, these cells should be the young reticulocytes. The morphologic picture of the reticulum appearing by supravital stain, however, is quite differtent from that of mitochondria as demonstrated by VALLEJO-FREIRE on the reticulocytes fixed with formol by electron microscope. The reticulum is irregular in shape and changes its morphologic picture according to the dyes used for the supravital stain as is seen in Figs. 5, 6, 8, 9 and 10. Moreover by drying the smear

in ether gas the reticulum appears as a fine granular substance (Fig. 11)[7]. This granular reticulum is generally seen on the reticulocytes supravitally stained only for a short time mixing with the brillant cresyl blue sailine solution, smeared, dried quickly and stained with Giemsa. The picture of the reticulum demonstrated by electron microscope on the reticulocytes stained supravitally with Nile blue, hemolyzed and removed the dye is rather similar to that of mitochondria (Fig. 7), however, it is irregular in shape suggesting the twining of some substances around the mitochondria.

Discussions

In the previous paper SENO and the collaborators asserted that the reticulum of reticulocytes shoud be an artifact but not a pre-existent structure on the basis of a number of cytological and cytochemical data[5, 6, 8, 9]. However, it has been clearly demonstrated that there exist mitochondria in the denucleated red cells which has been long believed to have no mitochondria. These cells, most of them at least, should be the reticulocytes. This fact seems to support the preexistence theory of reticulum which has been claimed by many authors. BRAUNSTEINER and others also concur the opinion that the reticulum is mitochondria itself[4]. However, the morphologic picture of reticulum is very different from that of mitochondria and yet the reticulum changes its picture according to the dye used for supravital stain or according to the staining period even in the case using the same dye. Furthermore, the reticulum appeared by supravital stain has several characteristics which have never been seen on the general mitochondria. For instance, the reticulum contains a quantity of ribonucleic acid showing a strong basophilicity, which disappears by affecting ribonuclease for a short period, and absorbing the ultra violet ray of 2650 Å[5, 6, 10]. Moreover, without supravital stain or the treatment with dehydrating or RNA-precipitating agents the reticulum does never appear on the reticulocytes. These characteristics are all of the cytoplasmic basophila as has been elucidated on the basophilic cytoplasm of various cells[7]. Then it should be said that the reticulum of reticulocytes stained by basic dyes in the fixed cells after the supravital stain is the coagulated endoplasmic reticulum, a different substance from mitochondria, especially concerning the granular reticulum appearing after affecting the basic dye for a short period or affecting ether gas. The reticulum seen in wet preparations by supravital stain will be the configurated structure of the mitochondria and the coagulated endoplasmic reticulum, because the lipophilic basic dyes as used generally for the supravital stain-

ing stains mitochondria as well as the endoplasmic reticulum, which contains a quantity of lipids, resulting in the coagulation of the latter. The picture of the reticulum demonstrated by electron microscope on the reticulocytes stained supravitally indicates that the reticulum can not possibly be the mitochondria itself. The reticulum should be formed by the entangling of the coagulated endoplasmic reticulum around the mitochondria.

Summary

1) The existence of mitochondria and the endoplasmic reticulum has been elucidated by electrion microscope on the thin section of the denucleated red cells as well as in the erythroblast.

2) The correlation between mitochondria and the reticulum of reticulocyte has been discussed referring the morphologic structure of reticulum and the former reports of SENO and collaborators.

3) The conclusion is that the reticulum in the reticulocyte should be an artifact formed by the coagulation of cytoplasmic basophilia entangling around the mitochondria.

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Explanation of Figures

Fig. 1. Three erythroblasts and two reticulocytes. In the cytoplasm of erythroblasts and in the reticulocytes there appear several mitochondria (M) having cristae or containing none. Besides these there are several vesicular structures, the endoplasmic reticulum (E). One reticulocyte is very small, probably being cut tangentially at the extremity. The nucleal membrane of one erythroblast is largely broken resulting in the mixing of the contents of nucleus with those of cytoplasm. This might be a stage of the denucleation process.

Fig. 2. An erythroblast (Ebl.). Several mitochondria (M) and numerous endoplasmic reticulum (E) are seen in the cytoplasm.

Fig. 3. A transversal section of a reticulocyte (Ret.) having a mitochondria (M). Ebl. is a neighbouring erythroblast.

Fig. 4. A reticulocyte. This one is cut rather thick. In this cell the endoplasmic reticulum appeared as a fairly elongated structure, but no mitochondria appeared.

Fig. 5. Reticulocytes stained supravitally on the Nile blue film. A wet preparation.

Fig. 6. A reticuiocyte stained with Giemsa after supravital stain with Nile blue. The reticulum is rather fibrous, though granular in a part of the structure.

Fig. 7. The electron-microscopic picture of reticulum stained supravitally with Nile blue, hemolyzed, and removed the dye. The structure in the upper part resembles to that of mitochondria but the reticulum appearing in the lower part shows entirely different picture from that of mitochondria comparing to those demonstrated by Vallejo-Freire on the fixed cells with formol.

Fig. 8. Reticulocytes stained supravitally on the neutral red film. A wet preparation.

Fig. 9. The same cells as those in Fig. 10 stained with Giemsa after smearing and fixing with methanol. The reticulum is rather grossly granular and far different from the picture of mitochondria.

Fig. 10. The reticulocytes stained supravitally on the film of brilliant cresyl blue. A wet preparation.

Fig. 11. The reticulocytes exposed to ether gas for 30 seconds in wet, fixed with methanol and stained with Giemsa. The reticulum appeared as fine granular substance.

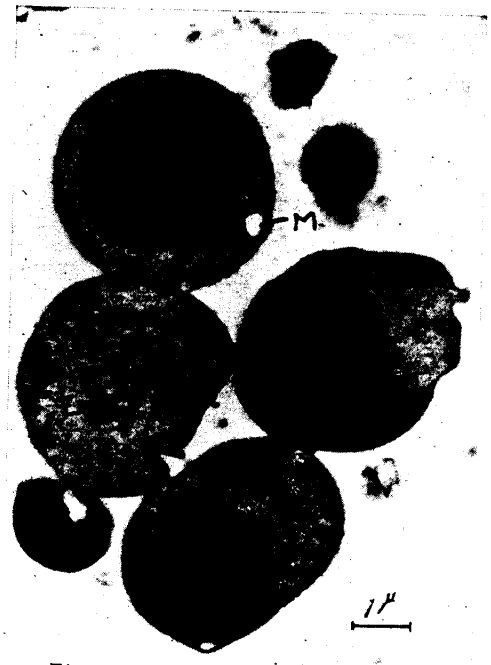

Fig. 1

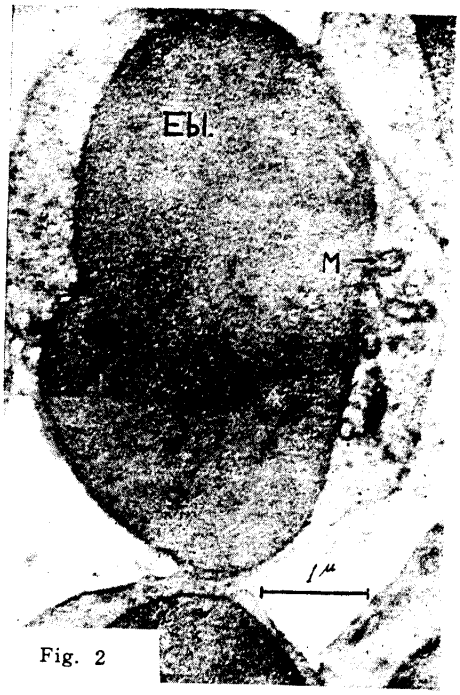

Fig. 2

Fig. 3

Fig. 4

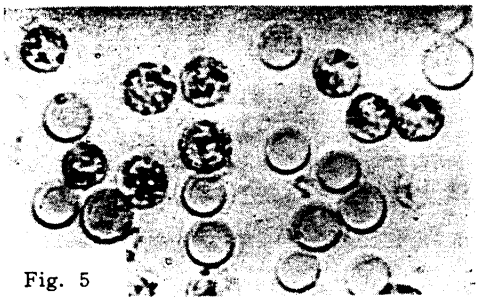

Fig. 5

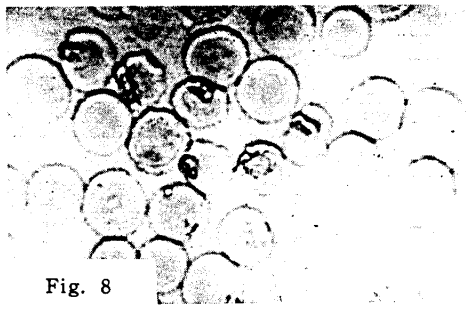

Fig. 8

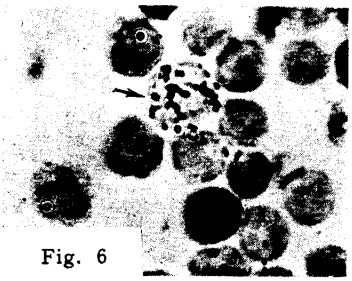

Fig. 6

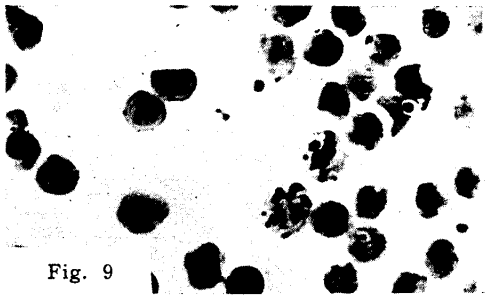

Fig. 9

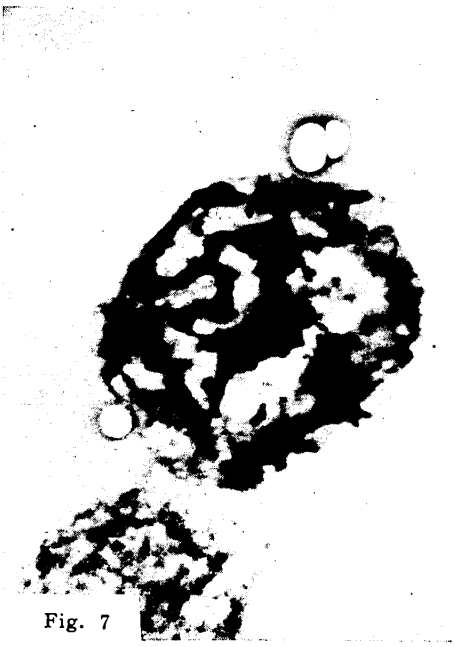

Fig. 7

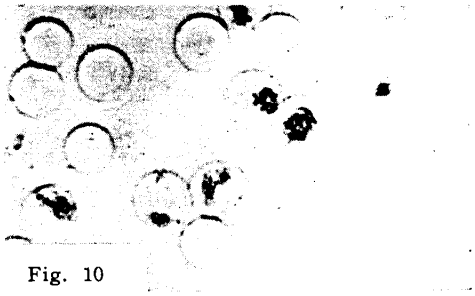

Fig. 10

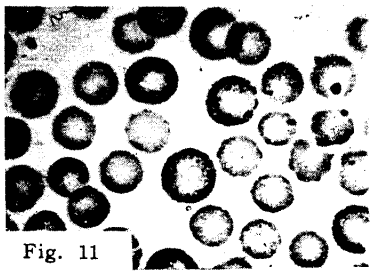

Fig. 11