the opportunity to gather more information on the subject.

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DEHYDRATION AND INFILTRATION

RECENTLY, in a series of papers too numerous to be cited here, there has been much discussion regarding the composition of fixing fluids designed to secure specific effects, mainly with plant tissues. The writer has done a great deal of similar experimentation, but the results have invariably been rather erratic and never wholly satisfactory. It was finally concluded that the trouble was caused by the fluids used following fixation and preceding infiltration with paraffin or balsam, and not to the fixing fluids themselves nor to the physiological condition of the tissues.

The tissues of all living organisms contain water and possess the capacity for absorbing water. The water contained in, say, vacuoles presumably does not react towards dehydrating fluids exactly as does the water in cell walls or in nuclei. The nature of the combination between water and the particular structure containing it varies according to the latter and may conceivably be altered during fixation.

Most technicians appear to take it for granted that the main if not the sole criterion of successful fixation is the apparent lack of changes during dehydration and infiltration. The writer, on the contrary, has become decidedly of the opinion that no blame can be placed upon the fixing fluid if disaster results during the post-fixation stages. In other words, "successful" dehydration and infiltration is not dependent upon "successful" fixation: the two processes are mutually exclusive.

The fluids commonly employed for dehydration are powerful desiccators. From the observation that the most satisfactory cases of infiltration resulted when only the free and not the combined water was removed from the tissues, it was apparent that it is possible to produce dehydration without causing desiccation. To state the case in another way: good infiltration occurs when the water is replaced, but the water-absorbing capacity of the tissue is not destroyed. The use of a fluid immiscible with water following, and in combination with, a dehydrating fluid which in itself produces desiccation only makes matters worse. Tissues become excessively hardened, plasmolysis occurs, and in the case of embedded material "cracking" results during sectioning on the microtome.

It seemed that what was required was a fluid miscible in all proportions with water, ethyl alcohol (in order to care for fixing fluids containing alcohol), paraffin and balsam and which would replace all free water yet cause no alteration in the water-absorbing capacity of the tissues. Practically all fluids in common use are automatically eliminated from consideration, as they fail to conform to one or more of these specifications. Normal butyl alcohol comes nearest to conforming, but the anhydrous product is miscible with water to the extent of not more than 8 per cent. by volume; experience has already demonstrated that it was very unsatisfactory with many plant tissues, the reason being that desiccation had taken place. Dioxane, first suggested by Graupner and Weissberger[1] for use on animal tissues, and tertiary butyl alcohol[2] now appear to be the most promising reagents. Each is miscible in all proportions with water, ethyl alcohol, paraffin and balsam or xylol-balsam, as well as with most of the fluids in common laboratory use (except that dioxane will not mix with pure glycerin unless 10 per cent. water is added). An absolutely anhydrous product must, of course, be used to insure perfect dehydration.

Sufficient experience with both dioxane and tertiary butyl alcohol has already been obtained to indicate that, when properly used, these fluids: (1) Eliminate the use of ethyl alchohol and all fluids commonly employed to precede infiltration with paraffin or balsam. (2) Give perfect preservation of the fixation image. Plastids, mitochondria and similar cell constituents are preserved with remarkable fidelity, whereas such things are generally dissolved or otherwise rendered invisible by the usual combination of absolute alcohol and clearing fluid. No plasmolysis nor shrinkage results. (3) Remove all free water but leave the water-absorbing capacity quite unaltered, which makes it possible to soften hard woody tissues by simply exposing one cut end of the piece of embedded material to the action of water for a short time. (4) Produce no hardening whatever, thus permitting perfect microtoming. (5) The most delicate materials, such as *Volvox,* fern prothallia, moss protonema, filamentous freshwater and marine algae, and fungal mycelia, intended for whole mounts, can be transferred from water through either of the fluids directly into xylol-balsam diluted with that fluid and this evaporated down to mounting consistency with no shrinkage, collapse or hardening. Stains are perfectly preserved.

In the experimental work, a variety of fixing fluids

[1] *Zool. Anzeiger,* 96: 204–206, 1931. The writer, however, is indebted to Miss Enid A. Larsen, of the School of Biology, Stanford University, for information concerning, and the experimentation with, dioxane.

[2] The dioxane was obtained from the Eastman Kodak Company (#2144 1,4-dioxane), the tertiary butyl alcohol (a Shell Oil Company product) from the California Botanical Materials Company of Palo Alto, in whose laboratories it is being extensively used. The writer is not aware that tertiary butyl alcohol has previously been used for the purposes noted above.

have been used preceding the employment of either or both dioxane and tertiary butyl alcohol and many surprising results have been noted. Much of the obloquy heaped upon certain killing and fixing fluids appears to be wholly gratuitous and should be laid instead against absolute ethyl alcohol, xylol, chloroform, benzene and similar fluids employed for "clearing" purposes.

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IMMUNOLOGICAL REACTIONS AND VISCOSITY

IN 1923, while at the Rockefeller Institute in New York, we devised a microviscosimeter of high sensitivity[1] which enabled us to follow continuously the changes of viscosity occurring in a sample of solution, as a function of time, temperature or as a consequence of a reaction. It occurred to us that this instrument was well adapted to the study of immunological reactions—immune serum plus antigen—and that it might be interesting to find out whether flocculation and precipitation were not preceded or accompanied by some variations in the viscosity of the mixture. We found that such was the case, and that the addition of one drop of specific antigen to 1 cc of immune serum determined a considerable but *momentary* increase in the viscosity of the mixture. The amplitude of the phenomenon reached, in certain cases, 300 per cent. of the original viscosity. In a few minutes, the viscosity comes back almost to its former value. This reaction is strictly specific. Although we did not publish it at the time, we described the phenomenon at the Pasteur Exhibition in Strasbourg, 1923. In 1933 we reported it before the Congress of Immunology (Rome, Convegno Volta) and in 1934 in the Ergebnisse der Hygiene.[2]

Late in 1934, we took it up again with Miss V. Hamon and applied it to the study of the diphtheria toxin-antitoxin reaction which had been shown by Ramon[3] to yield quantitative results, making it possible to titrate the antitoxic activity of a serum *in vitro*. We found that, under the conditions specified by Ramon, who kindly supplied us with toxin and antitoxin, the same increase in viscosity could be observed, and that, for a given ratio of concentrations of the two substances, a quicker and more important phenomenon took place. It was thus possible to titrate the antitoxin content of a serum by a new and entirely different method, which may prove to be more accurate than the flocculation method.

The same method was then applied to precipitins by our associate, Dr. M. Coppo,[4] in our laboratory. It was found that the increase in viscosity in this case was proportional to the concentration in precipitins of the serum (rabbits injected with horse serum). The reaction is extremely sharp. A few interesting results were obtained: The higher the titer in antibodies of the serum, the smaller the quantity of antigen required to obtain the maximal viscosity. For instance, if 1.5 cc of immune serum precipitating at 1/1000 is mixed with antigen, the maximum of viscosity will be attained when .7 cc of antigen is added. If the serum precipitates at a dilution of 1/15,000 it will only be necessary to add .01 cc of antigen to 1.5 cc of serum in order to obtain the maximal viscosity. And in that case the absolute value of the maximum will be much higher than in the first case. In addition, it was observed that the rapidity at which the viscosity increases, then decreases, is a function of the concentration in antibody of the serum. It was also found that, for a certain and different ratio antigen antibody, corresponding to an excess of antigen, a minimum of viscosity occurred, the amplitude of which was much smaller than that of the maximum.

Similar maxima and minima, when the serum is mixed with tannin in definite proportions, were observed in our laboratory by another of our associates, Dr. F. Seelich. A detailed account of the experiments will appear shortly.

This new reaction seems, therefore, to raise questions of interest, not only from the practical, but also from the theoretical standpoint.

LECOMTE DU NOÜY

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SCIENTIFIC APPARATUS AND LABORATORY METHODS

THE USE OF THE PHOTOELECTRIC CELL IN PHYSIOLOGICAL EXPERIMENTS

THE photoelectric cell, as ordinarily used, measures the density of light to which its total active surface is exposed.

In searching for a method of conveniently obtaining a record of the motion of a beam of light, it occurred to us that the photoelectric cell could be made to record a continuous curve of the arc traversed by the beam, rather than simply the fact that it impinged or did not upon the surface of the cell. It was found that this could be done by interposing between the

[1] Lecomte du Noüy, *Jour. Gen. Physiol.*, 5: 329, 1923.
[2] Lecomte du Noüy, *Ergebn. Hygiene, Bakter., Immunit., u. Exp. Therap.*, 15: 304–334, 1934.
[3] G. Ramon, *C. R. Soc. Biol.*, 86: 661 and 711, 1922.
[4] M. Coppo, *C. R. Soc. Biol.*, 118: 1307, 1935; 119: 165, 1935.