

of the heart, etc. The chapter on the nervous system has eighty odd pages of an exceptionally able discussion of the origin and development of the nervous system as a coordinating mechanism. It is illustrated by examples drawn from a great variety of nervous organizations from the neuromuscular apparatus of the protozoa and the nerve net of the coelenterates to the neurone and the synaptic systems of a wide range of invertebrate and vertebrate nervous systems. The segmental nature of the nervous system is presented by discussion of the functional behavior of a well-chosen series of invertebrates in which the chain ganglia are still distinct.

There are able discussions of several topics peculiar to comparative physiology, for example, the functions of the swim bladder as a static organ. However, the important problem of animal luminescence seems to be wholly neglected.

At the close of the volume are references to selected literature of value to the investigator in the field.

This volume should have a distinct influence in rescuing the subject of physiology from the restrictive dominance of the arts and to that extent should give back to practical medicine and to agriculture correspondingly broader training in the basic physiological sciences.

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## SCIENTIFIC APPARATUS AND LABORATORY METHODS THE COLLODION METHOD AND SERIAL SECTIONS

THE collodion (celloidin) method is admitted to give better effects than can be secured by the use of paraffin on a number of tissues, while for certain material, *e.g.*, grasshopper eggs, it is the only known means of securing satisfactory results. Nevertheless, there is a general reluctance to use collodion due chiefly to the belief that it is difficult to preserve the serial order of the sections by this method. In reality, mounting in serial order is very easily accomplished and, while the collodion method is slightly slower than the paraffin method, with some simplification of details it is easier, in many respects, to handle. This article contains little that is new, but the various points are so scattered through scientific papers that it seems desirable to make the whole procedure available.

*Preliminary steps.* The first part of the process is the same as for the paraffin method. Dehydration must be completed by the use of absolute alcohol since "clearing" oil is not used. The principle involved in clearing, however, is employed, *e.g.*, the tissue is saturated with a solution which is miscible with the

infiltrating substance, namely, a mixture of equal parts of absolute alcohol and ether.

*Infiltration and embedding.* The usual method of accomplishing these processes by two distinct steps is largely responsible for the prevalent idea that the collodion method is necessarily cumbersome. However, they may be combined in a very simple way by using a shell-vial or a similar vessel of suitable size as a container. It is desirable that the container should not have a neck in order to facilitate the later removal of the hardened mass.

Tri-nitro-cellulose under some of its trade names (collodion, celloidin, parlodion, etc.) is dissolved in equal parts of absolute alcohol and ether and used as the infiltrating medium. The solution, which ordinarily should be fairly thin, readily penetrates without heat tissues which are already saturated with the solvents. The time required varies widely. Usually the container is kept tightly closed for several days or in some cases even weeks or months. The cover is then slightly loosened to permit a very gradual evaporation of the solvents with a corresponding concentration of the collodion in the tissue. When the solution has become fairly viscous the tissue is oriented as desired. After the mass becomes firm it should be loosened about the edge so that it will contract away from the vial. When it has become sufficiently solid it can be removed easily. Evaporation should occupy several days; if sufficient time is not given the mass will not be of uniform density.

*Hardening and blocking.* The mass is trimmed, leaving about 1 mm. of collodion about the tissue and a flat base for mounting. It is then returned to the vial together with a piece of cotton saturated with chloroform for further hardening. The block may be stored in 70 per cent. or 80 per cent. alcohol indefinitely, but it should be hard enough for sectioning before it is placed in the alcohol. The necessity for again dehydrating, however, is obviated if the block containing the tissue is mounted on a proper support before placing in alcohol. The simplest procedure is to take the block directly from the chloroform vapor, stand the base for a moment in alcohol and ether to soften it, then transfer quickly to a fiberoid block, the top side of which has just received two or three drops of thick collodion. After not more than ten minutes' exposure to the air, in order that the collodion may set, the whole is placed either in chloroform vapor for further hardening, or, if the mount is small, directly in 70 per cent. alcohol, where it should remain for several hours before sectioning in order that the entire mount may become very firm.

*Cutting and mounting of sections.* Collodion sections are cut with the knife placed at the least possible angle to the direction of movement. The

knife is kept wet during the process, usually with 65 per cent. to 70 per cent. alcohol. This may conveniently be accomplished by arranging an automatic oil cup so that it will drop the alcohol on the knife at the desired rate. The cutting should be done with a quick, firm motion. If the block has been sufficiently hardened and the knife edge is in good condition every section should be perfect and the thickness of successive sections uniform. A small sable brush is best for handling the sections. The brush is kept wet in the alcohol on the knife and if the sections are to be mounted serially they are arranged near the back of the knife from right to left as they are cut, always keeping them moist. Several rows of the proper length to fit under the cover-glass may be so arranged in the relation to each other which they are to occupy on the slide. A thin piece of tissue paper is placed smoothly over the sections, being sure that there is sufficient alcohol to wet through the paper. With a uniform downward motion the paper is pulled off the knife, preferably over the back. The sections sticking flat to the paper are carried across to a chemically *clean* slip on which the paper is laid reversed so that the first section cut occupies the upper left hand corner and so that the sections are properly centered. The paper may be smoothed out with the addition of a small amount of alcohol if necessary. Several layers of absorbent paper are placed on top and the whole rolled lightly but firmly with some cylindrical object for about ten seconds. This, in addition to pressing the sections tightly against the glass, removes the 70 per cent. alcohol. The paper is then quickly peeled off, leaving the sections on the slide where they are instantly flooded with clove oil, which should remain until the sections are perfectly translucent. The clove oil will dissolve sufficient of the collodion to fasten the sections to the slip; after about eight minutes the surplus oil is drained off and the slide placed in 95 per cent. alcohol. After ten or fifteen minutes it is changed to fresh 95 per cent. alcohol to insure the complete removal of the clove oil. From this point on the preparation is treated the same as if it contained paraffin sections except that the collodion is not removed. Dr. Miriam J. Scott is authority for the statement that some of her slides, so prepared, were kept in 70 per cent. alcohol for two months without the loss of a section. An equally satisfactory method, if properly used, is to smear the surface of the chemically clean slip with a film of Mayer's albumen, place the sections on it as directed above, and, omitting the clove oil, immerse quickly in 95 per cent. alcohol for at least ten minutes. One small drop of Mayer's albumen is sufficient to prepare 25 or 30 slips. Any considerable amount of albumen precipitated under the sections

impairs the stain and lessens the probability of the sections remaining on the slip.

*Cautions.* (1) The block must be sufficiently hard to be quite rigid, otherwise its elasticity will interfere with cutting perfect sections such as are necessary for serial preparations. The proper degree of hardness should be obtained before placing in 70 per cent. alcohol.

(2) The slip must be chemically clean. It may be tested in this respect by placing a drop of distilled water on its surface. It is satisfactory if the water spreads uniformly and does not roll off when the slip is tilted without wetting the glass. (This is the most important of the precautions.)

(3) Just after the sections have been placed on the slide there is a moment when very precise work is necessary. After they are covered with the absorbent paper, they must be rolled long enough to remove practically all of the 70 per cent. alcohol except what is actually in the sections; when this condition has been obtained, speed is necessary in order to remove the paper and get the sections covered with the next medium before air gets between them and the slip, owing to the evaporation of the alcohol which is in the sections.

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## SPECIAL ARTICLES

### DIRECT EVIDENCE OF ATOM BUILDING<sup>1</sup>

THROUGH new and more precise measurements on cosmic rays than those heretofore made, Millikan and Cameron have just succeeded in bringing forth quantitative evidence that those rays represent the precise amount of energy which should, according to Einstein's equation showing the relation of mass to energy, be emitted in the form of ether waves when the primordial positive and negative electrons unite to create helium atoms and other light atoms such as oxygen and silicon, magnesium and iron.

Millikan and Cameron have investigated these rays through experiments in high mountain lakes, both in California and in Bolivia, and Millikan and Bowen have studied them with the aid of self-recording electroscopes sent up by sounding balloons which reached nine tenths of the way to the top of the earth's atmosphere.

The results obtained in such investigations during the past eight months constitute the first indubitable evidence that the cosmic rays on which they have been experimenting, instead of being spread like white light

<sup>1</sup> A report made in Pasadena to the California Institute Association on March 16.