11604

Effect of Lipid Solvents on Fourth Component of Complement.*

L. PILLEMER, S. SEIFTER AND E. E. ECKER

From the Institute of Pathology and the University Hospitals of Western Reserve University, Cleveland, Ohio.

Toda and Mitsuse[1] and Tokano[2] have influenced several investigators[3] to associate the fourth component of complement with a lipid fraction of serum. This is at such variance with the concept that the fourth component is the carbohydrate-complex carried by the end-piece,[4] that it was decided to repeat in detail the experiments of Toda and Mitsuse.

Methods and Materials. The methods advocated by Toda and Mitsuse for the inactivation of the fourth component by ether, chloroform, benzene, benzine and cadmium chloride were followed in detail, and without the slightest variation. All reagents were Merck Blue Label chemicals. Methods for complement titration and reactivations have already been given.[4]

Results. Under the exact conditions employed by Toda and Mitsuse, ether and chloroform destroyed complement irreversibly, but had no specific effect on the fourth component. Benzene and benzine did not inactivate dehydrated complement. Furthermore, the treatment of serum with cadmium chloride (1% in 0.9% NaCl) caused flocculation of the serum proteins, and no reactivation of either the supernatant liquid or the redissolved precipitate was possible. Varying the concentration of either the cadmium chloride or the serum also failed to reveal any specific effect on the fourth component.

It becomes evident that these findings do not confirm the work of Toda and Mitsuse, and that the fourth component is neither a lipid nor associated with lipids. Previous work from this laboratory[5] had also established that prolonged extraction of dehydrated, active complement with fat solvents does not inactivate complement; instead, an improvement of the lytic titer is obtained when 50% of

* Aided by a grant from the Commonwealth Fund.

1 Toda, T., and Mitsuse, B., *Z. f. Immunitätsforsch.*, 1933, **78**, 62.

2 Tokano, Y., *Z. f. Immunitätsforsch.*, 1936, **87**, 29, 72.

3 Osborne, T. W. B., *Complement or Alexin*, London, Oxford University Press, 1937.

4 Pillemer, L., Seifter, J., and Ecker, E. E., in press.

5 Ecker, E. E., Pillemer, L., and Grabill, F. G., PROC. SOC. EXP. BIOL. AND MED., 1938, **38**, 318.

the phospholipids is removed. Treatment of fresh serum under the same conditions results in an irreversible inactivation of the total complement due to protein denaturation.

While it is agreed that the phospholipids of the mid-piece may play a role in complementary activity,[6] it has not been possible to establish a lipid-fourth component relationship.

Summary. The fourth component of complement was not found to be in association with a lipid complex of serum.

11605

Effects of Irradiation on Leukemic Cells in Marrow Cultures.*†

EDWIN E. OSGOOD. (With the technical assistance of Evelyn Packham.)

From the Department of Medicine and the Division of Experimental Medicine, University of Oregon Medical School, Portland.

The experiments herein reported were undertaken to determine whether a small dose of irradiation would have a selectively greater effect than a large dose on a susceptible cell, such as the lymphocyte, in comparison with a less susceptible cell, such as the progranulocyte[1] (promyelocyte); whether an increase in dose of irradiation produced a corresponding increase in effect; and whether leukemic cells were more susceptible than nonleukemic cells of the same type.

The marrow culture technic[2] permits quantitative studies of the effect of irradiation on living normal or leukemic human cells. After an initial total and differential cell count, 50 cc of culture containing about 100,000,000 nucleated cells in a medium consisting of 65% balanced salt solution and 35% human cord serum is thoroughly mixed, and equal portions of about 8 cc are placed in each of several 30 cc vaccine vials. One of these is left as a control, and the others

6 Ecker, E. E., Jones, C. B., and Kuehn, A. O., in press.

* Aided by a grant from the Dazian Foundation. Dr. S. E. Rees and Dr. W. Haworth kindly irradiated some of these cultures.

† Presented before Section N of the American Association for the Advancement of Science, Seattle, Washington, June 19, 1940.

1 The cell nomenclature used is the same as that which is found in Osgood, E. E., and Ashworth, Clarice M., *Atlas of Hematology*, pp. 6-8, J. W. Stacey, Inc., San Francisco, 1937; and Osgood, E. E., *A Textbook of Laboratory Diagnosis*, pp. 161-164, Ed. 3, The Blakiston Company, Philadelphia, 1940.

2 Osgood, E. E., and Brownlee, Inez E., *J. A. M. A.*, 1937, **108**, 1793.