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The Alien Globulin-Albumin Ratio in Artificial Serum Mixtures.

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If 5 cc. 7.5% horse serum-globulin are added to 95 cc. normal dog serum, the mixture allowed to stand in the ice chest over night, and the proteins of the mixture then separated by half-saturation, followed by full-saturation with ammonium sulphate, titrations by means of specific rabbit precipitin indicate a quantitative recovery of the horse protein in the globulin fraction of the serum mixture.

If horse serum-albumin is similarly added to normal dog serum, 33⅓% of the horse protein is recovered in the globulin fraction, and 66⅔% is the albumin fraction. A similar apparent 33⅓% conversion of horse albumin into horse globulin takes place in heat-inactivated normal dog serum.

The above tests are preliminary to a study of the mechanism of the intravenous denaturization of foreign proteins.

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Is a Quantitative Precipitin Titration Possible?

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In order to estimate the specific protein content of an unknown solution, 2 methods of precipitin titration are in use. Successive dilutions of the unknown may be mixed with a constant amount of specific antiserum, and the maximum dilution giving a demonstrable precipitin reaction may be determined. This dilution is compared with the maximum dilution of a known or standard protein solution giving the same end-reaction. For example, if the unknown gives an end-reaction in the dilution 1:1000, while the corresponding reading with the standard solution is 1:10,000, the conclusion is drawn that the unknown contains 10% of the specific protein of the standard, interfering factors, of course, being experimentally ruled out.

The method favored by many botanists and zoologists, however, is to estimate the amount of precipitate in some arbitrary dilution, preferably by means of a hematocrite,[1] the assumption being that equal precipitates indicate equal amounts of specific protein.

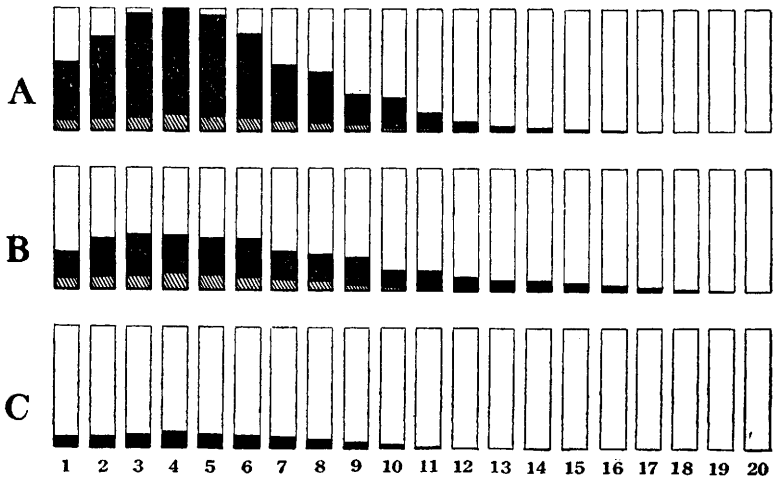
Applying these methods to a study of the parenteral history of alien proteins we have obtained contradictory and paradoxical results, the details of which have not yet been published.[2] As an illustration, the following data are cited.

If 2 cc. horse serum per kilo of body weight are injected intravenously into a normal dog, and if blood samples are withdrawn from this dog: (i) immediately before the injection, (ii) 15 minutes after the injection, and (iii) at the end of about 14 days, parallel titrations of the sera of the resulting blood samples by means of an ice-chest ripened (14 to 30 days) specific rabbit precipitin give the readings recorded in Fig. 1.

FIG. 1.

Attempted Titration of 14-day Parenteral Alien Proteins.

0.5 cc. successive dilutions (1:2) of the serum unknown, plus 0.5 cc. 20% rabbit antiserum; incubator 2 hours, ice chest over night. Each tube is now shaken to a uniform turbidity. Three of the maximum turbidities are mixed to form the 100% turbidity standard, the turbidities being read as percentages of this standard.



A—Serum from 15-minute canine blood sample.
B—Serum from 14-day canine blood sample.
C—Control, normal dog serum.

Cross hatched portions of A and B represent the "non-specific precipitate" (see C), which presumably must be subtracted from the total precipitate, to give specific protein reaction (black). Well ripened rabbit precipitin usually gives much less "non-specific precipitate" than indicated in C.

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

[2] Manwaring, W. H., *Science*, 1929, lxx, 2.

Comparison of the titers or end-reactions suggests that Sample B has at least 4 times the horse protein content of Sample A. Comparison of turbidities in the 9th to 12th dilutions suggests that A and B are of approximately equal horse protein content. Lower dilutions indicate that A has at least three times the horse protein content of B.

Our current quantitative precipitin technic apparently requires further study before it is applicable to complex protein mixtures.