

different arrangements were tried, one which is here-with described (Fig. 1) was found satisfactory in

amount of carbon dioxide given off by plants in the dark.

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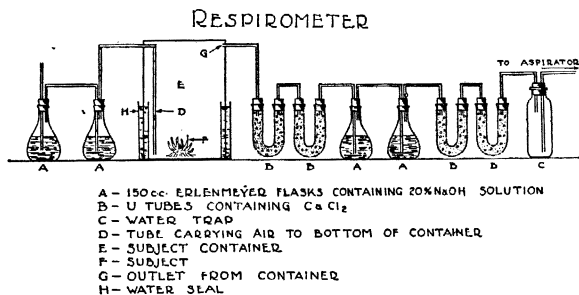


Fig. 1

every respect, including low cost for material and equipment.

The principal divergence from the customary respirometer is the subject container. This was made of galvanized iron. The air which has had the carbon dioxide removed in the pair of NaOH bottles (A) is drawn in at the top of the respiration chamber and carried to the bottom of the container in the metal tube (D). The air with the carbon dioxide of the asparagus respiration is then carried out of the container from the top through the opening (G). In this way there is a continuous change of the air in the container.

In using this container the greatest difficulty was experienced in making an air-tight seal which could be broken easily and frequently for examination of the asparagus root. Sealing wax, paraffin, grafting wax and similar materials had many objections. In no case could a tight seal be held for more than 12 hours, because the waxes withdrew from the galvanized iron container and the base, whether the base was metal or glass. To avoid this difficulty a base was made for the container, and in this base is a double-walled collar. The asparagus roots were set in this base. The side of the galvanized iron subject container fitted in between the walls. The double wall was then filled with water. No further trouble was experienced with air leaking through the container, and the respirometer has been in continuous operation for as long as 48 hours and no leaking has been detected.

An easy check for leaks is made by setting the respirometer up without putting a plant in the container. By introducing carbon-dioxide free air into the container and then passing the air through the entire apparatus the operator is able to take some carbon dioxide out at the aspirator, if there is any leaking in the system. None has been found after two interrupted 24-hour tests and one continuous 48-hour test.

This apparatus has the advantages of being easily and cheaply constructed, can be opened and closed easily and seemingly is satisfactory for measuring the

NEW FIXING FLUIDS FOR GENERAL PURPOSES

THE number of fixing fluids recommended up to date is admittedly very great. The excuse for the new formulae given here lies in the fact that all other fluids with which I am familiar, either harden the tissues too much or interfere with subsequent staining. Serial sectioning is often made very difficult, while macroscopic dissection becomes almost impossible. In addition, many fluids require prolonged washing. The duration of fixation is also often very limited, resulting in great inconvenience when one attempts to use the fluids on scientific expeditions. The new fluids recommended here represent the results of extensive experimentation extending over many years and are more or less free from the above-mentioned defects. Animals fixed in them remain soft and do not harden subsequently in 70 per cent. alcohol in which they may be left for many weeks. Washing is simple. All common stains may be used. Mallory's triple stain gives brilliant differentiation, though the picture is somewhat different from that obtained after fixation in Zenker's fluid. Complete penetration of all ingredients of the fluids is accomplished at the rate of one half millimeter per hour, but the nitric acid penetrates twice as rapidly. This was determined by an examination of pieces of liver at intervals of one hour. The surface of a smooth cut shows the fixed zone clearly. The penetration of paranitrophenol was determined by wetting the cut surface with ammonia and measuring the width of the zone in which the tissues turned yellow. Similarly, the penetration of cupric nitrate was determined with sodium sulfide.

The change in volume due to fixation was determined by measuring the displacement of water before fixation and after fixation. This was done by Dr. G. E. Pickford, who found an average swelling of about 10 per cent. in beef liver fixed for 24 hours in the phenol and the paranitrophenol mixtures, while in the case of the alpha dinitrophenol mixture the increase in volume was only about 5 per cent. A paranitrophenol mixture made up with 70 per cent. instead of 60 per cent. alcohol caused a swelling of about 20 per cent. Rat testes showed a shrinkage of about 5 per cent. after 24 hours fixation in the phenol mixture, but no appreciable change of volume after fixation in the paranitrophenol mixture. When transferred to 70 per cent. alcohol for 9 days, both showed an increase in volume; in the case of the testis fixed in the phenol mixture the increase amounted to about 13.5 per cent., in the case of that fixed in the paranitrophenol mixture to about 16 per cent. Transferred for 10 days into 80 per cent.

alcohol the first testis showed no further change in volume, while the second showed an increase to about 20 per cent. of its original size.

Phenol gives a peculiar elastic texture to the tissues, unlike anything produced by commonly used fixing fluids. Paranitrophenol comes nearest to phenol in this respect and, being stable in the mixture, is preferable where stability is desired. I have used both mixtures for arachnids, insects, myriapods, leeches, earthworms, flatworms, roundworms, older amphibian larvae and mammalian embryos. Professor J. S. Nicholas, of our department, is using my paranitrophenol mixture for rat embryos and fish in preference to sublimate or Bouin, while Professor W. R. Coe finds it very satisfactory for nemerteans. Macroscopic dissection of invertebrates and mammalian embryos fixed in either of these fluids is greatly facilitated.

No. 1. *Cupric-phenol fixing fluid.*

Stock Solution A.	
Distilled water	100 cc
Nitric acid, c.p., sp.gr. 1.41-1.42	12 cc
Cupric nitrate, c.p., crystals $\text{Cu}(\text{NO}_3)_2 \cdot 3\text{H}_2\text{O}$	8 grams

Stock Solution B.	
80 per cent. alcohol	100 cc
Phenol, crystals, c.p.	4 grams
Ether	6 cc

The stock solutions are perfectly stable and may be kept in glass-stoppered bottles. For use take: Solu-

tion A—1 part, Solution B—3 parts. The mixture does not keep and must be used within a few hours. For the same reason the duration of fixation must not exceed 48 hours. 12 to 24 hours will suffice in most cases. Wash in several changes of 70 per cent. alcohol.

The following fluids are perfectly stable and may be kept for months in glass-stoppered bottles:

No. 2. *Cupric-paranitrophenol fixing fluid.*

60 per cent. alcohol	100 cc
Nitric acid (as above)	3 cc
Ether	5 cc
Cupric nitrate (as above)	2 grams
Paranitrophenol, c.p., crystals	5 grams

Duration of fixation not limited by time, except as to the minimum time required for penetration at the rate of one half millimeter per hour. Wash in several changes of 70 per cent. alcohol.

No. 3 to No. 6: These fixing fluids have the same composition as No. 2, except that in place of 5 grams of paranitrophenol they contain 0.5 gram of one of the following nitroderivatives of phenol: No. 3—orthonitrophenol, No. 4—alpha (2:4) dinitrophenol, No. 5—beta (2:6) dinitrophenol, No. 6—picric acid (2:4:6 trinitrophenol). Fixation and washing as in No. 2, but Nos. 5 and 6 require longer washing and leave the tissues yellow.

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SPECIAL ARTICLES

VARIATIONS IN VISIBLE SOLAR LIGHT DURING SUBMARINE MEASUREMENTS

WHILE making measurements on the intensity of some of the components of visible solar light beneath the surface of ocean waters, variations in the intensity of the incident light were found to be of such magnitude, on some days, that considerable uncertainties are introduced into the results if these variations are unaccounted for. These variations are not ones which depend upon zenith distance, but are occasioned by other circumstances. They are of particular importance when the depth of water is such that very small currents result in the submerged photoelectric cell. These variations occur on days when the sky is cloudless and the atmosphere very clear to the eye.

The time required to make a series of measurements of illumination intensity beneath the sea with a photoelectric cell to a depth of 50 meters, say, is an hour or more if one-meter intervals are chosen as unit layers of the absorbing medium. During this time the illu-

mination intensity of the radiation incident upon the surface of the water changes slowly with the variation in the solar zenith distance. Also, the intensity of the visible light may vary within a few minutes by several per cent., even though there be a cloudless sky and a clear atmosphere. A study of these variations made during the summer of 1932 at the Friday Harbor (Washington) station of the Oceanographic Laboratories also show that a record of the total radiation intensity on a horizontal surface is not indicative of all changes which may occur in the illumination intensity.

Such variations are shown in Fig. 1. The total radiation was measured by means of an Eppley pyrliometer connected to an Engelhard recorder. This instrument had recently been calibrated by Dr. Herbert H. Kimball in charge of solar radiation for the U. S. Department of Agriculture. The illumination intensity was measured by means of a calibrated photoelectric cell.

The curves in Fig. 1 are characteristic of the obser-