IMPROVEMENTS IN THE TECHNIQUE OF THE CONCENTRATION OF ANTITOXIC SERA.

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The study of the factors influencing the heat-denaturation of serum proteins has led us to change the technique of my method (1916) for concentrating antitoxic sera. These changes involve a considerable shortening of the process and therefore a brief description of our present method of procedure may be useful to those engaged in serum concentration in other laboratories.

Our technique is as follows:

(*a*) *The preliminary treatment of the plasma.*

In order to ensure the desired ease of filtration at the various stages of the process of concentration, the pooled batches of *undiluted* oxalated or citrated plasma are subjected to one of the following alternative methods of treatment previous to their being heated.

1. (*a*) To the plasma is added a sufficient volume of ammonia to adjust the reaction to the value $p_H + 8{\cdot}0$. This may be accomplished as follows:

To 10 c.c. of the plasma is added the necessary volume (x c.c.) of a standard solution of ammonia to bring the hydrogen ion concentration [H] to the value $p_H + 7{\cdot}6$ using neutral-red as the indicator and Sörensen's solutions as standards. To another 10 c.c. of the plasma is added the necessary volume (y c.c.) of the same solution of ammonia to bring the [H] to the value $p_H + 8{\cdot}5$ (faint green tinge with three drops of a 0·1 per cent. solution of α naptholphthalein).

By the addition of $\frac{x+y}{2}$ c.c. of the ammonia solution to every 10 c.c. of the plasma the reaction is approximately adjusted to the value $p_H + 8{\cdot}0$.

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(*b*) If the operator cannot conveniently adjust the reaction, then to the plasma can be added not less than 0·25 and not more than 0·30 per cent. of cresylic acid or trikresol.

2. Two per cent. of solid sodium chloride is next added to the plasma treated as above.

(*b*) *The heating of the plasma.*

The heating is conducted in two stages as follows:

Stage 1. Into each of several jars of 22 litre capacity there are measured 14 litres of plasma. The jars are covered and placed in the heating tank containing water at 62°—64° C. whose temperature is maintained between these limits until that of the plasma has reached 57·5° C. The water in the tank is then allowed to cool to 57·5°—58° C., at which temperature it is maintained by means of a thermostat. The tank is covered with a closely fitting lid and *the heating is continued for a further period of four hours.*

Stage 2. At the expiration of the stated period for Stage 1, six litres of a saturated solution of ammonium sulphate are added to each of the jars. The ammonium sulphate content in each jar is thus brought up to 30 per cent. of saturation.

The temperature of the water in the tank is raised to 62°—63° C. and is kept between these limits until the temperature of the plasma-ammonium sulphate mixtures has reached 58° C. Throughout this operation it is essential to keep the mixtures thoroughly well stirred.

As soon as the required temperature has been reached, the jars are removed from the tank and the contents are allowed to cool to 45° C. at which temperature they are filtered. The precipitate thus separated constitutes the First Fraction precipitate.

(*c*) *The precipitation of the Second Fraction.*

The First Fraction precipitates, consisting of euglobulin, heat denaturated protein and a certain amount of pseudoglobulin, are washed with a solution of ammonium sulphate (30 per cent. of saturation), the volume of the latter being about one-half that of the plasma taken for concentration.

The washings from the First Fraction precipitates are filtered and added to the main bulk of the filtrates to which is then added the necessary volume of a saturated solution of ammonium sulphate *to bring the ammonium sulphate content up to* 46 *per cent. of saturation.*

The ensuing precipitate is filtered, pressed and dialysed in the usual way until free from sulphate. To the residue from dialysis is added 0·85 per cent. of sodium chloride and 0·35 per cent. of cresylic acid. This final product is stored in bulk in the cold room until required.

(*d*) *The recovery of antitoxin from the First Fraction Precipitates.*

During many years practical experience with the concentration of antitoxic sera in these laboratories it has been found that, no matter how carefully any given method be followed, there has always been a loss of antitoxin, the losses under ordinary circumstances ranging from 5 to 30 per cent. (average *ca.* 18 per cent.).

It has been ascertained that the "missing" antitoxin is not destroyed during the heating of the serum and of the serum mixtures at the temperatures employed, but that it is carried down with heat-denaturated pseudoglobulin in the First Fraction precipitates. The antitoxin is associated with the denaturated protein in such a manner that it cannot be recovered therefrom by extraction with ammonium sulphate (30 per cent. of saturation). However, it has been found that this "missing" antitoxin can be almost completely recovered by prolonged extraction of the First Fraction precipitates with brine in which the heat denaturated protein is soluble to some extent.

In order to reduce our losses to a minimum we have therefore reverted to the Banzhaf-Gibson plan of extracting the First Fraction precipitates with brine, which was long employed in this laboratory. At the same time the method was adopted of dealing with the First Fraction precipitates from a series of concentrations collectively and, since Dr MacConkey[1] had demonstrated that antitoxic sera saturated with salt could be kept at room temperature for months without any appreciable deterioration, his plan of allowing them to soak in brine until they could be conveniently treated, was employed, labour and expense being thereby saved.

Our procedure is as follows:

The First Fraction precipitates after having been washed with a solution of ammonium sulphate (30 per cent. of saturation), are placed in a coarse canvas bag. The bags are dumped into tubs containing a saturated solution of common salt with an excess of solid salt.

Into the same tub are successively dumped the similar precipitates from a series of weekly routine concentrations, the First Fraction precipitates from the concentration of 500 to 600 litres of plasma being

[1] Unpublished observations.

usually thrown into about 180 litres of brine. The tubs are kept covered and allowed to stand at room temperature until time can be spared to deal with the liquids. The brined extract of the precipitates is then filtered and to the filtrate is added 0·30 per cent. of glacial acetic acid. The ensuing precipitate is filtered, pressed and dialysed after 3 per cent. of finely ground washing soda has been added to the contents of each parchment dialysing bag. Care must be taken not to overpress the precipitates as in this eventuality great difficulty is experienced in dealing with the residues from dialysis.

The dialysis is continued until the contents of the dialysing bags are freed from salt. To the residues from dialysis are added 0·85 per cent. of solid sodium chloride and 0·35 per cent. of cresylic acid. The final product is stored in the bulk in the cold room until required.

The above technique presents the following practical advantages over that originally suggested by me (1916):

(1) The preliminary adjustment of the plasma obviates difficulties otherwise so often experienced in the filtration of the hot plasma ammonium sulphate mixtures and also of the final products.

(2) The shortening of the heating in Stage 1 makes it possible to complete the two stages of the heating process within 7 hours.

This is advantageous from the practical standpoint. In a working day of 8 hours' length, the operator can not only carry out the heating processes involved but can also leave the filtration of the First Fraction proceeding overnight.

(3) The precipitation of the Second Fraction by 46 instead of 50 per cent. of saturation with ammonium sulphate considerably reduces the amount of heat-denaturated albumin appearing in the final product. This is advantageous in two ways, for, not only is the degree of concentration enhanced but the colour of the final products is less pronounced.

(4) At a relatively small cost of labour, time and materials the total losses of antitoxin experienced during the concentration of sera can be reduced to a negligible quantity.

Thus, during a series of routine concentrations involving nearly 1500 litres of plasma, the loss with each individual concentration was 15—20 per cent. The subsequent treatment of the First Fraction precipitates with brine resulted in the recovery of an amount of antitoxin which reduced the total loss to 2·5 per cent. These results, based on animal tests, indicate that there had been practically no loss of antitoxin during the process of concentration.

Our present losses are considerably smaller than those experienced with the Banzhaf-Gibson method in which, in order to ensure clear and readily filterable end products, it has been found necessary to throw down the First Fraction precipitates with 33 or even with 36 per cent. of saturation with ammonium sulphate instead of with 30 per cent. as originally advocated.

Recent experiment has shown that, with the higher concentrations of ammonium sulphate thus adopted, the precipitated heat denaturated pseudoglobulin and attendant antitoxin are so influenced that their solubility in brine is considerably decreased. Under these conditions extractions of the First Fraction precipitates with brine does not reduce the loss to the minimum experienced by us in our present procedure.

The similar treatment of the First Fraction precipitates obtained in the Banzhaf (1913) method and in my modification of the latter likewise reduces the loss to a minimum.

In conclusion I desire to express my thanks to Mr Albert Riggs, Head Laboratory Assistant, for his co-operation and for the many valuable suggestions made by him during the working out of the details of the technique described above.

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