Role of chronic hepatitis in development of thalassaemic liver disease

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Masera, G., Jean, G., Gazzola, G., and Novakova, M. (1976). *Archives of Disease in Childhood*, **51**, 680. **Role of chronic hepatitis in development of thalassaemic liver disease.** Liver function and the presence of HBsAg and anti-HBsAg were studied in 90 hypertransfused thalassaemic children. Increased serum transaminases were found in 62 patients, and persisted for more than 6 months in 45 cases. Liver biopsy in this latter group led to a diagnosis of 14 cases of chronic persistent hepatitis, 9 cases of aggressive hepatitis, and 3 cases of hepatic fibrosis. In Italy thalassaemic children undergoing hypertransfusion therapy frequently encounter SH virus infection, with a consequent hepatitis that is generally anicteric and unrecognized unless systematically sought. In a liver already stressed by the concomitant iron overload, hepatitis infection might thus play a key role in the evolution of cirrhosis which frequently affects thalassaemics.

The picture of thalassaemia has markedly changed in the last few years because of a more correct transfusion therapy. Serious cardiopathy is seen later in the course of the disease, whereas more thalassaemics are succumbing to fatal hepatic cirrhosis (Beltrami and Nenci, 1969; Ellis, Schulman, and Smith, 1954; Giraud, Orsini, and Muratore, 1959; Panizon and Vullo, 1957; Witzleben and Wyatt, 1961). Hepatotoxic factors to which the liver of the thalassaemic child may be exposed include iron overload, chronic hypoxia, hepatic stasis, protein deficiency, and repeated viral infection, particularly with SH virus but knowledge is scanty on the role of each of these on the evolution of liver cirrhosis. What knowledge there is is based on necropsy findings, and only recently has liver biopsy *in vivo* allowed the systematic study of the development of liver disease (Risdon, Flynn, and Barry, 1973; Masera and Jean, 1973; Barry *et al.*, 1974). From these studies it has emerged that inflammatory changes develop in the liver compatible with a diagnosis of chronic hepatitis (Masera and Jean, 1973; Diebold and Camilleri, 1974). Furthermore, the observations of Economidou *et al.* (1970), Vierucci *et al.* (1972), Kattamis *et al.* (1974), and ourselves (Masera and Jean, 1973) have shown a high incidence of HBsAg and anti-HBsAg in hypertrans-

fused thalassaemic patients. While the data suggest that serum hepatitis is a frequent side effect of therapy in thalassaemia, it is not known whether the hepatitis is a limited episode or whether such infection is a decisive influence in the development of liver cirrhosis. The aim of this paper is to investigate the pathogenesis of histological lesions of liver in thalassaemia and to characterize their evolution in thalassaemic children.

Methods

The 90 children between 1 and 14 years of age included in this study, all of whom were homozygous for β-thalassaemia, have been followed during the course of treatment for at least 4 years. They were transfused with packed red cells every 20–30 days in order to maintain Hb above 7·5–8 g/dl. From 1970 all blood donors have been routinely examined for the presence of HBsAg.* During the first 2 years of observations, HBsAg, anti-HBsAg, and serum transaminases were determined two or three times a year in these patients. The methods used have varied during this 4-year period to take advantage of technical developments, and have included successively the immunodiffusion technique (Del Prete, Costantino, and Doglia, 1970) modified according to Prince, and two methods of immuno-osmoelectrophoresis (Costantino *et al.*, 1972).

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*In Italy the search for HBsAg in blood donors began in several medical centres in 1970, but from 1973 this testing has been performed in the majority of transfusion centres.

Where serum transaminases were much increased, further biochemical tests were made, total and direct serum bilirubin, alkaline phosphatase, serum proteins (total and fractionated), 45-minute BSP retention, immunoglobulin levels, and the presence of LE factor, antinuclear factors, smooth muscle antibodies (immuno-fluorescence-indirect method) and migration inhibition factor (MIF) (Soborg and Bendixen, 1967). Liver biopsy was carried out in patients with raised serum transaminase levels for more than 6 months (Chalmers and Alter, 1971); liver specimens were fixed in Bouin's liquid and in paraffin; these preparations were then stained with H & E with silver impregnation (Gomori) and by Perls's method.

The characteristic histological picture of a thalassaemic liver, with an intense haemosiderosis, progressive fibrosis, and the presence of haemopoietic foci, made it necessary to evaluate the inflammatory status of each specimen using precise parameters of aggresiveness and chronicity of the hepatic lesion (Desmet and De Groote, 1974; Baggenstoss *et al.*, 1972; Scheuer, 1968).

Indices of aggressiveness. The extension of necrosis was graded as follows: focal necrosis (cellular pleomorphism, Councilman's bodies); the bridging effect; submassive necrosis (involvement of portions of adjacent lobules with evidence of piecemeal necrosis).

Indices of chronicity. The disproportionate enlargement of the portal spaces from which fibrotic septa arise, interlobularly or taking the place of entire hepatic lobules, these septa being more or less infiltrated by inflammatory cells which tend to invade the surrounding parenchyma with phenomena of piecemeal necrosis; an increase in number of bile canaliculi.

On the basis of such elements we have classified our cases as either chronic persistent hepatitis, chronic aggressive hepatitis, or fibrosis. It is likely that this histological picture, due to a multifactorial pathogenesis, has a prognostic and developmental significance different from similar histological patterns seen in nonthalassaemic patients.

Results

Increase in serum transaminases. Systematic monitoring (every 20–30 days) of SGOT and SGPT levels led to the discovery of increase in these enzymes to more than three times the normal values in a large proportion of cases (62/90). 3 of the cases developed a typical icteric hepatitis, the features of which were no different from those of hepatitis in nonthalassaemic children. All 3 cases were HBsAg positive, the first being so for only a few weeks and the other 2 for 34 and 36 months, respectively. In the remaining 59 cases of anicteric liver disease there was no clinical evidence to suggest an acute liver involvement. In 45 of the 90 cases raised transaminases persisted for from 9 to 24 months.

Incidence of HBsAg and anti-HBsAg. Of the 90 cases examined, 9 (10%) were HBsAg positive. Transient evidence of HBsAg was found in 7 cases, but only in 2 cases was this antigen present a long time (34 and 36 months, respectively). Anti-HBsAg positivity was more frequently shown for prolonged periods (13 out of the 28 cases), yet this antibody was present for only a few weeks in the other 15 cases (Gazzola *et al.*, 1974).

Liver biopsy (Table I). Liver biopsy was performed in 26 patients who had evidence of abnormally high transaminase levels for more than 6 months. The lesions were multiple and varied somewhat in different biopsy specimens from the same patient.

Hepatitis (Fig. 1). In 23 out of the 26 cases inflammatory infiltrates and cellular necrosis were present. In 14 cases the diagnosis of persistent hepatitis was made since the inflammatory infiltrates were mainly in the portal spaces and in the fibrous tissue without invasion of the surrounding hepatic parenchyma. Frequently, small areas of focal necrosis with Councilman's bodies and mononuclear infiltrates were present within the hepatic lobule, but evidence of piecemeal necrosis was not seen. On the contrary, in 9 of the remaining cases piecemeal necrosis, bridging effect, areas of marked focal and/or submassive necrosis were observed accompanied by active or passive septa (Desmet and De Groote, 1974). In these cases the diagnosis of aggressive hepatitis was made.

Fibrosis (Fig. 2). In all 26 cases fibrosis was conspicuous, involving primarily the portal spaces from which fibrotic interlobular septa arise. Generally, the connective tissue bands, newly-formed or derived from the collapse of original reticulum, had mononuclear cell infiltrates. Only in 3 of those patients whose specimens were diagnosed as showing hepatic fibrosis were the fibrotic phenomena predominant over the inflammatory aspects. In these cases the bile canaliculi were increased in number, without, however, enough evidence of pseudolobular regeneration to permit a diagnosis of cirrhosis.

Siderosis. In all 26 cases massive siderotic pigmentation was noted (+ + + + according to the scale of Barry, 1974) and involved the hepatocytes, the Kupffer cells, and the macrophages in the portal spaces as well as in the fibrous tissue. In certain cases that had received desferrioxamine-B the siderosis was more prominent near the portal spaces than in the centre of the hepatic lobule.

TABLE I

Details of 26 biopsied patients

Case no.	Sex	Age (yr)	HBsAg	Anti-HBsAg	Icteric hepatitis	Serum transaminases*	Smooth muscle antibodies	MIF	Liver biopsy
1	M	14	+	−	No	13	+		HF
2	M	7	+	−	Yes	14	±	+	CPH
3	M	10	+ +	−	No	12	−	−	CPH
4	M	9	+ +	−	No	14	−	+	CPH
5	F	6	+ +	−	No	13	±	+	CAH
6	M	12	−	+	No	22	±	+	CAH
7	M	12	−	+	No	13	−	+	CAH
8	M	8	−	+	No	21	−		CAH
9	F	14	−	+	No	10		±	HF
10	M	10	−	+	No	24	−		CPH
11	M	9	−	+	No	16	−		CPH
12	F	13	−	+	No	18	+	+	CAH
13	F	12	−	+ +	No	14	−	+	CAH
14	F	14	−	+ +	No	9	−	+	CAH
15	F	11	−	+ +	No	13	−	+	HF
16	F	13	−	+ +	No	13	−	−	CPH
17	M	6	−	+ +	No	11	+	−	CPH
18	F	9	−	+ +	No	17	+	−	CAH
19	F	14	−	+ +	No	14	−	−	CPH
20	M	6	−	+ +	No	11	+		CPH
21	F	12	−	−	No	10		+	CPH
22	M	8	−	−	No	10		−	CPH
23	F	8	−	−	No	18	−	+	CAH
24	M	5	−	−	No	8	+ +		CPH
25	F	14	−	−	No	16	+ +		CPH
26	F	12	−	−	No	12			CPH

*Serum transaminases: length of the increase in months.
+, HbsAg or anti-HBsAg positive longer than 3 months; + +, HBsAg or anti-HBsAg positive transiently; CPH, chronic persistent hepatitis; CAH, chronic aggressive hepatitis; HF, hepatic fibrosis; MIF, migration inhibition factor.
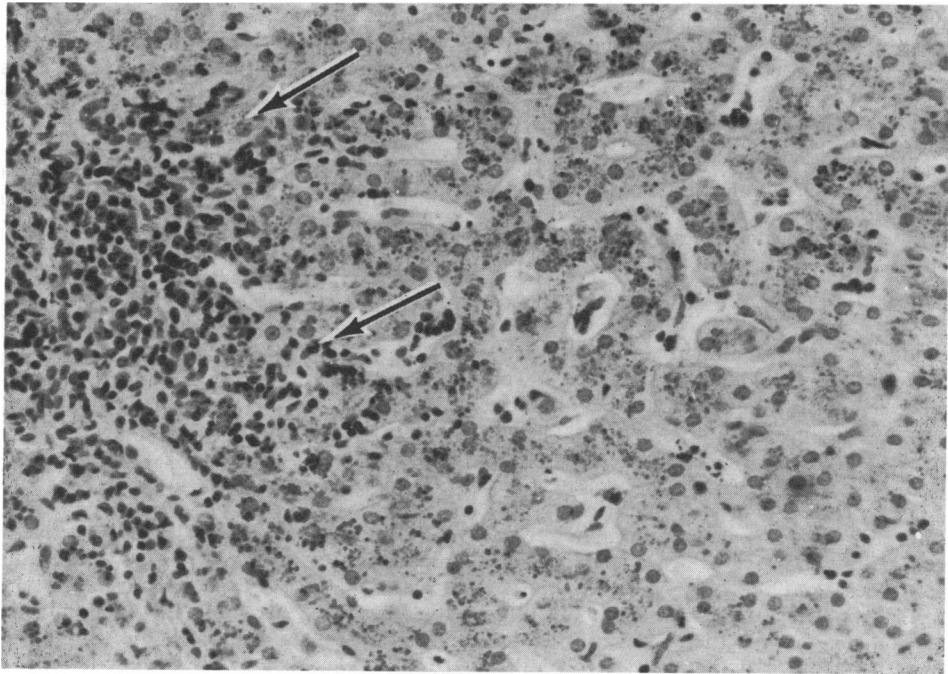

FIG. 1.—*Inflammatory infiltrate and piece-meal necrosis (arrows). Marked hyperplasia and hypertrophy of the Kupffer cells. Evidence of iron overload. (H. and E. ×260.)*
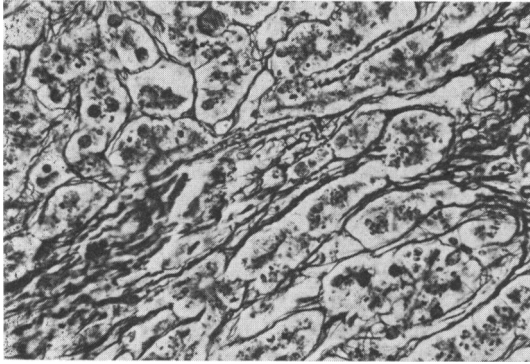
FIG. 2.—*Silver impregnation. Collapse of the reticulum and the initial formation of fibrotic septa. (Gomori's. ×165.)*

It is interesting to note that with H & E staining the Councilman's bodies appeared yellowish and not pink, indicating an even greater iron overload in the necrotic hepatocytes.

Areas of hepatic haemopoiesis (Fig. 3). Evidence of haemopoiesis was present in all cases. These areas were localized within the hepatic lobule and contained erythroblasts and megakaryocytes; their localization within the hepatic lobule and their composition (lacking myelocytes) being similar to that found in fetal liver (Diebold and Camilleri, 1974; Du Bois, 1963).

Summarizing conclusions, in hypertransfused thalassaemic patients signs of localized hepatitis, primarily in the portal spaces and periportal zones, are superimposed on a liver more or less extensively fibrotic. Less frequently the inflammatory infiltrate is located more towards the centre of the hepatic lobule, suggesting a histological picture like that described as chronic lobular hepatitis (Popper and Schaffner, 1971).

Correlation between histological findings and HBsAg and anti-HBsAg (Table II). Of the 14 cases with the histological picture of persistent hepatitis, only 3 were HBsAg positive and 6 anti-HBsAg positive. On the other hand, in 8 of the 9 cases diagnosed as aggressive hepatitis both HBsAg and anti-HBsAg positivity were found, an indication that these patients had been infected with SH virus.

Immunological investigations (Table I). Among the immunological tests made, smooth-
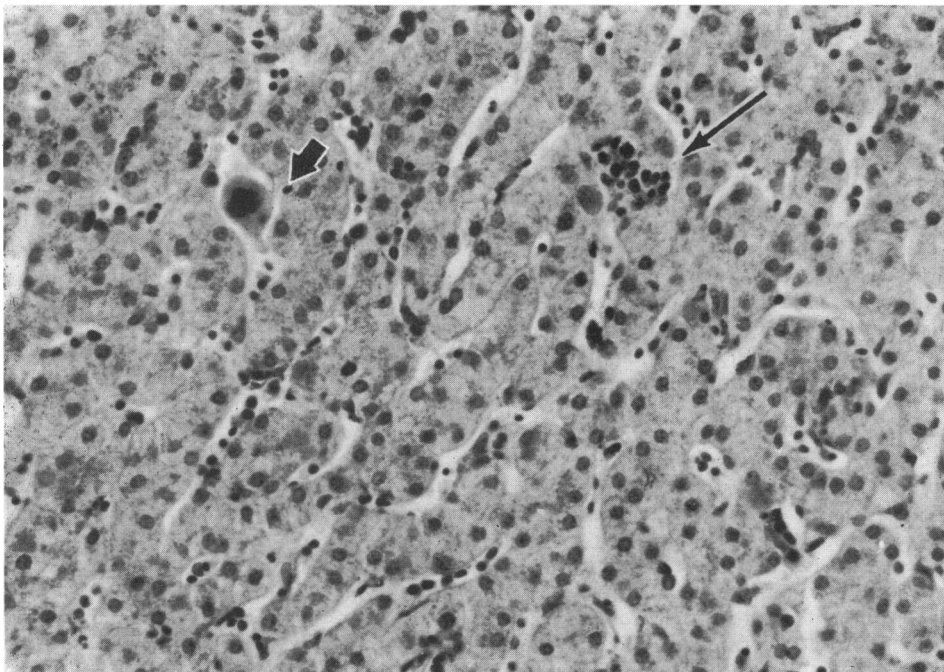

FIG. 3.—*Hepatic foci of haemopoiesis with clusters of erythroblasts (arrow) and with erythroblasts and a megakaryocyte (arrow head) along the hepatic sinusoids. (H. and E. ×260.)*

TABLE II

Correlation between histological findings and HBsAg and anti-HBsAg in 26 patients

	Liver biopsy		
	Chronic persistent hepatitis	Chronic aggressive hepatitis	Hepatic fibrosis
HBsAg	3	1	1
Anti-HBsAg	6	7	2
Negatives	5	1	–
Total	14	9	3

muscle antibodies were occasionally shown, but antinuclear factor was never noted. Interestingly, the migration inhibition factor was almost always positive in those cases with histological evidence of chronic aggressive hepatitis. It was not possible to formulate any conclusions on the modification of serum proteins since they are already considerably altered in thalassaemia, even in the absence of liver involvement.

Discussion

The study of the liver in hypertransfused thalassaemic children has generally been performed on post-mortem specimens (Beltrami and Nenci, 1969; Ellis *et al.*, 1954; Giraud *et al.*, 1959; Panizon and Vullo, 1957; Witzleben and Wyatt, 1961). These investigations have shown that the hepatic insufficiency of thalassaemics is due to the presence of haemochromatosis with fibrosis and hepatic cirrhosis. A few studies have been made using biopsy material (Risdon *et al.*, 1973; Masera and Jean, 1973; Barry *et al.*, 1974) but the initial stages of the hepatic lesions are poorly understood. Investigators have mainly concentrated on the massive deposits of ferrous pigment; sporadic reports have called attention to the mononuclear cell infiltrates, probably evidence of an inflammatory component in this hepatopathy (Beltrami and Nenci, 1969; Masera and Jean, 1973; Diebold and Camilleri, 1974; Necheles, 1974). Attempts to delay the development of hepatic cirrhosis have been largely through the use of iron-chelating compounds (Barry *et al.*, 1974). Yet the problem of cirrhosis should not be entirely attributed to pigment overload. In animal experiments it has not been possible to cause cirrhosis by administering massive doses of iron alone; only if another hepatotoxic factor (ethionine, carbon tetrachloride) is added to treatment will experimental cirrhosis develop (Goldberg and Smith, 1960; Kent *et al.*, 1964; Nath, Sood, and Nayak, 1972; Orfei *et al.*, 1968). These facts

prompted us to consider other factors which might exert harmful effects on the liver of hypertransfused thalassaemic children. Previous reports have shown frequent SH virus infection in thalassaemics (Economidou *et al.*, 1970; Kattamis *et al.*, 1974; Masera and Jean, 1973; Vierucci *et al.*, 1972) and undoubtedly repeated transfusion must expose the patients to infection with other hepatotropic viruses including cytomegalovirus (Prince *et al.*, 1971) and other unidentified viruses responsible for a hepatitis with a prolonged incubation (Finstone *et al.*, 1975). It must also be considered that thalassaemic children might be more susceptible to hepatitis caused by virus A.

The hypothesis of a hepatitis causing hepatic damage, at least in some thalassaemics, has been confirmed by our findings. HBsAg and anti-HBsAg were frequently shown in our thalassaemic patients, as in previous studies. Our findings relate to the studies carried out in Italy (Vierucci *et al.*, 1972), whereas in Greece (Kattamis *et al.*, 1974) the incidence of Au antigen in the normal population is higher (Italy: 1%, Ceppellini *et al.*, 1970; 2%, Rossi and Albrisi, 1974; Greece: 3%, Hadziyannis *et al.*, 1970) than in other countries. A transient or prolonged rise in serum transaminases was observed in 62 out of 90 of our patients, of which only 3 had symptoms of acute icteric hepatitis; the diagnosis of hepatitis would have been overlooked in the other cases if the appropriate investigation had not been undertaken.

In 23 of the 26 patients who underwent liver biopsy evidence of chronic persistent or aggressive hepatitis was found. In chronic aggressive hepatitis the presence of HBsAg or anti-HBsAg was shown in 8 of 9 patients. In some of the patients nonspecific immunological changes (smooth muscle antibodies, rheumatoid factor, migration inhibition factor) were noted such as are often seen in chronic aggressive hepatitis. These observations support the hypothesis that a chronic hepatitis contributes to the development of the liver disease in a large number of thalassaemic children who have received transfusion therapy.

The various forms of chronic hepatitis seen in thalassaemic children have special features which are probably due to the gross iron overload. This iron might favour extension of the hepatic necrosis caused by virus or might prevent regeneration of the hepatocytes primitively injured. The liberation of ferric pigment in the inflamed tissue would also lead to a more intensive fibroblastic proliferation, and so help to promote cirrhosis.

Regarding the therapeutic implications of these observations, chelation therapy is logical and its

efficacy proven (Barry *et al.*, 1974). Steroid therapy may be of some benefit in particular cases; for instance, those characterized by a histological picture of chronic aggressivity. At present we are studying serial liver biopsies of patients with chronic aggressive hepatitis under steroid therapy, and hope to determine whether this type of therapy is capable of slowing the course of liver disease towards cirrhosis.

Finally, in order to prevent infection, transfused blood components must not contain hepatitis virus. This requires strict selection and screening of blood donors, more precise detection of HBsAg and anti-HBsAg, and the use of frozen blood.

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