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Composition of Antigen-Precipitin Precipitate.

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In a previous study¹ on the composition of the precipitate produced by the reaction of an antigen with its anti-serum, we used hemoglobin as the antigen, because we had a specific method for the determination of small amounts of this protein.

On account of the difficulty of preparing anti-hemoglobin serum, hemoglobin is not a suitable antigen for the further study of the problem. We have, therefore, looked for some other protein which is a stronger antigen than hemoglobin and which also lends itself to quantitative determination in a mixture of proteins. Iodo-albumin has been found to serve our purpose, and we will report some results which have been obtained with it.

The iodo-albumin is prepared as follows: 100 cc. of 5% sodium carbonate was added to 400 cc. of 2.5% crystallized egg-albumin solution. After cooling to 0°, the solution was mixed with an iodine solution prepared by dissolving 5 g. of iodine in 150 cc. 10% KI solution. The mixture was allowed to stand in the ice-box for 24 hours. The iodo-albumin formed was precipitated by neutralizing the solution and then adding a slight excess of acetic acid. The precipitate was washed twice with water by centrifuging. It was then dissolved in 200 cc. N/10 Na₂CO₃ to give an approximately 1% solution. It is free from iodide and iodine and has a yellow color. The iodo-albumin prepared in this way contained 14.1% nitrogen and 6.9% iodine.

Iodo-albumin is a good antigen. No difficulty was encountered in preparing the anti-serum. Rabbits were immunized by giving 3 series of injections. In each series, intravenous injection of 5 to 10 cc. of 1% iodo-albumin was given daily for 3 days. Rabbits were then rested for 3 days before another series was started. The sera were tested on the 4th day after the last injection.

The general analytical procedure used in the previous study was followed in the present work. The precipitate, after 2 washings with 0.8% NaCl, was dissolved in 1 cc. N/10 NaOH and diluted, according to the amount of the precipitate, to a suitable volume

¹ Wu, H., Cheng, L. H., and Li, C. P., *PROC. SOC. EXP. BIOL. AND MED.*, 1927, **xxv**, 853.

(5-20 cc.). Aliquot portions of the solution were taken for N and I determinations. The N was determined by the same micro Kjeldahl method used in our previous study. The iodine was determined by a colorimetric method to be described elsewhere. The amount of antigen-N was calculated by multiplying the iodine figure by 2.04.

TABLE I.
Analyses of precipitates produced by the reaction of iodo-albumin with its anti-sera.

Rabbit No.	Amount serum, cc.	Conc. of iodo-albumin 1.43 mg. N per cc.	Iodo-albumin in ppt.		Total N in ppt.		Iodo-alb.-N in total -N %
			I mg.	N mg.	Total mg.	per cc. serum mg.	
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(a)	8	1/30	0.0584	0.116	1.02	0.126	11.7
(b)	8	"	0.0535	0.110	0.94	0.118	11.7
(c)	8	"	0.0508	0.104	0.93	0.116	11.2
913	8	1/10	0.0762	0.156	1.55	0.194	10.0
	6	1/15	0.0761	0.156	1.42	0.236	11.0
	6	1/20	0.0957	0.196	1.73	0.289	11.3
	6	1/30	0.0692	0.142	1.30	0.216	10.9
	6	1/60	0.0442	0.091	1.01	0.168	9.0
914	11	1/20	0.0345	0.069	0.70	0.063	9.9
	10	1/30	0.0409	0.082	0.80	0.080	10.3
	10	1/45	0.0393	0.079	0.87	0.087	9.1
	10	1/60	0.0383	0.077	0.85	0.085	9.1
915	12	1/20	0.0829	0.169	1.69	0.141	10.0
	12	1/30	0.0885	0.181	1.77	0.147	10.2
	12	1/60	0.0738	0.151	1.57	0.130	9.7
	14	1/90	0.0653	0.133	1.52	0.109	8.8
916	10	1/10	0.1326	0.271	2.56	0.256	10.6
	6	1/20	0.0809	0.165	1.70	0.284	9.7
	6	1/30	0.0621	0.127	1.51	0.251	8.4
	6	1/60	0.0352	0.072	1.10	0.184	6.5
	13	1/90	0.0492	0.101	1.62	0.125	6.2

* (a), (b), (c) Precipitate washed 2, 3, 4 times respectively with 0.8% NaCl.

The results of some analyses are shown in the accompanying table. It will be noted that the iodo-albumin content of the precipitate is approximately constant for different sera when the concentration of the antigen used is higher than 1/60 of 1%. With lower concentrations, however, the iodo-albumin content tends to decrease.

These results confirm our findings with hemoglobin. The approximate constancy of the antigen content of the precipitate strongly suggests a chemical reaction between the antigen and the precipitin (protein), but the question cannot be decided without further study. We are planning to use horses for the preparation of large quantities of immune sera and to study the reaction of the isolated precipitin (protein).