

Ringer-Locke solution, giving a concentration of dissolved hemoglobin approximating that of the normal plasma proteins (5 to 7 per cent.). The red cells are taken from beef, cat, dog or human blood, twice washed with Ringer-Locke solution, and, after the last centrifugalization, hemolyzed by the addition of distilled water or by ether. For best results absolutely fresh blood must be used. Beef cells are best adapted for the work; they readily hemolyze when added to distilled water, and their potassium content approximates that of normal blood plasma. The final solution is made isotonic with normal blood. The stromata are removed by the centrifuge. The oxygen capacity of the solution when finally prepared is about half that of normal cat or dog blood.

This solution may be introduced into the bodies of cats or dogs through a cannula placed in the jugular vein, and the blood simultaneously removed through a carotid cannula. In this manner, by continuous bleeding, all but the last traces of the normal blood may be swept out of the body, and the concentration of the red blood corpuscles may be reduced to the vanishing point. By this method we have observed:

(1) After complete removal of the normal blood from cats or dogs under veronal anesthesia and replacement by hemoglobin-Ringer the hemoglobin in solution appears to be able to carry oxygen, and furnish base for the transport of carbon dioxide much as when enclosed within the red blood cells. The respiratory movements continue, usually increasing in rate as the experiment progresses. The heart beat remains strong. Reactions to such drugs as adrenalin occur as in the normal animal. Circulatory and respiratory reflexes persist. Other reflex actions are present; the pupil of the eye, for instance, constricts upon illumination. The oxygen consumption continues with little or no change during and for up to two hours after the removal of the normal blood. A similar constancy in oxygen consumption has been observed in heart-lung preparations of the dog.

(2) The hemoglobin in solution exerts a colloidal osmotic pressure, which prevents the edema which follows perfusion with ordinary Ringer-Locke.

(3) The completeness of the removal of the red cells has been checked by histological examination. All but the last traces of cells are removed from the various tissues when hemoglobin-Ringer amounting to eight times the normal blood volume has been passed through the body. The bone marrow is readily swept clear. The spleen alone holds some red cells for a longer time.

(4) Blood volume may be directly determined by collection and measurement of all red cells removed from the body. The most dependable values are secured from previously splenectomized animals.

(5) After the removal of the normal blood under ether anesthesia the animals may regain consciousness and exhibit an essentially normal behavior for several hours. Our best experiments of this type have been with cats. They are able to walk, run, see and hear. They are able to jump to the floor from a considerable height, judge distances correctly and make their way about in a normal manner. All postural and equilibratory reflexes appear to be normal. When dropped upside down they land on their feet. Respiratory rates are, however, higher in these animals than normal. They show a marked tendency to sleep, but are easily aroused and may become quite active, for a brief period, only to lapse into sleep again rather suddenly.

(6) Such animals finally die, after five or six hours, not because the hemoglobin is unable to carry on its respiratory function, but because it leaves the blood stream with much greater ease than do the normal plasma proteins. It appears in urine and feces, and is in part removed by cells of the reticulo-endothelial system. Its concentration within the blood-vessels falls considerably, the blood volume appears to diminish and the animal dies from oxygen lack and respiratory failure.

We conclude from these experiments that the chief function of the vertebrate red blood corpuscles is to hold hemoglobin within membranes impermeable to it, so that it can not leave the blood stream. In other respects hemoglobin appears to be able to carry out its respiratory rôle in solution much as it does within the red cells, sustaining every vital function, even the more complicated activities of conscious life.

A full account of these experiments will appear shortly in the *Journal of Cellular and Comparative Physiology*.

WILLIAM R. AMBERSON
ARTHUR G. MULDER
FREDERIC R. STEGGERDA
JAMES FLEXNER
DAVID S. PANKRATZ

COLLEGE OF MEDICINE,
UNIVERSITY OF TENNESSEE

THE BEHAVIOR OF FROG EGGS IN AN ELECTRICAL FIELD

Numerous investigations have been made of the cataphoretic properties of living cells, but until recently the most reliable data have concerned bacteria and blood corpuscles. The electrokinetic potential of these small cells has been found to be negative. The studies of Mr. Katsuma Dan, of this laboratory, have demonstrated that sea-urchin eggs also bear a negative charge.¹

¹ K. Dan, *Anat. Rec.*, 51: 28, 1931. Extensive paper to appear shortly.

The behavior in an electrical field of the egg of the frog *Rana pipiens* reveals marked electrokinetic properties in this cell, which is enormously larger than the microscopic cells whose charge has been determined. Freshly stripped eggs were introduced into a trough 8 mm wide and 5 cm long, containing pond water, pH 6.8. A potential of 10 volts was applied by a Zn-ZnSO₄-agar system in which the agar bridges completely occupied the ends of the trough, so that the lines of current flow were parallel to the walls and floor of the chamber. The current intensity used was 1 milliampere.

When the current is passed, the egg within its jelly layers begins almost immediately to move toward the cathode, at the rate of about 10 microns per second. This occurs regardless of whether the animal or the vegetal pole of the egg faces the cathode. The egg migrates as far as possible within the jelly, often distending it at the cathodal end, and sometimes being flattened against this end by its pressure.

When dry eggs, *i.e.*, those with jelly unswollen, are introduced, or any eggs whose jelly is not too sticky, the whole egg, jelly and all, moves toward the cathode. This motion begins after the migration within the jelly, and, although it is irregular because of the fact that the egg lies on the bottom of the chamber, it has about the same or even a higher velocity. Observed values range from 8 to 19 microns per second. As the jelly moves, the egg appears eccentrically located within it, at the cathodal end. Because of the narrowness of the trough, the movement can not be ascribed to endosmotic current due to the charge upon the walls, for the currents in both directions impinge upon the egg. The fact that the jelly too migrates to the cathode precludes the possibility that electroosmosis through the jelly is the cause of the movement of the egg within it. The truly "cataphoretic" nature of the migration can further be verified by using eggs whose jelly has been removed with KCN. Eggs so treated move toward the cathode very clearly, although they lie on the bottom of the chamber.

In all cases the jelly swells to many times its normal volume during the passage of the current. When dry eggs are introduced, the jelly does not swell uniformly, as it does when the eggs are simply placed in water. There occurs immediately a very rapid swelling at the anodal end, none at the cathodal. Since the egg has migrated during the course of the swelling, it appears pressed against the original thin layers at the cathodal end, backed at the anodal end by a great swollen mass of jelly. Within the inner jelly layer at the anodal end, large sacs or vacuoles are seen to form, evidently by the pressure of the water moving anodally.

Cataphoretic movement of cytoplasmic granules

within plant cells has been observed by Hardy² and others. Hardy clearly demonstrated movement toward the cathode. A similar movement can be seen within the frog egg. When the current is passed through water in which the egg lies, there appears, after a short time, a narrow clear margin beneath the membrane at the anodal end, and this clear space widens until it occupies about one tenth the diameter of the egg. The appearance is the same whether the animal or vegetal pole or the equator of the egg faces the anode. The contents of this clear space are easily distinguishable from the yellowish yolk. If now the direction of the field is reversed, the granules move back through the clear space, obliterating it. Then a similar space is formed at the new anode. This behavior on reversal demonstrates that the phenomenon is a true movement of granules toward the cathode, and not a breakdown of colored material at the anode.

These observations, repeated many times on eggs from different frogs, are purely qualitative, since the condition of free suspension demanded for cataphoresis measurements is not satisfied. They do, however, indicate a positive charge upon the egg as a whole and upon the internal granules, a charge whose magnitude may be exactly determined in later studies.

D. MAZIA

UNIVERSITY OF PENNSYLVANIA

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² W. B. Hardy, *Jour. Phys.*, 47: 108, 1913. Older literature in Heilbrunn, "Colloid Chemistry of Proto-plasm," 1928.