



FIG. 1

third with gravel and for the remaining upper two thirds with fine sand. The water filters slowly downward, leaves by way of a glass tube and returns to the reservoir.

The cork stopper of the reservoir should contain four openings: one for the tube returning water from the filter, one for the supply pipe (b.) conveying water to the aquarium, one for the air tube (a.) and one to equalize the air pressure within the reservoir. A cover may be placed over the top of the aquarium to prevent excessive evaporation, especially if the system is for sea water.

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IMPROVEMENT OF PARAFFIN SECTIONS BY IMMERSION OF EMBEDDED TISSUES IN WATER

IN a recent article in *SCIENCE*, Slifer and King¹ suggested a modification of Petrunkevitch's² technique for softening tissues too brittle for sectioning by the paraffin method. The work of these investigators was carried out on grasshopper eggs and entailed the use of a 4 per cent. solution of phenol in 80 per cent. alcohol and subsequent soaking of the embedded tissues in water. Either of these two processes without the other failed to give satisfactory results.

Difficulties encountered for several years in this laboratory in the sectioning of rats' adrenals led to the investigation of the applicability of the technique

described by Slifer and King to adrenal and other tissues. The adrenal glands used in our studies had to be treated by special methods. The glands were fixed in Wislocki's³ modification of Kohn's fluid containing potassium dichromate and formalin, washed in formalin, and passed through graded solutions of alcohol to 70 per cent. The adrenals were then bleached in hydrogen peroxide and alcohol for several hours. This part of the process frequently rendered the glands extremely brittle. They were next passed through graded solutions of alcohol to absolute alcohol and cleared in a mixture of benzyl benzoate and methyl salicylate. The brittleness of the adrenals increased in proportion to the time the glands remained in the mixture. Some which were left in this fluid for two years or more crumbled when they were sectioned.

In view of these difficulties it seemed advisable to utilize the Slifer and King method in our work. In the case of the adrenals, the glands were exposed to a 4 per cent solution of phenol before or after bleaching in hydrogen peroxide or clearing in the oil mixture. As it would have been impractical to cut the adrenals in half, since the obtaining of serial sections for reconstructions was desired, surfaces of the glands were not exposed to water. It was hoped that the phenol treatment alone would suffice to soften the embedded material. The glands failed to section, but it was observed that if water was applied to the surface of the block before each section was cut, that section came off perfectly. This same effect, however, occurred even though the glands had not been previously treated with phenol. It seemed reasonably certain that if a surface of the embedded glands could be exposed to water, the laborious necessity of moistening the block for each section could be eliminated. The crux of this problem lay in the fact that exposure of a large surface would entail the loss of important sections.

The following method afforded a solution to the problem. Rats' adrenals were prepared by the technique outlined above. These included fresh material and material which had been preserved in the clearing mixture for several years. None of the glands was treated with phenol at any time in the course of preparation. After the glands were embedded, paraffin was shaved off in such a way that one surface of each gland was just scratched. Whatever section would be lost by this procedure could be accounted negligible for accurate reconstructions. Each block was then immersed in water and allowed to soak for varying periods of time. When the allotted time period for each block had elapsed, the

¹ Eleanor H. Slifer and R. L. King, *SCIENCE*, 78: 366, 1933.

² A. Petrunkevitch, *SCIENCE*, 77: 117, 1933.

³ G. B. Wislocki, *Bull. Johns Hopkins Hosp.*, 33: 359, 1922.

exposed surface was carefully dried and dipped into melted paraffin. Care had to be exercised in this manipulation to avoid stratification and formation of bubbles between the hot and cold layers of paraffin. The block was cooled and trimmed as usual. Glands which had been soaked for a sufficiently long time sectioned perfectly, and the ribbon was flawless. The minimum length of time required for the water treatment varied between three days and two weeks, depending upon the size of the gland. Soaking for longer periods was not harmful to the tissues.

This method did not in the least impair the staining properties of the sections nor did it affect the histologic aspect of the tissues. Actual quantitative studies involving reconstructions proved the loss of tissue was so slight as to be insignificant.

The results of the work with the adrenals were so encouraging that our studies were extended to other tissues which, although prepared by ordinary histologic methods for sectioning, presented similar difficulties. Several blocks of spleen which previously it had been impossible to section were subjected to the water treatment. Quite satisfactory sections were then obtained from this material. Sections of human autopsy material, including intestine, ovary, liver, kidney and stomach, were noticeably improved. In Table 1 are listed the various types of tissues tested in this study. The results in every case were distinctly satisfactory.

An interesting feature was the absence of electrification of sections. It was noted that bone tissue, not

TABLE 1

Tissue	Animal	Fixative	Number of specimens
Adrenals	Rat	Wislocki	50
Intestine	Human (autopsy)	Zenker	3
Stomach	Human (autopsy)	Zenker	2
Liver	Human (autopsy)	Zenker	1
Ovary	Human (autopsy)	Zenker	1
Kidney	Human (autopsy)	Zenker	1
Kidney	Kitten	Bouin	3
Basisphenoid	Rat	Bouin	50
Hypophysis	Rat	Bouin	10
Muscle	Kitten	Bouin	2
Tongue	Kitten	Bouin	2
Spleen	Rat	Bouin	2

materially softened by water, sectioned much more satisfactorily after water treatment, by virtue of the elimination of static charge.

It is conclusively shown by this series of investigations on various types of mammalian tissues that exposure to water of a small area of embedded material will obviate (1) difficulties consequent to brittleness, and (2) electrification of sections.

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SPECIAL ARTICLES

MONOCYTES AS AN INDICATOR OF CERTAIN STATES OF BLOOD SERUM

It is already known that cell colonies can be used for detecting certain characteristics of blood serum. The mode of activity of living tissues depends in a large measure on the nature of their medium. Any anatomical structure consists of humors as well as of cells. The morphology of the cells is almost meaningless, if not related to the chemical constitution of the humors. Conversely, the composition and the physicochemical conditions of an organic fluid remain without great significance unless expressed in terms of their structural and functional effects. For instance, the transformation of blood monocytes into cells closely resembling macrophages, clasmatocytes, epithelioid cells and fibroblasts is indicative of the presence around the cells of definite media. Likewise, the serum of old, starved or sick animals is characterized by its retarding or accelerating effects on the growth of colonies of fibroblasts. Fibroblasts have often been used as an indicator of the state of the

humors.¹ The value of the growth index of a given serum depends on ill-defined chemical changes undergone by an animal under the influence of physiological or pathological agencies. In old age or in animals having an abscess, the growth index becomes low.² It generally rises during starvation or in certain infections. In experimental tuberculosis, as Swift has shown,³ the serum is growth-inhibiting at the beginning of the disease and growth-stimulating during the period of leucocytosis.

The colonies of fibroblasts react against the variations of blood serum by changes in their rate of growth. But they are not very sensitive to those variations, much less so than blood monocytes are.

¹ A. Carrel and A. H. Ebeling, *Jour. Exp. Med.*, 34: 599, 1921; 38: 419, 1923. L. E. Baker and A. Carrel, *Jour. Exp. Med.*, 45: 305, 1927.

² A. Carrel and A. H. Ebeling, *Compt. rend. Soc. biol.*, 90: 170, 1924. A. Carrel, *Compt. rend. Soc. biol.*, 90: 333, 1005, 1924.

³ H. F. Swift, J. K. Moen and E. Vaubel, *Jour. Exp. Med.*, 60: 149, 1934.