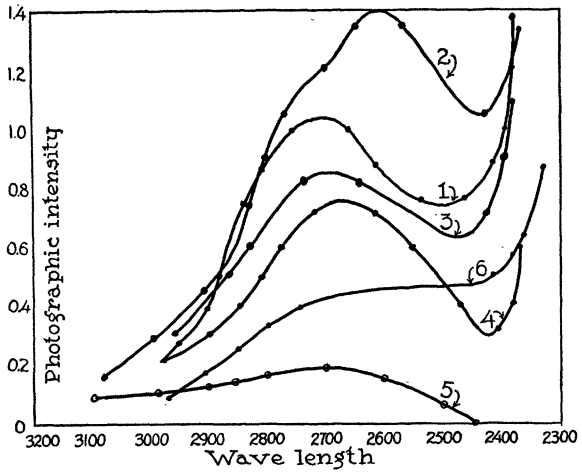

FIG. 1. Absorption of ultra-violet light by bacteriophages and control materials. Bacteriophages were all prepared with *Escherichia communior* grown in 2 per cent. peptone water. (1) Non-lytic filtrate of host organism. (2) Bacteriophage C16. (3) Bacteriophage C36. (4) Bacteriophage C13. (5) Bacteriophage C13 purified by modified Kligler-Olitzki technique. (6) Sterile 2 per cent. peptone water.

cally the same amount of nitrogen and had roughly the same titer, the relative degree of absorption was distinct for each one. All the maxima, however, were found to lie between 2,600–2,700 Å and the minima to fall at about 2,450 Å. The findings suggested that absorption parallels particle size. It will be noted, however, that an exception occurred with the non-lytic filtrate, which fell midway between bacteriophages C16 and C36.

There was significant difference in the quantitative absorption between the crude and purified bacteriophage C13. No change in the wave-length of maximum absorption occurred with the loss of extraneous nitrogenous material.

The determinations with the sterile medium were not entirely satisfactory for technical reasons, but the curve indicates that less ultra-violet was absorbed by it than by the filtrates.

No correlation between the titers of the bacteriophage preparations and their absorption spectra was apparent. Absorption, also, seemed to be independent of nitrogen content, which, with the exception of the purified bacteriophage, was practically constant.

SUMMARY

The absorption of ultra-violet light by three bacteriophages C13, C16 and C36, prepared with a strain of *Escherichia communior* has been determined. Each bacteriophage preparation showed a characteristic absorption curve, when the wave-length was plotted against the photographic density. Crude bacteriophage preparations absorbed more light than one such preparation obtained in a purer state, but the wave-length of maximum absorption remained the same.

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THE CHEMICAL CONSTITUTION OF BENZOYL GLUCURONIC ACID

THE chemical constitution of benzoyl glucuronic acid has been a subject of controversy ever since the compound was first isolated in the form of the sodium salt by Magnus Levy[1] in 1907. The benzoyl derivative of glucuronic acid is excreted in the urine in relatively large quantities when dogs are fed benzoic acid. Conjugation of aromatic acids with glucuronic acid is one of the important detoxicating mechanisms of man and certain mammals. Benzoyl glucuronic acid itself has been studied more extensively perhaps than any of the other conjugated glucuronides, yet its chemical constitution has never been definitely established.

Magnus Levy believed the derivative to be an ester in which the benzoyl radical is attached to the first or aldehydic carbon atom of the uronic acid. This explanation was generally accepted until 1926, when Quick[2] first isolated the conjugated derivative as the free acid. On the basis of certain polarimetric changes which benzoyl glucuronic acid underwent in faintly alkaline solution, or in the presence of sodium cyanide solution, Quick objected to the formula assigned by Magnus Levy and suggested that the compound is a benzoyl ester substituted not on the first, but on one of the remaining carbon atoms of the uronic acid. This postulation has since been questioned by Pryde and Williams[3] who maintain that the structural formula of Magnus Levy is the more probable. The suggestion, however, is not accepted by Quick[4] with the result that the exact constitution of this biologically important substance still remains uncertain.

The preparation of 1 bromo, 2, 3, 4, triacetyl glucuronic acid methyl ester by the author[5] has made possible the laboratory synthesis both of conjugated glucuronides and of other derivatives of glucuronic acid substituted on the first, or aldehydic carbon atom. If, therefore, the naturally occurring benzoyl glucuronic acid is a β ester substituted in position one as supposed by Magnus Levy, the triacetyl methyl ester derivative of the naturally occurring substance should be identical with the synthetic derivative prepared by

1 A. Magnus Levy, *Biochem. Zeit.*, 6: 502, 1907.
2 A. Quick, *Jour. Biol. Chem.*, 69: 549, 1926.
3 J. Pryde and R. T. Williams, *Biochem., Jour.*, 27: 1210, 1933.
4 A. Quick., *ibid.*, 28: 403, 1934.
5 W. F. Goebel and F. H. Babers, *Jour. Biol. Chem.*, 111: 347, 1935.

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

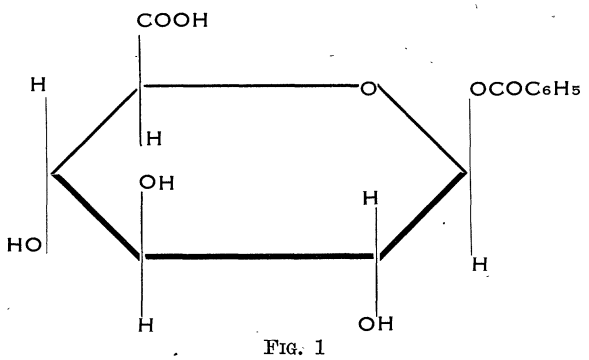

FIG. 1

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.