

acid per plant been found to occur at night, but instead, losses of 15 to 20 per cent. have been observed. It is thus evident that neither the total gain nor loss at night can be estimated by the methods which have been employed and that the total losses are probably considerably greater than have been estimated. The results thus suggest that vitamin C may play a much

more important part in the economy of the plant than has been previously attributed to it.

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SCIENTIFIC APPARATUS AND LABORATORY METHODS

AN APPARATUS FOR VACUUM DRYING IN THE FROZEN STATE¹

IN the fractionation of thermo-labile biological products it often becomes necessary to recover small amounts of solids from comparatively large volumes of solution. Vacuum drying in the frozen state, as recommended by Flosdorf and Mudd² and Link, Eggers and Moulton,³ is often preferred, particularly if the material is protein in nature.

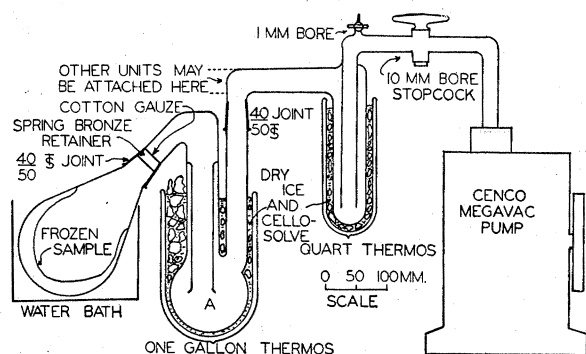


Fig. 1

The apparatus shown in the diagram is designed so that each receiver (A) is capable of receiving 500 to 600 cc of the distillate within a period of twelve hours. A Cenco Megavac pump serves to create the vacuum. A mercury vapor diffusion pump is not necessary. The water bath surrounding the flask containing the sample is left at room temperature and serves to prevent ice formation on this flask during evaporation. An acetone bath chilled by solid carbon dioxide is used to freeze the sample to be evaporated, while methyl cellosolve is used in place of acetone in the vacuum bottles in order to minimize loss through evaporation. A short spring bronze cylinder is used to hold a piece of cotton gauze in the neck of the sample flask to prevent loss of dry particles due to rapid vapor currents.

The apparatus is constructed of pyrex glass, is

¹ This work was supported in part by a grant from Armour and Co.

² E. W. Flosdorf and S. Mudd, *Jour. Immunol.*, 29: 389, 1935.

³ G. K. K. Link, V. Eggers and J. E. Moulton, *Bot. Gaz.*, 102: 590, 1941.

simple, compact and sturdy and has become nearly indispensable in our laboratory. It has been used to recover biologically active materials from as much as one liter of solution in a period of twenty-four hours with a single change of the receiver and recharging with solid carbon dioxide after twelve hours. Solutions containing the gonadotropic or thyrotropic fractions from the pituitary, choline esterase, bacterial toxins, whole blood, minced tissues and various extracts have been shown to retain their solubility and biological activity under such treatment. Solutions treated with toluene according to the method of Railton, Cunningham and Kirk³ yield sterile preparations.

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ELIMINATION OF DEHYDRATION IN HISTOLOGICAL TECHNIQUE

THE classical methods for preparing tissues for microscopic study have a number of disadvantages. One of the more important is the extensive dehydration of the specimen by passage through increasing concentrations of alcohol. Another, consequent upon this, is the solution of cellular fat in the alcohol; this requires, for fat stains, the setting aside of special blocks of tissue and the use of the frozen section technique.

In the search for an improved method which would avoid dehydration, we thought of the recently synthesized group of resins, the polyvinyl alcohols, more familiar in the trade as PVA. These substances are readily soluble or dispersible in water, forming sols which penetrate easily into tissues. They can be made to form gels of a consistence comparable with that of celloidin. Sections may be cut as thin as four to five micra without difficulty. These stain excellently with hematoxylin-eosin, Masson's trichrome, Weigert-van Gieson; staining for fat is done with Sudan III on sections cut from the same block. The polyvinyl alcohols in solution are considered stable to the action of bacteria and most fungi; in our tests, the gels as well

³ I. R. Railton, B. Cunningham and P. L. Kirk, *SCIENCE*, 94: 469, 1941.

allowed no such growth. Finally, the cost of the entire process is well within the capacity of the ordinary laboratory, if not cheaper than present methods.

A solution containing 20 per cent. by weight of grade RH-393 PVA is prepared by suspending the powder in cold water (about 20° C.), breaking up the lumps, then stirring well while heating in a steam-bath to a temperature of 75–85° C. To the cooling solution is added 20 per cent. of glycerine by weight. Washed formalin-fixed tissue, without further preparation, is placed in this material in covered shallow dishes. Infiltration of ordinary tissues, as heart, lung, liver, spleen, etc., using pieces of average size, is as good when they are put directly into 20 per cent. PVA as when they are run through 5 per cent. and 10 per cent. first. The dishes are kept at room temperature but for a daily exposure of two hours to a temperature of 56° C. in the oven. Solidification takes place in 8 to 9 days. The total time may be shortened by cautiously uncovering the dish toward the end. The trimmed block of hardened PVA is attached to the fibre carrier-block with paraffin or with cement. The cut sections unroll in lukewarm water and are mounted immediately for staining. The medium is not washed away, but stains no more than does celloidin. The remaining procedures are as usual.

This is a preliminary report. Further experimentation in progress is aimed at eliminating heat and at shortening the procedure. The protean qualities of this plastic make such improvements highly probable.

We should like to thank E. I. du Pont de Nemours and Company for supplies of PVA and for considerable advice.

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X-RAYS FROM RADIO TUBES

IN 1937 Simons, Clark and Klein¹ described a simple apparatus for the generation of x-rays from an old 01-A radio tube, the total cost of the equipment being something like \$25. The purpose of the present note is to describe a simplified form of the apparatus, which can be assembled at an expense of six or seven dollars and which is remarkably effective for making radiographs of various specimens.

The materials required are:

Two Ford model T ignition coils (KW brand).

A step-down transformer, from 110 to 12 volts (if unavailable, two 110–6.3 volt transformers commonly used in radio circuits can be substituted with the 110-volt sides connected in parallel and the 6-volt sides in series).

An old 01-A radio tube.

¹ *Radiology*, 29: 721, 1937.

A small wedge of sponge rubber.

Some No. 22 wire for hook-up connections.

A small portion of a metal foil.

The two spark coils are connected in series by connecting the two terminals nearest the vibrators together. The 12-volt source is connected to the two terminals on the ends opposite the vibrators. The 110-volt end of the transformer is connected with a 110-volt line source. One of the vibrators is turned down tight so that it does not operate. A wedge of sponge rubber is slipped under the other vibrator to produce the maximum frequency possible. This adjustment takes about five minutes. One high tension lead is connected with the four prongs of the base wired together and the other with the foil wrapped around the upper part of the radio tube. The foil should not cover the portion of the tube facing the flat side of the plate element inside where the x-rays originate. The radio tube should be mounted on an insulated support, for the voltage is about 16 KV to ground with about 32 KV between the two high-tension terminals. A small glass tumbler, with a strip of adhesive tape, makes a good support. Radio tubes displaying a green fluorescence produce an x-ray beam of greater intensity than tubes showing a blue fluorescence.

For making radiographs at a distance from the radio tube to the object of four to six inches and with Agfa non-screen x-ray film held in double black paper envelopes, the time of exposure is from two minutes for thin objects to five minutes for thicker ones.

Excellent radiographs of objects such as seeds, fountain pens and other fabricated objects, gems, flowers, bones, etc., are easily obtained. The apparatus is also particularly well adapted for microradiographs, in which the radiograph of small specimens is registered on a fine-grained photographic emulsion and enlarged as described by Clark and Shafer.²

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² *Transactions of the American Society of Metals*, p. 732. 1941.

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