Report of the Biochemical Methods Committee Meeting, Washington, D.C.

THE BIOCHEMICAL METHODS COMMITTEE (BMC) meeting was held on March 30 from 3–7 PM. Those in attendance were: George Rouser (chairman); members Robert Witter, Donald Therriault, Gerald Feldman, and P. P. Nair; consultant Gerald Simon and visitor Richard Doughtie of the Smalley Committee.

The past activities of the BMC were reviewed. The approach to date has been largely:

1) An attempt to define the most profitable areas for expenditure of effort. This was facilitated by working with the Education Committee. Short Courses on thin-layer and gas-liquid chromatography were organized and conducted (Rouser and Feldman as chairmen, respectively). From these it was concluded that the BMC could contribute by evaluating and standardizing adsorbents for chromatography (TLC, GLC and liquid-solid columns).

2) An attempt to prepare a standardized silicic acid for column chromatography. Initially, Nicholas Pelick was to prepare K. K. Carroll's acid-washed Florisil (a type of silicic acid) which was to be tested by K. K. Carroll and Robert Anderson. This project went closely according to plan and Supelco is now selling substantial amounts of the product.

3) A blood lipid subcommittee under the Chairmanship of Robert Witter was established. The subcommittee has sent out samples for cholesterol determination by the Abel-Kendall method as described by the Committee. Data were received and turned over to the AOCS Statistics Committee from which a report has not been received to date.

4) Development of a classification scheme for lipids was begun over 2 years ago by Leonard Norcia who circulated several different classifications to members of the BMC who commented upon them at length. It was agreed that the project was desirable and a classification scheme would aid the work of the Committee. However, the Chairman of the BMC felt the scheme should be revised completely along a new line which was then prepared and discussed during the period March 4–5, 1967 (in Philadelphia) with Leonard Norcia. Dr. Norcia is now preparing the first completely revised scheme for presentation to the Committee.

At the March 30, 1968 meeting the following conclusions and recommendations were made after extensive discussion.

I. The BMC recommends evaluation and standardization of GLC adsorbents. This has been and will continue to be conducted by the AOCS Committee on Gas Chromatography with Gerald Feldman acting as the representative of the BMC.

II. The Blood Lipid Subcommittee is to continue its work as previously planned.

III. The first draft of the lipid classification scheme will be completed as soon as possible.

IV. The BMC will concentrate most of its efforts on evaluation of adsorbents for column and thin-layer chromatography. Adsorbents will be selected on the basis of favorable reports in the scientific literature (i.e., journal publications) which must come from different laboratories. Using this guiding principle, the following procedure will be followed:

A. *Thin-Layer Chromatography:* Applied Science adsorbents, Merck Silica Gels, and Silica Gel-magnesium silicate according to Rouser et al. (JAOCS 1964) will be tested and compared for use with less polar lipids (sterols, sterol esters, triglycerides, fat-soluble vitamins, etc.) by P. P. Nair, R. Witter, and D. Therriault. The results will be presented to the other members of the Committee who will then perform similar checks in their own laboratories. The same adsorbents will be tested and compared for use with polar lipids (phospholipids, glycolipids) by G. Rouser, G. Simon, D. Therriault, and R. Anderson who will present their results to the BMC after which the other members will conduct similar studies in their own laboratories for verification of previous results.

In testing TLC adsorbents, every effort will be made to find the optimum conditions for each adsorbent and to compare adsorbents using these optimum conditions. All chromatograms will be photographed and all conditions will be recorded for inclusion in the report from each laboratory.

In the case of polar lipids, two-dimensional TLC will be emphasized because maximum resolution of components of complex mixtures is obtained with the procedure. Standard lots of adsorbents will be purchased by the BMC and distributed. Adsorbents will be spread with a fixed-distance (0.25 mm) spreader, heat-activated, cooled and spotted under conditions of controlled humidity and developed in lined or unlined chambers. Neutral and basic solvents will be used in the first dimension and followed by acidic solvent mixtures in the second dimension. Standard text mixtures of phospholipids and glycolipids (prepared by N. Pelick) will be used by all laboratories. The most complex mixtures will contain cerebroside and sulfatide as glycolipids and the following phospholipids: phosphatidyl choline, phosphatidyl ethanolamine, phosphatidyl serine, phosphatidyl inositol, diphosphatidyl glycerol (cardiolipin), lysophosphatidyl choline, lysophosphatidyl ethanolamine, phosphatidic acid and sphingomyelin. Some lipid classes present special problems in resolution and mixtures of these will be provided also (sphingomyelin + lysophosphatidyl ethanolamine; phosphatidyl serine + phosphatidyl inositol + phosphatidic acid; diphosphatidyl glycerol + phosphatidyl ethanolamine + cerebroside and others as the need arises).

B. *Column Chromatography:* For separation of less polar lipid classes, Unisil (silicic acid), Florisil (magnesium silicate), and acid-washed Florisil (a type of silicic acid) will be compared by K. K. Carroll, P. P. Nair, R. Witter, D. Therriault, and R. L. Anderson.

For separation of polar lipid classes, silicic acid (Unisil) and various preparations from the Brown Co. and H. Reeve Angel of diethylaminoethyl (DEAE) and Brown Co. triethylaminoethyl (TEAE) cellulose will be evaluated and compared by D. Therriault, J. Turner, G. Simon, G. Feldman, and G. Rouser. Column effluents will be tested for the presence of adsorbent "fines" and other impurities by elution of the column without application of a sample. A standard mixture of phospholipids and glycolipids will then be applied and the elution sequence under test followed. Weights of each fraction and the lipid classes observed by TLC will be recorded. Performance will be evaluated in terms of reproducibility, completeness of separation, presence of adsorbent "fines," and the extent of production of artifacts through hydrolysis, oxication, etc.

V. A procedure for quantitative determination of phospholipids by two-dimensional TLC and phosphorus assay will be presented and tested by J. Turner, D. Therriault, G. Simon, and G. Rouser. The steps will be as follows:

a) TLC separations will be compared.
b) The phosphorus determination procedure will be evaluated; and
c) Test mixtures will be separated by TLC and the phosphorus content of spots determined.

GEORGE ROUSER, Chairman
Biochemical Methods Committee