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### Blood Plasma Volume Determination by Injection of Anti-Crystalline-Egg-Albumin-Serum.

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The blood plasma volume of an animal is usually found by determining the dilution in the plasma of a known amount of an injected test substance following its introduction into the blood stream. Dyes and other chemical substances are generally employed. The dilution of the material in the plasma is found (1) colorimetrically,<sup>1, 2</sup> (2) spectroscopically,<sup>3</sup> or (3) by chemical means.<sup>4</sup> There is a considerable variation in the figures obtained for blood plasma volume by these methods. This variation may be due partly to loss of the injected foreign material from the blood stream and partly to the difficulty of determining the dilution of the substance introduced. The injection of an immunologically distinct blood or serum would, presumably, be more harmonious with the blood and tissues of an experimental animal than a foreign dye or other chemical substance. The dilution of such a blood could be determined by means of immunological tests. This has been resorted to by several investigators for the determination of blood volume. The first to present this method was von Behring.<sup>5</sup> His procedure consisted in finding the dilution of a known amount of an antitoxic serum after its introduction into the blood stream. Later, Todd and White<sup>6</sup> employed isohemolysins to determine the dilution of blood from another animal of the same species after its injection. Ashby<sup>7</sup> described a similar method which made use of isohemagglutinins for finding the blood volume of man.

These immunological methods have not been widely used, although they would seem potentially advantageous since a native

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<sup>1</sup> Keith, N. M., Rowntree, L. G., and Geraghty, J. T., *Arch. Int. Med.*, 1915, **16**, 547.

<sup>2</sup> Hooper, C. W., Smith, H. P., Belt, A. E., and Whipple, G. H., *Am. J. Physiol.*, 1920, **51**, 205.

<sup>3</sup> Graff, S., and Clark, H. T., *Arch. Int. Med.*, 1931, **48**, 808.

<sup>4</sup> Meek, W. J., and Gasser, H. S., *Am. J. Physiol.*, 1918, **47**, 302.

<sup>5</sup> von Behring, E., *Münch. med. Woch.*, 1911, **58**, 655.

<sup>6</sup> Todd, C., and White, R. G., *Proc. Roy. Soc. Lond.*, 1912, **84B**, 255.

<sup>7</sup> Ashby, W., *Med. Clinics North Am.*, 1920, **3**, 783; *Arch. Int. Med.*, 1925, **85**, 516, 632.

animal protein is administered. This is in part perhaps because the immune reactions concerned seem to lack sufficient accuracy for determining the dilution of the injected blood. We<sup>8</sup> have recently described a method for the quantitative estimation of the milligrams of precipitin antibody in rabbit anti-crystalline-egg-albumin-serum which may satisfy this requirement. The work reported here is an application of this method to the determination of the blood plasma volume of rabbits.

*Method.* A known volume of a precipitin-rich rabbit anti-crystalline-egg-albumin-serum is injected into the marginal ear vein of a normal rabbit immediately after the same amount of blood has been drawn from the recipient's heart. At intervals of 5, 15, and 60 minutes after the injection, 3 cc. blood samples are taken from an ear artery of the injected animal. By means of the neutralization method<sup>7,8</sup> for estimating precipitins, the mg. of precipitin per cc. is determined for the injected serum and for the serum of the blood samples taken from the recipient rabbit after it received the anti-serum. The plasma volume of the injected rabbit is found by the formula:

$$\text{Blood plasma volume} = \frac{\text{Mg. precipitin per cc. of injected antiserum} \times \text{cc. of antiserum injected}}{\text{Mg. precipitin per cc. of recipient's serum post injection}}$$

TABLE I.  
Data on Blood Plasma Volume Determinations.

Sex	Weight	Antiserum Injected	Precipitin Injected	Precipitin (Mg. per cc.)			Plasma vol. (15 min.)	Plasma vol. (15 min.) Wt. of rabbit
				5 min.	15 min.	60 min.		
M	gm.	cc.	mg. per cc.					%
M	2080	15	0.90	.213	.213	.189	63.0 cc.	3.1
M	2130	10	1.00	.119	.119	.119	82.0 cc.	3.8
M	2230	15	1.30	.213	.236	.213	82.5 cc.	3.7
M	2480	15	0.47	—	.084	—	82.5 cc.	3.3
M	2640	15	1.04	.189	.189	.143	82.5 cc.	3.1
F	1760	12	1.00	.166	.166	.143	72.0 cc.	4.0
F	2070	15	0.52	.094	.119	.094	64.5 cc.	3.1
F	3040	15	0.61	.107	.107	.107	85.5 cc.	2.8

The plasma volume of the 8 animals in this series is found to average 3.4% (that is, about one-twenty-ninth) of the body weight. The precipitin content of the 3 blood samples taken at intervals from each animal after the injection of the precipitating antiserum shows that the mixture of the antiserum and the blood of the in-

<sup>8</sup> Culbertson, J. T., *J. Immunol.*, in press.

jected animal is complete within 5 minutes and that the injected precipitin is retained quantitatively in the blood stream for at least 15 minutes after its introduction. The method here described seems to be a reasonable procedure for blood plasma volume determination and to possess certain advantages over other methods. A more extensive report of this work will be made elsewhere.

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**Tobacco Sensitiveness in Thrombo-Angiitis Obliterans.**

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(Introduced by George Baehr.)

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The etiology of thrombo-angiitis obliterans is unknown. The current theories as to the cause are specific infections, endocrine disturbances, toxic factors, such as ergotism and tobacco. Thus far there is no decisive evidence in favor of specific infection. The rôle of tobacco has been stressed by Buerger, Erb, Meyer, L. F. Barker, Silbert and others. Statistically Barker has shown that the excessive use of tobacco is much more common in patients with thrombo-angiitis than in a control group of similar ages. The disease has been reported in individuals who do not use any tobacco. In the large experience of Silbert, however, no typical instances of thrombo-angiitis obliterans have ever been observed in non-smokers. Silbert has noted clinically that cessation in the use of tobacco has often definitely arrested the progress of the disease. While it is a fact that patients suffering from thrombo-angiitis are almost universally excessive smokers, it is also true that many equally extensive users of tobacco never develop thrombo-angiitis obliterans. If, therefore, tobacco plays an etiologic rôle, the question arises why this agent should affect one group of individuals, and not all others.

It occurred to one of us (J. H.) to investigate this problem from the point of view of specific hypersensitiveness to tobacco. Typical cases of thrombo-angiitis observed for a number of years in the Thrombo-angiitis Clinic were selected for investigation. This report gives the results obtained in 68 cases of thrombo-angiitis varying between the ages of 24 and 56, and 122 controls. All the control