diet containing tricaproin. For this reason an attempt is now being made to feed a diet containing much more butter fat with the hope that more striking differences can be secured. Until further work is done the writer wishes to withhold any definite conclusion regarding the effect of the ingestion of butter fat on the chemical composition of body fat.

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Quantitative Studies on Precipitins.

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Since the quantitative precipitin method of Boyden and Baier[1] has proved to be fairly simple, rapid, and reliable, it was thought advisable to investigate further some properties of this reaction itself. The volume of precipitate obtained in the reaction was studied as affected by (1) quantity of antigen, (2) temperature of the reaction mixture, (3) time and rate of centrifugation, and (4) length of incubation.

Only simple protein antigens have been used in this study (crystalline egg albumin).[2] All protein concentrations have been found by making modified Kjeldahls[3] on samples. The antisera were obtained from rabbits by intravenous injections of the antigen. They were bled from the heart 10 days after the last injection. Calibrated instruments only were used in making all dilutions of antigen and antiserum in buffered saline.

The technique in performing the reaction consisted in preliminary titer determinations (ring test) to ascertain the strength of the antiserum. Following this the quantitative nature of the reaction was studied, using van Allen thrombocytocrits as stated[1] with suitable modifications for particular experiments. The experimental results are shown graphically.

The quantitative technique employed has verified some of the conclusions of earlier workers with regard to antigen-antibody

* The writer desires to thank Dr. A. A. Boyden of this Department for his guidance and advice both in the experimental work and in the writing of this paper.

[1] Boyden, A. A., and Baier, J. G., *J. Immunol.*, 1929, xvii, 29.

[2] Hopkins and Pinkus, *Physiol.*, 1898, xxiii, 130.

[3] Folin, O., and Wright, L. E., *J. Biol. Chem.*, 1919, xxxviii, 461.

equilibrium. Fleishman and Michaelis[4] recognized that the formation of precipitate increases at first with addition of precipitinogen, then decreases and with a certain excess of the antigen approaches zero. Zinsser[5] also noticed the solvent action of excess of antigen. Opie[6] has drawn the same conclusions using crystalline egg albumin as antigen.

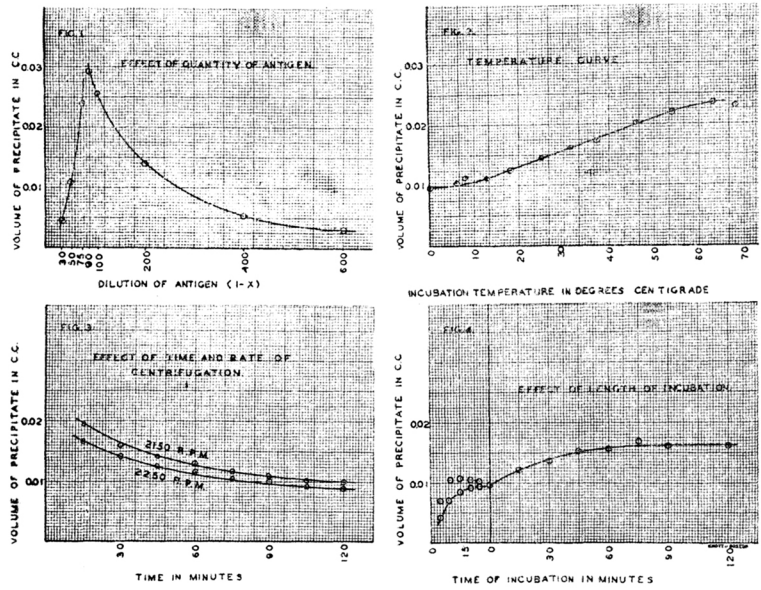

Figure 1 shows the definite effect of antigen dilution on precipitate volume. The antiserum was constant (0.5 cc. of a 1:5 dilution) for all readings, while the antigen was diluted as shown using 0.5 cc. amounts. The curve rises and falls sharply in the region of the maximum precipitate, so sharply in fact that the maximum point can scarcely be found with certainty with the error involved in the readings.

Figure 2 shows, for the first time, the effect of the temperature of incubation on the volume of precipitate. The antigen and antiserum were constant for all readings (0.5 cc. of a 1:150 dilution of antigen and 0.5 cc. of a 1:5 dilution of antiserum). Surprisingly enough the greater part of the curve is a straight line. The point 37.5° C. is in no sense normal for the reaction. Slavish use of

[4] Fleishman and Michaelis, *Biol. Z.*, 1907, iii, 425.

[5] **Zinsser, H., "Infection and Resistance," The Macmillan Co., 1914.**

[6] Opie, E. L., *Immunol.*, 1923, viii, 1.

this temperature in precipitin work is apparently unjustified as a higher temperature may prove more practicable.

Figure 3 shows the volume of precipitate as influenced by time and rate of centrifugation. The antigen and antiserum were diluted as in Figure 2. The curve is self-explanatory. The greater the rate of centrifugation in similar centrifuges the greater the packing of the precipitate and the less the absolute volume. Obviously, for absolutely comparable results equal centrifugal forces must be employed.

Figure 4 shows the effect of time of incubation on precipitate formation. The antigen and antiserum were diluted as in Figure 2. In the routine technique employed all tubes are centrifuged for 30 minutes after the incubation period. The time of centrifuging, however, is really a part of the incubation period. To get data regarding the volume of precipitate with less than 30 minutes' total incubation it was necessary to centrifuge for less than 30 minutes and calculate the volume for 30 minutes' centrifugation from previous data. Thus the portion of the curve to the left of the Y axis has been indirectly obtained from the points shown above the curve, while that to the right shows the volumes after varying times of incubation plus 30 minutes' centrifuging. The fact that in earlier stages of the reaction a certain rate of precipitate formation is just balanced by the rate of deposition in the thrombocytocrits brings with it a possibility of further studies throwing light on the nature of the reaction itself.

The quantitative data obtained were studied statistically. The probable errors were no larger than those reported in the paper of Boyden and Baier already cited. This means that the results obtained are fairly consistent.

Summary and Conclusions: 1. The quantitative technique described by Boyden and Baier has been successfully employed in a study of the precipitin reaction. 2. The relation of antigen to antibody has been shown quantitatively, using simple protein antigens, to confirm the results of others (Fleishman and Michaelis, Dean and Webb, Opie, Zinsser). 3. The volume of precipitate obtained in one hour's incubation was directly proportional to the incubation temperature. 4. An incubation of one hour at 37.5° C. gives very nearly the maximum precipitate. An incubation of one and one-half hours at the same temperature gives a practically complete precipitation. 5. The rate of centrifugation affects the volume of precipitate formed during a given time and must be considered wherever comparable results are desired.