5—THE ESTIMATION OF SERUM PROTEIN CONCENTRATIONS*

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Towards the completion of the work it was realized that the results obtained by the nesslerization method described in Parts 2-4 of this series of papers tended to differ from those obtained by the micro-Kjeldahl digestion of a larger quantity of serum followed by titration (Young, Bishop, Hickmans and Williams, 1949; Young, Poyner-Wall, Humphreys, Finch and Broadbent, 1950; Poyner-Wall and Finch, 1950). It was therefore decided to compare a series of results obtained by the two methods.

Comparison of Results Obtained by Nesslerization and by Titration

Although the digestion mixture which was used for the nesslerization method was suitable for the rapid digestion of 0.005 ml. of serum, the presence of the phosphoric acid made it unsatisfactory for the prolonged digestion of 0.1 ml. of serum. This digestion was therefore carried out with a mixture of the same composition as that used by Levin, Whitehead, and Oberholzer (data to be published) so that the results from the two hospitals taking part in the Medical Research Council investigations could be correlated.

Method of Comparison. The comparison was first carried out by taking one sample of serum and repeating the estimation, by nesslerization, of the total nitrogen in 0.005 ml. of serum 28 times, i.e. on 2.5 ml. of a solution of 0.1 ml. of serum in 50 ml. of water. The total nitrogen in 0.1 ml. of the same serum was estimated four times by the titration method. For these comparative results, the total nitrogen has been converted to grammes of protein per 100 ml. but no correction has been made for the non-protein nitrogen. The results by nesslerization showed a wide scatter but the four results obtained by the titration method were all within 0.10 g. protein per 100 ml. of each other. The average of these four results, viz. 6.75 g. protein per 100 ml. has been taken as correct. Fig. 1A is a frequency distribution diagram of the differences between the individual results

by nesslerization and the average of the four results by titration. It shows that 20 out of 28, i.e. 71% of the results by nesslerization were within ± 0.25 g. protein per 100 ml. of the average titration value. It also shows that there were more results by nesslerization below the titration value than above it and that the biggest differences were always due to low results by the nessler method. The average of the 28 results by nesslerization was 6.53 g. protein per 100 ml. and the standard deviation of the results about this average was 0.39 g. The deviation of the results by nesslerization about the average of the titration results (6.75 g. protein per 100 ml.) was 0.45 g.

A second comparison of the two methods was carried out by making a duplicate estimation of the total nitrogen, by each method, on 71 different samples of serum. The duplicate estimations by the titration method did not vary by more than 0.13 g. protein per 100 ml. In all except three cases, the duplicate estimations by the nessler method did not vary by more than 0.20 g. protein per 100 ml. and none varied by more than 0.40 g. protein per 100 ml. The comparison has been made on the average of the duplicate estimations and the titration result has been assumed to be correct in each case. Fig. 1B is a frequency distribution diagram of the difference between the results by the two methods. Thirty-seven of the 71, i.e. 52%, of the results by nesslerization were within ± 0.25 g., and 51 of the 71, i.e. 72%, were within ± 0.35 g. of protein of the titration value. Although section A of the figure presents a rather different picture from section B, because in the second series there was a greater percentage of the results within ± 0.05 g. of the titration value, it can be seen that again there were more results below the titration value than above it and that the biggest discrepancies were due to lower results by the nesslerization method. There was no apparent relationship between the discrepancy in the results by the two methods and the level of the serum protein being estimated.

Conclusion

The average of a series of serum protein concentrations estimated by the nesslerization method (Hickmans, 1948) was slightly lower (0.22 g. protein per 100 ml.) than the average of the same series determined by a titration method. Since the error of the

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FIG. 1.—Comparison of serum protein concentrations determined by nesslerization with the 'correct' result determined by titration. A. These estimations were made on a single sample of serum. The average of four results by titration is assumed to be correct. B. These estimations were made on 71 different sera. For each sample the average of duplicate estimations by titration is assumed to be correct.

nesslerization method is not a consistent one, no correction can be applied to individual results which have been obtained by this method. If, however, the serum protein concentrations of a sufficiently large number of cases have been estimated, the effect of individual errors on the average of the results is reduced and it is possible to assess the findings. Nevertheless, when small variations in serial specimens are to be estimated, the titration method is preferable in spite of the fact that it requires a little more serum.

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REFERENCES

- Hickmans, E. M. (1948). *Pediatrica Internazionale*, **1**, 331.
 Young, W. F., Bishop, E. A., Hickmans, E. M., and Williams, Y. J. (1949). *Arch. Dis. Childh.*, **24**, 250.
 —, Poyner-Wall, P., Humphreys, H. C., Finch, E., and Broadbent, I. (1950). *Arch. Dis. Childh.*, **25**, 3.
 Poyner-Wall, P. and Finch, E. (1950). *Arch. Dis. Childh.*, **25**, 129.