TABLE II.

RESULTS AVERAGED BY SERIES. EFFECTIVE DILUTION OF SERUM EXPRESSED IN DECIMALS.

Series.		Week.				
		1	2	3	4	5
I.....	Heated.....	.01250	.00083			
	Control.....	.01050	.00046			
II.....	Heated.....	.00520	.00078	.00020	.00010	.00012
	Control.....	.00380	.00023	.00008	.00008	——
III.....	Heated.....	.01500	.00025	.00024	.00027	.00050
	Control.....	.01000	.00013	.00017	.00021	.00035
IV.....	Heated.....	.00034	.00017	.00017	.00010	——
	Control.....	.00500	.00514	.02600	.00008	.00010
V.....	Heated.....	.10000	.01500	.01500	.01660	.02000
	Control.....	.10000	.01400	.01000	.01100	.02000

In general our results confirm those of Graziani and suggest that a moderately high atmospheric temperature (29°–32° C.) tends slightly to decrease the power of agglutinin formation in the rabbit. In Series IV alone this was not indicated. Here both control rabbits gave abnormal results. No. 93 showed a marked drop in agglutinating power during the third week; while No. 141 never formed any powerful agglutinins and died after the third week. With the exception of this series there are sixteen weekly averages of heated and control rabbits compared in Table II. In these sixteen cases the effective dilution for heated and control animals was on two occasions the same while in the other fourteen instances a consistently larger amount of serum was needed to produce agglutination in the case of the heated animals.

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Improved methods for the quantitative determination of plasma proteins.

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The blood is drawn into a tube containing an amount of potassium oxalate sufficient to make 0.2 or 0.3 per cent. oxalate solution, and is centrifuged twenty minutes.

Fibrin.—5 c.c. of plasma are run into a beaker containing 100–150 c.c. 0.8 per cent. NaCl and 2–5 c.c. of a 2.5 per cent. $CaCl_2$ solution. The $CaCl_2$ may be in amounts from 2–25 equivalents of the oxalate, but about five equivalents are best. When coagulation is complete, the fibrin is filtered, the clot washed with 0.8 per cent. NaCl, and the nitrogen determined by Kjeldahl. The above is an adaptation of Howell's method for determining the activity of thrombin.[1] The filtrate from the clot may be tested for complete precipitation by addition of a solution containing thromboplastic substances.

Albumin and Globulin are calculated from the following three determinations:

Total nitrogen is determined on a 1–2 c.c. sample.

Non-protein nitrogen is determined in the filtrate obtained after precipitating the plasma with nine volumes of trichloracetic acid.

Nitrogen of Globulin Filtrate:—Globulin and fibrin are precipitated by adding to 5 c.c. of plasma 20 c.c. of H_2O and 25 c.c. of saturated ammonium sulfate solution. 20 c.c. of the filtrate are mixed in a Kjeldahl flask with 3 gm. MgO "Merck's Reagent" and 350 c.c. of 50 per cent. alcohol. The solution is distilled until the distillate gives a negative test to red litmus. This takes about one hour and reduces the volume to about 20 c.c. The nitrogen, representing albumin plus non-protein nitrogen, is then determined by Kjeldahl, using 25 c.c. H_2SO_4. When the digestion mass becomes light brown, the sides of the flask are washed down with a few c.c. of water and ten more c.c. H_2SO_4 added.

Calculation:

Filtrate N—Non-protein N = Albumin N.
Total N—(Filtrate N + Filbrin N) = Globulin N.

[1] *Am. Jour. Physiol.*, 1910, XXVI, 453.