

sure chamber.¹¹ At the end of this period, the mortality percentages were recorded and the thyroid glands of all animals were dissected and weighed. The results are shown in Table 1.

The data indicate that treatment with thiourea for 12 or more days enables the rats to survive a reduction of the atmospheric pressure to 200 mm of Hg, whereas the majority of the untreated controls succumb. Thiourea injections made a short time (5 hours) prior to exposure to low pressures have no apparent beneficial effect. Preliminary experiments performed with males have shown that these are also benefited by thiourea provided the treatment is applied for at least 12 to 14 days. It is to be observed that the animals on a thiourea ration for 12 or more days possess heavier thyroids than do the untreated rats or those

given the drug for shorter periods of time. This enlargement of the thyroid gland, a condition which is readily reversible when thiourea is withdrawn, is indicative of the development of a hypothyroid condition.^{6,7} We are of the opinion that this state of functional hypothyroidism (chemical thyroidectomy) is responsible for the increased tolerance to reduced atmospheric pressures shown by rats treated with thiourea.

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SCIENTIFIC APPARATUS AND LABORATORY METHODS

A NEW METHOD FOR PRESERVATION OF HUMAN AND ANIMAL TISSUES BY THE USE OF A TRANSPARENT PLASTIC

A GOOD preservation of form of human and animal organs can be obtained by imbedding them in a transparent plastic, "Plexiglas." A method for imbedding insects and similar structures in "Plexiglas" has been proposed by Dr. Charles Sando,¹ but this procedure could not be applied successfully to fleshy animals or organs of animals and humans without considerable loss of form and color. The present process has been used so far in the case of relatively small organs (approximately 8×5×5 cm) and to slices of larger organs (10×10×2 cm). There appears to be, however, no difficulty in applying the method to considerably larger objects.

The advantages of this method of tissue preservation are: (1) the remarkable preservation of size, shape and texture and the satisfactory retention of color; (2) the ease of handling of the finished specimen, which is permanent and practically unbreakable; (3) the avoidance of distortion of vision due to the curvature of the surface of containers and the preserving fluids generally employed.

Preliminary to the imbedding, it is necessary to dehydrate the tissue. This is accomplished by placing the fresh organ or piece of tissue on a layer of frozen water (5 to 10 mm in thickness) contained in a suitable receptacle, and cooled to -20° C. or below, by covering the organ with cold distilled water and freezing the whole mass as rapidly as possible at a temperature of -20° C. or below. The mass of ice without and within the organ is then removed by condensation of water vapor at low temperature *in vacuo*.²

¹¹ M. Dubin, *Quart. Jour. Exp. Physiol.*, 24: 31, 1933.

¹ H. G. Knight, *SCIENCE*, 86: 333, 1937.

The tissues thus obtained are very light and porous and appear to have lost a great deal of the color and texture. At this point the specimen may be readily trimmed with a sharp scalpel. The dried tissues should be promptly sealed or processed so as to avoid absorption of moisture. They should be carefully brushed with a camel's hair brush, to remove any loose particles or dust and similar foreign material.

The next step in the process is the saturation of this tissue with the liquid acrylic ester (monomeric ethyl methacrylate).³ This is attained by pouring the ethyl methacrylate in a large-mouthed receptacle, placing the tissue in it and then producing a vacuum (about 700 mm of Hg.). This is easily accomplished by setting the open receptacle containing the specimen in a vacuum-desiccating jar with stout walls. In a period of one half hour to one hour, the air will be entirely replaced by the ethyl methacrylate, and upon readmitting air into the jar, the tissue will sink in the fluid. In the case of highly porous materials, it may be necessary to repeat this vacuum treatment several times before all trapped air has been replaced by the monomer. The colors of the tissue appear to have returned, but they are somewhat duller than normal and the natural texture is still lacking at this point of the process.

The subsequent steps have to deal with the imbedding of the tissue by polymerization of the monomer. In view of the fact that prolonged immersion of the tissue in the liquid ethyl methacrylate causes bleaching, it is desirable to hasten the polymerization by the addition of a catalyst, such as benzoyl peroxide, and

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² Max M. Strumia and John J. McGraw, *Jour. of Lab. and Clin. Med.*, 28: 9, 1140-1153, June, 1943.

³ Obtained from Rohm and Haas Company, Philadelphia 5, Pa.

by cautious use of heat. The simplest technic may be summarized as follows:

(1) Dissolve benzoyl peroxide in ethyl methacrylate, in the proportion of .2-.3 per cent.

(2) After adding a porous clear boiling chip, bring a sufficient amount of this mixture to boil over a hot plate. Avoid an open flame, as the material is inflammable. Boil the material (approximately 115° C.) for about one minute and then cool immediately with running water to about 50° C. Polymerization is an intensely exothermic reaction, and cooling must be done rapidly, otherwise the heat production will be so intense as to cause boiling over and solidification in the form of an opaque mass. Allow the ethyl methacrylate to remain in a closed receptacle (avoid rubber) at plus 50° C. until complete solidification is obtained. About 20 per cent. shrinkage in the volume of the material occurs during polymerization and allowance must be made for this loss.

(3) Remove the piece of tissue saturated with ethyl methacrylate from the liquid monomer, place it upon the base of "Plexiglas" thus prepared, and immediately cover with the ethyl methacrylate-benzoyl peroxide mixture which has been polymerized previously to a syrupy consistency. Polymerization to syrupy consistency is accomplished exactly as outlined under 1 and 2, but the process is stopped when the proper viscosity is obtained. The trapping of air bubbles during this procedure should be carefully avoided. If this occurs, remove by means of a vacuum in a desiccator as previously outlined.

(4) Close the container and place at about 50° C. and allow polymerization to proceed until the mass is thoroughly solid. Polymerization occurs more rapidly at higher temperatures, and more heat may be applied gradually until a temperature of about 70° C. is reached in two to three days. It is desirable, when polymerizing at temperatures higher than 40-50° C., to reduce the height of the layer of the liquid monomer to about 10 mm. Successive layers may be added as desired.

(5) The glass receptacle is then broken and the mass of solidified acrylic resin containing the imbedded tissue is removed. The block is generally clear enough for viewing the specimen without further work. It may, however, be readily cut and polished so as to obtain a more finished product. For large flat slices of organs, a form can easily be obtained by properly binding plates of thick glass with parchment paper.

Any object made by casting an acrylic resin may "craze" unless it is given an annealing treatment. This "crazing" is seen initially as an iridescent effect on the surface of the material and may eventually result in a network of fine cracks extending some distance into the solid mass. In order to prevent "crazing,"

after any cast object has been machined to the approximate finished shape, we recommend that it be subjected to a temperature of 100-115° C. for one-half hour.

The organs thus imbedded have the original size and shape and retain the normal texture to a remarkable degree. The color is somewhat less brilliant than it was originally, but if the period of polymerization is not too long, it may be satisfactorily preserved. The color has been found to be permanent in mounted specimens even on continuous exposure to sunlight for several months.

Fatty tissues are not so suitable for this type of imbedding. Some organs, such as the liver, have a tendency to release pigment, which causes the imbedding mass to have a slight discoloration. This does not seem, however, to interfere with the general appearance and preservation of the specimen. If the tissue is thin, a certain degree of transparency is attained, which in many cases is desirable.

This process offers what appears to be a most promising method of preservation of normal and pathological specimens for teaching and museum purposes.

Further studies on this procedure are in progress. For the present, the method outlined appears most applicable for the preservation, without shrinkage or loss of color and texture, of small organs, embryos, little animals, fleshy insects and tissues with delicate structure.

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BOOKS RECEIVED

- COITH, HERBERT. *So You Want to Be a Chemist?* Pp. x + 128. McGraw-Hill Book Company.
- Estadística. Pp. 192. Inter-American Statistical Institute. 60¢.
- EYRING, HENRY, JOHN WALTER and GEORGE E. KIMBALL. *Quantum Chemistry*. Illustrated. Pp. vi + 394. John Wiley and Sons. \$5.00.
- FREUD, ANNA and DOROTHY T. BURLINGHAM. *War and Children*. Pp. 191. International University Press. \$1.50 paper cover; \$2.00, cloth cover.
- KOEHLER, W. A. *Principles and Applications of Electrochemistry*. Pp. v + 573. Illustrated. John Wiley and Sons. \$5.00.
- STROUD, ROBERT. *Stroud's Digest on the Diseases of Birds*. Illustrated. Pp. ix + 483. L. G. Marcus and Company. \$5.00.
- SUTER, CHESTER M. *The Organic Chemistry of Sulfur*. Illustrated. Pp. v + 858. John Wiley and Sons. \$10.00.
- The Harvey Lectures*. Series XXXIV. 1938-1939. Illustrated. Pp. 279. The Williams and Wilkins Company.