TABLE 1

DISINTEGRATION OF THROMBOPLASTIC PROTEIN BY FREEZING IN PRESENCE OF ETHER

Fraction	Centrifugal characteristics: Duration of centrifugation	Centrifugal characteristics: Centrifugal force	Centrifugal characteristics: Sedimentation	Electrophoretic characteristics*: Mobility	Electrophoretic characteristics*: Area	Proportion of starting material	N	P	Thromboplastic activity†	Phosphatase activity‡: Phosphatase units per mg	Phosphatase activity‡: Initial activity per mg
	min.	*g*		*($u \times 10^5$)*		*per cent.*	*per cent.*	*per cent.*	*γ*		*A100*
Thromboplastic protein	30	5,000	No	7.59	100		7.6	1.6	0.008	1.56	4.49
	90	31,000	Complete								
A	30	1,900	Almost complete			50.4	8.3	1.4	0.003	2.73	5.58
B	30	1,900	No			7.5	8.1	1.5	0.008	2.91	4.36
	90	31,000	Complete								
C	90	31,000	No	3.34	25	15.8	12.1	0.70	Inactive up to 6γ	2.02	4.52
				6.55	46						
				8.07	29						
D (Control experiment)	30	1,900	No			98.1	7.2	1.6	0.003	0.9	1.83
	90	31,000	Almost complete								
E (Control experiment)	90	31,000	No			1.7			0.03	0.7	1.20

* The experiments were carried out in borate buffer of pH 8.5. The computation of mobilities and relative areas is based on the descending boundaries.

† Expressed as smallest amount clotting 0.1 cc of rooster plasma within 30 minutes. The experiments were carried out at 30.6° by mixing 0.1 cc of fresh rooster plasma (normal clotting time above 80 minutes) with 0.03 cc of the solution of the protein in borate buffer, pH 8.5.

‡ The determinations were carried out in the presence of Mg ions. For the experimental arrangement and the definition of the units, compare.[2]

protein could be separated by high-speed centrifugation (Fraction B). The supernatant then was found to contain a considerable proportion of a mixture of non-sedimentable proteins (Fraction C) which, while quite active as phosphatase, was devoid of thromboplastic activity. A lipid fraction (rich in acetal phosphatides) amounting to 18 per cent. of the starting material, *i.e.*, roughly one third of the total lipids of the thromboplastic protein, was recovered from the combined ether extracts. A control experiment carried out simultaneously with the omission of ether failed to reveal an appreciable aggregation or disruption of the protein or other gross changes due to the freezing: the sedimentation of the protein (Fraction D), almost negligible at 1900 g, became practically complete at 31,000 g. The supernatant contained only traces of protein (Fraction E). Fraction D showed a higher thromboplastic and a lower phosphatase activity than the untreated protein; but this effect of freezing on the phosphatase potency was not observed to that extent with other preparations.

The view of the structure of lipoprotein complexes, based on x-ray evidence, as thin protein layers inserted between bimolecular lipid leaflets,[8] appears to permit the assumption that these units could arrange in a regular manner to form large complexes whose size would perhaps be limited by the intracellular spaces in which their formation takes place. The importance of the lipids in maintaining uniformity of particle size and electrophoretic mobility could thus be understood. The isolation of a fraction (consisting of three electrophoretically distinct components) having marked phosphatase, but no thromboplastic activity (Fraction C) is indicative of the far-reaching changes produced by even the partial removal of the lipids from the ostensibly homogeneous complex, once the protective water barrier is frozen away. It should be of interest to apply this technique to some of the animal viruses which, as isolated from infected tissues, are reported to occur in form of, or attached to, lipoproteins of very high particle weight.

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[8] K. J. Palmer, F. O. Schmitt and E. Chargaff, *Jour. Cell. and Comp. Physiol.*, 18: 43, 1941.

THE OPTICAL ROTATION OF CELLULOSE AND GLUCOSIDES IN CUPRAMMONIUM HYDROXIDE SOLUTION

THE high (levo) optical rotation of cellulose in cuprammonium hydroxide solution is believed to be a property of a complex formed by a copper-containing radical and free hydroxyl groups of cellulose. Neither the composition of the copper radical nor the points of its engagement with cellulose have been known with certainty. The following experiments (Table 1) show that complexes of similar high rotation are formed when appropriately substituted simple glucosides are dissolved in cuprammonium hydroxide solution. The levo-rotatory complex appears to be a cyclic structure involving hydroxyl groups on glucose carbon atoms

2 and 3. This conclusion is in agreement with the speculations of others regarding the structure of the copper-cellulose complex.[1] The formation of a highly levo-rotatory complex does not require that the glucosidic units be linked together in a polysaccharide.

β-Methyl glucoside dissolved in cuprammonium hydroxide solution does not show a levo rotation. However, β-methyl-4-methyl glucoside, which possesses the same free and substituted positions as cellulose, shows optical activity remarkably like that of cellulose. Similar behavior is exhibited by α- and β-methyl glucosides substituted in positions 4 and 6. In these cases only hydroxyl groups on positions 2 and 3 are available for engagement with the copper radical.

When positions 2 and 4 of a methylglucoside are substituted the levo-rotating complex is not formed, indicating that a free hydroxyl group on position 2 is essential for the complex formation. Likewise when positions 3, 4 and 6 are substituted the levo-rotatory complex is not formed indicating that a free hydroxyl group on position 3 is also essential. Formation of the levo-rotatory complex in glucopyranosides appears to require that hydroxyl groups on carbon atoms 2 and 3 be free while that on 4 must be substituted. It is immaterial whether position 6 be free or substituted. Finally the possibility that the complex involves linkage of the 2 position of one glucoside molecule with the 3 position of another was investigated. A solution containing equal parts of 2,4- and 3,4,6-substituted glucosides dissolved in cuprammonium hydroxide solution showed no indication of complex formation.

All the glucose derivatives considered in this communication are believed to have the pyranoside structure. Since the magnitude of the optical rotation in cuprammonium hydroxide solution is dependent upon the relationship between concentration of copper and carbohydrate, all observations were made on approximately 0.03 Molar glucoside solutions or 0.5 per cent. cellulose solutions. Aqueous solutions of similar concentration were employed. A description of the synthesis and properties of β-methyl-3-methyl-4,6-ethylidene glucoside as well as observations on the optical rotation of other polysaccharides and substituted glucosides will be published in another communication.

TABLE 1

THE OPTICAL ROTATION (Hg BLUE LINE) IN WATER AND CUPRAMMONIUM HYDROXIDE SOLUTION OF CELLULOSE AND SOME METHYLGLUCOSIDES*

Substance	Solvent†	$[\alpha]_{436}^{25}$	$[M]_{436}^{25}$
Cellulose (Purified cotton fiber)‡	Cupra	−1200°	−194,400°
	Water-Triton B(1:1)	− 46°	− 7,500°
		Dif.	−186,900°
β-Methyl-4-methyl glucoside	Cupra	−1008°	−209,700°
	Water	− 36°	− 7,500°
		Dif.	−202,200°
β-Methyl-4,6-ethylidene glucoside	Cupra	−1058°	−234,800°
	Water	− 163°	− 36,200°
		Dif.	−198,600°
α-Methyl-4,6-benzylidene glucoside	Cupra	− 608°	−171,500°
	Water	+ 159°	+ 44,800°
		Dif.	−216,300°
β-Methylglucoside	Cupra	+ 67°	+ 13,000°
	Water	− 62°	− 12,000°
		Dif.	+ 25,000°
α-Methylglucoside	Cupra	+ 432°	+ 83,800°
	Water	+ 306°	+ 59,400°
		Dif.	+ 24,400°
α-Methyl-2,4-dimethyl glucoside	Cupra	+ 275°	+ 61,000°
	Water	+ 308°	+ 68,400°
		Dif.	− 7,400°
β-Methyl-3-methyl-4,6-ethylidene glucoside	Cupra	− 128°	− 30,200°
	Water	− 126°	− 29,700°
		Dif.	− 500°

* The Hg blue line (436 mμ) was isolated for aqueous solutions by use of Corning filters 511 and 038. For cuprammonium solutions it is only necessary to use filter 038 since the longer wave-lengths are absorbed by the solution.

† The cuprammonium hydroxide solution contained 15 gm. copper, 240 gm. ammonia, and 1 gm sucrose per liter. All observations on cuprammonium solutions were made in an 0.5 dm tube. The rotation of the solvent was +0.09° (0.5 dm).

‡ It is impossible to give a correct figure for the rotation of cellulose in water solution. The present value was obtained by dissolving acid-treated cotton fiber in Triton B and diluting with an equal volume of water. Triton B is an aqueous solution of trimethyl benzyl ammonium hydroxide supplied by Röhm and Haas Company, Inc.

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SCIENTIFIC APPARATUS AND LABORATORY METHODS

A STILL FOR THE CONTINUOUS PRODUCTION OF DOUBLE DISTILLED WATER

THIS apparatus has been used for the production of all-glass distilled water for over a year and has proved very efficient. The water level in the distilling flask is maintained by means of a simple float valve made from a cork and a rectangular brass rod about 1 cm wide and 2 mm thick. This is faced at one end with a piece of gum rubber about 3 mm thick. A weather-stripping cement[1] is used to fasten the rubber to the brass. This is hinged so that when the large cork is horizontal the inlet tube (a quarter inch brass tube) is closed. The box *A* for the leveling device is made from ⅛ inch brass plates. The distillation flask

[1] "Natural and Synthetic High Polymers," p. 291. By Kurt H Meyer. Interscience Publishers, Incorporated, New York, 1942.

[1] 3 M weatherstrip cement sold by Minnesota Mining and Manufacturing Company, St. Paul, Minn.