All these experiments have been duplicated.

Chow and Wong[3] have just reported the inactivation of complement by iodine, thus substantiating our previous findings.

Summary. Iodoacetate, a compound known to form mercaptides with sulphydryls, has been shown to inactivate guinea-pig complement. The inactivated complement was partially regenerated with ascorbic acid and glutathione (SH) but not with saturated H_2S water or KCN by our experimental procedure. This would indicate that the action of the iodoacetate on guinea-pig complement is not wholly upon thiol grouping, and that it may have other effects on the protein molecule. The fact that the iodoacetate-treated complement could be reactivated with two natural occurring reductants points to the possibility of a specific action of these two agents.

From these observations it must be assumed that iodoacetate acts primarily upon the protein and possibly on the 4th component of complement.

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Serum Lipoids and Complementary Activity.*

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Ottolenghi and Mori,[1] and Guggenheimer[2] showed that complement treated with ether became inactive after the solvents were allowed to evaporate. Toda and Misuse[3] reported later that the factor removed by extraction with chloroform or ether was the same as the 4th component. They further claimed that a similarity exists between the 4th component and the lipoid fraction since cadmium compounds, known to combine with lecithin, inactivate this component. Benzene inactivated their complement and the inactive serum was regenerated by the fraction soluble in benzene but not by the 4th component. Since this heat-stabile fraction did not reacti-

3 Chow, Bacon F., and Wong, Sam C., PROC. SOC. EXP. BIOL. AND MED., 1938, **38**, 120.

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1 Ottolenghi, D., and Mori, M., *Centralbl. f. Bakt. I*, 1905, **38**, 338, 468.

2 Guggenheimer, H., *Z. f. Immunitätsf.*, 1910, **8**, 295; 1911, **11**, 393.

3 Toda, T., and Misuse, B., *ibid.*, 1933, **78**, 62.

vate ammonia-treated complement they postulated a hypothetical 5th component. Tokano,[4] however, could not demonstrate the existence of such a 5th factor but noted that ether or chloroform disturbed the 4th component. Hardy and Gardiner[5] removed lipoids from diphtheria antitoxin by means of alcohol and ether in the cold and showed that the lipoid-free globulin became soluble in saturated NaCl solution and that the antitoxic properties of the serum remained unaltered. Hartley[6] extracted lipoids by a similar method from rabbit antihorse, antihuman, antityphoid, and diphtheria-antitoxic serums, demonstrating a loss of precipitability of the extracted serums. Agglutination by the antityphoid serum and neutralization by the diphtheria antitoxin remained unchanged.

Horsfall and Goodner[7] more recently observed that the removal of lipoids from Type I antipneumococcal horse serum caused a loss of type-specific agglutination and precipitation, and that in the case of rabbit serum a marked reduction of these properties occurred.

In summing up, it may be stated that except for the work of Hardy and Gardiner, Hartley, and Horsfall and Goodner, little attention has been paid to the question of protein-denaturation during the process of lipoid-extraction. This is particularly emphasized in the problem of the relationship between lipoids and complementary activity.

The methods employed were fundamentally the same as advocated by Hartley, and Horsfall and Goodner except that the time of extraction was lengthened.

Method A. In this technic the spongy powder of 1 cc. active, dehydrated complement was extracted with 1 cc. cold absolute alcohol at −10°C. for a period of 24 hours. The dried complement was centrifuged and the supernate decanted. The residue was taken up in 10 cc. of cold absolute ether, agitated and kept at −10°C. for 48 hours, centrifuged and the supernate decanted. The residue was then dried *in vacuo* until free of all ether and dissolved in 1 cc. 0.85% saline.

Method B. The same procedure was followed except that the dried complement was shaken intermittently for 24 hours with cold absolute alcohol (kept at −10°C.). This was followed with cold petroleum ether for 24 hours and after removal of the petroleum ether the residue was again extracted with cold absolute ether for

4 Tokano, Y., *ibid.*, 1936, **87**, 29.

5 Hardy, W. B., and Gardiner, S., *J. Phys.*, 1910, **40**, 68.

6 Hartley, P., *Brit. J. Exp. Path.*, 1925, **6**, 180.

7 Horsfall, F. L., and Goodner, K., *J. Exp. Med.*, 1935, **62**, 485.

another 24 hours. The supernate was again decanted and the residue dried *in vacuo*.

Extractions were also made at 5°C. and at 20°C. Finally, the time of extraction was tripled (72 hours) for each solvent in both methods and at −10°C.

The phospholipid contents of the serums were determined as follows: The extractions were made according to the method of Bloor[8] with an alcohol-ether mixture. An aliquot of this was reduced to dryness in a pyrex test-tube and ashed with sulfuric acid and superoxol. The phosphates were then determined by the method of Fiske and Subarrow.[9] By these methods it was found that not more (usually less) than 50% of the phospholipids were extractable, indicating that the lipoids may be firmly bound to proteins or carbohydrates.

Fresh complement was further extracted by these methods but the complement was invariably inactivated.

Extractions of the same serums were also performed under anaërobic conditions but this did not prevent inactivation.

All complement titrations were made according to the method described by Ecker, Pillemer, Wertheimer and Gradis.[10]

Table I summarizes the results secured in a typical experiment.

TABLE I.
Initial Titers of Active, Dehydrated Complements Following Partial Removal of Phospholipids.

Methods	Temperature		
	−10°C.	5°C.	20°C.
Control	.03	.03	.03
Method A	.02	.03	—
" B	.02	.03	—

Even after prolonged extraction for a period of 9 days by methods A and B at −10°C. no change of titer occurred. The original titer of the dehydrated complement was 0.03 cc. of 1:30 and remained the same.

From these results it is evident that partial removal of the lipoids did not interfere with the activity of the complement. In fact, the removal of the lipoids often showed an improvement of initial titers which was strikingly noted in the case of an active dehydrated dog-complement with a normal initial titer of 0.05 cc.

8 Bloor, W. R., *J. Biol. Chem.*, 1928, **77**, 53.

9 Fiske, C. H., and Subarrow, Y., *ibid.*, 1925, **66**, 375.

10 Ecker, E. E., Pillemer, L., Wertheimer, D., and Gradis, H., *J. Immunol.*, 1938, **34**, 19.

of a 1:10 dilution. Following the method-B extractions the initial titer increased to 0.02 cc. of a 1:15 dilution.

The activity of active, dehydrated complement remained unimpaired after 7 days' extractions with chloroform, benzene and pyridine at a temperature of 4 to 5°C.

All the experiments were repeated with dog complement in the active, dehydrated state with no diminution of initial titers.

The recovered lipoids showed no hemolytic powers by themselves, and often exhibited anticomplementary properties when returned to the extracted residues prior to titrations.

Since it was shown by Ecker, Pillemer, Wertheimer and Gradis[10] that the initial complement-titers of guinea pigs, fed a vitamin-C deficient diet, were markedly lowered, it was considered of interest to compare the phospholipid contents of their serums and the serums of normal guinea pigs. The average phospholipid content of the serums of 50 normal guinea pigs, determined as phosphorus, was 2.14 mg. per 100 cc. of serum. The serums of 7 guinea pigs fed a vitamin-C deficient diet for 27 days gave an average of 2.04 mg. per 100 cc. of serum. This would seem to indicate that the serum phospholipids are not materially decreased during the course of vitamin-C deficiency. The initial complement-titers of vitamin-C deficient guinea pigs showed a decline of 75%, falling from 0.02 to 0.08 cc. of a 1:30 dilution. From these results it may be concluded that the phospholipid content of the serums plays no rôle in the reduction of initial complement-titers noted in the serums of guinea pigs fed a vitamin-C deficient diet.

Summary. Active, dehydrated complement showed no reduction of power when extracted in the cold with absolute alcohol, ether and petroleum ether. It was not possible to remove more than 50% of the total phospholipids of active, dehydrated complement by the methods described. Fresh complement was readily denatured by the solvents employed, namely, absolute alcohol, absolute ether, and petroleum ether. Treatment of active, dehydrated complement with chloroform, benzene and pyridine led to no change in initial titers. No evidence of a 5th component in the sense of Toda and Misuse was found. No relationship was found between the phospholipid contents of the serums of guinea pigs fed a vitamin-C deficient diet and their lowered complementary activities.