that of untreated antibody protein. The only noticeable difference lay in its solubility in various saturations of ammonium sulfate solution. The absence of any gross chemical change in spite of the marked immunological change is not surprising. It has now been repeatedly demonstrated that only slight alteration of chemical groups or of their stereo-chemical relationship may destroy all the immunological properties of protein. By analogy, it might be assumed that the protein of microörganisms subjected to the same action may also be little changed. But in this case, the preservation of antigenicity would be more complete. This may explain why organisms treated with photosensitization would make better vaccine.

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Chemical Nature of Component Involved in the Reaction Between Iodine and Complement.

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Chow and Wong have reported[1] that iodine destroys the complement activity of normal sera and that there exists a definite stoichiometric relationship between the amounts of iodine used and complement inactivated. It seemed to be of interest to find out the chemical nature of and the component involved in this process. This may either be an addition of iodine to ethylenic linkages or replacement of hydrogen atoms attached to the carbon atoms, with the result that iodine will be bound to complement. It may also be a simple oxidation of complement by iodine, which in turn, is reduced to free unbound iodide. In order to arrive at some conclusion, we have determined quantitatively first the bound iodine, if any, to the inactivated serum protein, and secondly, the free iodine in the mixture. The present communication gives the result of such a study.

1. Determination of non-dialyzable organic iodide in the inactivated serum protein. To 50 cc of fresh hog serum (having a titer of 21 units per cc) were added 10 to 15 cc of N/10 iodine solution. One-half cc of the resulting solution was found to contain less than

[1] Chow, B. F., and Wong, Sam C., PROC. SOC. EXP. BIOL. AND MED., 1938, **38**, 120.

one unit of complement per cc. Therefore, the inactivation of complement was almost complete. The inactivated hog serum was dialyzed in a collodion tube of small porosity against distilled water at 0°C until free from iodide. Five cc of N/10 sulphuric acid were added to effect a complete solution of the protein that was precipitated out during dialysis. The total volume was then made up to 100 cc with water, *i. e.*, two times the original volume. Three 20 cc samples were pipetted into separate nickel crucibles, and their iodine content determined according to the method of McCullagh.[2] The results are given in Table I.

TABLE I.
Determination of Iodine Bound to the Inactivated Serum Protein.

Expt	Sample of hog serum cc	Units of complement per cc	Total iodine N/10 necessary for complete inactivation, cc	Total iodine equivalent to N/10 found after dialysis, cc	% iodine dialyzed
1	50	21	7	.06	99.15
2	50	30	10	.15	98.50
3	50	35	12	.10	99.17

2. Determination of iodide in the protein-free filtrate. A typical experiment was as follows: One cc of fresh hog serum (titer of 33 units per cc) was diluted ten fold with 0.85% sodium chloride solution. To each of four 2.0 cc samples of the diluted hog serum were added 5 cc of N/10 iodine solution in distilled water. To one of the mixtures, 0.6 cc of 10% sodium chloride solution was added to make the solution isotonic, in order to determine the percentage of complement activity destroyed by this amount of iodine. To the other three was added 1 cc of 2/3 N sulphuric acid and 1 cc of 10% sodium tungstate solution. The suspensions were centrifuged and 4 cc of the supernatant were used for the determination of iodide, in the usual manner, by oxidizing it to iodate with bromine. The results of several such experiments with the sera of different species of animals are given in Table II.

3. Determination of the component which is inactivated by iodine. To different samples of 0.5 cc of iodine inactivated guinea pig or hog serum was added 0.5 cc of 1:5 each of the following: To the first sample, heat inactivated, to the second, ammonia inactivated, and to the third, yeast inactivated guinea pig or hog serum; in each case, the hemolytic power of the mixture was found to be restored. To other samples of 0.5 cc of the same was added 0.5 cc of 1:10

[2] McCullagh, R., *J. Biol. Chem.*, 1934, **107**, 35.

TABLE II.
Determination of Iodine in the Protein Free Filtrate.

Expt	cc original serum used	Units complement inactivated	Iodine added equivalent to N/100, cc	Total iodine in supernatant equivalent to N/100, cc	% recovery of iodide
		a. Hog serum.			
1	.2	4.7	.32	.288	90
2	.2	4.7	.32	.294	92
3	.2	4.7	.32	.300	94
		b. Human serum.			
1	.5	12	.32	.266	83
2	.5	12	.32	.288	90
3	.5	12	.32	.304	95
		c. Guinea pig serum.			
1	.5	30	.32	.294	92
2	.5	30	.32	.304	95
3	.5	30	.32	.310	97

either of water soluble fraction or water insoluble fraction of the given pig or hog serum, the hemolytic power of the mixture was again restored. However, when either one of water soluble or insoluble fractions was treated with iodine, the ability of the fraction to reactivate untreated fraction was destroyed.

From the results presented in the above experiments, it seems clear that in the first place, only a small amount of iodine was found in the dialyzed serum, and that iodine could be quantitatively accounted for as iodide. Therefore we are compelled to conclude that inactivation of complement is not an addition to protein, but an oxidation reaction. In the second place, serum oxidized by iodine was found to be able to reactivate sera which have been previously inactivated by yeast[3] and ammonia.[4] Furthermore, it has also been found that when complement is inactivated by these various means, the amount of iodine reduced by the inactivated complement is the same as the original serum. Hence, it may be concluded that iodine component is qualitatively different from the third or fourth component.

[3] Osborn, W. W. B., *Complement or Alexin*, Oxford University Press, 1937, p. 16.

[4] Gordon, J., Whitehead, H. R., and Wormall, A., *Biochem. J.*, 1926, **20**, 1028, 1036, 1044.