THE DISTRIBUTION OF ANTIBODY TO CRYSTALLINE EGG ALBUMIN IN THE SERUM OF INJECTED RABBITS

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Introduction

Antibodies are always found in the globulin portion of an antiserum, but their distribution in the different globulin fractions obtainable depends on the antigen and on the species of animal which has been immunized.

As crystalline egg albumin and homologous antiserum have been used extensively for quantitative investigations of the precipitation reaction, the work to be described was undertaken to discover the distribution of antibody to this antigen in the serum of injected rabbits. Several globulin fractions were obtained by precipitation at different concentrations of ammonium sulphate, and whilst in all, the antibody was fairly uniformly distributed, the euglobulin fractions had a slightly higher content than the pseudoglobulin.

Methods

In order to provide sufficient material, antisera from different rabbits were pooled, two batches of pooled antisera being examined. After the antisera had been mixed, the contents of serum albumin and serum globulin present were determined. The total nitrogen of the antiserum was estimated and the value for albumin nitrogen plus non-protein nitrogen was obtained as follows: 7 c.c. of saturated magnesium sulphate solution and 1·2 g. of finely powdered magnesium sulphate were added to 1 c.c. of serum, the mixture was well shaken and left to stand overnight in a tightly stoppered 25 c.c. flask; the precipitate was then filtered off and the nitrogen was determined in an aliquot part of the filtrate. The globulin nitrogen was calculated by subtracting the value obtained for albumin nitrogen plus non-protein nitrogen from the total nitrogen.

The different globulin fractions of the antiserum were prepared by the addition of the appropriate amount of saturated ammonium sulphate solution. The precipitates obtained were dissolved and reprecipitated twice, as described by Taylor *et al.* (1932) and finally redissolved and dialysed in collodion membranes against 0·85 per cent saline at 0° C. until the dialysate gave no precipitate on the addition of saturated barium chloride. The concentrations of the globulin solutions so obtained were calculated from nitrogen determinations

of the dialysed proteins, supplemented by measurements of the refractive indices, the factors used for conversion of these values into globulin concentrations being those given by Adair & Robinson (1930) for horse serum globulin.

The antibody contents of the pooled antiserum and of the globulin fractions obtained were determined by the method of optimal proportions (Dean & Webb, 1926; Taylor *et al.* 1932), the results being recorded as optimal ratios which express the proportions by volume of antiserum and of a standard 1 per cent solution of crystalline egg albumin found to yield the most rapid particulation. Taylor *et al.* (1934) and Hooker & Boyd (1935) have shown that optimal proportions of crystalline egg albumin and homologous whole antiserum are the proportions in which the two reagents neutralize each other, and that the relationship of the antigen nitrogen to the nitrogen in the precipitate, the precipitate N/antigen N ratio, from a neutral mixture, is of the order of 1 to 11; Heidelberger & Kendall (1934) and Culbertson (1935) give the value as 1 to 12. Both the optimal ratio and the precipitate N/antigen N ratio were obtained for the unfractionated antiserum and for the globulin fractions, all of which flocculated readily when mixed with antigen in the optimal proportions titrations, flocculation taking place in 20–30 min. with the dilutions used.

To obtain precipitates for nitrogen determinations, mixtures of the two reagents in optimal proportions were incubated for 2 hours at 37° C. and then left in the refrigerator overnight. Next day the mixtures were centrifuged and the supernatant fluids were decanted and examined for residual antigen and antibody by ring tests, as described by Taylor *et al.* (1934, p. 121); neither was ever found, the mixtures were apparently neutral. The precipitates were washed twice by suspension in 0·85 per cent saline and centrifugalization, dissolved in glass-distilled water with the addition of a little dilute soda, and analysed for nitrogen. All the nitrogen determinations mentioned above were carried out by the method of Parnas & Wagner (1921).

The preparation of crystalline egg albumin and the production of antisera have been described by Taylor *et al.* (1932). The egg albumin contained 15·6 per cent of nitrogen. Each optimal proportions titration was performed at least twice, at room temperature, and the ratio assigned was the mean of the results expressed to the nearest 0·5. 0·85 per cent saline was used throughout as diluent.

EXPERIMENTAL

First batch of pooled antiserum

The whole serum contained per c.c. 11·2725 mg. of nitrogen, of which 5·5189 were albumin plus non-protein nitrogen and 5·7536 mg. were globulin nitrogen, i.e. the pooled antiserum contained 3·80 per cent of globulin if the value 15·13, which Adair & Robinson (1930) found for the percentage of nitrogen in horse globulin, be used.

Total globulin was prepared by half-saturation of the serum with ammonium sulphate, two reprecipitations and dialysis. A 5·36 per cent solution resulted, this was diluted with saline until the concentration was that of the globulin in the untreated serum, 3·80 per cent. A refractometric reading of this diluted solution gave the percentage as 3·884.

Euglobulin was prepared by one-third saturation of the serum with ammonium sulphate, two reprecipitations and dialysis. The concentration of the solution obtained was 2·74 per cent.

Pseudoglobulin was prepared by raising to half-saturation the concentration of ammonium sulphate in the filtrate from the euglobulin precipitate. After two reprecipitations and subsequent dialysis a 2·47 per cent solution was obtained.

Eu 2 fraction. When the euglobulin precipitate was dissolved for the first time in water, and the solution was reprecipitated by one-third saturation with ammonium sulphate, the filtrate from the precipitate was tested with ammonium sulphate to see if any globulin remained in solution. On raising the concentration of ammonium sulphate to one-half saturation, a considerable precipitate was formed and termed the Eu 2 fraction. It was collected, dissolved and dialysed, a 2·564 per cent solution being obtained.

Second batch of pooled antiserum

The whole serum contained per c.c. 10·754 mg. of nitrogen, of which 5·469 mg. were albumin plus non-protein nitrogen and 5·284 mg. were globulin nitrogen, i.e. 3·49 per cent of globulin.

Table I

Fraction	Optimal ratio 1% antigen to antiserum	Mixture for N determination: Anti-serum c.c.	Mixture for N determination: Antigen mg. N	Precipitate N mg.	Ratio Precipitate N / Antigen N	Antiserum N in precipitate from 1 c.c. of 1% solution of fraction	Optimal ratio of 1% solution of fraction
			First experiment				
Whole serum (globulin 3·80%)	1 to 13·5	1	0·115	1·3738	11·9	0·331	51·3
Total globulin (3·88%)	1 to 12	1	0·1300	1·5127	11·6	0·355	46·7
Euglobulin main (2·74%)	1 to 21	1	0·0743	1·1655	15·7	0·398	57·5
Pseudoglobulin (2·47%)	1 to 24	1	0·0650	0·8512	13·1	0·318	59·3
Euglobulin 2 (2·564%)	1 to 14	1	0·1114	1·3748	12·3	0·494	35·8
			Second experiment				
Whole serum (globulin 3·49%)	1 to 11·5	1	0·1356	1·5792	11·65	0·414	40·1
Euglobulin main (1·323%)	1 to 36	3	0·1300	1·8998	14·60	0·447	47·5
Pseudoglobulin (1·38%)	1 to 32	3	0·1463	1·7724	12·10	0·393	44·2
Euglobulin 2 (1·91%)	1 to 15	1	0·1040	1·0969	10·5	0·520	28·65
Euglobulin A (0·181%)	1 to 200	3·5	0·0273	0·3062	11·2	0·440	36·2
Euglobulin B (0·538%)	1 to 60	2	0·0520	0·5859	11·3	0·496	32·3

Euglobulin fractions. As shown in Table I, in the first experiment the highest antibody content appeared to be associated with the Eu 2 fraction, that with a solubility in ammonium sulphate slightly lower than that of pseudoglobulin. In the second experiment, the euglobulin was further fractionated, in order to discover whether the least soluble fraction contained no antibody; this was not the case. Starting with 145 c.c. of pooled antiserum, the first precipitate appeared after the addition of 49 c.c. of saturated ammonium sulphate solution; a further 1 c.c., to make 50 c.c., was added, and a fraction, designated Eu A, was obtained. After two reprecipitations very little remained; the dialysed solution contained 0·2736 mg. of nitrogen per c.c., i.e. 0·181 per cent of globulin.

The precipitate of the Eu A fraction, after solution in water, had been reprecipitated by adding 1/2·9 of its volume of saturated ammonium sulphate solution. After filtration, more ammonium sulphate was added to the filtrate to bring the concentration to one-third saturation, and a precipitate was obtained, which was dissolved and reprecipitated twice, dialysed and called Eu B; it contained 0·8134 mg. of nitrogen per c.c., i.e. 0·538 per cent of globulin.

The concentration of ammonium sulphate in the filtrate from the Eu A fraction was made up to one-third saturation. The precipitate which resulted was dissolved and reprecipitated twice and, after dialysis, yielded a solution which contained 2·0015 mg. of nitrogen per c.c., i.e. 1·323 per cent of globulin. This was called the main euglobulin fraction.

As was the case in the first experiment, increasing the concentration of ammonium sulphate in the filtrate from the first reprecipitation of the main euglobulin fraction to half-saturation yielded a considerable precipitate, which we called as before the Eu 2 fraction. After two reprecipitations and dialysis the solution contained 2·8897 mg. of nitrogen per c.c., i.e. 1·91 per cent of globulin.

The pseudoglobulin was prepared as in the first experiment by raising the concentration of ammonium sulphate in the filtrate from the main euglobulin fraction to half-saturation. After two reprecipitations and dialysis, a solution was obtained which contained 2·0847 mg. of nitrogen per c.c., i.e. 1·38 per cent of globulin.

Details of the experiments made to determine the precipitate N/antigen N ratio for the two batches of pooled antisera and the globulin fractions obtained are given in Table I. In the penultimate column are the amounts of antiserum nitrogen in the precipitates resulting from the mixture of 1 c.c. of 1 per cent solutions of the fractions and the optimal amounts of antigen: it is assumed that all of the antigen is in the precipitate. The last column gives the optimal ratios of 1 per cent solutions of the fractions; a high ratio indicates a low antibody content, a low ratio the reverse. The antibody content of the second batch was greater than that of the first. The results show that the antibody is fairly uniformly distributed throughout the globulin fractions prepared; in both cases the Eu 2 fraction had the highest antibody content.

After dialysis, the albumin from both batches of antiserum was examined for antibody, but none was detected.

Two experiments were carried out in order to see if the globulin fraction that we have designated Eu 2 could be obtained from normal rabbit serum. In each experiment, 30 c.c. of normal serum which had been heated for 30 min. at 57° C. were precipitated by the addition of 15 c.c. of saturated ammonium sulphate solution, the precipitate was filtered off, pressed between filter papers, then dissolved in 20 c.c. of water and reprecipitated by the addition of 10 c.c. of saturated ammonium sulphate solution. The precipitate was again filtered off, the volume of the filtrate was measured and the concentration of ammonium sulphate therein was raised to half-saturation, when some turbidity was manifested and a small precipitate was deposited after the mixture had been kept for some hours in the refrigerator. The bulk of the precipitate obtained was in no wise comparable with that which appeared immediately in the two experiments with pooled antisera on increasing the ammonium sulphate concentration to half-saturation in the filtrate from the second precipitation of the main euglobulin fraction.

The figures recorded in Table I show that there is no appreciable difference between the ratios for precipitate N/antigen N given by the untreated antiserum and by the antiserum total globulin precipitated by ammonium sulphate. According to the theory put forward by Boyd & Hooker (1934), an increase in the molecular weight of the antibody should increase this ratio, and an unchanged ratio indicates that the state of aggregation of the antibody is unaltered by precipitation with ammonium sulphate. In the case of the euglobulin and pseudoglobulin fractions some differences in the ratios have been observed, but it is not possible to draw definite conclusions as to the state of aggregation, because antibodies of different solubilities and molecular weights might be present.

Summary

Two batches of pooled antisera obtained from rabbits injected with crystalline egg albumin have been fractionated with ammonium sulphate in order to investigate the distribution of the antibody to this antigen in the serum proteins.

The albumin fractions contained no antibody.

All the globulin fractions examined contained antibody, and flocculated readily when mixed with appropriate dilutions of the antigen.

The distribution of antibody throughout the serum globulin was fairly uniform, the ratio of the antibody content to the protein concentration being the smallest in a fraction with a solubility in ammonium sulphate slightly lower than that of pseudoglobulin.

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