

Inactivation of Complement by Iodine.

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Complement may be partially or completely inactivated by a number of chemical reagents.¹⁻⁴ However, practically nothing is known about the nature of reaction which takes place between them. In this paper, we shall report a quantitative chemical reaction between complement and an oxidizing agent, namely, iodine.

The procedure of a typical experiment for the inactivation of complement in serum by iodine is as follows: A suitable amount (1.0 to 3.0 cc.) of freshly prepared guinea pig serum was pipetted into a series of eight 10 cc. volumetric flasks, to which varying amounts of 0.0100 N iodine solution were subsequently added. After standing at room temperature for 5 minutes, they were made up to volume and the units of complement were determined by the standard method. The results given in Table I show a proportionality between units of complement inactivated and the amount of iodine used except in flasks 2 and 3 in which the number of units of complement destroyed is small and the usual method of determination is not sufficiently sensitive to detect the difference. We have also determined the amount of iodine necessary for the complete inactivation of complement in a number of guinea pigs' sera, and found that the ratio of complement to iodine (C) is also constant as shown in Table II.

TABLE I.
Comparison of Degree of Inactivation with Amount of Iodine Added.

Flask No.	I ₂ N/100 added (B) cc.	Total units of complement found	Units of complement destroyed (A)	$\frac{A}{B} = C$
1	0.0	100	0	—
2	0.1	92	8	80
3	0.2	88	12	60
4	0.4	59	41	102
5	0.6	40	60	100
6	0.8	24	76	95
7	1.0	5	95	95
8	1.1	0	100	—

¹ Gordon, J., Whitehead, H. R., and Wormall, A., *Biochem. J.*, 1926, **20**, 1028, 1036, 1044.

² Osborn, T. W. B., *Biochem. J.*, 1933, **27**, 1425.

³ Gordon, J., and Thomson, F. C., *Brit. J. Exp. Path.*, 1933, **10**, 249.

⁴ Wadsworth, A. D., *J. Immunol.*, 1937, **33**, 297.

TABLE II.
Ratio of Units of Complement Inactivated to Iodine Used.

A. Guinea pig serum					B. Rat serum					C. Hog serum				
Vol. serum used cc.	Total units of complement found		Iodine N/100 used		Vol. serum used cc.	Total units of complement found		Iodine N/100 used		Vol. serum used cc.	Total units of complement found		Iodine N/100 used	
	A	B	A	B		A	B	A	B		A	B	A	B
2	200	110	1.8	1.4	1	30	22	90	4.8	3	41	2.9	18	18
2	170	105	1.6	0.9	1	20	22	90	5.6	3	36	1.8	16	16
2	200	110	1.8	2.5	1	66	26	20	1.2	1	20	1.2	17	17
2	240	105	2.3	2.7	2	76	28	15	1.2	1	15	1.2	13	13
2	240	100	2.4	2.4	2	50	21	84	5.2	3	84	5.2	16	16
2	200	100	2.0	1.5	2	32	22	41	2.9	2	41	2.9	14	14
2	150	100	1.5	2.7	2	54	20	36	1.8	1	36	1.8	20	20
2	172	107	1.6	2.9	2	76	26	75	5.0	3	75	5.0	15	15
2	164	102	1.6	2.4	2	66	28	60	4.6	2	60	4.6	13	13
2	200	105	1.9	2.8	2	74	26	78	4.5	3	78	4.5	17	17
2	266	102	2.6	2.6	2	66	25	32	1.8	1	32	1.8	18	18
2	280	112	2.5	3.0	2	66	22	28	2.0	1	28	2.0	14	14
3	300	106	2.8	3.7	2	74	20	35	2.2	1	35	2.2	16	16
2	290	105	2.8	1.9	2	50	27	37	2.4	1	37	2.4	16	16
3	300	103	2.9	3.5	3	90	26	39	2.6	1	39	2.6	15	15
2	240	100	2.4	2.9	2	76	26	28	1.8	1	28	1.8	15	15
2	228	100	2.3	3.6	2	76	31	30	2.2	1	30	2.2	14	14
1	80	100	0.8	3.0	2	66	22	32	2.6	1	32	2.6	13	13
				3.0	2	66	29	14	1.0	1	14	1.0	14	14
				3.0	2	80	27	15	1.1	1	15	1.1	14	14
				4.3	3	100	23							
				4.3	3	100	23							
				3.8	2	80	21							
				3.8	3	100	26							
Mean		104					24							15

The complement in normal serum of other species of animals is also inactivated by iodine. The animals studied include rat, hog, duck, man, dog, and chicken. The value of the ratio of complement to iodine for rat and hog have been found to be also constant (Table II).

Gordon, Whitehead and Wormall¹ reported that inorganic salts can inactivate complement. In order to show that the inactivation by iodine is not due to a specific effect of iodine or iodide, the inactivated serum was dialyzed to remove iodide and any remaining iodine. The serum so treated was found to possess no alexic activity. Therefore, the inactivation of complement by iodine must be a chemical reaction, most probably an oxidation. In fact, the results just reported indicate that there is a stoichiometric relationship between the amount of iodine consumed and the amount of complement inactivated. This indicates that the inactivation of complement proceeds so rapidly that the first portion of iodine added is almost completely reduced by complement and only the excess of the reagent is reduced by other substances in serum.

Our finding suggests a simple and rapid method of determining the amount of complement in guinea pig serum. The actual method and its significance will be reported in another communication.

Conclusions. 1. Complement in sera of different species of animal can be inactivated by iodine. 2. The amount of iodine necessary to inactivate a definite amount of complement in the serum of a species of animal, is a constant. 3. That constant varies in different species of animal.

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Effects of Chronic Injection of Rats with Extract of Pituitaries of the Same Species.

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Until about a year ago studies dealing with the production of loss of sensitivity to anterior pituitary extracts in animals subjected to chronic administration of these extracts had quite uniformly shown that this phenomenon apparently was one of species specificity. It appeared that the sera of such animals exerted a protec-