Medicine. — *On hypoproteinemia.* By E. GORTER.

(Communicated at the meeting of December 27, 1941.)

My interest in the problem of hypoproteinemia was roused by a new method of investigation, in which use is made of the property which many substances have, such as fats, lipoids, amines, alcohols, but also proteins, of easily spreading in a monomolecular layer of constant thickness and dimensions.

When we are in possession of an apparatus to determine the area at varying pressure, it is easy to determine the area occupied by a certain small quantity of protein. In this way it can also be easily determined what the area is of a certain quantity of a serum spread in a monomolecular layer.

When the circumstances are well chosen, and the maximal spreading is measured, which is obtained by placing 0.1 n HCl in the tray, then easily reproducible results are obtained. In order to reduce the square metres found to milligrams the method should be tested by examining how much area is occupied by one milligram in a monomolecular layer. We have lately done this again together with Ir. P. C. BLOKKER [1]). It was then seen, that

1 mg serum-albumin spreads 1.04×10^4 cm².
1 mg serum-globulin spreads 0.93×10^4 cm².

By globulin we have always meant the protein, which is precipitated when a serum is half saturated with ammonium sulphate.

With the spreading method it is not possible to determine fibrinogen which occurs in plasm but not in serum, because it does not spread unless a small quantity of trypsin is added.

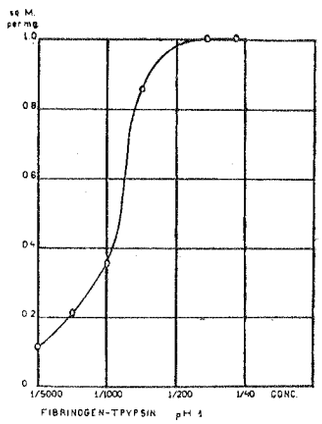

Fig. 1.

It can now be easily understood how serum proteins are determined. Blood is taken from the patient under certain precautions to prevent contamination with spreading substances. The syringe is boiled in water free from fat, the skin is cleaned with alcohol and ether, the glassware is made free from fat with bichromate and sulphuric acid. The blood is coagulated and after some time the serum is collected by centrifuging.

0.1 cm³ of this serum diluted 10 times is measured and this dilution serves to determine all proteins (albumin as well as globulin). In another tube 0.1 cm³ is pipetted and then 0.1 cm³ of a saturated watery solution of ammonium sulphate is added. There is a precipitation of the globulins. This precipitate is washed with a half-saturated solution of ammonium sulphate, and finally dissolved in 1 cm³ water. This solution is used to determine the percentage of the globulins. For the determination of the protein in these solutions the tray of the apparatus of LANGMUIR is filled with 1 n HCl. After cleaning 5 mm³ of the protein solution is blown out at the watersurface from a capillary pipette. The protein then spreads in a layer of ca. 10 Å thickness over part of the area, it is spread maximally. This area may be measured and determined while placing it under increasing pressure. Thus we obtain a pressure area curve, from which by extrapolation to pressure zero we determine the area under zero pressure. From the figures found we calculate the area of 1 cm³ of the protein solution. It is only necessary to divide by the factor which indicates how many square metres is occupied by 1 mg of the protein examined. The albumin is found by deducting the area of the globulins from that of the total proteins.

The problem of hypoproteinemia can be studied by this or by a different method. The spreading method has the advantage that it gives exact results, even when the quantity of blood is very small. It stands to reason that especially the children's specialist and the patient appreciate this advantage.

Clinic of hypoproteinemia.

Now I must at once place you in medias res and will do this by reading to you a number of case histories of patients in whom we found hypoproteinemia. This enables me to illustrate why as medical men we are interested in this problem and by pointing out to you the great differences between the diseases characterised by a loss of blood proteins. I can make it clear that the examination must extend to the entire patient. For the sake of clarity I have collected some figures concerning the patients in tables, but what is communicated about etiology, course and details of the diseases is not made superfluous.

The first patient has the picture of hypoproteinemia in its simplest form. It is a boy of 2½ years, his complaints are a puffy face, swollen arms, legs and abdomen. He has gained much in weight. He urinates less than usual, smaller quantities and less often. He is listless and feels ill, possibly he is feverish. His appetite is poor. He has vomited much and complains of thirst.

These symptoms have existed only 5 days. He has not been ill before. He has only coughed since the beginning of February, following a bad cold. The cough resembled whooping cough a little.

The family is healthy, he is the second of three children. The diet has been a little one sided. It consisted principally in bread, rusks and much vegetables, with little milk, little meat, very little fruit and not always potatoes.

During his first year he was given cod liver oil. Although it is not possible to make quite sure, the food seems especially to have been poor in proteins, and vitamins A and B have probably been scanty.

Examination shows a puffy face, praetibial edema, a strongly inflated abdomen, so considerable edema. This will soon disappear on a saltless diet, while in 12 days the bodyweight decreases from 13.8 kg to 11.04 kg.

On examination of the heart there is not a single symptom of insufficiency of the heart muscle. The liver is not enlarged, there is no abnormality in the urine, there is no protein, the sediment does not contain cells or crystals.

The WASSERMANN reaction is negative.

Protein in Blood.

Data	Alb.	Glob.	Fibrin.	Total	Ureum	Cholesterol
25/4	3.0 %	1.1 %	0.37 %	4.5 %	260 mg/l	172 mg %
3/5	3.5 %	1.2 %	—	5 %		
7/5	4.0 %	1.4 %	—	5.5 %		

Haem. 13.3 gr %; erythr. 4.700.000.

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

We have considered this case as hypoproteinemia with the usual consequences, caused by insufficient proteinfeeding. In young babies this hypoproteinemia is frequently found with insufficient feeding.

The protein percentage is much too low: 4.5 % (instead of at least 6 %), but it increases rapidly to 5.5 %. The decrease of the globulin is greater than of the albumin. The hypoproteinemia is not combined with high figures for cholesterol and lipoids. The kidneys function normally: the ureum of the blood is low.

I will now tell you about a case in which the decrease of the proteins was caused by considerable albuminuria.

The girl, 3½ years of age, became ill in the early part of May with fever. It had also been noticed that her face was swollen and that the urine was brown. Her legs had also become swollen. For some months she has been in a local hospital, but had improved little. She had never been ill before, and the family is also healthy. Her diet cannot be considered as deficient. As cause of the symptoms we find a very great quantity of protein in the urine.

Protein in Blood.

Data	Alb.	Glob.	Total	Ureum	Rest N	Cholesterol	Lipoids
3 Dec.	2.9	1.7	4.6 %	180 mg %		421 mg %	1.76 g %
9 Dec.	2.7	1.6	4.3 %				

Data	K	Na	Chloride	Ca	P
11 Dec.	15.2	320.6	421	7.8	4.5 mg %

You recognize some of the phenomena found in the first patient, and seen almost regularly in hypoproteinemia.

In these cases of nephrosis — a degenerative kidney disease — the figures for the total protein have much decreased. Mostly it is a decrease of albumin. Moreover we see a strong increase of the cholesterol and the lipoids of the blood as additional symptoms.

I now arrive at a third group of diseases in which hypoproteinemia is found with its consequences, but the cause of which is not known.

Because of this the picture has been called essential hypoproteinemia by COPE and GOADBY [1]).

The first case they call by this name concerns a man of 20, with edema, without albuminuria, with a urea-clearance of 70 %.

Protein in blood: albumin 3.13, globulin 1.61. Total 4.6 %.

Protein in Blood.

Diet	1934	Alb.	Glob.	Fibrin.	Tot.	Rest. N	Ureum	Ca	P
	—/5	3.13 %	1.51 %	— %	4.6 %	—			
Low protein	8/6	2.90 %	1.69 %	0.45 %	4.58 %	23	250 mg/l	10.1	4.1
High protein	18/6	2.67 %	1.89 %	0.80 %	4.56 %	40	560	8.8	5.0
Liver	24/6	2.76 %	1.51 %	0.66 %	4.26 %	44		8.9	3 5
Normal	4/8	1.94 %	1.77 %	— %	3.70 %	37			
Normal	17/9	2.50 %	1.64 %	— %	4.10 %				

Our patient whose case we have called by the same name is a girl of 10.

This girl has frequently a swollen face, legs and abdomen. When she rests and gets

[1]) LANCET, 1935, May 4.

little salt she urinates much and the edema disappears. It returns when she exerts herself. Otherwise the child does not feel ill and there is no other symptom of disturbed heart or kidney functions. She has never before had such disturbances. Of infantile diseases she has only had measles, whooping cough and chickenpox, otherwise she has not been ill either.

This returning edema has been observed in the child from the age of 3 years onwards. No such cases of edema occur in the rest of the family.

On examination we find considerable edema of the entire body. She has a puffy face, praetibial edema, ascites. There is also marked swelling of the coniunctiva bulbi.

To our surprise there is no indication of disturbed kidney function. The urine never contains protein, there is no sediment. We looked in vain for any symptom of insufficientia cordis. The bloodpressure is 105/85. The ureum and the rest N of the blood is low. The urea clearance is 63 %. The quantity of cholesterol is low, that of the lipoids is normal. The inorganic composition of the blood is also normal.

Protein in Blood

Per.	Date	Alb.	Glob.	Fibr.	Tot.	Ureum	Rest N	Cholesterol	Lipoids
I	30/9	3.9	1.3	0.38	5.2 %				
	1/10	—	—	—	—	390 mg L. 360	36.7 mg %	90 mg %	593 mg %
II	6/10	3.5	1.2	—	4.7 %				
VI	7/11	3.8	1.2	—	5.0 %				
VII	14/11*)	3.7	1.0	—	4.7 %				
VII	17/11	3.6	1.2	—	4.8 %				
VII	18/11*)	3.3	1.1	—	4.4 %				
	4/12	4.0	1.1	—	5.1 %	320 mg L.	31 mg %	146 mg %	562 mg %
VIII	18/12	3.6	1.2	—	4.8 %				

Per	Date	K	Na	Chlorides	Date	Ca	P
I	1/10	17.7	368 mg %	398 mg % 427 mg %	29/9	8 mg %	3.1 mg %
	11/12	15.8	316 mg %	424 mg %			

*) Injection lyoph. serum 4 and 6 cc: Hem. 14.5 gr %; Erythroc. 4.370.000.

The only abnormality is a decrease of the protein percentage of the blood. There is no anemia: hemoglobin 14.5 g %. Erythrocytes 4.370.000.

Course. During her stay in the clinic we can confirm the observation of the parents that under the influence of rest and a diet with little salt the edema disappears rapidly, but that it rapidly returns when salt is given. We have also ascertained, that a diet rich in protein does not improve the illnes.

Epricrisis.

As noteworthy features of this case of hypoproteinemia we would mention the following facts: absence of any symptom of a disease of heart or kidneys, absence of the increase of cholesterol and lipoids in the blood serum and of the increase of ureum and rest nitrogen, and especially the pertinacity with which the low percentage of proteins in the blood is maintained in spite of the high protein percentage of the diet and the injection of lyophile serum in the blood. Moreover it is striking that the decrease of the protein is especially a decrease of globulin, to a less extent of albumin, so that the proportion albumin : globulin is usually 3 : 1. This proportion also changes very little. The fibrinogen percentage is normal.

Recapitulating the course of the illness: the edema has existed from the age of three years and is unchanged, the girl is now 10 years old. There has been no previous illness

	Diet	
	of Periods II [1]) — V	of Periods VI — VII — VIII
Eggs	(1)	(1)
Sugar	37½ gr	37½ gr
Butter	24 gr	24 gr
Bread	200 gr	240 gr
Rice	45 gr	45 gr
Gravy	18 gr	18 gr
Meat	37½ gr	75 gr
Vegetables	—	—
Potatoes	200 (250) [1]) gr	250 gr
Fruit	50 gr	100 gr
Red currant juice	50 cc	50 cc
Gelatin	—	5 gr
Liver	—	100 gr
Curds [2])	—	150 gr 300 gr
Serum of oxen [3])	—	— 60 cc

and intercurrent diseases such as measles, whooping cough and chickenpox have left no traces.

From all this we conclude that there is essential hypoproteinemia. The disease does not occur in the family, both father and mother have a normal protein percentage.

From this and many other clinical observations we can at least deduce, what will be the consequences of hypoproteinemia.

The consequences of hypoproteinemia.

Of one symptom the edema, it is certain that it is caused by hypoproteinemia.

It appears that the chance of it is the greater as the serum albumin is lowered. All the other consequences of hypoproteinemia, except increased susceptibility for certain infections, are doubtful. The influence of protein in the bloodplasm and the great influence of serum albumin in the production of edema are accounted for by the osmotic pressure, depending on the proteins, being lowered. Normally there is a certain proportion between the hydrostatic pressure in the small vessels and the osmotic pressure of the plasm. Owing to this some liquid is pressed out in the arterial part of the capillary vessels, as here the bloodpressure is higher than the osmotic pressure of the proteins, whereas in the venous part of the capillaries the liquid enters the vessels, because here the blood pressure has decreased below the osmotic pressure of the proteins. In humans or in animals with hypoproteinemia this osmotic pressure is much too low, so that difficulties arise for the transition of liquid from the tissues to the blood vessels.

It is clear that the condition worsens when the liquid in the tissues also contains protein. Generally this is not the case. The difference between the osmotic pressure caused by the protein in- and outside the blood current is decisive for the liquid resorption in the venous capillaries.

That albumin has the greatest influence is because it has the smallest molecular weight,

[1]) Not in Period II.
[2]) Contains 250 mg % NaCl.
[3]) Contains 585 mg % NaCl and 6.2 % protein, 4.2 % alb. and 2.0 % glob.

Causes of hypoproteinemia.

While from the case histories communicated some circumstances may be deduced which cause the loss of proteins in the blood, we can mention the following facts from experimental physiology which shed more light on this matter.

Purely theoretically our knowledge of this problem is insufficient. For we do not know with certainty where the plasm proteins are formed, although there are many arguments in favour of the liver being the principal organ for the formation. This is certain in the case of fibrinogen, the protein we shall not discuss, it is less sure for serum albumin and not very probable even for serum globulin.

The best investigations were made with drogs. They can be given hypoproteinemia by daily tapping their blood (e.g. ¼) and reinjecting the erythrocytes in salt solutions. The quantity tapped daily is regulated so that the animal constantly retains 4 gr protein per 100 cc blood plasma. It is now seen that such an animal rapidly recovers its proteins even when it gets no food. Moreover the quantity of plasm has to be tapped to keep the protein percentage low always becomes smaller until it becomes constant, see fig. *a* in J. C. MADDEN and G. H. WHIPPLE: Physiological review, **20**, 207 (1940).

This is an indication that there is a protein depot on which may be drawn. But such a dog may also be examined as to the effect of various proteins of the diet on the protein percentage. It is seen that serum protein which is given to drink is most effective, and that there are even proteins, such as zeine which cannot help to form proteins of the plasm. Perhaps this is owing to the lack of some amino acids in these proteins. An indication of this would be that some proteins inactive in these experiments, improve noticeably by the addition of one of more amino acids.

The protein percentage of a plasm can also be increased by injecting certain bacteria (pneumococci). Here it has been possible to determine quantitatively that only protein is formed which may be absorbed by the pneumococci. Soon a maximum is reached that remains constant. This increase concerns the globulins.

Can these data help us in diagnosing our patients and especially in our attempt to improve their condition?

Treatment of hypoproteinemia.

It is clear how a patient with starvation edema is to get rid of his hypoproteinemia.

It stands to reason that this protein rapidly increases by a diet rich in proteins (3 g per kg).

It is not possible to improve the hypoproteinemia of patients in a simple way with nephrosis. The loss of protein with the urine cannot be remedied.

But for essential hypoproteinemia the improvement is more difficult still, if not impossible. We have seen the slight influence of a diet rich in proteins. Perhaps the depot has been drawn upon in this patient too and this will first be replenished. We also see that serum protein does not act much better than the other proteins. Possibly we must continue this much longer, although it will be difficult for the patient to take the liquid any longer in the great quantities required. Yet recovery may perhaps be expected. For plasm, even after concentration to ¼ volume, may be injected intravenously. This plasm must be from humans and must be dried under high vacuum at a temperature of minus 180° C. The white powder which is then obtained dissolves in a small volume of water and this concentrated serum called lyophile serum, can very well be given. Our patient too stood the injections very well. But she was given too little to expect any result. The protein has little changed in this process. Even the complement, a very labile substance, which can be kept unchanged for a week at most by preserving it below 0° can then be kept for a year. Serum dried in this way dissolves very easily, in contradistinction with serum dried in the ordinary way.

We found that when dissolved again it still spreads in exactly the same way as before the process, whereas denatured protein does not spread and will spread again only after

the addition of a small quantity of trypsin, just as the fibrinogen discussed in the introduction.

Summary.

Speaker gives a description of the method of determining proteins in the bloodserum by spreading in a molecular layer. He mentions the results of a series of determinations in which, together with Ir. P. C. BLOKKER he has used the spreading method and that of KJELDAHL. After this the m^2 found can be reduced to mg.

A number of patients have been examined by this method. As an example of various diseases in which too low a protein percentage of the blood, hypoproteinemia, was found, the author describes a case of *starvation edema*, a case of *nephrosis* and a case of essential *hypoproteinemia*. The results are given of determinations of protein, lipoid, cholesterol and inorganic substances of the bloodserum.

A summary is then given of the *consequences* of hypoproteinemia, based on clinical experience.

When the literature about *animal experiments* is consulted we find: that the *diet*, especially the sort of proteins has an evident effect on hypoproteinemia, which is the consequence of loss of plasm in dogs.

Especially serum protein, given per os, causes the blood protein percentage to increase. It also appears that any animal forms a protein depot in the tissues from which — even when it does not get food — the protein of the plasm is rapidly replaced. The blood protein does increase, but now it is the globulin that increases at different immunizations.

The treatment of hypoproteinemia begins with *restriction of the salt percentage* in the diet. When salt is given the edema reappears. A *diet* is chosen with a high protein percentage and especially serum proteins are given. Intravenously great quantities of concentrated *lyophile* serum are injected.

Medicine. — *Determination of serum albumin and globulin by means of spreading.* By E. GORTER and P. C. BLOKKER.

(Communicated at the meeting of December 27, 1941.)

In the laboratory of the children's hospital of the "Academisch Ziekenhuis" at Leiden, albumin and globulin have of late years been determined almost exclusively by means of spreading, as with this method there is the great advantage that the determination can be done with very little serum.

In a manner previously communicated [1]) the number of m^2 is determined that 1 cc of the protein solution occupies under certain circumstances on 0.1 n HCl and this figure is then reduced to the weight percentage of protein by dividing it by the so-called spreading factor, i.e. the number of m^2 that 1 mg protein occupies under those circumstances. 0.90 is used as spreading factor of albumin as well as of globulin. It is a well known fact that the spreading factor of nearly all proteins on 0.1 n HCl is approximately of this magnitude, e.g. 0.90 m^2/mg for casein, 1.00 m^2 for ovalbumin, 1.13 m^2 for haemoglobin, ca 0.90 m^2 for globulin and euglobulin and ca 1.04 m^2 for pseudoglobulin, see a.o. [2]).

In the first publication [1]) about the determination of serum globulin and albumin by means of spreading we found a spreading factor of 0.90—0.95 m^2/mg for albumin, but for globulin the factor was only 0.60—0.62 m^2/mg. After that it seemed worth while again to test the magnitude of these factors very carefully. Therefore we compared the magnitude of the spreading with the quantity of protein calculated from nitrogen determinations by the KJELDAHL method. The nitrogen determinations were made according to the micro-method described in detail by ABDERHALDEN–FODOR [3]) in which air, free from ammonia, is sucked through the solution containing the destroyed substance, and then through 0.01 n HCl.

It was found however that, contradictory to ABDERHALDEN–FODOR's instructions, it was necessary to boil the solution gently. In this way the total nitrogen percentage and the non protein nitrogen percentage of the sera examined were determined. As the separation of albumin and globulin in the spreading method was always made with ammonium sulphate and as this salt has many advantages over other salts sometimes used for this purpose, the nitrogen determinations of the globulins were also made with the globulins obtained by this method of separation. It was therefore necessary completely to remove the ammonium sulphate before the destruction. This was done by the method of CULLEN and VAN SLIJKE [4]), in which the solution is boiled with MgO and 50 % alcohol until all ammonia has disappeared. In order to be able to use as little MgO as possible, the quantity of ammonium sulphate present in the globulin obtained by the separation was determined in some cases and in further experiments more than double the amount of MgO corresponding to this quantity of ammonium sulphate was taken. Control experiments with ovalbumin solutions free from ammonium sulphate proved that the addition of ammonium sulphate had no influence on the ovalbumin nitrogen percentage obtained by the method described.

From the nitrogen percentage of the total protein and of the globulin fraction the total protein resp. globulin percentage was calculated by multiplication by 6.30. The albumin

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[1]) E. GORTER and F. GRENDEL, Biochem. Z., **201**, 391 (1928).
[2]) C. HOOFT, J. de Physiol., **36**, 652 (1938).
[3]) E. ABDERHALDEN and A. FODOR, Z. physiol. Chem., **98**, 190 (1917).
[4]) G. E. CULLEN and D. D. VAN SLIJKE, J. biol. Chem. **41**, 587 (1920).