SCIENCE, November 5, 1926.[1] My own modifications of the technique are in connection with the special material used and the exceptional use of quite thick sections, together with the study of these in a strong reflected surface light, rather than by transmitted light from below through cleared sections. For special purposes it is possible of course to stain relatively thin frozen sections by this technique, and clear and study as usual, but the quicker method with thick sections seems preferable for the objects of this study.

The following steps of procedure are recommended.

(1) Material: Pieces of cord and brain-stem from cadavera in which formalin has been introduced beneath the dura and allowed to stand are most favorable. Some specimens which were evidently embalmed late showed fatty degeneration more or less markedly. Pieces of cord which have remained *in situ* during the dissection of the body can be removed to alcohol and put through this method from 95 per cent. alcohol. Fresh cords make excellent material after thorough hardening, poorly fixed and hardened specimens crumbling on cutting. Old bottled formalin or alcoholic material may often be used with good effect.

(2) Cutting: Sections should be smoothly cut free-hand, one or more millimeters thick, with a sharp, flat, thin razor blade. The thicker sections are preferable, though frozen sections of about 100 micra which do not curl or tear are quite usable. As has been said, it is feasible to cut thinner frozen sections, double stain, clear and mount in balsam, but these have restricted usefulness.

(3) Mordanting: Sections should stand in 95 per cent. alcohol for at least 5 minutes, then be transferred to Cole's mordant made as follows: 50 per cent. alcohol, 20 cc; ferric chloride, 1 grm; glacial acetic acid, 2 cc. Sections remain in this mordant for at least 5 minutes. For some material this process of mordanting is sufficient when prolonged till the details of structure have developed out sharply. The sections can then be removed to alcohol and studied without further treatment, but most material gives better results when stained as follows.

(4) Staining: First prepare Cole's "stock hematoxylin solution" as follows: absolute alcohol, 20 cc; sodium hydrosulphite (same as sod. bisulfite), 0.2 grm; distilled water, 5 drops; hematoxylin crystals, 1 grm. Use light brown crystals, not dark crystals of hematoxylin. A useful discussion of the staining will be found in Dr. Cole's original article.

Now add 5 drops of this just described stock solution to 10 drops of tap-water, and follow this with 1 drop of ammonium hydroxide. Before using let this mixture stand to ripen for 30 seconds. Now add 5 cc of 95 per cent. alcohol to this ripened staining solution and flood the section (which has been taken from its alcoholic bath) to cover its smooth-cut surface. Allow the stain to act for at least 5 minutes. This over-stains and must be differentiated.

(5) Differentiation: This should be accomplished by destaining through the action of 0.4 per cent. hydrochloric acid. The microscopic details of the gray matter can be thus brought out sharply in contrast to a darker background. It is sometimes well to stroke the surface with cotton or lens paper to remove sediment deposited from the stain. When sharp definition is obtained rinse off in slightly alkaline 95 per cent. alcohol and study by strong reflected light.

A very instructive picture of the grosser features and relations of the gray figure, compared in different regions, is secured by diluting the stain and using it briefly without differentiation. In this case the gray figure and septa stand out black against the lighter matter of the funiculi, but little detail is shown within the gray figure.

Dr. Cole's original paper contains interesting discussion of the various applications and problems connected with the use of his stain which does not seem to be known as well as it deserves.

(6) Lighting: Reflected light of strong intensity should be used, preferably concentrated on the surface of the section by a condensing lens. Under this lighting the section should be studied with a hand lens, binocular spectacles or a low power 48 millimeter objective on a compound microscope.

Of course the mordant and stock solutions will be prepared in advance for class work, and each student will follow the technique in watch-glasses and begin study of the specimens in a few minutes. Once stained, sections may be kept in alcohol for later examination.

This paper was finished at the Wistar Institute. I take pleasure in here thanking the director and staff of the institute for many helpful kindnesses while I worked there as guest this winter.

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[1] SCIENCE, 64: 452–3.

CELLULOID CASES FOR MICROSCOPES, MICROTOMES AND BEAM-BALANCE CASES

BELL jars placed over microscopes are hazardous both to microscope and bell jar, because bell jars are cumbersome and heavy. A cone or cone frustum made of celluloid will answer as well, cost much less, be far more convenient and have a longer life than a glass bell jar.

Purchase a sheet of celluloid from a mail-order house or auto-supply store. Cut a sheet of stiff paper to form a cone to fit the microscope; use this as a

pattern for celluloid. For a large microscope piecing may be necessary. Use celloidin, or better, Dupont Duco Cement, permitting the first application to partly dry, apply a second layer of cement, hold in place with weights or pressures for ten minutes and the job is done. (I have such a cone fitted over a Leitz research microscope standing on a Chambers micro-dissection apparatus, all beautifully visible yet dust-proof.) A cone frustum would be better looking but slightly more difficult to make, though it is merely a matter of fitting in the top. It also would eliminate the piecing necessary for the cone.

By folding the celluloid over a wire frame to give better rigidity I have made a celluloid case to cover a Thoma-Jung microtome. Dr. E. P. Bartlett, seeing this, conceived the idea of making dust-proof cases for beam balances. These are folded and cemented like paper boxes.

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DEVELOPMENT OF A PERMANENT BLUE COLOR FOR COLORIMETRIC PHOSPHORUS DETERMINATION

THE blue color used as a standard for Dénigés colorimetric method for the determination of phosphorus is very unstable. The color fades rapidly and a new color standard must be prepared rather frequently. By reducing a solution containing 2.5 grams of ammonium molybdate in 100 cc of 10 n. sulfuric acid a permanent blue color can be developed. The solution is reduced by stannous chloride. A dense blue color is formed which can be diluted to the desired intensity by adding 10 n. sulfuric acid. With proper dilutions a series of standards can be prepared which represent definite readings of phosphorus in parts per million.

The blue color developed under the latter condition is of a slightly different hue from the color of the reduced standard phosphorus solution but this slight difference in color is not enough to be objectionable. As a matter of fact, this permanent blue color compares as well to the unknown blue color as to the blue standard phosphate color.

The shades of blue color vary with the higher concentration of both ammonium molybdate and sulfuric acid. With the mentioned amount of ammonium molybdate in a slightly lower concentration than of 10 n. sulfuric acid a bright yellow color is produced upon reduction.

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SPECIAL ARTICLES

AN EFFECT OF SHORT ELECTRIC WAVES ON DIPHTHERIA TOXIN INDEPENDENT OF THE HEAT FACTOR

ABOUT thirty-five years ago D'Arsonval and Charrin found that high frequency currents of 200,000 cycles per second diminished the strength of diphtheria toxin. This effect was obtained without elevation of temperature to a level which would by itself affect the toxin. Since that time little has been done to develop this finding. Recent advances in short wave technique have given new impetus to the study of the biological action of these waves. It is readily accepted from many recent papers that such electrical waves may produce tremendous changes through the indirect medium of temperature elevation. Before any result is ascribed to the specific action of short electric waves, heat effect through conductivity and eddy currents must be ruled out. The chief import of this paper is to show that radiation of the type used here is capable of producing changes in biological substances independent of a heat effect.

Throughout, the wave-length was 1.9 meters and the substances to be radiated were placed between two condenser plates of a resonating circuit. The amperage in the resonating circuit was .95 to 1.2, and the frequency was 158,000,000 cycles per second.

From the beginning, and so far in this work—eliminating heat effect—completely negative results were obtained in attempts to sterilize milk and to destroy bacteria both *in vivo* and *in vitro*. Radiation *in vivo*, both as general radiation of the whole animal and local radiation to the site of injection, produced no changes in the course of streptococcus infections in guinea-pigs. In addition, no effects of the radiation could be detected on the precipitin titer of the pneumococcus antisera from rabbits.

The study of diphtheria toxin was made in two series of experiments. First series: One sample of toxin was chilled in ice water to 7° C., then exposed to radiation until the temperature had risen to 38°–40°C. (about four minutes). When such a temperature was attained the sample was taken out of the high frequency field and chilled again in the ice water. This process was repeated until the total time of radiation was fifteen to sixty minutes. A control sample was kept at the identical temperatures with the same rate of heating and cooling by alternate chilling in ice water and immersion in a small heated water bath. The temperature attained did not affect