author has employed a loose form of statement—for instance, he speaks of "physiological sensations" (p. 162), and he employs the term color throughout in an equivocal and confusing fashion, sometimes referring to color-stimulus and sometimes to color-sensation; a few inaccuracies of statement are also to be found, of which perhaps the most serious is the assertion that the extreme peripheral region of the retina is totally color-blind (p. 71; p. 258). Although the book will be of doubtful service to elementary students, it may safely be recommended to more advanced workers as a supplement to the earlier and more critical summaries by Mrs. Ladd-Franklin and others in Baldwin's "Dictionary," and by Rivers in Schäfer's "Text-Book of Physiology."

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SPECIAL ARTICLES

THE MAMMALIAN ERYTHROCYTE—A BICONCAVE DISC[1]

The existence of "bell"- or "cup"-shaped red corpuscles in mammalian blood has been recorded frequently since the early observations of Leeuwenhoek (1719).[2] The serious proposal that the cup, and not the classic biconcave disc, is to be considered normal is, however, a comparatively recent teaching which has been received with considerable skepticism. Since these concavo-convex corpuscles may be found in drawn blood, in fixed tissues, and even in circulating blood, the issue obviously hinges on the determination of which is the normal and which the derived form—one or the other representing a modification.

1. *Examination of Drawn Blood.*—It has been claimed (Weidenreich, '02,[3] et seq.; Lewis '04)[4] that drawn blood examined immediately on a warm slide is favorable for the demonstration of cups. According to this view, the assumption of the familiar disc-shape depends on an almost instantaneous change due to the evaporation and concentration of plasma before the preparation can be made and examined.

That the disc-form is normal has been asserted by Jordan ('15)[5] working with blood, diluted with physiological solutions, in culture slides, and by Löhner ('10)[6] who employed a cabinet of sufficient size to contain a microscope and to permit the free use of his hands, introduced through appropriate openings. Within this apparatus, heated to 38° C. and saturated with moisture, blood was drawn from the finger and examined. Löhner reports that the blood corpuscles were "stets und ausschliesslich" biconcave discs.

In ordinary warm slide- and cover-preparations, made as quickly as possible, I have observed a few cups only, but have never followed the transformation of these into discs as the newer hypothesis suggests. The momentary exposure to air necessitated in making ordinary preparations may be practically eliminated by utilizing the following method. Superimposed cover glasses, separated by a hair, are fused at one point by heat; if an edge be now applied to a needle prick in the finger, and the finger squeezed, the issuing blood is drawn in by capillarity. Such preparations, examined quickly, have never yielded evidence for the general existence of the cup-shape. A few cups may usually be found, whereas scores of indubitable discs appear.

Since the experiments of Ranvier, in 1875,[7] it has been known that graded temperatures can alter disc-shaped corpuscles to shallow cups, thick-walled cups or even to spheres—*e. g.*, typical cups are found exclusively when blood is warmed to 55° C. (Zoth).[8] Is it possible that some investigators, who advocate the

[1] From the Anatomical Laboratory of the Northwestern University Medical School, Contribution No. 43, July 2, 1916.

[2] Leeuwenhoek, A., "Epistolæ physiologicæ," epistola 44, 1719.

[3] Weidenreich, F., *Arch. f. mik. Anat.*, Bd. 61, pp. 459–507, 1902.

[4] Lewis, F. T., *Jour. Med. Research*, Vol. 10 (N. S., 5), pp. 513–517, 1904.

[5] Jordan, H. E., *Proc. Soc. Exp. Biol. and Med.*, Vol. 12, No. 7, pp. 167–169, 1915.

[6] Löhner, L., *Arch. f. gesam. Physiol.*, Bd. 131, pp. 408–424, 1910.

[7] Ranvier, L., "Traité technique d'Histologie," 1st ed., Paris, 1875.

[8] Zoth. Vide Löhner, *op. cit.*

cup shape, have heated unduly their slides and covers in overzealous attempts to maintain normal(!) conditions?

Experimentation in which various "physiological solutions" are used for the dilution of blood may ever, though perhaps unjustly, be subjected to criticism. At best these are artificial media, the tonicity and colloidal constitution of which may or may not simulate blood plasma.

To preclude such criticism natural serum must be used. Accordingly, I had 20 c.c. of blood drawn from my basilic vein. This was defibrinated by whipping and centrifuged quickly; thus an examining medium was obtained, identical with blood plasma except for the loss of one of its minor protein constituents —fibrin.

By utilizing an electrically heated warm-stage, hollow-centered life slides, cover glasses, as well as the air of the cell itself, may be maintained constantly at body temperature. A drop of serum was placed on a finger, previously cleaned with alcohol, and the finger pricked through the drop. The droplet of blood, thus diluted, was touched to a cover and suspended, as a hanging drop in the life cell. Vaseline served to seal the cell, the air in which could be kept saturated with moisture by introducing previously a drop of water and sealing. A few seconds only are required to make such preparations; if a large drop of serum be used the loss by evaporation prior to sealing is inconsiderable, whereas further evaporation in the cell can not occur.

A microscopic examination of blood prepared according to this technique reveals numerous isolated corpuscles. A favorable place for scrutiny is near the center of the drop. Here sinking corpuscles revolve slowly, showing alternately their concave faces. *Usually a few cups can be found, whereas quantities of discs are seen in every field.* This experiment may be varied by filling shallow concave slides with serum into which the drop of diluted blood is introduced. Evaporation is prevented by sealing with a cover and vaseline.

Human sera, kindly furnished by three of my colleagues, gave results identical with those already described, both when corpuscles were examined in their own serum and in each of the other sera. Similar tests have also been made with .85 per cent. and .9 per cent. saline, and with Tyrode's solution. More cogent proof concerning the primary shape of the mammalian erythrocyte, to be derived from the study of drawn blood, I can not imagine.

Various dilutions of human serum with distilled water were next prepared. When a droplet of blood is mixed with a drop of diluted serum containing ca. 40 per cent. water and examined as before, typical cups are found almost exclusively; in dilutions containing ca. 65 per cent. water deeply dimpled spheroids appear; perfect spheres result when the water content is ca. 70 per cent. In concentrated serum erythrocytes crenate. It is evident, therefore, that the shape of a corpuscle is, at least in part, a function of the concentration, *i. e.*, the osmotic pressure, of its medium. In progressively hypotonic solutions corpuscles imbibe increasing amounts of water, ultimately becoming spheres and laking. In hypertonic media, water is given up and crenation results. It is interesting to note that between wide limits these form changes are repeatedly reversible—for example, crenated corpuscles may be restored to the disc- or cup-shape and then recrenated.

The importance of these dilution phenomena on the question of the normal shape of erythrocytes seems to me paramount. *Since the form of a corpuscle depends on the concentration of its medium, how can the cup-shape be normal when human serum must be diluted one third to produce this type?*

Experimentation with the serum of cats and dogs has given comparable results, both with their own and with human corpuscles. The rat, guinea-pig and rabbit have afforded variable pictures, which I believe indicate that the rodent's blood plasma may possess individual variability in its tonicity, thereby rendering this group of animals unfitted for experimentation of this kind.[9]

[9] Details will be given in a later contribution of which present paper constitutes a preliminary note.

2. *Observations on Circulating Blood.*—Weidenreich reported having observed cup-shaped corpuscles in the mesentery of the rabbit ('02) and in the wing of the dormant bat ('03).[10] Lewis ('04) drew similar conclusions from a study of the guinea-pig's omentum, whereas Triolo ('05)[11] recorded finding complete spheres in this animal. Jolly ('05 et seq.),[12] working on the wing of bats restored from hibernation, and Schäfer ('12)[13] on certain mammals (sp. ?) maintain that discs occur. Jordan ('09)[14] found both types, in approximately equal numbers, in the cat.

To avoid the pressure on the vessels caused by the ordinary use of a cover glass and an oil immersion objective, I employed Tyrode's solution (without a cover glass) as in the water-immersion objective of former days. A Leitz no. 4 dry objective and a no. 12 compensating ocular, with the draw tube set at 190 mm. also gave satisfactory magnification.

The omenta of 8 cats and 2 dogs were studied for periods of from 1 to 4 hours. The animals used were in a state of deep surgical shock, the anesthetic having been stopped 2 to 4 hours previously. Regions of the omentum where temporary stasis has caused corpuscles to adhere in clumps or agglutinated masses I do not consider favorable. Ordinary circulation is much too rapid to enable one to make accurate observations. It is sometimes possible, however, to find a bifurcation of medium sized vessels in which the rapid flow selects one limb almost exclusively, separate corpuscles, nevertheless, being intermittently "kicked off" into the slowly moving plasma of the other limb. Such a situation, where the flow is rapid and normal (to find which has sometimes necessitated an hour or more of diligent search) I regard as most favorable for study.[15] Criticisms of pressure from the microscope and of observing capillaries so small that the corpuscles must adjust themselves to their exiguous confines are obviated.

Erythrocytes emerging from the main stream in the way indicated were found to be almost exclusively discs; most of these corpuscles are revolving when first seen and it is easy to be certain of their biconcavity. In such situations I have observed, and have shown to my colleagues, hundreds of discs with only an occasional cup- or saucer-form.

In *anesthetized* guinea-pigs and rabbits cups were very common, and in a dog, under ether anesthesia, a great preponderance of cup-shapes were observed. Is the anesthetic responsible for the cup-shape? The following experiment is highly suggestive. A hanging drop preparation of human blood, or of the blood of a cat or dog, diluted with serum is made. If a drop of ether or chloroform be introduced into the bottom of the cell, the drop takes on the vapor and the discs are seen to change rapidly first to shallow cups, then to deep cups and spheres.

I believe that my observations indicate that the erythrocytes of normal circulating mammalian blood are biconcave discs, the burden of proof resting on those who have used anesthetized animals to show that the anesthetic held in the blood is not responsible for the preponderance of cups observed.

3. *Action of Fixitives.*—Many workers have recorded that mammalian tissues, preserved in various standard fixatives, contain cup-shaped erythrocytes. Should great weight, however, be given evidence of this sort? These corpuscles are plastic structures of extreme delicacy, mere contact with adjacent corpuscles or with obstacles sufficing, when gentle streaming is induced, to cause excessive and varied temporary distortions. Fixation is essentially a coagulation process and it has been shown that the so-called best fixatives actually diminish the diameter of the corpuscle. If, therefore, the reagent does not act on all sides of an erythrocyte simultaneously is not a buckling of the side first fixed to be expected? Indeed

[10] Weidenreich, F., *Ergeb. d. Anat. u. Entwickl.*, Bd. 13, pp. 1–94, 1910.

[11] Triolo, *Gazz. d. ospitali, Milano,* Vol. 26, p. 393, 1905.

[12] Jolly, J., *Comp. rend. soc. biol.,* T. 58, pp. pp. 481–483, 1905.

[13] Schäfer, E. A., "Quain's Anatomy," Vol. 2, Pt. 1; 11th ed., Longmans, Green & Co., London, 8vo, 11 and 739 pp., 1912.

[14] Jordan, H. E., *Anat. Anz.,* Bd. 34, No. 16 u. 17, pp. 406–412, 1909.

[15] For making these observations I can particularly recommend the dog's omentum.

a biconcave shape would invite this alteration. It seems plausible that the delicately constructed and highly flexible erythrocyte is more easily subject to distortion, through the action of reagents, than are ordinary tissues for it is not supported by contiguous cells or by intercellular cement.

The following experiment of Löhner ('11),[16] which I have corroborated, is interesting from this viewpoint. If a droplet of blood be drawn by capillarity between cover slips,[17] fused at one point, discs are observed. If now 1 per cent. osmic acid be drawn in cautiously from one side only, many cups, some wedge-shaped discs, discs, and distorted forms are seen.

A limited number of cup-shaped erythrocytes undoubtedly exist in normal blood. Possibly they represent corpuscles, whose structure is such that unequal tensions with respect to the osmotic balance exist; perhaps they are old (or young?) corpuscles. In anemias the presence of many cups have been reported, and in fevers it is said crenation may occur. May it not be that the blood of certain individuals contains "normally" excessive numbers of cup-shaped corpuscles? Is it possible that this explains why some of our most careful workers have been led to describe this form as normal?

The evidence gained from the examination of drawn blood, diluted in human serum, and from the study of circulating blood in non-anesthetized living mammals justifies, I believe, the conclusion that the biconcave disc represents the normal shape of the mammalian erythrocyte—the concavo-convex cup being merely an occasional modification.

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THE PENETRATION OF BALANCED SOLUTIONS AND THE THEORY OF ANTAGONISM

ANTAGONISM has been explained by Loeb and by the writer on the ground that antagonistic substances prevent each other from entering the cell. As the writer has repeatedly pointed out,[1] this explanation encounters a difficulty in the fact that antagonistic substances penetrate the cell in a balanced solution (although the penetration is much slower than in unbalanced solutions). The proof of this has been obtained by the writer by means of the method of plasmolysis[2] as well as by determining electrical resistances[3] and it has recently been confirmed by Brooks[4] by means of the method of tissue tension as well as of diffusion through a disk of living tissue.

It is obvious that antagonistic substances must penetrate in a balanced solution since otherwise the cell could not obtain the salts necessary to its existence.

As a way out of this difficulty the writer has suggested[5] that the slow penetration of salts may produce effects quite different from those produced by rapid penetration, just as the precipitation of colloids may be brought about by the rapid addition of salts while it does not take place when they are added slowly.

This difficulty completely disappears if we adopt the standpoint recently advocated by the writer in developing a dynamical theory of antagonism.[6] From this point of view we regard the slow penetration of salts in balanced solutions not as the cause but as the result of antagonism, or rather we may regard both the slow penetration and the increased length of life (or growth, etc.), by which we measure antagonism, as the results of certain life processes which are directly acted on by the antagonistic substances.

The essential feature of the explanation lies in the behavior of these life processes rather than in the manner or rate of penetration.

It is assumed that these life processes consist of consecutive reactions of the type

$$A \rightarrow M \rightarrow B$$

[16] Löhner, L., *Arch. f. gesam. Physiol.*, Bd. 140, pp. 92–108, 1911.

[17] Blood should occupy part of the capillary space only.

[1] SCIENCE, N. S., 34, 189, 1911; 35, 115, 1912; 36, 576, 1912. *Plant World*, 16, 135, 1913.

[2] SCIENCE, N. S., 34, 189, 1911.

[3] SCIENCE, 35, 115, 1912; 36, 576, 1912. *Am. Jour. of Botany*, 2, 93, 1915.

[4] Unpublished results.

[5] SCIENCE, N. S., 34, 189, 1911; 35, 115, 1912; 36, 576, 1912. *Plant World*, 16, 135, 1913.

[6] *Proc. Am. Phil. Soc.*, 55, 1916.