THE EFFECT OF OUABAIN (1 : 500,000) ON THE POTASSIUM CONTENT OF FROG'S SARTORIUS MUSCLE

Experiment	Time of exposure	Potassium per 100 gm. muscle		Potassium loss
		Control	Ouabain	
	hours	*mgm.*	*mgm.*	*per cent.*
3/31/37	5–1/2	290	242	17
4/3/37	4–3/4	217	174	20
4/7/37	6	308	231	25
4/9/37	6–1/2	293	231	21
4/12/37	7	315	238	24
6/9/37	6	195	99	49
6/11/37	6	227	147	35
6/12/37	7	191	119	38
Average: ..		**255**	**185**	**29**

muscles exposed to a 1:500,000 ouabain concentration in Ringer's solution uniformly show a loss of potassium as compared with the companion control muscles kept in Ringer's solution alone, the average loss in the eight experiments being 29 per cent. The potassium loss in the last three experiments is greater than in the earlier ones, possibly representing a temperature or seasonal variation.

It is to be noted that the concentration of ouabain employed is greater than that obtaining in therapeutic doses. However, striated muscle is relatively resistant to digitalis action, and at the end of the period of exposure to ouabain, these muscles showed no contracture and gave a good contraction when stimulated. The study is being extended to include cardiac muscle and also potassium metabolism in animals receiving therapeutic amounts of the digitalis glucosides.

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SCIENTIFIC APPARATUS AND LABORATORY METHODS

UNIFORM TISSUE SECTIONS FOR WARBURG TECHNIQUE[1]

WHEN the metabolism of a section of excised surviving tissue is to be measured and related to unit weight of tissue, there must be a limiting section thickness which is dependent upon the magnitude of the metabolism and the diffusion constant of the reacting substances. Warburg[2] has discussed this subject and from theoretical considerations determined that, if "the tissue section is to breathe in all its parts, it must be thinner than 4.7×10^{-2} cm, if it breathes in pure oxygen, and thinner than 2.1×10^{-2} cm if it breathes in air."

This paper is concerned with a simple method for the preparation of satisfactory tissue sections. Two razor blades of the Gillette type are separated by a thin metal strip and attached to a handle, as shown in Fig. 1.

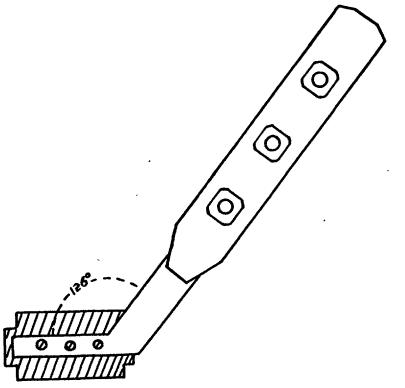

FIG. 1. Sketch showing construction of cutting instrument.

Immediately after removal from the animal, the tissues to be sectioned are laid on a filter paper moistened with physiological salt solution and held in position with the thumb and forefinger of one hand. The cutting instrument is dipped in the salt solution and the excess solution shaken off. Holding the razor blade edges at an angle approximately 45° to the plane of the filter paper, the instrument is drawn across the tissue with sufficient downward pressure to permit a clean cut. The cut section of tissue is removed from between the razor blades and weighed on a microtorsion balance. While the tissue is suspended on the balance, it is possible to clip off a portion of the tissue with a small pair of scissors so as to give a tissue section of desired weight. In this manner, it is possible to place tissues of approximately the same weight and thickness in a series of Warburg vessels. Since the amounts of tissue in a series of vessels are nearly equal, the manometer readings show the trend of results while the experiment is in progress.

Experimental proof that tissue sections cut in this manner are thin enough to permit the tissue to respire in all its parts was obtained in the following manner. By placing metal strips of different thicknesses between the razor blades, albino rat liver sections of varying thicknesses were made and floated in a Petri dish over coordinate paper. The tissue sections were trimmed to equal areas, approximately 100 square millimeters, placed in the Warburg vessels, and oxygen consumption measured in a phosphate buffer solution of pH 7.3 containing 0.1 per cent. dextrose. Folding

[1] From the Bureau of Chemistry and Soils, U. S. Department of Agriculture, at the Department of Pharmacology, Stanford University School of Medicine, San Francisco, Calif.

[2] O. Warburg, *Biochem. Zeitschr.*, 142: 317, 1923.

of the tissue sections was prevented by small platinum hooks anchored to the central absorption chamber. "KOH-papers" were placed in the central absorption chambers of the Warburg vessels as recommended by Dixon and Elliott[3] to facilitate absorption of carbon dioxide evolved during respiration. Upon completion of the experiment the tissues were dried to a constant weight at 105° C, and the wet weight calculated by multiplying the dry weight by 5, the factor used by Warburg.[2]

In four typical experiments the cutting edges of the instrument were separated by a strip of metal 0.3 mm thick. The dry weights of the tissue sections were 7.4, 7.5, 7.4 and 7.2 mgm, corresponding to wet weights of 37.0, 37.6, 37.3 and 36.0 mgm, respectively, when multiplied by the factor of 5. The wet weights obtained by direct weighing were in substantial agreement, being 41.0, 40.5, 40.0 and 40.5 mgm, respectively. The calculated thickness of the tissue sections were within the limit specified by Warburg, being 4.1×10^{-2}, 4.05×10^{-2}, 4.0×10^{-2} and 4.05×10^{-2} cm, respectively. The Q_{0_2} values, based on dry weight, were of the proper magnitude, namely, -10.0, -10.5, -9.7 and -9.9 cu mm. When the cutting edges were separated by approximately 0.6 mm, the thickness of the tissue exceeded the maximum value specified by Warburg, and a Q_{0_2} value of -7.2 cu mm showed that the tissue did not respire in all its parts.

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AMMONIUM FLUORIDE FUSION: A RAPID MEANS OF DETERMINING POTASSIUM IN SOILS

ORIENTATION data in the study of soil fertility problems require quick and reasonably reliable methods for determination of the major plant food elements in soils. In the case of total potassium a less time-consuming procedure than is now available for accurate estimation of the element would be of distinct advantage in many investigations.

Studies by Shead and Smith[1] showed that it was feasible to decompose refractory silicates with fused ammonium fluoride, with reduction of the time required for fusion to as low as approximately ten minutes. Although their work was limited to the determination of silica in glass sand, their data showed that other constituents were not lost; and since potassium would likewise remain in the non-volatile residue, the procedure offered promise for the determination of potassium.

In preliminary experiments with application of an adaptation of Shead and Smith's procedure to a number of tropical soils, the time required for determination of potassium has been reduced as much as one half; and approximately 80 to 92 per cent. of the value for K, as determined by a modification of the classical J. Lawrence Smith method,[2] has been obtained.

The technical grade of ammonium bifluoride suggested by Shead and Smith has proved somewhat more satisfactory than the fluoride, and it is believed, therefore, that the technique required to make this method of decomposition applicable to all kinds of soils can be developed to such a point that the time required for the determination can be materially reduced, and the accuracy of the determination of potassium improved. Details of the procedure as developed will be published at a later date.

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[1] A. C. Shead and G. F. Smith, *Jour. Am. Chem. Soc.*, 53: 483–486, 1931.

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