

gress in 1911 made a specific appropriation for the Bureau of Soils, U. S. Department of Agriculture, for "exploration and investigation within the United States to determine a possible source of supply of potash, nitrates and other natural fertilizers." At the same session in 1911, the Geological Survey was authorized to make exploratory borings to determine the possible existence of potash deposits within the United States. In 1916 another item was added in the appropriation act for the "investigation and demonstration within the United States to determine the best method of obtaining potash on a commercial scale."

The book under review is the second of a series of two books by the same author describing the research, exploration and development work on potash that followed the enactment of these appropriation acts. The first of these two books entitled "Potash: A Review, Estimate and Forecast," covers the period 1911 to 1926. The second of the series begins where the first left off and covers the fifteen-year period following 1926. The first chapter reviews the progress that has been made in the production of potash during this period in various countries of the world as well as in the United States. It reviews also the purposes

and activities of the American Potash Institute, which was organized in 1935. The second chapter outlines the uses of potash in agriculture and in the chemical industries and gives statistical data on American and World Trade in potash salts between 1926 and 1941. In the third chapter detailed information is given on the technology of potash production at Searles Lake and in the New Mexico field.

This book is one of the American Chemical Society Series of Scientific and Technologic Monographs and it is in keeping in every respect with the high standards set by the society for this series of publications. The author is recognized as having taken a leading part in all research and development work on potash since this was first undertaken in 1911. The present book, as with the first of the series, can, therefore, be recommended without reservation to those who are interested in securing the most authoritative information available on the history and development of the American potash industry.

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## SPECIAL ARTICLES

### THE DISINTEGRATION OF MACROMOLECULAR TISSUE LIPOPROTEINS<sup>1</sup>

THE thromboplastic protein (inducing the formation of thrombin) isolated by the ultracentrifugal fractionation,<sup>2</sup> or, in considerably less pure form, by the fractional salt precipitation<sup>3</sup> of beef lung extracts is a macromolecular lipoprotein. As has been described previously,<sup>2</sup> preparations may be obtained under proper conditions that exhibit homogeneity of sedimentation and electrophoretic mobility, have the extremely high particle weight of 170 million (from rate of sedimentation), and appear in electron micrographs as spheres with a diameter of 80 to 120  $\mu$ . Apart from their extremely high thromboplastic potency (as little as 0.008 $\gamma$  being demonstrable by clotting tests), these preparations were distinguished by a marked phosphatase activity.

The thromboplastic protein of beef lungs resembles in certain outward respects, *e.g.*, its analytical composition and content of acetal phosphatides, the submicroscopic particles isolated from a number of tissues.<sup>4</sup> As is true of most natural lipoproteins,<sup>5</sup> the

lipids forming part of the thromboplastic protein<sup>3, 6</sup> are held vigorously and can be removed only by exhaustive extraction with alcohol-ether which renders the protein moiety insoluble and inactive.

It was recently shown by McFarlane<sup>7</sup> that a large proportion of the serum lipids, ordinarily not extractable with ether, may be transferred into the ether phase, when ether-containing serum is frozen below  $-25^{\circ}$  and allowed to thaw. The application of a similar technique to the thromboplastic protein and other lipoproteins gave interesting results.

In a typical experiment, summarized in Table 1, a solution of 330 mg of the thromboplastic protein in 15 cc of borate buffer of pH 8.5 was mixed with 10 cc of ether (free of peroxides and alcohol), kept for 4 minutes at  $-30^{\circ}$ , and permitted to thaw. This operation was, after replacement of the ether layer by fresh solvent, repeated six times. From the aqueous phase, freed of the remaining ether by careful evacuation, a large proportion of the protein could be isolated by centrifugation at a low speed as Fraction A which exhibited somewhat higher thromboplastic and phosphatase activities than the original material. A small amount of practically unaltered thromboplastic

<sup>1</sup> This work has been supported by a grant from the John and Mary R. Markle Foundation.

<sup>2</sup> E. Chargaff, D. H. Moore and A. Bendich, *Jour. Biol. Chem.*, 145: 593, 1942.

<sup>3</sup> S. S. Cohen and E. Chargaff, *Jour. Biol. Chem.*, 136: 243, 1940.

<sup>4</sup> A. Claude, *SCIENCE*, 97: 451, 1943.

<sup>5</sup> E. Chargaff, "Advances in Protein Chemistry," vol. 1, 1944 (in press).

<sup>6</sup> S. S. Cohen and E. Chargaff, *Jour. Biol. Chem.*, 139: 741, 1941.

<sup>7</sup> A. S. McFarlane, *Nature*, 149: 439, 1942.

TABLE 1  
DISINTEGRATION OF THROMBOPLASTIC PROTEIN BY FREEZING IN PRESENCE OF ETHER

Fraction	Centrifugal characteristics		Electrophoretic characteristics*			Proportion of starting material	N	P	Thromboplastic activity†	Phosphatase activity‡	
	Duration of centrifugation	Centrifugal force	Sedimentation	Mobility	Area					Phosphatase units per mg	Initial activity per mg
	min.	g		( $u \times 10^5$ )		per cent.	per cent.	per cent.	$\gamma$		$A_{100}$
Thromboplastic protein	30	5,000	No	7.59	100		7.6	1.6	0.008	1.56	4.49
A	90	31,000	Complete								
	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 6 $\gamma$	2.02	4.52
				6.55	46						
				8.07	29						
D	30	1,900	No			98.1	7.2	1.6	0.003	0.9	1.83
Control experiment	90	31,000	Almost complete								
	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.<sup>2</sup>

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,<sup>8</sup> 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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#### 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

<sup>8</sup> K. J. Palmer, F. O. Schmitt and E. Chargaff, *Jour. Cell. and Comp. Physiol.*, 18: 43, 1941.