16095

Stratification of the Erythrocytes of Man by Ultracentrifuging.*

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The shape and structure of the mammalian erythrocytes have attracted the attention of many investigators.[1-4] The chief problems involved seem to center around a possible explanation for the bioconcave form assumed by the erythrocytes when floating freely in the plasma, and on efforts to deduce an architecture suitable to account for the function and physiological behavior of these cells.

Beams and Hines[5] found that it was possible to stratify the rat erythrocytes by ultracentrifugation. Similar studies dealing with the physical properties of the erythrocytes of man have been made and are herewith reported.

Material and Methods. Freshly drawn blood from the finger of an adult man was placed in the rotor of the ultracentrifuge which was subsequently sealed. After standing until the blood had clotted the centrifuge was started and operated at a pressure which delivered a centrifugal force of approximately 400,000 times gravity. After centrifuging for 20 to 30 minutes the blood was fixed in Champy's fluid for 12 hours. Paraffin sections were made of the clot which contained many erythrocytes in its meshes. These sections were bleached in potassium permanganate and oxalic acid and subsequently stained in Heidenhain's hematoxylin or Mallory's triple stain. Several other staining procedures were also tried, including supravital methods.

Results. The cells in Fig. 1 and 2 have been stained in Heidenhain's hematoxylin. They are slightly elongated and their typical biconcave appearance seems to have been altered. The centrifugal halves of the cells stain sharply with the hematoxylin while the centripetal portion remains unstained. It is evident that the erythrocytes possess a membrane which is capable of resisting considerable mechanical distortion. Fig. 3 and 4 show erythrocytes in profile that have been stained in Heidenhain's hematoxylin. The hematoxylin-stained material has been concentrated at the centrifugal end of the cells without greatly distorting their biconcave appearance. Because these cells were free to shift their positions relative to the centrifugal field during the process of removal from the centrifuge, they are not all oriented in the same direction in the photographs.

In Fig. 5 the cells were stained in Heidenhain's hematoxylin and acid fuchsin. In the isolated cells of the lower half of Fig. 5 separate layers may be observed: a hematoxylin-staining layer at the centrifugal end, a non-staining area in the middle, and an acid fuchsin staining portion at the centripetal end. Similarly, 3 distinct layers may be observed following staining in Mallory's triple stain (Fig. 6). Here the centrifugal portion of the cells is stained orange, the middle red, and the centripetal end a light blue. The fact that they are more or less selectively stained by different dyes indicates that they are of a different chemical composition.

Discussion. The erythrocytes seem to contain at least three distinct substances which differ in their relative specific gravities and staining reactions. However, the chemical composition of these substances is at present unknown. Supravital preparations did not reveal clear evidence of stratification, although it was previously observed for the erythrocytes of the rat that the hemoglobin in some

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1 Winthrobe, M. W., *Medicine*, 1930, **9**, 195.

2 Krumbhaar, E. B., *Special Cytology*, Paul B. Hoeber, New York, 1932, **2**, 555.

3 Ponder, E., *Protoplasma Monographien*, Berlin, 1934, **6**, 66.

4 Isaacs, R., *Handbook of Hematology*, Paul B. Hoeber, 1938, **1**, 5.

5 Beams, H. W., and Hines, E. B., *Anat. Rec.*, 1944, **90**, 155.

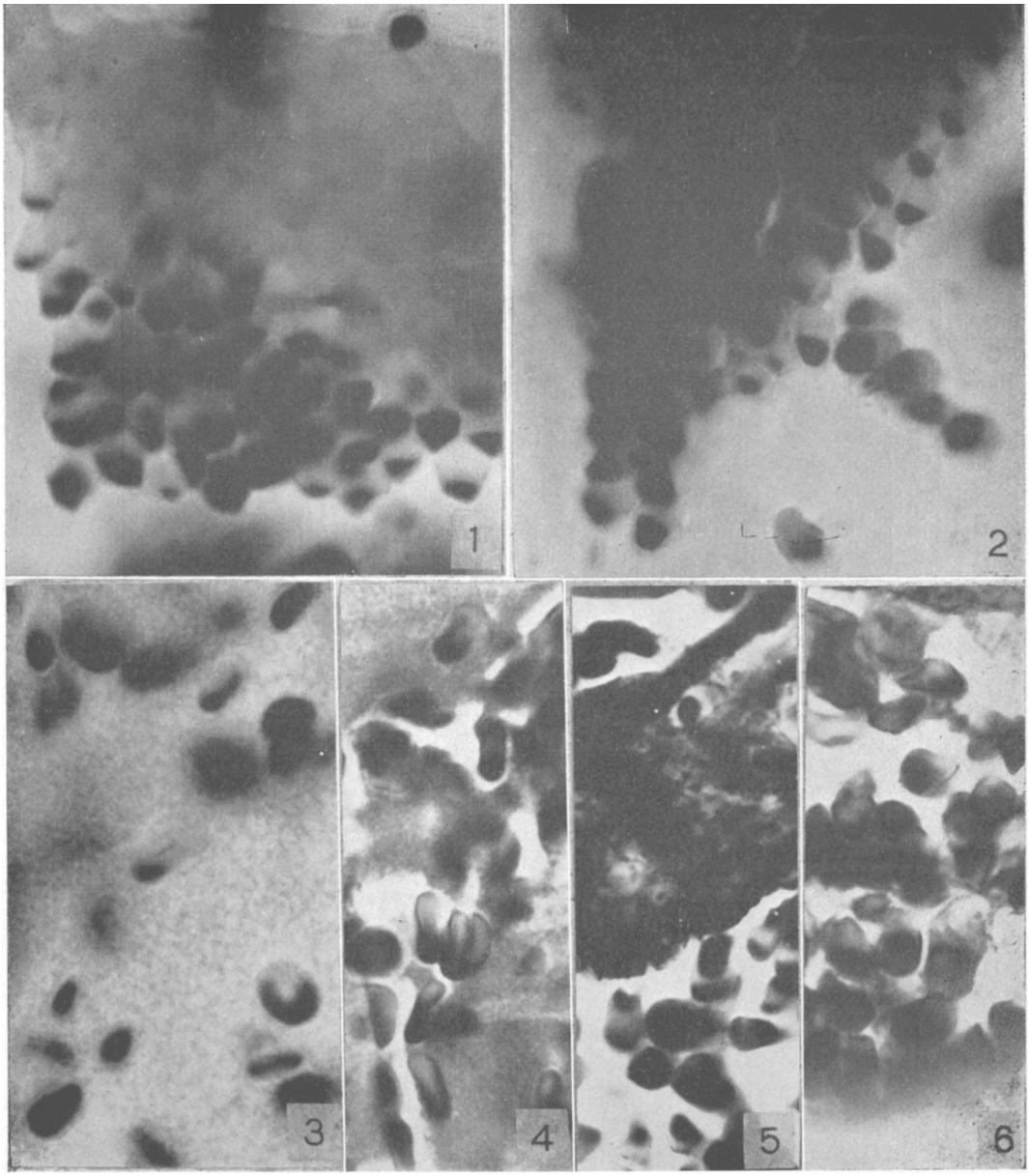

FIG. 1-6.

Centrifugal ends of cells for the most part directed toward the bottom of plate. All cells magnified approximately 1250 ×. 1 and 2. Stratified erythrocytes, hematoxylin stained. 3 and 4. Similarly treated cells in profile, normal shape evident. 5. Hematoxylin and acid fuchsin stained, 3 layers present. 6. Mallory's stained, three layers evident.

of the cells was displaced. The absence of a sharply visible stratification in the living unstained erythrocytes is not surprising because no internal structure can be seen in the normal cells with direct illumination, dark field, or with ultraviolet light.[3] Although the appearance of the stratified layers may be somewhat altered by different staining procedures, it is not likely that they represent artifacts any more than do stained structures of other cells treated in a similar way. No evidence of a stained reticulum was observed

in these studies, although we have observed it in normal uncentrifuged erythrocytes treated by other methods.[6]

Summary. Three distinct substances which differ in their relative specific gravities have been stratified by high centrifugal force in the erythrocytes of man. These substances seem to differ chemically for they may be differentially stained by certain dyes. The stretching of certain of the erythrocytes by centrifugal force indicates that they possess a membrane of considerable elasticity.

[6] Jordan, H. E., Kindred, J. E., and Beams, H. W., *Anat. Rec.*, 1930, **46**, 139.

16096

Influence of Epinephrine on Vasoconstrictor Action of Kidney Extracts.*

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Perfusion of rabbit's ears with saline extracts of minced kidney is usually associated with vasoconstriction. Minute amounts of histamine or histamine-like compounds present in the minced organ are thought to be responsible for this effect. When these are removed by dialysis, acetone-ether treatment and other methods involved in fractionation of the crude preparation, the vasoconstrictive action as determined with the rabbit's ear or the dog's tail, is lost.

It is reported, further, that the vasoconstrictive action of some kidney fractions as demonstrated by intravenous injection in the living animal may be absent when tested by the perfusion method.[1,2,3] Important conclusions, it will be recalled, have been drawn from this discrepancy as determined by the two methods of testing the same extract.[2,3] Little if any attention on the other hand has been given the reports of Schaumann,[4] Burn and Tainter[5] and more recently of Morton and Tainter.[6] These authors observed that various sympathomimetic amines are vaso-inactive when perfused through isolated peripheral blood vessels. When, however, subthreshold amounts of epinephrine are added to the perfusate, strong vasoconstriction follows.

In the light of these findings the vasoactivity of various kidney extracts has been re-examined.

As test object the vessels of the isolated rabbit's ear were selected. This is a technique devised by Pisemski and Krawkow,[7] modified by Schlossman,[8] Kahlson and von Werz,[9] and further by Brandt and Katz.[10] More recently it has been extensively employed by Page[11] who incorporated pulsating pressure. It is the general opinion that this method adequately permits measurement of vasoconstrictive substances in perfusion fluids.

Materials and Methods. In general, the experiments followed the technique described by Landis[12] and by Morton and Tainter.[6] A constant, non-pulsating perfusing pressure

* Aided by a grant from the Commonwealth Fund.

[1] Hessel, G., *Klin. Wchnschr.*, 1938, **17**, 843.

[2] Braun-Menendez *et al.*, *Renal Hypertension*, translated by Dexter, L., Chas. C. Thomas, Springfield, Ill., 1946.

[3] Page, T. H., and Helmer, P. M., *J. Exp. Med.*, 1940, **71**, 29, 495.

[4] Schaumann, O., *Arch. Path. Pharmacol.*, 1928, **138**, 208.

[5] Burn, J. H., and Tainter, M. L., *J. Physiol.*, 1931, **71**, 169.

[6] Morton, M. C., and Tainter, M. L., *J. Physiol.*, 1940, **98**, 263.

[7] Krawkow, N. P. *et al.*, *Z. f. die ges Exp. Med.*, 1922, **27**, 127.

[8] Schlossman, E., *Arch. f. Exp. Path. u. Pharmacol.*, 1927, **121**, 160.

[9] Kahlson, G., and von Werz, R., *ibid.*, 1930, **148**, 173.

[10] Brandt, G., and Katz, G., *Z. f. Klin. Med.*, 1933, **123**, 23.

[11] Page, T. H., *Am. Heart J.*, 1942, **23**, 336.

[12] Landis, E. M., *et al.*, *Am. J. Physiol.*, 1943, **139**, 26.