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Study of Bile Proteins in Difuse Chronic Hepatopathies

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A study is made of the protein constitution of the bile in difuse chronic hepatopathies. Immunoelectrophoresis and immunodiffusion techniques are used for this aim. Seventeen specific antiserums have been used in characterizing the various proteins. The quality of the proteins found is independent of the quantity of total proteins. Noteworthy in the qualitative study is the absence of IgM-Immunoglobulin, alfa-2-Macroglobulin, as well as fibrinogen and alpha-1 and beta-lipoprotein. Even though it may be early for drawing conclusions, it is interesting to note that proteins with a high molecular weight are absent while those of a low molecular weight are present.

The term «albuminocholia» was introduced more than a century ago to define the presence of this protein in the bile, its existence being interpreted as a pathological fact. From that time onward, this «albuminocholia» was believed by French authors to originate from an inflammatory process of the biliary tract, in as much as the German school attributed it to the parenchymatous affectation of the liver. Thus it is that for EPPINGER (5), «albuminocholia» was a consequence of the destruction or abnormal permeability of the hepatocites and the cause of biliary lithiasis, in as much as it could constitute the nucleus of the calculous formation. However, today we cannot always consider «albuminocholia» as the result of a pathological process, since a large variety of proteins exists in normal bile. We should therefore speak of a *physiological proteinocholia*, and study along with this a *pathological proteinocholia*, which may originate from inflammatory processes of the biliary tract or a parenchymatous hepatic lesion. In this sense, we have known for a long time how alcoholic intoxication, for example, increases the permeability of the hepatic cell, giving rise to an increase in bile proteins.

A more recent acquisition is the knowledge of possible biliary disproteinemias. Thus, SOTGIU *et al.* (6) have discovered, in cases of acute viral hepatitis, a disminution in the ammount of albumin in the bile, and also an increase in the globulinic

fraction. This gives rise to the problem of explaining the nature of proteins of the bile and their antigenic identity with that of other tissues. Today we accept the existence in the bile not only of serum proteins but also from other origins, such as the specific ones of the liver. It is also highly interesting to note the nature of proteins found in the bile, in increased quantities, in those inflammatory processes of the biliary tract, since according to several authors, the presence of these in the bile would be closely related to the formation of biliary calculosis, as mentioned before.

It is therefore interesting, as regards the data it may provide, to study the proteins of the bile in difuse hepatic diseases, which is the subject of this paper.

Materials and Methods

This study is composed of the analysis of 18 biles. Of these, 12, were of patients with postnecrotic cirrhosis; 3, chronic agressive hepatitis; 1, subacute hepatonecrosis; 1, acute viral hepatitis; and 1, Dubin-Johnson syndrome. In all cases the samples were aseptically obtained by direct puncture of the vesicle under peritoneoscopic control.

The protein content of each sample was determined, as well as their bilirrubine content. Likewise, in all cases a culture in agar-blood was performed to observe any possible bacterial growth.

For the qualitative study of proteins, the samples were centrifuged at 5,000 r.p.m. for not less than five minutes, and maintained at a temperature never below five degrees Centigrade. Later the samples were subjected for 24 hours to dialysis against phosphate buffer *p*H 8.33 with the aim of eliminating the greatest possible quantity of pigments and biliary salts. Following this, and with a working temperature between 4 and 6 degrees Centigrade, the sample was concentrated 20 to 30 times by negative pressure, or in a solution of gum arabic at 50 % in barbital buffer (*p*H 8.6, ionic strength 0.1). The product, thus obtained, was kept at 10 and 15 degrees below zero until being used.

The methods of immunoelectrophoresis and immunodiffusion of Ouchterlony were used to separate and identify the various proteins components of the bile. For this aim an universal technique was followed. For the characterization of the various bile proteins the following specific antiserums were used: 1) Global-antiserum. 2) Anti-prealbumin. 3) Anti-albumin. 4) Anti-IgG-immunoglobulin. 5) Anti-IgM-immunoglobulin. 6) Anti-IgA-immunoglobulin. 7) Anti-IgD-immunoglobulin. 8) Anti-β-lipoprotein. 9) Anti-α-1-lipoprotein. 10) Anti-haptoglobin. 11) Anti-fibrinogen. 12) Anti-α-2-macroglobulin. 13) Anti-β-2-glycoprotein. 14) Anti-α-1-glycoprotein acid. 15) Anti-α-2-SH-glycoprotein. 16) Anti-α-1-antitrypsin. 17) Anti-transferin. 18) Anti-celuroplasmin (Behringwerke A. G. Marburg Lahn. Germany).

Results

Table I shows the proteins obtained in each case of hepatic disease studied. As can be seen, the number of total proteins varies from one case to another, but with no relationship to the quality of proteins found. The cultures performed were all sterile except for case 17, an agressive chronic hepatitis, in which abundant colonies of an alpha hemolitic streptococi were isolated.

With regard to the proteins studied, the almost constant presence of the following is noteworthy: Prealbumin, albumin, IgG-Immunoglobulin, IgA-Immunoglobulin, Haptoglobin, β-2-glycoprotein, α-1-antitrypsin, α-1-glycoprotein antacid, α-2-SH-glycoprotein, and transferin. As we see in Table I, IgM-Immunoglobulin was negative in all cases except in samples 9, 17, 24 and 28, while IgD-Immunoglobulin was always absent. For its consistency, the ab-

Table I. *Serum proteins in bile by Immunodiffusion. Liver diseases.*

Bile sample	Disease	Proteins content g %	Proteins																
			Prealbumin	Albumin	IgG-Immunoglobulin	IgM-Immunoglobulin	IgA-Immunoglobulin	IgD-Immunoglobulin	β-Lipoprotein	α_1-Lipoprotein	Haptoglobin	Fibrinogen	α_2-Macroglobin	β_1-Glycoprotein I	α_1-Glycoprotein Ant.	α_2-SH-Glycoprotein	α_1-Antitrypsin	Transferin	Ceruloplasmin
2	Postnecrotic Cirrhosis	—	+	+	+	—	+	—	—	—	+	—	—	—	+	+	+	+	+
6	Postnecrotic Cirrhosis	2.0	+	+	+	—	+	—	—	—	+	—	—	+	+	+	+	+	—
7	Postnecrotic Cirrhosis	1.1	+	+	+	—	+	—	—	—	+	—	—	+	+	+	+	+	+
9	Postnecrotic Cirrhosis	1.0	+	+	+	+	+	—	—	—	+	—	—	+	+	+	+	+	+
12	Postnecrotic Cirrhosis	1.8	+	+	+	—	+	—	—	—	+	—	—	+	+	+	+	+	—
13	Postnecrotic Cirrhosis	—	+	+	+	—	+	—	—	—	—	—	—	—	+	—	+	—	—
14	Postnecrotic Cirrhosis	0.5	+	+	+	—	+	—	—	—	—	—	—	+	+		+	+	+
15	Postnecrotic Cirrhosis	1.3	+	+	+	—	+	—	—	—	+	—	+	+	+	+	+	+	+
16	Postnecrotic Cirrhosis	2.5	+	+	—	—	—	—	—	—	—	—	—	+	+	+	+	+	—
19	Postnecrotic Cirrhosis	6.5	+	+	+	—	+	—	—	—	+	—	+	+	+	+	+	+	+
27	Postnecrotic Cirrhosis	2.3	+	+	—	—	+	—	—	—	+	—	—	+	+	+	+	+	+
30	Postnecrotic Cirrhosis	2.5	+	+	—	—	+	—	—	—	—	—	—	—	+	+	+	—	—
17	Chronic Agrr. Hepatitis	4.0	+	+	+	+	+		—	—	+	—	—	+	+		+	+	+
20	Chronic Agrr. Hepatitis	1.3	+	+	+	—	+	—	—	—	+	—	—	+	+	+	+	—	—
26	Chronic Agrr. Hepatitis	2.5	+	+	+	—	+	—	—	—	—	—	—	+	+	+	+	+	+
24	Subacute Hepatonecrosis	1.6	+	+	+	+	+	—	—	—	+	+	+	+	+	+	+	+	+
28	Acute Hepatitis	3.0	+	+	+	+	+	—	—	—	—	—	—	+	+	+	+	+	+
10	Dubin-Johnson Syndrome	2.9	+	+	+	—	+	—	—	—	+	—	—	+	+	+	+	+	+

solute absence of lipoproteins (alpha-1 and beta) in all cases studied was highly interesting. Fibrinogen was negative as well, in all cases except in the one with subacute hepatonecrosis. α-2-macroglobulin did not exist except in biles samples 15 and 19 of post-necrotic cirrhosis and in 24 of subacute hepatonecrosis. With regard to ceruloplasmin, its almost constant presence is interesting, except in cases 6, 12, 13, 16 and 30, corresponding to postnecrotic cirrhosis. It was also negative in case 20, an agressive chronic hepatitis.

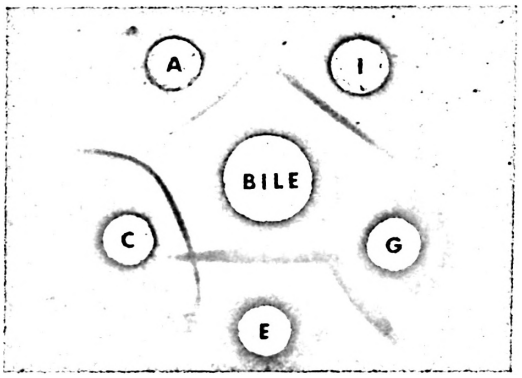

Fig. 1. *Immunodiffusion of Ouchterlony. Bile 19.*
A: Prealbumin. C: Albumin. E: IgG-Inmunoglobulin. G: IgM-Immunoglobulin. I: IgA-Immunoglobulin.

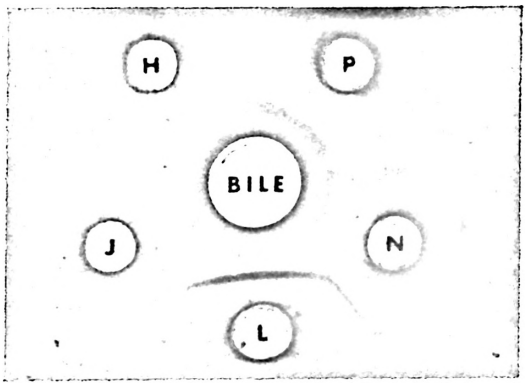

Fig. 2. *Immunodiffusion of Ouchterlony. Bile 19.*
H: Beta-lipoprotein. J: Alpha-1-lipoprotein. L: Haptoglobin. N: Fibrinogen. P: Alpha-2-macroglobulin.

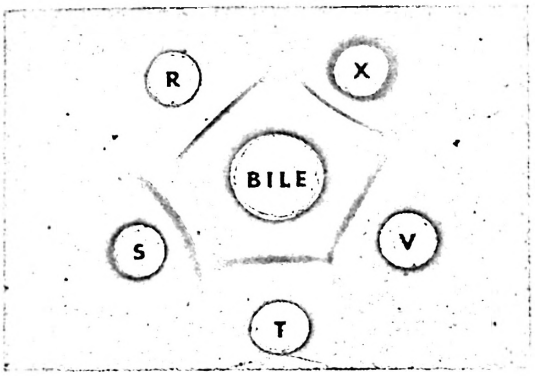

Fig. 3. *Immunodiffusion of Ouchterlony. Bile 19.*
R: Beta-2-glycoprotein I. S: Alpha-1-glycoprotein antacid. T: Alpha-1-antitrypsin. V: Transferin. X: Ceruloplasmin.

Discussion

From our studies, and those published in the literature, we may state it is still early to draw conclusions and more complicated on the problem are necessary. However, we do have data of irrefutable value which may be of importance in pathogenics of difuse hepatic disease.

In hepatic cirrhosis, the interpretation of ous result is obscure and difficult. Generally speaking, the proteins shown are similar to cholelithiasis (4), but with two outstanding features: the almost total absence of IgM-Immunoglobulin and alpha-2-Macroglobulin. In this sense, we must conclude that these two proteins along with beta-Lipoprotein, which is also absent in these cases, are those with a higher molecular weight, and that nevertheless all proteins of a low molecular weight are found in the bile. That is to say, it might be some impediment by the cirrhosis liver to let pass high molecular weight proteins, a situation which does not exist in normal bile (3, 7) and cholelithiasis (4).

This fact is in agreement with the statement of CAMERON and HOU (1), who feel that the passage of bacteria, viruses and large inert molecules from the portal or

systemic blood to the bile is only possible when there is no existing damage of the hepatic cell or the Kupffer cells. Likewise, CHENDEROVITCH *et al.* (2), have found in the bile polyvinylpirrolidon, which had been expermentally injected into the portal vein. This fact makes one think that, under normal conditions, with a structurally healthy liver, high molecular weight plasma proteins can pass to the bile, but with hepatic lesions they do not, which is totally in agreement with our results.

To draw draw conclusions on the presence or absence or certain serum proteins in the bile of patients carrying difuse hepatopathy is still premature, for which, and awaiting new information on this subject, we present our results without venturing any speculations.

Resumen

Se estudia la constitución proteica de la bilis en las hepatopatías difusas crónicas. Para ello se emplean técnicas de inmunoelectroforesis e inmunodifusión. En la caracterización de las diferentes proteínas se han utilizado 17 antisueros específicos. La cualidad de las proteínas encontradas es independiente de la cantidad de proteínas totales. En el estudio cualitativo, llama la atención la ausencia de IgM-inmunoglobulina, de α-2-macroglobulina, así como de fibrinógeno y de α-1 y β-lipoproteína. Aun cuando es pronto para sacar conclusiones, tiene interés el hecho de que están ausentes preferentemente aquellas proteínas con un mayor peso molecular, mientras que las de bajo peso se hallan presentes.

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