condensing 1 bromo, 2, 3, 4 triacetyl glucuronic acid methyl ester with silver benzoate.

Benzoyl glucuronic acid was isolated according to the method of Quick from the urine of dogs which had been fed daily doses of benzoic acid. The pure derivative crystallizes from water as needles melting at 184–185°. $[\alpha]_D^{25°} = -26.8°$ in H_2O (C = 0.6 per cent.). The methyl ester of benzoyl glucuronic acid was prepared in excellent yields by treating a methyl alcoholic solution of the free acid at −10° with a slight excess of diazomethane. The compound crystallizes from water as needles melting at 190–191°. $[\alpha]_D^{25} = -16.3°$ in CH_3OH (C = 1.5 per cent.) $C_{12}H_{13}O_6COOCH_3$. Calculated OCH_3 9.92. Found OCH_3 10.13.

The acetyl derivative was prepared by acetylation of benzoyl glucuronic acid methyl ester with pyridine and acetic anhydride at 0°. Triacetyl monobenzoyl

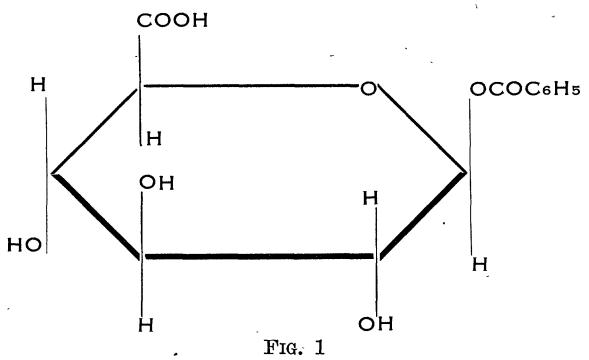

FIG. 1

glucuronic acid methyl ester is formed in yields of 88 per cent., and only one product is obtained from the reaction mixture. When recrystallized from methyl alcohol the substance is obtained as needles melting at 145°. $[\alpha]_D^{28°} = -16.6°$ in $CHCl_3$ (C = 1.5 per cent.) $C_{20}H_{22}O_{11}$. Calculated C 54.77, H 5.06, OCH_3 7.07. Found C 55.10, H 5.26, OCH_3, 7.12.

Synthetic 1 benzoyl 2, 3, 4, triacetyl glucuronic acid methyl ester was prepared by condensing 1 bromo 2, 3, 4, triacetyl glucuronic acid methyl ester (1 mol) with silver benzoate (3 mols) in anhydrous chloroform at 61°. The derivative is formed in yields of 75 per cent. When recrystallized from methyl alcohol the synthetic derivative was found to be identical with triacetyl monobenzoyl glucuronic acid methyl ester prepared from natural benzoyl glucuronic acid. The melting point, crystalline structure, specific rotation and analysis of the two substances are identical. A mixed melting point of the two derivatives shows no depression.

It has previously been shown that 1 bromo 2, 3, 4, triacetyl glucuronic acid methyl ester is a pyranose derivative having the β configuration.[6] The substitution product, 1 benzoyl 2, 3, 4, triacetyl glucuronic acid methyl ester, may therefore be considered as having the same ring structure and configuration. Since the derivative obtained both synthetically and from natural sources is the same, the parent substance, benzoyl glucuronic acid, may be assigned the following structural formula, in which the benzoyl group is attached to the first carbon atom.

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ON THE MECHANISM OF THE ACTION OF DIGITALIS GLUCOSIDES ON MUSCLE

IN a recent communication,[1] an account was given of certain functional changes which occur in the sartorius muscle of the frog, maintained in oxygen, as a result of previous exposure to a low concentration of ouabain. These changes are, first, an augmentation in both twitch tension and initial heat production, followed later by an abrupt fall in both tension and heat, with an accompanying fall in efficiency, and finally complete loss of excitability. The effects described do not appear as long as the muscle is maintained in the ouabain-Ringer's solution, and after they have developed recovery follows re-immersion in the solution which produced them. This was considered evidence for the formation of a diffusible substance responsible for the toxic effects described, and it was pointed out that the changes are consistent with those caused by the action of potassium escaping from the interior of the cell. It should be emphasized that these effects occur only when the muscle is removed from its fluid environment, and thus do not represent the classical digitalis effects which develop in the intact animal or when the muscle is kept in fluid or perfused. If the digitalis glucosides cause the muscle cell to lose potassium, this process might enter into the mechanism of the production of muscle shortening and other digitalis effects by causing an increase in the calcium-potassium ratio inside the cell. The interest in this possibility is emphasized by the fact that there are in the literature a large number of observations showing the similarity between the effects on the heart of digitalis and calcium, and it is well known that digitalis action is greater in the presence of a high concentration of calcium in the blood or perfusion fluid. The phenomenon of delayed action and accumulation would also receive a satisfactory explanation.

We have carried out some preliminary studies on the changes in potassium content of the frog's sartorius muscle occurring as the result of soaking for several hours in a solution of ouabain. The figures from eight experiments are given in the table. The

[6] R. D. Hotchkiss and W. F. Goebel, *ibid.*, 115, 285, 1936.

[1] *Jour. Pharmacol. and Exper. Therap.*, 60: 101, 1937.

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.

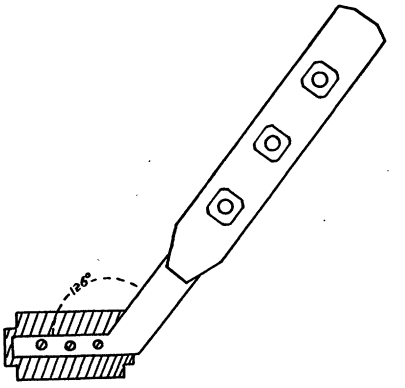

FIG. 1. Sketch showing construction of cutting instrument.

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.