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DEPARTMENT OF THE ARMY
Fort Detrick
Frederick, Maryland

ON THE CLINICAL APPLICATION OF A NEW ELECTROPHORESIS PROCESS

II. LIVER DISEASES

(Following is the translation of an article by H. Knedel, Central Clinic, Goppingen, Germany, published in the German language periodical Med. Wochenschrift 5, pages 707-709 (1951). Translation performed by Constance L. Lust.)

In increasing measure interest in medical-biological research has recently focused on the proteins of human serum and plasma. While the constituents of blood have been intensively studied for some time the difficulties of methodology have slowed the trend to use the analysis of proteins and peptides in clinical cases. It was some newer physio-chemical, analytical methods, especially electrophoresis according to A. Tiselius (1), which have opened exciting possibilities leading to important, experimental research findings.

General use of this method in the clinic was hindered for two reasons: 1) The electrophoretic apparatuses developed for the separation of protein solutions are prohibitively expensive for most clinicians; 2) Only one serum sample can be sutdied at a time so that a larger number of investigations are not possible.

There is obviously a need for a simple, reproducible instrument suitable for a larger series of experiments. The instrument should also be inexpensive. Such an instrument was recently described by Grassmann and Hannig (2,3,6). Knedel (5,6) reported about its clinical applications. Wieland and Fischer (7) showed for amino acids and Turba and Enenkel (8) for proteins that these substances can be separated on filter paper treated with a buffer solution. The proteins and amino acids may be made visible by staining. In carrying this principle further, this investigation focused on developing a dye which would stain proportionally to the concentration of the protein on paper. This together with a special method to quantitate the process of the electrophorotic separation is now described and shows great promise as a commonly useful, routine method to be used in hospitals and clinics.

Methods

Whatman number 1 paper, 4 x 30 cm. is soaked in Veronal-sodium acetate buffer according to Michaelis (1); 0.005 - 0.01 ml of fluid to be investigated is applied in a 3 cm. band. The filter paper is placed in an electrophoresis chamber and the separation occurrs in 14 hours at a constant voltage of 110 and 2.0 mA. The protein migrate according to their characteristics toward the anode Following the strip is dried, 10 minutes in a dye bath of amido black 10B (saturated soln.) in methanol

and 10% acetic acid; washed in methanol 10% HAc to destain the areas of paper that contained no protein. These areas are later only paleblue. The dried, dyed strip is then treated (submerged) in parafilm oil and bromonaphthalein to make it transparent, and mounted in a special glass holder which is fitted into the recording apparatus. The intensity of light transmitted is proportional to protein-dye complex and is recorded on a photocell, which transmitts an impulse to a recorder. The graph (profile) is plotted onto graph paper for further calculations.

Grassmann and Hannig (10) have determined the optimal conditions for exact dying conditions; reproducibility of recording and other errors. A particular advantage of this process over optical methods with cell systems is that the overall process occurrs in to distinct, separate stages 1)electrophoretic separation, 2) quantitative evaluation. This allows one to do any number of samples with a single recording system. In figure 1 is presented a electrophoresis diagram which was obtained with this procedure.

We have performed 1200 electrophoretic studies so far, of which more than 300 were on patients with liver disease. Since we only have limited space here we will only make some basic comments on the values obtained during the disease conditions.

In clinical diagnosis reactions that detect changes in the composition of serum have long been of importance. The development of tests to detect changes is constantly going on, and even today the goal to be able to surely and quickly detect patholigical changes in serum has not been reached. Most turbidity and floculation tests show an increase in the gamma globulin fraction. A positive test may be explained that in these cases a reaction takes place between the spheroprotein with its symmetrically distributed charge and the other protein reagents. All these tests contain a number of unreliable factors, temperature, concentration of reagents, subjectivity of the observer. Sometimes globulins are also displaced during illnesses and this adds further confusion. We have repeatedly observed in our electrophoresis that this method is more sensitive for detecting changes than other methods which measure elevation of gamma globulin during pathological conditions.

There are less uncertainties with electrophoresis, especially so in the measurement pathologically altered serum factors. There are continuous attempts to designate one of serum altered tests as a specific method to dilineate specific liver illnesses. Riva (13) stated that it is hardly possible to expect that a liver specific protein reaction can be determined. Up to now no liver specific test for pathological protein alterations has been devised - inclucing electrophoretic procedures. The clinical findings at the bedside will always play a major role in pointing the direction of an illness. Only then will the methods to assay serum protein alterations come into play in order to quantitate a disturbance in the liver. Electrophoresis may give the kind of protein as well as the amount of change.

On the basis of over 300 electrophoretic patterns on liver illnesses which were all correllated with serum tests and histological studies on liver tissue, we are able to compare the serum tests and electrophoresis. The electrophoretic separation of proteins of serum is of much more value diagnostically and prognostically.

In chronic diffuse liver parenchyma illnesses we saw with the electrophoretic pictures marked gamma globulin elevations. Walmann, Wunderly and de Nicola (14) recently pointed out heterogeneity of GG in serum of ill patients; this becomes even more marked by these procedures. The more chronic the process the more heterogeneous are the protein patterns of serum. With electrophoresis the heterogeneity of a fraction showed up as a different distance in the mobility as well as in others in the width of the band. In agreement with the above named authors we could determine that damage to protein function in liver in chronic, diffuse processes is always manifested by wide-band, rounded off Gamma fraction. The height of the band is usually less than its width.

$$\text{Quotient} = \frac{\text{Height of Gamma band}}{\text{base width of b band}} = \text{less than 1.0.}$$

The picture of figure 2 representing an electrophoretic pattern of a long term liver cirrhosis shows this clearly.

If in the pattern several GG subfractions show up, then to calculate the quotient one must use the base width of all the GG fractions. This is because the separation of several subfractions is a sign of heterogeneity.

Here we will not discuss the changes in GG in biliary and xanthomatotic cirrhosis.

In acute liver parenchyma illnesses (Hepatitis) we saw together with an elevation in GG an increase in alfa-2 globulin; at this time the "normal" liver tests were still negative. When subsequently the alfa-2 band decreased and the GG became more clear, the turbidity and floculation tests became positive.

The electrophoresis diagram obtained when occlusion-ikterus is suspected is of interest. In all our cases with occlusions we saw only very minimal elevations of GG. If the obstruction is long term than one would expect that the electrophoretic pattern might be able to illustrate pathologic changes in the protein pattern of liver cells.

Kalk and Wildhirt (15) recently reported on a divergence between liver function tests. Occassionally GG levels appear not to be altered but these may be a simultaneous change in another fraction; particularly alfa-2 globulins. This pathologic condition we can detect very clearly by using paper electrophoresis. On the other hand it must be noted that there are indeed cases of liver disease where no serum alterations can be detected. The authors of reference(16) reported on asymtomatic portal cirrhoses. Since we have discussed the altered serum protein as an expression of liver disfunction, it appears to us that the divergence

between the serum protein picture and the histological studies in no way diminishes the method of electrophoresis. There are occassionally liver diseases of great seriousness in which protein alterations are not manifested.

Summary

A new electrophoretic method is discussed, and its potential clinical applications in liver diseases.

Table 1

fraction	international standard		physiol. range
	Serum	Plasma	
Albumin	63,5	60,3	± 2,8
α^1-Globulin	4,8	4,6	± 0,7
α^2- "	7,3	7,2	± 1,3
α^3- "			
β^1- "			
β^2- "	12,7	12,1	± 1,9
β^3- "			
γ^1- "			
γ^2- "	11,5	11,0	± 2,9
ψ (Fibrinogen)	—	3,1	± 0,6

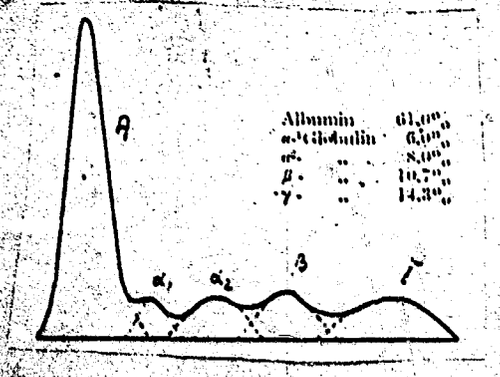

Figure 1

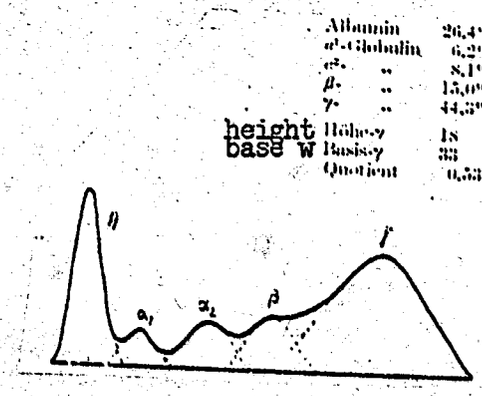

Figure 2

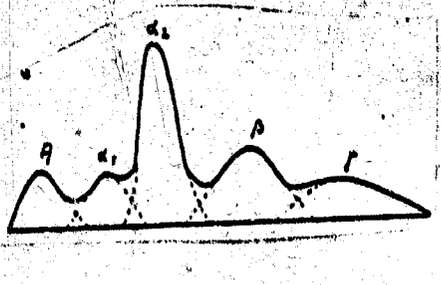

Figure 3

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Table LI

Electrophoresis study of 11 patients with cirrhosis of liver

Nr.	Name des Patienten	Albumin %	Globulin % α_1	α_2	β	γ	A/G quotent		diagnosis
1.	R. Sei. ♀	49,8	3,0	5,6	8,8	32,8	0,95	0,63	histolog; beginning cirrhosis
2.	E. Hob. ♂	50,0	1,8	4,7	7,2	36,3	1,00	0,6	" " "
3.	K. Kri. ♀	38,6	3,5	6,5	12,4	39,0	0,63	0,52	hist. liver cirrhosis
4.	M. Sch. ♀	35,6	3,4	4,0	10,9	45,3	0,55	0,58	" " "
5.	N. Paw. ♂	28,7	5,2	6,6	13,5	46,0	0,4	0,46	Clinical; early cirrhosis
6.	F. Fre. ♂	43,3	3,8	7,3	6,8	39,1	0,76	0,66	Clinical; splenomegale, liver cirrhosis
7.	A. Lat. ♂	43,2	2,8	6,0	6,3	41,6	0,77	0,68	Clinical; " plus ascity
8.	J. Kar. ♂	27,3	8,2	12,8	10,5	41,2	0,38	0,35	Clinical; early cirrhosis
9.	G. Hie. ♀	33,6	4,5	9,0	15,8	37,1	0,5	0,45	advanced cirrhosis
10.	L. Hom. ♀	39,0	3,3	5,7	8,0	45,0	0,66	0,64	" "
11.	H. Sch. ♂	28,5	4,9	6,1	8,5	52,0	0,4	0,42	

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