Medicine. — *Simplification of the determination of serum albumin and globulin by the spreading method.* By E. GORTER and J. J. HERMANS.

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In the determination of serum albumin and globulin by means of spreading as developed by GORTER and BLOKKER [1]), total protein and globulin are measured directly, while the albumin content is calculated from the difference between the spreading areas for both. To spread the globulins, these are precipitated, washed three times in the centrifuge and finally solved in 1 % sodium chloride.

It is obvious that this procedure takes up much time, and we have therefore tried to simplify matters by spreading the albumins directly from the centrifugate, so that the globulins instead of the albumins are determined indirectly. All experiments were carried out with only one tenth the amount of serum mentioned by GORTER and BLOKKER: 0.10 cc of serum are blown from a pipette into 1.0 cc of 1 % NaCl-solution and thoroughly mixed. 5 mm^3 of the mixture are spread on HCl 0.1 N and the area determined. Using the value 1.01 m^2/mg, the protein content is calculated. Further, 0.10 cc of serum are mixed with 0.10 cc of saturated ammoniumsulphate. After centrifuging at 4000 revolutions per min. for about ten minutes, 0.10 cc of the centrifugate are diluted with 1.0 cc of distilled water; 10 mm^3 of this mixture are spread on HCl 0.1 N. With the value 1.04 m^2/mg this leads to the albumin content of the serum.

In this case particular care must be taken to spread as slowly as possible. If the liquid is blown from the pipette too rapidly, it tends to disappear under the surface on account of its high salt content [2]). We want to emphasize this point since its neglect may lead to results which are too low by as much as 20 % or even more. It is quite simple, however, to acquire the technique of spreading sufficiently slowly, and no errors are to be feared once this point is taken into account. It may be said at this juncture that, satisfactorily though the method worked if applied to serum, no reproducible results could be obtained with cerebrospinal liquid. Here the protein content is too low in comparison with the ammonium sulphate present, causing the direct spreading of albumin to break down in almost all cases examined.

The difference between total protein and albumin gives the figure for globulin. If the method is reliable, the direct determination of globulin must tally with that calculated. To show this, the globulins are washed three times with half-saturated ammonium sulphate, finally dissolved in 0.95 cc of 1 % sodium chloride and spread on HCl 0.1 N (10 mm^3 or 20 mm^3 as the case may be). The spreading factor used here is 0.93 m^2/mg. It is assumed that the precipitated globulins with the adhering solution of ammonium sulphate, when dissolved in 0.95 cc of NaCl-solution, make up to very nearly 1 cc, or, in other words, that the final dilution is 10 times. This assumption doubtless is attended with some uncertainty in the results obtained, and it is even difficult accurately to estimate the error involved. Yet it would seem that this error will not be larger than, say, 5 % and that the very small amount of serum needed for the determination amply makes up for this small loss of accuracy.

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1) E. GORTER and P. C. BLOKKER, Proc. Acad. Amsterdam.

2) It is clear that we may also dilute the centrifugate with 2 cc of water instead of 1 cc and then spread 20 mm^3 instead of 10. And so on. There is a limit, however, to this procedure. For, if a large quantity of liquid is to be spread slowly, the pipette used must be of small crosssection. In the long and narrow pipettes, however, the surface of the inner wall becomes so large that adsorption of proteins on to this wall begins to play a part. For this reason we have chosen the figure 10. If necessary one may go as far as 20.

All experiments were carried out in duplo. The table gives albumin, globulin and total protein as determined by means of spreading. The fourth column gives the sum of albumin and globulin, showing that the agreement is quite satisfactory. This agreement is the more convincing if we remember that the experimental error involved in the determination of any of the serum proteins mentioned by the spreading method may amount to some 2 %.

Proteins in human serum [3]).

	Albumin %	Globulin %	Total protein %	Total protein calculated (alb. + glob.)
1	4.6	2.10	6.8	6.7
2	4.5	1.92	6.4	6.4
3	4.5	1.60	6.3	6.1
4	4.15	1.30	5.6	5.4
5	4.2	1.75	6.3	6.0
6	6.0	1.65	7.4	7.6
7	5.5	1.70	7.2	7.2
8	5.1	1.60	6.6	6.7
9	4.8	2.0	6.6	6.8
10	5.9	1.65	7.6	7.6
11	5.3	1.74	7.0	7.0
12	4.0	2.1	6.0	6.1

We may conclude that serum albumin may be determined by spreading directly, calculating the globulin content of the serum as the difference between total protein and albumin. In this way the tedious manipulations involved in washing the globulins are avoided, thus reducing the time of an experiment from about 2 hours to, say, three quarters of an hour. There is, of course, one drawback to this method. The globulins represent only $^1/_5$ to $^1/_3$ of the total proteins in serum. An error of 2 % in the determination of albumin may sometimes give rise to an error of 8 % in the globulin calculated. For clinical purposes this is usually of no great consequence. It shows, however, that a direct determination of globulin is to be recommended if the globulin content is wanted with greater precision. Even then, however, our method will be useful since it affords a simple means to check the results obtained.

[3]) To avoid confusion, it is to be noted that the results mentioned in this table apply to patients, all children, with a variety of diseases and are not to be considered as average values for normal human serum.