Medicine. — *The determination of protein in liquor by means of the spreading method.* By E. GORTER and J. J. HERMANS.

(Communicated at the meeting of October 31, 1942.)

The protein content of liquor is determined by the spreading method. To that end quantities as large as 100 cmm must be brought on to the surface. It is shown in a series of experiments that this is of no consequence to the results obtained provided the spreading is performed with sufficient care, and the protein content of the liquor is not too low.

As regards the determination of globulin and albumin separately, the method can give no more than a rough estimate.

The application of the method shows that the protein content of liquor is a valuable information in clinical examination.

It has been shown by GORTER and BLOKKER [1]) that the protein content of human serum may be found from the spreading area on 0.1 n. HCl. To that end serum is diluted ten times, and 5 mm^3 of the dilute solution are spread on the tray.

In the present paper similar experiments with liquor will be discussed. Now, the protein content of normal liquor varies between 10 and 40 mg %, while that of serum is of the order of 7 %. Consequently if the spreading is performed on a tray of similar dimensions, it is obvious that quantities of the order of 100 mm^3 of undiluted liquor will have to be spread. Thus the question arises whether the spreading method is applicable to these large quantities of protein solution of low concentration. In the spreading technique employed the solution is slowly blown out on to the surface, and it is by no means to be relied upon without further investigation that spreading is complete if the protein concentration is low.

Experiments with ovalbumin. For this reason some preliminary experiments with ovalbumin were carried out. A solution of ovalbumin in water, containing about 40 mg protein per cc, was diluted 5, 10, 20 and 100 times respectively. Of these solutions 5, 10, 20 and 100 mm^3 were spread on HCl 0.1 n. The force-area curves proved to be identical within experimental error, i.e., within a few per cent.

A similar result was obtained on acetate-veronal buffers 0.0033 molar of $p_H = 3$ and $p_H = 5$. Yet this result is not to be considered as applicable to all proteins at any p_H. Further experiments with ovalbumin showed (a) that p_H should not be too high and (b) that salts must not be present in the protein solution to such an extent as to make the density of the solution considerably larger than that of the liquid in the tray. As regards (a): at large p_H values spreading becomes more and more incomplete if the protein concentration is decreased. As regards (b): a large concentration of salts impedes spreading from protein solution, probably because the solution when flowing from the pipette tends to disappear under the surface on account of its high density [2]). The subject was not studied by us in great detail, and the effects mentioned may weigh differently with different proteins. For the present, however, we are only interested in the behaviour at $p_H = 1$.

Experiments with serum and liquor. Since we are here concerned with the proteins of

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[1]) E. GORTER and P. C. BLOKKER, Proc. Ned. Akad. v. Wetensch., Amsterdam, **45**, 151 (1942).

[2]) Compare also: E. GORTER and J. J. HERMANS, Proc. Ned. Akad. v. Wetensch., Amsterdam, **45**, No. 8 (1942).

human liquor, the following experiments were carried out with serum, liquor, and mixtures of serum and liquor.

Human serum was diluted 20, 40, 80, 160 and 320 times. The amount of liquid spread was 5, 10, 20, 40 and 100 mm^3 respectively. The protein content of the serum as calculated from these determinations was the same with all dilutions except the highest. In another series we even succeeded in spreading 100 m^3 of a 400-fold dilution without loss of protein. It is clear that this upper limit will to a certain extent depend on spreading practice and may also change slightly with the serum used. Similar results were obtained with ox-serum; a summary is given in table I.

TABLE I.

Human serum			Human serum			Ox serum		
Dilution	mm^3 spread	% Protein found	Dilution	mm^3 spread	% Protein found	Dilution	mm^3 spread	% Protein found
20	5	7.7	10	5	6.8	20	5	8.6
40	10	7.4	40	20	6.6	40	10	8.5
80	20	7.5	200	100	6.8	80	20	8.6
160	40	7.9	400	100	6.7	160	40	8.8
320	100	5.8	800	200	3.8	320	100	6.5

The protein concentration in serum diluted 200 times is about 40 mg %, which is just about the protein content of normal liquor. We may conclude that the total protein content of abnormal liquor ($>$ 40 mg %) can be safely determined by means of spreading. With normal liquor the spreading value may sometimes be too low unless spreading is performed with great care. Since, however, the protein content of normal liquor may vary between 10 and 40 mg %, from a clinical point of view even a hundred per cent error in this region would be of only minor importance.

Finally, it could be shown that the results obtained with serum also apply to liquor. To that end serum was diluted 200 times, and then mixed with different amounts of liquor. Of these mixtures 100 mm^3 were spread. It was found that the spreading area was additive within experimental error. Moreover, in two experiments the protein content of liquor was determined by a micro-Kjeldahl and was found to agree with the spreading value.

So far the determination of total protein. Our attempts to determine albumin and globulin separately were not successful. In serum the globulins are precipitated by half-saturated ammonium sulphate, centrifuged off and redissolved in NaCl 1 %. In this solution the globulin can be determined by the spreading method [1]), while moreover the direct spreading of the centrifugate yields information about the albumins [2]). It was found, however, that this procedure failed if applied to liquor. This is due to several factors. To begin with, the solubility of globulins depends on the protein concentration. If globulin is precipitated from dilute serum, the percentage found is too low. This factor diminishes the value of almost all other methods for the determination of globulin in liquor. Further, the salt content of the centrifugate, containing the albumins, is much too high to allow of direct spreading. In the case of serum, this centrifugate may be diluted 10 or 20 times, thus reducing the salt content to a value where it does no longer impede spreading. In the present case this is impracticable since the albumin content of liquor is too low.

Consequently, the figures mentioned for globulin in the tables below only give a rough estimate. Their significance is mainly derived from the fact that normal liquor contains practically no globulins at all.

Determination in liquor. Resuming we may say that total protein in liquor may be determined by the spreading method, while globulin can only be estimated roughly. If necessary, the liquor is centrifuged or filtered; then 100 mm^3 are slowly blown out of a

pipette on to HCl 0.1 n. It is essential to use long pipettes of uniform cross-section, since it appeared impracticable to spread sufficiently slowly from 100 mm^3 pipettes with spherical widening such as they are commonly used in blood analysis. It is clear that smaller amounts must be spread in abnormal cases where the protein content is high.

In table II some results are recorded. By far the most are normal values (below 40 mg %). In all cases of meningitis the protein content is too high, varying between remarkably wide limits.

In the tables III, IV and V three particular cases have been studied in more detail. The parallellism between protein content and clinical estimation is obvious.

TABLE II.

Patient	Age (years)	mg % Protein	Number of cells	Diagnosis	Symptoms and further particulars on day of examination.
C	2	17.5		otitis media	convulsions
B	3	42		tuberculous meningitis	symptoms not very convincing
D	2	115	1700/3	tuberculous meningitis	glucose = 18 mg %; NaCl = 430 mg%
H	6	34		observation for encephalitis	slight tremor in hands
Z	3	9.1		convulsions	febris e sausa ignota; convulsions; NaCl = 619 mg %
P.V.	6	14.5		encephalitis?	NaCl = 680 mg%
K.V.	4	19	18/3	encephalitis?	ataxia, nystagmus
R.	1	29	22/3	meningismus, pneumonia	temperature normal, fluid on cultivating sterile.
P.	3	57 50	842/3 195/3	meningitis due to influenzabacilli	temperature almost normal, fluid sterile, 2 days later
D.M.	3/4	15		Jackson's epilepsy	preceded by encephalitis
C.M.	3	14		hydrocephalus communicans	
E.M.	1/6	3000		meningitis due to influenzabacilli	6 days before death
J.M.	2	18		acute enteritis	suspected of convulsions
D.K.	1/4	6.3	5/3	atrophia	debilitas mentis; high temperature
K.K.	8	50	301/3	acute cerebrospinal meningitis	Kernig slightly positive
		33	51/3		5 days later, Kernig negative

TABLE III. N. de H. no. 944 1941—'42.
Age 5 months; meningitis due to influenza bacilli, treatment sulfapyridin.

Date	mg% Protein	mg% Globulin	Number of cells	Particulars
17—3—42	132	18	7000/3	sulfapyridin from day of admission, 300 mg p. KG. during 1rst fortnight. Cerebrospinal fluid turbid; day of admission: 6th day of illness. All symptoms of acute meningitis, temp. 39.6°.
18—3—42	140	18	3764/3	temperature 39.0°
19—3—42	146		1728/3	sensorium free. Temp. 38.3°
23—3—42	123		968/3	condition unchanged. High fever since 3 days.
25—3—42	120			temp. 39.4° on the 24th and 39.6° on the 25th.
26—3—42	98		1788/3	
27—3—42	95		1400/3	temp. lower; condition not improved.
28—3—42	91	trace	1200/3	200 mg sulfapyridin per K.G. during 3 weeks.
30—3—42	113		2200/3	first day without fever, temp. remains normal since that date during a fortnight. Many bacteria.
31—3—42	106			
1—4—42	112	±10		
2—4—42			1648/3	
4—4—42	104			
7—4—42			1364/3	
8—4—42	162			
9—4—42	140		280/3	
10—4—42	207		624/3	Cheyne-Stokes respiration, opisthotomus.
11—4—42			5900/3	beginning of relapse
12—4—42			6000/3	
15—4—42	680	±85	14000/3	cerebro-spinal fluid very turbid
16—4—42	695	±200	23500/3	one day before death.

TABLE IV. v. d. G. no. 855 1941—'42.

Age 5 weeks; acute cerebrospinal meningitis, treatment with sulfapyridin.

Date	Liquid examined	mg % Protein	mg % Globulin	Number of cells	Particulars
4—3—42	ventricular fluid	290	50	5200/3	fontanelle bulging. 6th day after admission, 7th day of illness. Symptoms of meningitis very slight on admission, gradually developing of characteristical symptoms of meningitis. Lumbar punction gives turbid liquid containing many meningococci. Since day of admission temp. has been 39.2° during 4 days, from now on it remains almost completely normal. Treatment: sulfapyridin from 5th day after admission, 300 mg p. KG.
7—3—42	„	310		974/3	Liquor less turbid, sterile. Clinically little improvement.
10—3—42	„			2390/3	
14—3—42	„			1416/3	Treatment: sulfathiazol.
16—3—42	„	215	32	456/3	Distinct improvement, stiffness of neck diminished, tension of fontanelle considerably less.
19—3—42	„	176		182/3	Some stiffness of the neck.
23—3—42	spinal fluid	141	trace	232/3	Stiffness of the neck has disappeared
8—4—42	„	117			general condition satisfactory.

TABLE V. K. v. L. no. 1035 1942.

Age 6 years; meningitis due to pneumococci, treatment with sulfapyridin.

Date	mg % Protein	mg % Globulin	Number of cells	Particulars
28—3—42				treatment with sulfapyridin, 300 mg per KG. weight.
30—3—42	216		20200/3	marked symptoms of meningitis. 3rd day after admission. 8th day of illness. During the 3 first days of stay in hospital, temperature very high.
31—3—42	204	44	3120/3	improved. Temp. still high 39.5°. Pneumococci cultivated from blood and spinal fluid.
1—4—42	140	33	692/3	Culture sterile. Gradual fall of temp. Temperature remains normal during 17 days.
2—4—42			294/3	
4—4—42	82		188/3	stiffness of the neck slight. Kernig positive.
8—4—42	48		42/3	no stiffness of the neck. Kernig slightly positive.
12—4—42	35		30/3	general condition satisfactory.
15—4—42	44		13/3	
17—4—42	24		10/3	agranulocytosis, 3 days later cured.