ON THE CONCENTRATION OF SERUM BY MEANS OF SODIUM SULPHATE.

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The method most commonly employed for the separation of antitoxic globulins is that by means of Ammonium Sulphate, and this was the process used in these laboratories for several years until, at the end of 1917, the supply of the necessary salt was cut off. We were then thrown back on Sodium Sulphate, a salt we had used before the War but which had not given us satisfactory results. A few fresh trials, however, showed us the faults of our previous technique and proved that Na_2SO_4 could satisfactorily replace Am_2SO_4 and so, since February 1918, we have used only the former salt. Changes in detail have been made from time to time and we have now established a routine method which has for some time given us satisfactory results.

The object of this communication is to describe the process as it may be of use to others who are interested in the same subject. To those who are conversant with the literature of the concentration of serum it will be obvious that the method is based on the work of S. N. Pinkus (1901–1902) and on that of R. Gibson (1905).

Before describing the routine method it may be as well to mention that, as is the case with ammonium sulphate, "fractional" precipitation by Na_2SO_4 allows of the antitoxic globulins being separated out and a considerable, say eight to ten times, concentration realised. But it must be borne in mind that the precipitation by Na_2SO_4 must be carried out at a temperature above 33° C. because below this temperature the concentration of the salt necessary for the precipitation cannot be obtained.

At 33–40° C. the addition per 100 c.c. of plasma or serum of 10–11 gms. Na_2SO_4 will throw down the euglobulin and the further addition per 100 c.c. of filtrate of 8·5–7·5 gms. of sulphate will separate the pseudoglobulin, the albumen remaining in the filtrate. The precipitation limits are not sharp and some of the antitoxin is carried down with the euglobulin and a little may pass on with the albumen. If, then, we take into account only the fraction defined by the limits 11 and 18·5 gms. of Na_2SO_4 we can obtain high concentration, but a fairly large loss of antitoxin must be expected. If it is considered worth while much of this loss can be recovered by appropriate treatment of the precipitate, and in our routine process we make this our aim, but when all has been done it will be found that if a minimum loss of antitoxin is desired one must be content with a concentration of four to five times the original volume. We,

here, are satisfied with this increase, and it is only in special cases that we work for the higher degree.

As an example of the possibilities of this fractional process, I mention that we have obtained from an antitetanic serum containing 250 units per c.c. a solution of antitoxic globulins of a titre of 2200 units per c.c.

The usual routine proceeds as follows:

Plasma[1] or serum is warmed in a water bath to 33–37° C. and then anhydrous sodium sulphate is added and dissolved until the density reaches 1175, which represents about 18·5 gms. of sulphate per 100 c.c. of plasma or serum. The mixture is then filtered through chain cloth at a temperature of 33–37° C. and allowed to drain thoroughly. In the precipitate will be found euglobulin, pseudoglobulin and sulphate; in the filtrate, albumen and sulphate.

The precipitate is re-dissolved in water at 33–37° C. in the water bath, made up to the original volume of the plasma and the precipitation and filtration repeated.

The albumen filtrates may be discarded. The precipitate of euglobulin and pseudoglobulin is now dissolved in cold water and made up to twice the original volume of plasma. When completely dissolved common salt (table salt[2]) is added to saturation. The density should then be 1200–1205. The mixture is filtered through chain cloth. The precipitate is again dissolved up in water, made up to the original volume of plasma and the process of saturation with salt, and filtration repeated.

The residue on the filter cloths (euglobulin) may be discarded, or digested and used for media making, or used as a fertilizer. The two portions of filtrate (solutions in brine of pseudoglobulin) are mixed together and the globulin is precipitated by the addition of 0·3 per cent. of glacial acetic acid.

The precipitate is separated by filtration through chain cloth and allowed to drain thoroughly.

The cloths with the precipitate are opened out flat and placed together face to face (precipitate inside) in pairs between towels to remove the excess moisture—the towels being changed as often as may be necessary.

When fairly dry the cloths, still in pairs, are placed between dry towels, and these between boards in a press, there being alternately a board and a pair of cloths.

Slight pressure only is put on at first and then gradually increased as the

[1] The plasma or serum may be diluted with water if preferred. The ordinary reaction of plasma or serum is a quite suitable one.

[2] The table salt used is supplied by Messrs Bumsted and Co., who have kindly sent us the following recent analysis:

Sodium chloride	99·86 %
Sodium sulphate	0·11 %
Calcium sulphate	free
$MgCl_2$	„
Sodium carbonate	0·01 %
Sodium bicarbonate ...	0·02 %
Insoluble	none

precipitate becomes drier. When properly dried the precipitate is like crumbling cheese—neither pasty nor hard and gluey.

Great care must be taken not to overpress as this spoils the result.

The dried precipitate is removed from the cloths, weighed out in lots of 800–1000 gms. and placed in the centre of moistened circular (24 ins. diameter) sheets of vegetable dialysing parchment. Powdered washing soda is then sprinkled over it in the proportion of 3 per cent. of the weight of the precipitate. The parchment paper is folded up into the form of a bag and suspended in a dialysing tank through which water gently flows.

When free from excess of salts—which usually takes place in about 48 hours and which may be ascertained by the dipping refractometer[1]—the pseudoglobulin solution is removed from the dialysing bags and measured. Then 0·7 per cent. of a mixture of equal parts of cresylic acid and ether (Banzhaf, 1921) is added and the solution is stored in sterile 4 litre corked bottles in the cold room until required.

Examples of Results.

Batch 1. *Diphtheria antitoxin.*

550 litres of plasma were mixed together and treated as above at the rate of about 100 litres per week. The results of the several weeks' work were mixed together and tested:

Original 550 litres at 250 a.u. per c.c. = 137,500,000 a.u.
Concentrated 96·5 litres at 1400 per c.c. = 135,100,000 a.u.

Batch 2.

Original 500 litres at 350 a.u. per c.c. = 175,000,000 a.u.
Concentrated 168·5 litres at 1000 per c.c. = 176,925,000 a.u.

Tetanus antitoxin.

Original 110 litres at 200 a.u. per c.c. = 22,000,000 a.u.
Concentrated 23·3 litres at 1000 a.u. per c.c. = 23,300,000 a.u.

Antidysentery serum.

Original 200 litres neutralising 500 M.L.D.'s (rabbit) per c.c.
= 100,000,000 M.L.D.'s.
Concentrated 50 litres neutralising 2000 M.L.D.'s (rabbit) per c.c.
= 100,000,000 M.L.D.'s.

Antiscorpion serum and antiplague serum can be concentrated in the same way.

The question may be raised as to how it comes about that in diphtheria antitoxin, batch 2, mentioned above, the solution after concentration contains a larger total number of units than were apparently present originally.

[1] A portion of the liquid in the dialysing bags is diluted with an equal quantity of distilled water, boiled and filtered. The salt content of the filtrate is estimated with the refractometer and compared with a filtrate obtained by treating normal serum in the same manner.

It stands to reason that no process of this sort can be carried out without some loss actually occurring. The figures given above are not meant to show that no loss has taken place. The sera are not tested closer than 50 units. Thus the titre of the original plasma in batch 2 was 250 units but not 300, while that of the concentrated solution was tested to be 1050 units but not 1100 units per c.c. The possible differences in the actual exact titres would easily account for the apparent excess recovered, but the maximum possible loss could not have been more than 11·5 per cent.

The degree of concentration obtained depends upon the pressing and drying of the pseudoglobulin precipitate and the greater the concentration the higher will be the percentage of protein in the final product.

We have found that sodium sulphate has the following advantages over ammonium sulphate:

1. It is non-toxic and therefore it is not so necessary to remove the last traces of it[1].
2. It is not such a good food for micro-organisms and so there is not the same liability to contamination and it is not necessary to put any preservative in the dialysing bags.
3. The rate of dialysis is quicker (*cf.* Banzhaf, 1908–9).
4. With equal concentration of protein the final product is not so viscid.
5. The cost is less.
6. The sodium salt does not attack metal so strongly.

The description may give the impression that the process is complicated and laborious but we do not find it so in practice. We have adopted it only after a lengthy trial and comparison with other methods. During the war, when we were using ammonium sulphate in various ways, we came to the conclusion that the old original Gibson process was the one which gave the best and most reliable final product, and now it will be seen that after wandering in many devious by-paths we have found our way back to, and are content to travel on, the old road, but substituting sodium for ammonium sulphate.

I am much indebted to Mr Albert Riggs, head laboratory assistant, for his enthusiastic co-operation in the work and for the very careful observations he made during experiments, observations which resulted in valuable practical improvements in the technique.

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[1] 1 c.c. of a 7 % solution of sodium sulphate injected intraperitoneally into a mouse did not cause death but 1 c.c. of a 10 % solution killed in 1½ hours. (Cf. also F. Greenwald, 1918, *Journ. Pharmac. and Experiment. Therapeutics*, vol. xi. p. 283.)