

# Ammonium Sulfate Fractionation of Sera: Mouse, Hamster, Guinea Pig, Monkey, Chimpanzee, Swine, Chicken, and Cattle

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Optimal  $(\text{NH}_4)_2\text{SO}_4$  concentrations were sought for serum fractionation in order to obtain the gamma globulin as free as possible from other serum components while maintaining a reasonable recovery. Various ammonium sulfate concentrations were used to fractionate sera from mice, hamsters, guinea pigs, monkeys, chimpanzees, swine, chicken, and cattle. All precipitates and supernatants were analyzed by electrophoresis to study the effects of various treatments on the composition of these materials. Approximately 75% of all the gamma globulins were recovered when each serum was fractionated with its optimal sulfate concentration. These optimals were determined to be as follows: three precipitations in 35% saturated ammonium sulfate (SAS) for hamster, chimpanzee, swine, and chicken serum; one precipitation in 35% SAS followed by two in 40% SAS for mouse and guinea pig serum; one precipitation in 30% SAS and then two in 40% SAS for monkey serum; and one precipitation in 30% SAS followed by two in 35% SAS for cattle serum.

In immunology, serum fractionation methods are designed primarily to separate the gamma, or immunoglobulins, from the other serum proteins and to recover the antibodies in good yields. A salt such as ammonium sulfate is frequently used either alone or as a preparative step for more elaborate techniques. Serum fractions prepared by  $(\text{NH}_4)_2\text{SO}_4$  precipitation are utilized in immunofluorescence studies in many areas of microbiology.

In a previous paper in this journal (3), data were presented on the  $(\text{NH}_4)_2\text{SO}_4$  fractionation of rabbit, sheep, horse, and goat sera. An optimal  $(\text{NH}_4)_2\text{SO}_4$  concentration for fractionation was determined for each animal, and in each case it was less than the routinely used 50% saturation. The same methods have now been applied to eight additional animal sera frequently involved in immunological studies: mouse, hamster, guinea pig, monkey, chimpanzee, swine, chicken, and cattle.

## MATERIALS AND METHODS

**Sera.** All studies were done with serum pools rather than with individual sera. The normal sera used were from a number of mice, hamsters, and guinea pigs, 3 African green monkeys, 19 chickens, and 6 cattle. The immune sera were from six swine immunized against hog cholera and six chimpanzees, some experimentally infected with *Treponema pallidum* and some infected with *Neisseria gonorrhoeae*.

**Ammonium sulfate.** A stock solution of saturated ammonium sulfate (SAS) was prepared and stored at room temperature (approximately 25 C). Working solutions of 60, 70, 80, and 90% SAS were prepared (vol/vol) fresh as needed from the stock saturated solution. Equal volumes of these solutions and various antisera resulted in reaction mixtures of 30, 35, 40, 45, and 50% SAS.

**Fractionation.** The procedure followed for fractionation was detailed in a previous paper (3).

**Protein.** Protein concentrations were measured by the biuret method (2) with a Beckman DB spectrophotometer. Protein compositions were determined by cellulose acetate strip electrophoresis (CASE) with the Beckman Microzone (1) equipment and procedure, with a slight modification. The preparation of uncleared membranes for densitometric analysis and the interpretation of the densitometer tracings have been described (3).

## RESULTS

**Preliminary data.** The whole sera electrophoresis profiles of the eight species are shown in Fig. 1. They are rather easily distinguished from each other and from the rabbit, sheep, horse, and goat serum profiles presented earlier (3). The closest resemblance is between monkey and chimpanzee serum profiles, but even there the electrophoretic position of the protein peaks is different. All of the gamma globulin peaks were cathodic under the conditions tested, and the albumins migrated distinctly

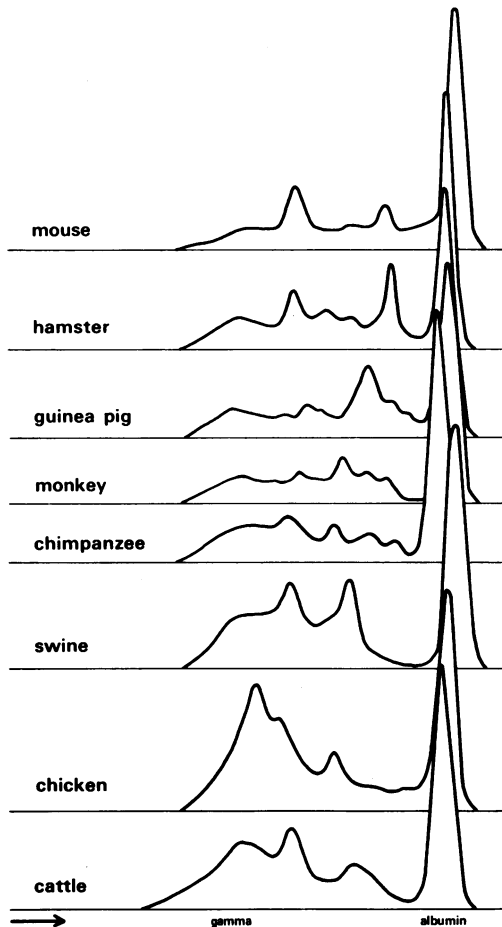


FIG. 1. Electrophoretic profiles of pooled sera.

different distances toward the anode. With such variety in the composition of whole serum, it was not unrealistic to anticipate differences among the ammonium sulfate fractions.

**Hamster, chimpanzee, swine, and chicken serum studies.** The CASE profiles of hamster, chimpanzee, swine, and chicken sera and their fractions obtained after three precipitations in various percent SAS solutions are shown in Fig. 2. The 50% SAS fractions are very crude, and in the hamster serum fraction, gamma globulin was not even a major component. With each of these four animal sera, the optimal fraction was obtained after three precipitations in 35% SAS.

Hamster serum had prominent alpha and beta globulin peaks which became minor after three precipitations in 35% SAS, but a lesser globulin peak ( $\alpha_2$  or  $\beta_1$ ) in the whole serum profile was a major problem after fractionation and could not be further reduced. The optimal

35% SAS fraction was 55% gamma globulin, free of albumin.

Chimpanzee serum fractionation presented no special problems, and the optimal 35% SAS fraction was 76% gamma globulin with only 0.4% albumin.

The swine and chicken sera each had a beta globulin that could not be removed under these conditions. The 30% SAS fractions of swine and chickens contain no albumin and a higher percent gamma globulin than the 35% SAS fractions, but more than 50% of the gamma globulins were lost in the supernatants. The optimal 35% SAS fractions of swine and chicken sera were 44% and 58% gamma globulin, respectively.

**Mouse serum studies.** The CASE profiles of mouse serum and its various fractions are shown in Fig. 3. The prominent beta globulin in the serum profile could not be removed under these conditions. The 50% fraction was not much of an improvement over whole serum. The 40% SAS fraction was less than 50% gamma globulin. Most of the albumin and alpha globulins were eliminated after three precipitations in 35% SAS, but so was a large portion of the gamma globulin. However, the first 35% precipitation was superior to the first 40%, and a single precipitation in 35% SAS followed by two precipitations in 40% SAS gave a fraction which was 58% gamma globulin with only 0.4% albumin.

**Guinea pig, monkey, and cattle serum studies.** The CASE profiles of guinea pig, monkey, and cattle sera and their various fractions are shown in Fig. 4. As usual, the 50% SAS fractions were extremely crude, and the fractions obtained with less concentrated sulfate solutions were more desirable. The 35% SAS fractions were acceptable, but the optimal fractions were obtained by using two different concentrations of ammonium sulfate. With guinea pig serum, more gamma globulin was lost at 35% than at 40%, but a first precipitation in 35% SAS followed by two precipitations in 40% SAS gave a fraction which was 51% gamma globulin with no albumin. With monkey serum, a single precipitation in 30% SAS followed by two precipitations in 40% SAS gave a fraction which was 74% gamma globulin with only 0.5% albumin. Cattle serum, after one precipitation in 30% SAS followed by two precipitations in 35% SAS, provided a fraction containing 90% gamma globulin and no albumin.

**Recovery of gamma globulin.** When the optimal ammonium sulfate concentrations were used to fractionate mouse, hamster, guinea pig,

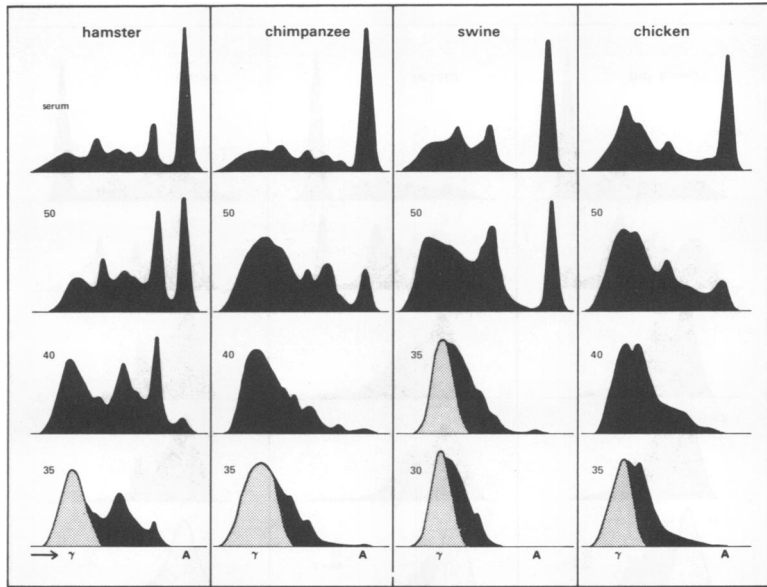


FIG. 2. Electrophoretic profiles of animal sera and their fractions after three precipitations in various percent saturated solutions of  $(NH_4)_2SO_4$ .

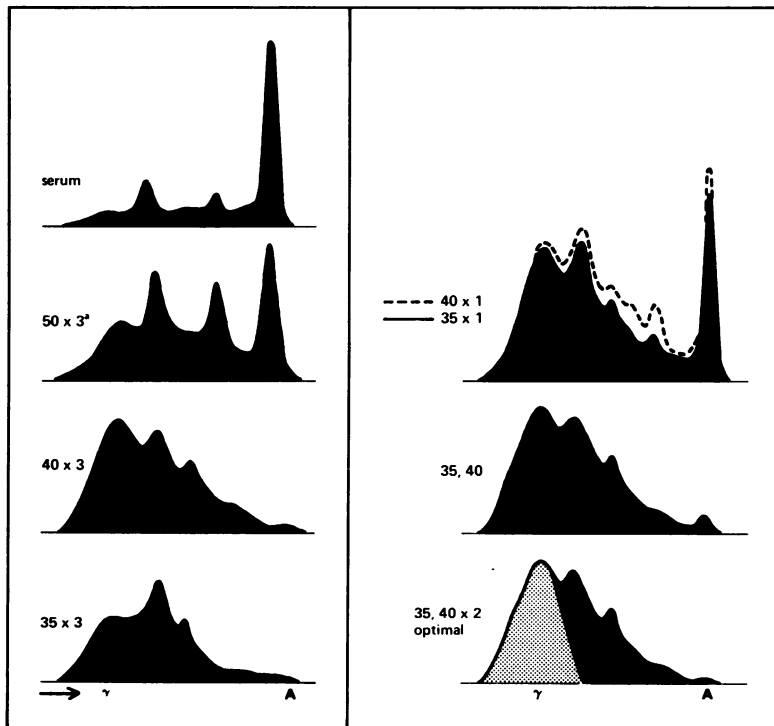


FIG. 3. Electrophoretic profiles of mouse serum and its  $(NH_4)_2SO_4$  fractions. <sup>a</sup>Three precipitations in 50% saturated  $(NH_4)_2SO_4$ .

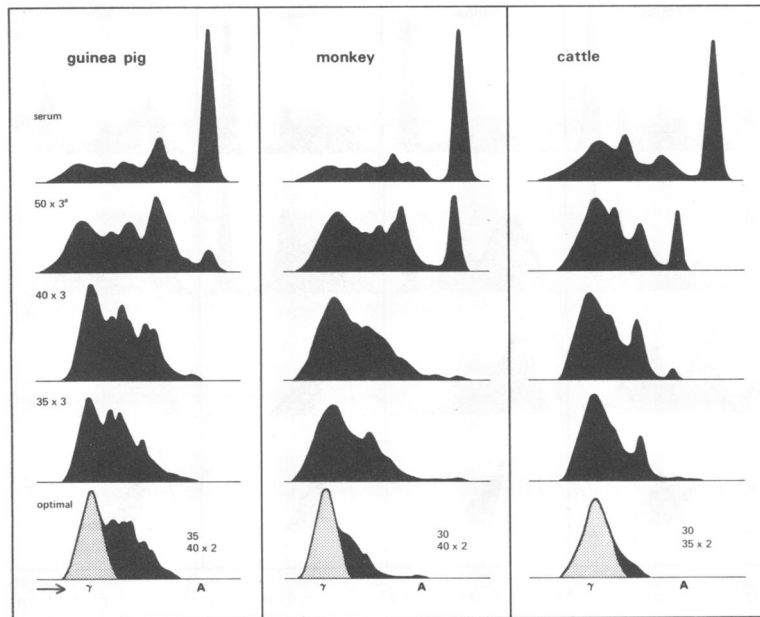


FIG. 4. Electrophoretic profiles of guinea pig, monkey, and cattle sera and their various  $(\text{NH}_4)_2\text{SO}_4$  fractions. <sup>a</sup>Three precipitations in 50% saturated  $(\text{NH}_4)_2\text{SO}_4$ .

monkey, chimpanzee, swine, chicken, and cattle sera, an average of 75% of the total milligrams of gamma globulins per milliliter was recovered in the final fractions (Table 1). The lowest gamma globulin recovery was 66% from hamster serum, and the highest was 97% from chimpanzee serum.

### DISCUSSION

The composition of whole sera from animals is so diverse that some variations in procedures were necessary to obtain optimal fractions. This was certainly true of the first four animals studied (3), and species-dependent variables have also been noted by others (4, 5).

During this study, each of the precipitates and first supernatants was analyzed by CASE. None of the supernatants from precipitations in 50% SAS contained gamma globulin, which indicates that all of the gamma globulins together with other serum proteins were precipitated from each of the eight sera in 50% SAS. As the concentration of ammonium sulfate was decreased, fewer of these other proteins precipitated with the gamma globulins. When the  $(\text{NH}_4)_2\text{SO}_4$  concentration was reduced to 40% saturation, some gamma globulin was not precipitated and was lost; below 40% saturation an appreciable amount was lost from some of these

sera. Repeated precipitations with a given concentration of ammonium sulfate served primarily to reduce the percentage of albumin.

With most of these sera very little precipitate formed in 30% SAS, and its composition was not desirable. However, with monkey and cattle sera, the fraction from one precipitation in 30% SAS was such an improvement that, even though some gamma globulin was lost, it was worth the sacrifice. With each of these sera the second and third precipitations were carried out in a higher percent SAS to minimize loss of gamma globulin, i.e., 40% SAS for monkey and 35% SAS for cattle serum. Three precipitations of swine serum in 30% SAS gave a fraction which was 53% gamma globulin with no albumin, but the recovery of gamma globulin was only 32%.

Although the sera of only two of the eight species were from immunized animals, the procedures should give equivalent results with normal and immune sera. Earlier studies with both normal and immune sera (3) indicate that the electrophoretic migration of the gamma globulins, as measured by CASE, is not altered by immunization, although the total amount of gamma globulin is increased. If the antibody titer is reduced in antiserum fractions obtained by using optimal  $(\text{NH}_4)_2\text{SO}_4$  concentrations, it is probably a reflection of loss of gamma globu-

TABLE 1. Recovery of gamma globulin from animal sera after fractionation with  $(\text{NH}_4)_2\text{SO}_4$ 

| Sera and $(\text{NH}_4)_2\text{SO}_4$ fractions | CASE      |                |             | Total protein (mg/ml) | Gamma globulin |                 |
|---|-----------|----------------|-------------|-----------------------|----------------|-----------------|
|   | Gamma (%) | Beta-alpha (%) | Albumin (%) |                       | mg/ml          | Recovery (%)    |
| Mouse Serum                                     | 8.5       | 41.5           | 50.0        | 64                    | 5.4            | NA <sup>a</sup> |
| 35, 40 (2) <sup>b</sup>                         | 58.0      | 41.6           | 0.4         | 7                     | 4.1            | 76              |
| Hamster Serum                                   | 13.0      | 45.0           | 42.0        | 64                    | 8.3            | NA              |
| 35 (3)  | 55.0      | 45.0           | 0.0         | 10                    | 5.5            | 66              |
| Guinea pig Serum                                | 15.0      | 39.0           | 46.0        | 55                    | 8.3            | NA              |
| 35, 40 (2)                                      | 51.0      | 49.0           | 0.0         | 12                    | 6.1            | 74              |
| Monkey Serum                                    | 14.0      | 33.0           | 53.0        | 66                    | 9.2            | NA              |
| 30, 40 (2)                                      | 74.0      | 25.5           | 0.5         | 9                     | 6.7            | 73              |
| Chimpanzee Serum                                | 18.0      | 33.0           | 49.0        | 78                    | 14.1           | NA              |
| 35 (3)  | 76.0      | 23.6           | 0.4         | 18                    | 13.7           | 97              |
| Swine Serum                                     | 21.0      | 41.0           | 38.0        | 72                    | 15.0           | NA              |
| 35 (3)  | 44.0      | 55.0           | 1.0         | 24                    | 10.6           | 71              |
| Chicken Serum                                   | 26.0      | 41.0           | 33.0        | 47                    | 12.0           | NA              |
| 35 (3)  | 58.0      | 41.5           | 0.5         | 15                    | 8.7            | 72              |
| Cattle Serum                                    | 28.0      | 34.0           | 38.0        | 81                    | 22.7           | NA              |
| 30, 35 (2)                                      | 90.0      | 10.0           | 0.0         | 18                    | 16.2           | 71              |

<sup>a</sup> Not applicable.

<sup>b</sup> One precipitation in 35% SAS followed by two consecutive precipitations in 40% SAS.

lins during fractionation. The electrophoretic profiles of gamma globulin in the original sera and in the optimal fractions appear identical.

Many of the various alcohol and column fractionation procedures used to obtain gamma globulin from sera will yield much cleaner preparations than some of those shown in this paper. However, salt fractionation is a very simple and inexpensive procedure that can be carried out in any laboratory. It is also useful as a preparative step for fractionation by more elaborate techniques. Use of the recommended optimal  $(\text{NH}_4)_2\text{SO}_4$  concentrations should improve the efficiency of the endeavor.

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