

6467

Removal of Intravenously Injected Antigen by Circulating Precipitin.

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Numerous investigators have reported that a specifically immunized animal eliminates intravenously injected antigen more rapidly than does a normal animal.^{1, 2, 3} Evidence has been presented to show that the precipitin antibody is of importance in the disposition of the foreign material.⁴ Virtually nothing has been established, however, as to whether the circulating or a fixed-tissue precipitin plays the major rôle in this elimination. The attempts to determine the relative importance of the circulating precipitin and the fixed-tissue precipitin in the removal of the injected antigen have been hindered by the lack of suitable methods for quantitatively estimating this antibody in an antiserum. Recently, we⁵ proposed a procedure, which we called the "neutralization method," for quantitatively titrating the precipitin against crystalline egg albumin. This method seems to overcome the difficulty in estimating precipitin and appears advantageous for investigating the relative importance of the circulating and fixed-tissue antibody in eliminating antigen.

We have been able by the neutralization method to determine quantitatively the effect on the circulating precipitin of the intravenous injection of antigen into an immunized rabbit. The amount of antibody, expressed in milligrams of nitrogen per cc. of the serum, present just before and one hour after injecting a known amount, in milligrams of nitrogen, of crystalline egg albumin has been found experimentally. The total circulating precipitin of the rabbit has been calculated by multiplying the amount of precipitin determined per cc. of serum by the number of cc. in the animal's plasma volume. The plasma volume has been considered, from determinations we have recently offered,⁶ to approximate 3.4%

¹ Weill-Halle, B., and Lemaire, B., *C. R. Soc. Biol.*, 1906, **61**, 114.

² Smith, G. H., and Cook, M. W., *J. Immunol.*, 1917, **2**, 421.

³ Hempl, H., *J. Immunol.*, 1917, **2**, 141.

⁴ Glenny, A. T., and Hopkins, B. E., *J. Hyg.*, 1922, **21**, 142; 1923-24, **22**, 12, 208.

⁵ Culbertson, J. T., *J. Immunol.*, in press.

⁶ Culbertson, J. T., *PROC. SOC. EXP. BIOL. AND MED.*, 1932, **30**, 102.

of the body weight of the rabbit. Data, suggesting the essential importance of the circulating precipitin in removing the intravenously injected antigen, are presented in Table I for 8 rabbits of a longer series studied in this regard.

TABLE I.
The effect of the intravenous injection of antigen on circulating precipitin.

Plasma volume (cc.)	Antigen injected (mg.N.)	Antibody plasma vol. before test injection (mg.N.)	Antibody plasma vol. 1 hr. after test inject. (mg.N.)	Antibody neutralized by test inject. (mg.N.) Exper. (Diff. columns 3 and 4)	Theoretic. (Antibody the antigen would neutralize "in vitro")
64	1.51	49.64	28.22	21.42	19.63
66	3.02	41.14	6.83	34.31	39.26
72	1.51	60.86	32.64	28.22	19.63
73	1.51	50.02	33.02	17.00	19.63
81	3.02	33.66	None*	33.66*	39.26
89	0.76	46.92	37.40	9.52	9.88
91	1.51	59.50	35.70	23.80	19.63
95	4.53	54.74	None*	54.74*	58.89

* Antigen detected in the blood taken 1 hour after the injection of antigen.

The experimental data show that the mean difference between the amount of precipitable antibody in the plasma of the rabbit prior to the test injection and that after the injection is approximately equal to the amount of antibody which would be precipitated by the given amount of antigen in the test tube. The ratio of union of antigen with antibody is apparently the same both for the union *in vitro* and for the combination in the circulation of the immune animal. After an amount of antigen greater than that calculated to neutralize the precipitin of the plasma of the rabbit is introduced into the circulation, free antigen but no precipitin is present in the blood. After an amount of antigen less than that necessary to neutralize the precipitin of the plasma volume is introduced, no antigen is present in the blood and the precipitable antibody of the plasma is reduced in proportion to the quantity of antigen injected. This result indicates that the antibody immediately available for the disposition of intravenously injected antigen is contained entirely in the blood plasma.