SCIENTIFIC APPARATUS AND LABORATORY METHODS

A QUICK METHOD OF PRESERVING CATS FOR DISSECTION

THE customary techniques for preserving cats for student dissection are by embalming or by injection of formalin through the femoral or carotid arteries. All these require a certain amount of dexterity and considerable time, especially as the best results are obtained with anesthetized specimens or by using gravity pressure. It is probable that analogy with the medical school dissecting-room is responsible for these divergencies from the technique used for lower vertebrates. Where there are large classes and the injecting must be done by the instructors alone or with student assistance, the time spent in this way may be a real burden. The following method, devised to meet this difficulty, can be used on freshly killed specimens, and permits satisfactory dissection of the digestive, urino-genital, muscular and autonomic nervous systems.

Open the abdominal and thoracic cavities with a median incision, starting well posteriorly, avoiding the milk glands of the nursing females. Cut through the skin on the outside of each thigh from the knee to the gluteal region, and pull up the flaps of skin on each side. With a hypodermic syringe inject from 200 to 400 cc of 10 per cent. formalin into the left ventricle (according to the size of the animal), until bubbles appear at the nostrils. Immerse the animal in 5 per cent. formalin. The cats can be injected in an average time of five to eight minutes apiece. Later, when the students skin their individual cats, perhaps one out of four specimens will show slight discoloration under the skin, which will disappear before the next laboratory period, if the animal is replaced in the 5 per cent. formalin. One specimen out of ten, perhaps, will have a definite decayed spot and is best discarded. After a few days, the formalin may be diluted to 3 per cent. In dissecting the muscles, it is helpful to rub glycerine on the parts being studied.

This is a satisfactory rough-and-ready method; it is not, in any sense, a museum technique.

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A CULTURE MEDIUM FOR FREE-LIVING FLAGELLATES

THE following culture medium has been tried out for two years and may be of general use to other laboratories. Whole wheat is weighed into five-gram lots, which are then put into large test tubes and 25 cc of tap water added. These are then plugged with cotton, capped with lead foil and autoclaved at fifteen pounds for two hours, which very thoroughly macerates the wheat. Tap water is again added up to 50 cc, and desired percentages of this are used after shaking. After opening a tube it is necessary to sterilize again in an Arnold sterilizer, as bacterial growth is quite vigorous in the mixture. However, a tube may be used day after day, if sterilized daily.

Varying percentages of this afford a very good medium for many protozoa. Bacterial feeders as Chilodon, Paramecium, Oicomonas and others thrive. Ochromonas, Chilomonas and several of the smaller Euglenas (E. gracilis, E. quartana, E. mutabilis) have been grown in great abundance in various dilutions and there are several species of Amoeba which likewise occur or are capable of being cultured in large numbers. It has proved best, however, for Entosiphon and Peranema. Both of these forms are easily grown in quantities sufficient for classroom use; isolation cultures of the former have been carried for over a year on this medium. In general it seems much better than cracked boiled wheat, which is often used.

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SPECIAL ARTICLES

CONCERNING PROTOPLASMIC CURRENTS ACCOMPANYING LOCOMOTION IN AMEBA

INVESTIGATORS of the mechanics of ameboid locomotion have generally agreed as to the existence of currents in the protoplasm of the progressing ameba, but there has been much disagreement as to the direction and general relations of such currents. One of the most serious contradictions is that between the observations of Rhumbler[1] and those of Jennings.[2] Rhumbler described a system of currents which was entirely in accord with his view that surface tension is an essential agency in ameboid locomotion. He asserts that the deeper protoplasm (endoplasm) flows forward. Any given portion of it, having attained a superficial position at the advancing front of the animal, then turns and moves backward at a relatively

[1] Rhumbler, L., 1898. "Physikalische Analyse von Lebenserscheinungen der Zelle." *Arch. für Entwickelungsmech. der Organismen*, 7, pp. 103–350, plates VI, VII; 100 figs. in text.

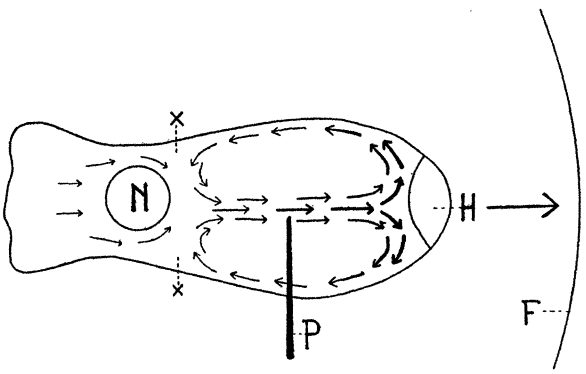
[2] Jennings, H. S., 1904. "The Movements and Reactions of Amoeba." Carnegie Institution Publication No. 16, pp. 129–234, 78 figs. Washington, D. C.

superficial level. Thus, at the anterior end of the ameba the axial forward flow is continually dispersing itself into the peripheral backward flow. The effect is that of a fountain-like system of currents at the anterior end, involving continual transformation of endoplasm into exoplasm. The necessary compensatory relations exist at the rear of the animal. Jennings, unable to corroborate Rhumbler's observations, asserts that all the protoplasm, both axial and superficial, is flowing forward, but at different velocities, the axial flow being more rapid than the peripheral flow. He therefore suggests that Rhumbler suffered an optical illusion, mistaking the relatively slower forward motion of the superficial currents for an absolute backward motion.

The observations reported in this present note were made incidentally in connection with other work on amebae. The circumstances were such that it was not practicable to turn aside for an intensive study of the current system. Nevertheless such observations as were made were so definite and seemed so free of opportunity for misinterpretation that a brief report of them is justifiable, even though it must lack much detailed information which could be desired.

The amebae upon which the observations were made were taken from hay infusion cultures which the junior author had started by inoculation from cultures maintained by Dr. James A. Dawson, of Harvard University. The amebae were of the "limax" type and were extraordinarily large. The optical conditions under which we observed them seemed especially good. The animal was placed upon an ordinary slide, with cover glass, and viewed with a Leitz No. 7 dry objective. Stationary "landmarks" or reference points for detection of motion were afforded by the edge of the field and by the common device of a hair "pointer" secured at the image level within the ocular. The setting of the field at the time when the most satisfactory observations were made, and the direction of the observed currents are shown in the figure. The ameba, whose length was equal to half or more than half of the diameter of the field, was progressing steadily across the field. The slide was so placed that the anterior end of the ameba was near the edge (F) of the field, and the pointer (P) was superposed transversely to the long axis of the ameba, usually about midway of its length, otherwise at any region where it was desired to observe motion. Internal movements of the richly granular protoplasm were perfectly easy to see.

Under these conditions currents were observed as follows. (The description refers, except as otherwise noted, to the currents observed when the microscope was focused at about the middle level of the ameba. The varying thicknesses of the arrows in the figure indicate roughly the relative velocities of the currents.) A broad column of the deeper protoplasm was, in relation to the animal itself, flowing forward, its velocity being greater at the axis of the animal and less in the more peripheral regions of the column. The velocity of the absolute forward movement of the axial protoplasm—that is, its velocity as judged in relation to the fixed pointer—greatly exceeded the velocity of the forward motion of the animal as a whole. That is, in any given time, the distance covered by a particular granule in relation to the pointer was much greater than the distance covered by the most anterior point of the ameba in relation to the edge of the field. At a rough estimate we should judge that the former exceeded the latter by two or three times. At the same time, upon either side of the central forward-flowing column the movement of granules, observed in relation to the pointer, was backward, and this in spite of the fact that the animal was meanwhile moving steadily in the opposite direction. It follows, then, that this peripheral lateral protoplasm was, *with reference to the animal itself,* moving backward faster than the animal was moving forward.



At the anterior tip of the ameba is a zone of perfectly hyaline and homogeneous looking protoplasm (H). The extent and shape of this zone remain constant and it is sharply delimited from the granular protoplasm. Just behind this hyaline zone is a region within which granules delivered by the forward axial flow may be observed to swing off laterally in curving orbits, or (as ascertained by vertical focusing) to move either up or down, to begin then their return backward via the lateral and peripheral flow. It is just here at the apex of the axial forward current that granules attain their greatest velocity. They move at relatively high speed in the centrifugal flow at the anterior end of the ameba and then, as they swing over into the peripheral backward current, the velocity diminishes. Within the peripheral backward current the velocity decreases with distance from the

anterior end until, at the region marked "x" in the figure, little or no indication of movement could be seen.

The posterior region of the ameba (behind and around the nucleus) seemed to be one of comparative stagnation. No strongly marked currents could be observed there. Behind the nucleus there were some indications of weak forward flow. Central to the regions "x – x" could occasionally be seen granules moving slowly centripetally to rejoin the forward axial current and there was some evidence of forward flow around the sides of the nucleus. The stagnation at the region "x" is apparently due either to the dying out of the peripheral backward currents from in front of that region, or to their counteraction by weak forward currents from behind.

When the microscope was focused at or near the axial level of the animal, the opposed central and peripheral currents were always perfectly clearly seen. Focusing at levels either above or below the central forward-flowing mass of protoplasm gave scarcely less satisfactory evidence of backward currents similar in velocity to the lateral currents.

It follows from all this that the relations between the axial and the peripheral currents are markedly different at the two ends of the animal. At the anterior end the transition from the axial to the peripheral flow is sharply localized within a small territory and the currents run at high speed. At the posterior end the compensating transition between the two sets of currents is effected by means of comparatively sluggish flow which is diffused throughout a territory representing one fourth or one third the length of the animal.

Such currents as we have described must involve continual interchange of the deeper and more superficial protoplasm, as conceived by Rhumbler.

In one observation, made by the senior author, the nucleus afforded a vivid exhibition of the mechanical effect of the opposite flow of the central and peripheral currents. Apparently because of the mobility of the protoplasm about it, the nucleus is constantly subject to slight alterations in position. At the moment of the observation it happened to be lying just lateral to the axis of the ameba and perhaps a little farther forward than usual, so that the surface of its medial hemisphere was contiguous with the forward-flowing axial current and the surface of its lateral hemisphere was contiguous with the backward-flowing peripheral current. Within the few seconds during which the nucleus remained in this location, its precise vertical relations within the ameba could not be ascertained. Whatever these may have been, the resultant of the effects of all the currents impinging upon the surface of the nucleus was to impart to the nucleus a rotary motion whose most conspicuous component was an apparent counter-clockwise rotation upon the vertical axis. The velocity of the rotation was rather less than that of the currents which caused it. The rotation ceased when the nucleus moved over more nearly into an axial position and, as if coming more strongly under the influence of the axial current, was swept slightly forward. In this new location it lingered for some time with slight fluctuations as if pressed by the current and yet somehow sufficiently strongly held that it could not drift down stream. In watching the nucleus one is perplexed by the fact that it is so freely movable within limits which are in no way visibly defined. Apparently freely immersed in a labile and actively flowing protoplasm, why is its position not completely at the mercy of the currents? It must possess some highly elastic anchorage. It is as if a slightly buoyant sphere, immersed in a strongly flowing stream of water, were anchored by relatively slender and extremely elastic rubber bands. Conklin,[3] in his study of the protoplasm of the egg of Crepidula, finds evidence for the existence of a highly elastic spongioplasm which constitutes a "tenuous framework" holding other cell structures in their normal positions. Mechanical agencies, such as centrifugal force, may produce great change in the configuration of cell organs, with corresponding distortion in the framework. Yet, upon removal of the disturbing agency, the elasticity of the spongioplasm is adequate to restore displaced cell organs to their normal locations. The position and movements of the ameba nucleus could be accounted for on the ground of such an elastic spongioplasmic anchorage opposing the stresses due to the protoplasmic currents.

The currents here described were seen in at least ten individuals, and the observations were distributed over four days. In no case were currents seen otherwise than as described here. We do not claim to have settled the problem of ameboid locomotion. We do not claim that such currents as we have described exist in all amebae at all times. But we shall not be easily persuaded that we have been the subject of optical illusion. If, in the particular amebae which we observed and at the times of our observations, the protoplasmic currents were not proceeding as we have described them, then we shall no longer be justified in trusting our optical sense to inform us correctly concerning the directions of traffic currents in a city street. It is scarcely exaggeration to

[3] Conklin, E. G., 1917. "Effects of Centrifugal Force on the Structure and Development of the Eggs of Crepidula." *Journ. Exper. Zool.*, 22, pp. 311–420, 124 figs.

say that our view of the former was no less clear and convincing than is our daily view of the latter.

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THE INCREASE IN THE CALCIUM OF HENS' BLOOD ACCOMPANYING EGG PRODUCTION[1]

IN connection with our work on the influence of ultraviolet light on egg production we have had occasion to determine the calcium content of the blood of a fairly large number of normal laying hens. Pre-chickens ranging in age from day-old chicks to mature laying pullets. Determinations were also made on the blood of some mature hens which were out of production because of the molting period. Other determinations were made on blood of hens that had passed through the molting period and had come into production. The determinations were made by a slightly modified Kramer-Tisdall method. The summary of the results obtained are shown in the accompanying table:

These results show quite clearly that the calcium content of the blood of hens during the period of egg production is about double that during the periods of non-production.

Age	No. of birds	Condition	Mg Ca per 100 cc of plasma		
			High	Low	Average
1 day	25		Blood pooled		12
1 mo.	6		Blood pooled		12
2 mo.	6		Blood pooled		13
3 mo.	6		Blood pooled		13
4 mo.	6		Blood pooled		14
5 mo.	10	Immature pullets	15	12	13
5 mo.	10	Mature pullets not in production	25	15	20
5 mo.	10	Mature pullets in production	34	25	27
7 mo.	3	Capons	13	13	13
7 mo.	10	Mature cockerels	15	13	14
18 mo.	10	Molting hens not in production	18	11	14
18 mo.	3	Mature hens after molting in production	35	29	31

vious experiments had shown that the amount of calcium and inorganic phosphorus in the blood of normal growing chicks was quite uniform and about the same as that of other normal animals. From this we expected to find a similar uniformity in the calcium content of the blood of mature hens. Instead of this uniformity, however, we found surprisingly great variations in the calcium content of their blood. In a lot of ten hens we found values ranging from 13 mg per 100 cc of blood to 32 mg per 10 cc. Trap-nest records were not available on these hens, so an absolute correlation of the blood calcium and egg production could not be made. It appeared, however, that the variation in the calcium content of the blood was due to the variation in egg production. The high values were obtained in the case of the hens which were in production and the low values from hens which appeared as if they were not in production.

In order to obtain reliable information on the relation of egg production to the calcium content of the blood, a series of determinations was made this year on the calcium content of the blood from normal

[1] Contribution No. 129, Department of Chemistry, Kansas State Agricultural College.

During the time this work was under way Riddell and Rheinhart[2] published the report of their work showing that there was a marked rise in blood calcium in pigeons at the time of egg production. The results of our work agree with theirs both in the fact that egg production is accompanied by a large increase of blood calcium and also in the fact that there is no increase in the blood calcium in male birds accompanying sexual maturity.

The increase in the blood calcium of the laying hen seems to be due to the same interplay of hormones that bring about the development of secondary sexual characteristics. At least the increase in blood calcium and the development of the characteristics by which egg production may be judged parallel each other, so that one can easily select a high or low blood calcium hen by observing these characteristics. Work is now under way to determine the relation of activity of the parathyroids to the increase of blood calcium.

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[2] *American Journal of Physiology*, Vol. 74.