

## 13365 P

**A New Serologically Active Phospholipid from Beef Heart.**

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Alcoholic extracts of beef heart have long been used as antigens in the complement-fixation test for syphilis, but the substances responsible for the serologic activity of such extracts have not been identified. The isolation of a new phospholipid is here reported and evidence is presented that the new substance is the active component of the antigenic extracts. Details of its preparation and chemical properties will be published elsewhere.

The substance was prepared by precipitating alcoholic extracts of beef heart with cadmium chloride, removing lecithin from the precipitate by a recently described method<sup>1</sup> and fractionating the lecithin-free cadmium compounds by precipitation from ether with alcohol and acetone. The purest preparation of the cadmium-free phosphatide contained 4.18% of phosphorus and no nitrogen. It was subjected to repeated purification in the form of the cadmium salt without essential change in chemical or serologic properties and is therefore considered to be a single substance.

An abbreviated complement-fixation technic<sup>2, 3</sup> based on the quantitative methods of Wadsworth, Maltaner, and Maltaner<sup>4</sup> was employed in testing the serologic activity. High-titered sera from syphilitic patients, diluted in pooled, filtered, normal sera, were used. Control tests with pooled sera from nonsyphilitic persons were negative.

In testing the complement-fixing activity of lipid fractions that have been freed of lecithin, it is necessary to add both lecithin and cholesterol. In the absence of lecithin, such fractions are markedly anticomplementary; in the absence of cholesterol, the purified fractions have not been found to fix complement. The amount of added lecithin was so adjusted that a 1:10 saline dilution of the antigen

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<sup>1</sup> Pangborn, M. C., *J. Biol. Chem.*, 1941, **137**, 545.

<sup>2</sup> Wadsworth, A. B., *Standard Methods of the Division of Laboratories and Research of the New York State Department of Health*, 2d ed., Baltimore, The Williams and Wilkins Company, 1939, pp. 213-267.

<sup>3</sup> Kent, J. F., *J. Immunol.*, 1940, **39**, 307.

<sup>4</sup> Wadsworth, A., Maltaner, F., and Maltaner, E., *J. Immunol.*, 1938, **35**, 105, 217.

was not anticomplementary. Alcoholic solutions were made up to contain 0.03% of the test substance, 0.05% of lecithin, and 0.4% of cholesterol, and were diluted with 0.85% sodium chloride by the method used for the routine antigen.<sup>2</sup>

The lecithin used was purified by the petroleum ether-alcohol method.<sup>1</sup> Lecithin so purified is not anticomplementary and fixes no complement in the presence of syphilitic or normal sera, either alone or when mixed with cholesterol. Lecithin from eggs and from beef heart gave identical results.

Comparisons of antigenic activity were based upon the linear relation of syphilitic serum to complement. A series of serum dilutions was titrated, using several antigen dilutions with each quantity of complement; the range of antigen dilutions was so chosen as to ensure a maximum reaction with each amount of serum.<sup>3</sup> From the observed percentages of hemolysis given by each serum quantity with its optimum dilution of antigen, the number of units\* of complement required for 50% hemolysis was determined,<sup>2, 3</sup> and the values so obtained were plotted as ordinates against quantities

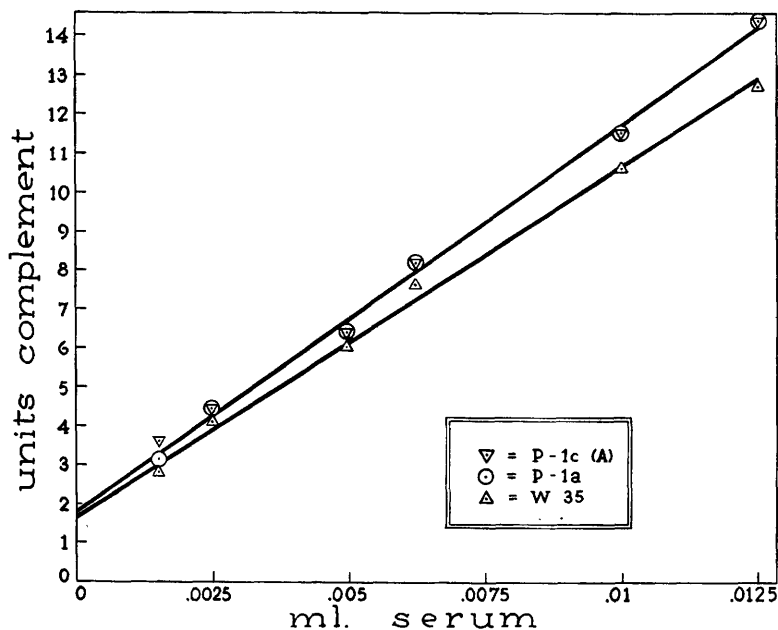


FIG. 1.  
Titration of syphilitic serum with purified phospholipid.

\* One unit of complement is the amount required for 50% hemolysis of 0.1 ml of a 5% suspension of sheep erythrocytes sensitized with an equal volume of optimally diluted amboceptor.<sup>2</sup>

of serum as abscissae. Equally active antigens simultaneously titrated in this way with the same serum and the same pool of complement should give identical serum-complement curves; any significant difference in slope indicates a difference in the complement-fixing activity of the antigens under the conditions of the experiment.

The figure presents a titration in which 2 samples of the purified phosphatide are compared with the standard antigen W35, which was prepared for routine diagnostic use.<sup>2</sup> Of the 2 preparations, P-1c(A) was the more highly purified; it was obtained from P-1a by repeating the purification of the cadmium salt. The serologic activity was evidently not altered by the final purification, since the 2 preparations gave identical titration curves.

The figure shows that the activity of the purified substance is closely comparable to that of the routine antigen, and it may be concluded that the new phosphatide is responsible for the complement-fixing activity of beef heart extracts with syphilitic sera. Chemical and serologic studies of the substance are being continued.

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#### Effect of Pseudopregnancy on Mammary Carcinoma Incidence in Mice of the A Stock.

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The inbred A stock of mice has a high incidence of spontaneous mammary carcinoma when there occurs a hormonal stimulation of the mammary tissue resulting from breeding. Females kept as virgins, however, have a relatively low incidence of breast cancer. The incidence of breeding females of a selected subline (F38-F58 generations) was found to be 96.3% and for virgin females, 4.9%.<sup>1, 2</sup>

Fifty pedigreed females, 2 months of age, of a selected subline (F38-F58 generations) obtained from Dr. J. J. Bittner were placed 4 in a cage with a vasectomized A stock male. Oestrus smears were made on mice after discovery of vaginal plugs. All experimental mice were known to be in a state of pseudopregnancy at least once.

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<sup>1</sup> Bittner, J. J., *Pub. Health Rep.*, 1939, **54**, 1642.

<sup>2</sup> Bittner, J. J., *Cancer Research*, 1941, **1**, 113.