DNA synthesis been at a much higher rate than normal for the past 48 hours but mitosis had also developed and the liver had increased in size.

There are other factors involved in the response; changes are described also in cell walls and lyosomes of regenerating liver. The relationship between these components is not clear at present.

It seems therefore that one may consider the proliferative response in terms of at least these separate aspects, each of which may become deranged in different circumstances and after the exhibition of different toxins.

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Dr J R Hobbs
(Department of Chemical Pathology, Royal Postgraduate Medical School of London)

Serum Proteins in Liver Disease

The absolute diagnostic value of serum electrophoresis is very limited. However, in cases of known liver disease, uncomplicated by other diseases, the electrophoretic pattern can be helpful. After describing six patterns useful in the differential diagnosis of jaundice (Fig 1) I shall discuss the results of immunoglobulin studies.

Pattern 1: Surprisingly, a normal serum electrophoretic pattern can be very informative when seen in a patient who presents with jaundice, for it thereby largely excludes pre-existing cirrhosis. There is one important exception, when the serum is collected after a massive blood transfusion, for then the patient's original plasma is largely replaced by normal plasma.

Pattern 2: If the α_2-globulin is increased hæmolytic jaundice is unlikely, for about half the increase in α_2-globulin which occurs nonspecifically in most illnesses is due to an increase in haptoglobins. In the presence of intravascular hæmolysis, hæmoglobin becomes bound to haptoglobins and *in vivo* the complex is rapidly removed so that the α_2-globulin in fact tends to decrease. At the same time, the α_1-globulin increases so that in hæmolytic jaundice the α_1-globulin is characteristically greater than the α_2-globulin. Where the hæmolysis is autoimmune an increase in γ-globulin is usually seen and there is also absence of β-γ fusion, which is discussed later.

Pattern 3: If the first strip appeared normal, serum electrophoresis should be repeated about two weeks after the onset of the jaundice. If the γ-globulin has not risen then an infective ætiology is unlikely and this has been useful in attributing the jaundice to drugs where simple biliary obstruction can be excluded. When obstruction has been severe and continued for some weeks, then α_2-globulin elevation is usual. This is because the lipoproteins largely run in the α_2 position on cellulose acetate. The lipid of long-standing biliary obstruction contains a uniquely high proportion of phospholipid and is characteristically denatured to a white precipitate by freezing and thawing. If a previously clear jaundiced serum comes out of the deep freeze looking like sour milk, it is likely to be from a patient with biliary obstruction (Dangerfield & Hurworth 1962).

Pattern 4: Where a γ-globulin increase is seen after one or two weeks, this is useful as a pointer to an infective ætiology. It is not diagnostic of virus hepatitis, for it is also seen with the jaundice of glandular fever, infective cholangitis and serum sickness. With the clinical background it can be reassuring, especially when it can be further shown to be mostly due to slow γM-globulin without any change in the β-γ demarcation.

Pattern 5: This is the classical pattern of chronic liver disease. The albumin is reduced, the γ-globulin is increased, and in a given patient these tend to vary inversely. Franklin *et al.* (1951) showed that the increase of γ-globulin also involved fast γ_1-globulin and Demeulenaere & Wieme (1961) stressed that this resulted in a characteristic filling in of the gap normally present between β- and γ-globulins (*see* Pattern 1). This β-γ fusion is largely due to an increase in γA globulin which mainly has γ_1-mobility. To this should be added the lack of rise of α_2-globulin. In nearly all ill patients an increase in α_2-globulin is to be expected and this is largely due to haptoglobins and α_2-macroglobulin which are made in the liver. With liver disease this can be impaired, so that the α_2-globulin does not show the expected increase, and this is a useful point in distinguishing cirrhosis from chronic infection where albumin can be decreased and a broad increase in γ-globulin can mimic β-γ fusion, but where α_2-globulin is usually raised. As already mentioned, intravascular hæmolysis can have a similar effect in preventing α_2-globulin increase, so that the electrophoretic strip should be interpreted in the light of all the findings.

Pattern 6: The classic pattern of cirrhosis, however, contrasts with that seen in juvenile cirrhosis.

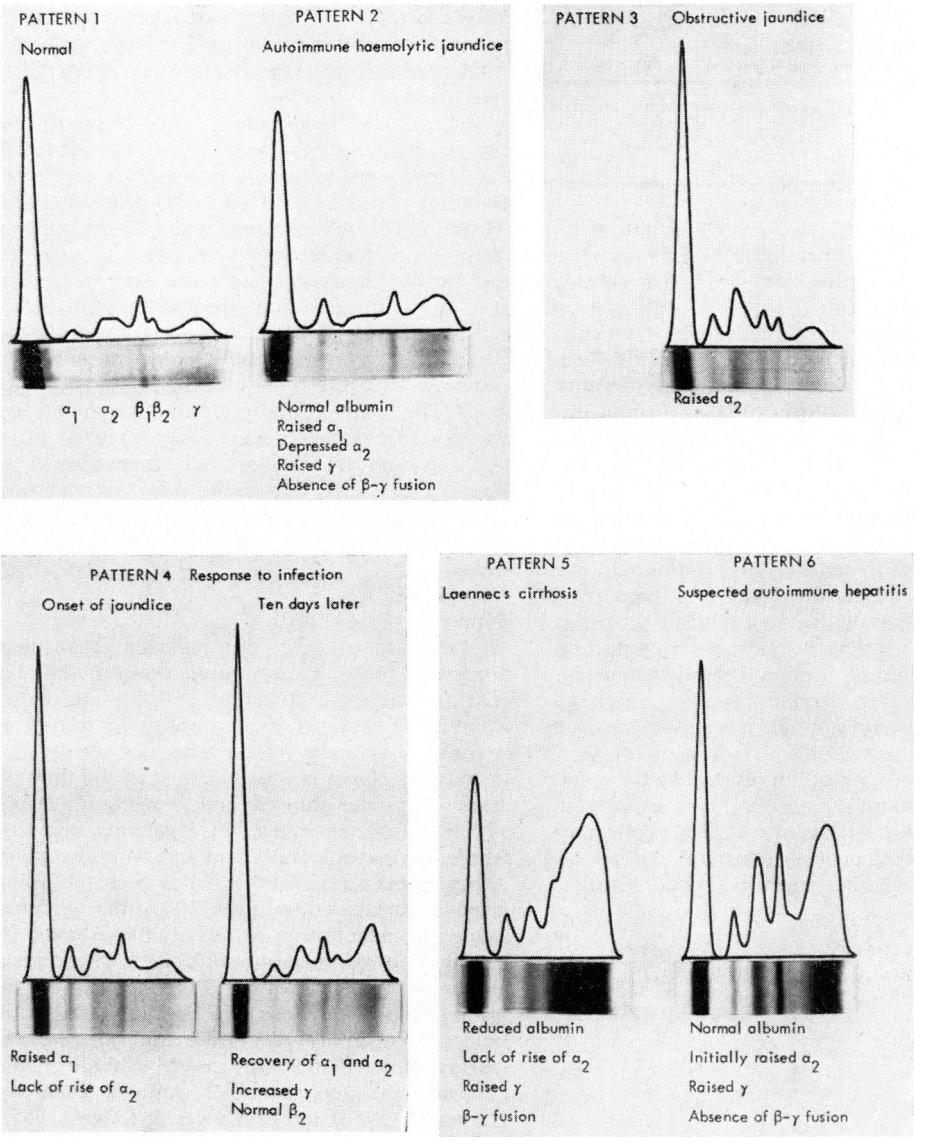

Fig 1 *Six patterns of serum electrophoresis on cellulose acetate which can be helpful in the differential diagnosis of jaundice*

Waldenström (1950) described this condition, which was recently reviewed by Sherlock (1966). The albumin level can be well maintained, the α_2-globulin is initially raised and there is usually a disproportionate increase in γ-globulin present from the onset. While I was in Professor Baron's department at the Royal Free Hospital, a change over to electrophoresis on cellulose acetate enabled sera to be examined within a few hours of separation. It became evident that instead of the classical β-γ fusion, many sera from patients with cirrhosis showed the opposite – absence of β-γ fusion. Without knowing the diagnosis, the electrophoretic patterns of patients were separated into these two groups. I then reviewed the diagnoses with Dr Gordon Cooke and Table 1 shows that we found the absence of β-γ fusion to be a reliable criterion in distinguishing 'juvenile cirrhosis' from cirrhoses other than biliary.

Immunoelectrophoresis showed that the absence of β-γ fusion was due to an increase in largely slow γG-globulin, no increase in γA-globulin and a reduction in β_1C-globulin, a component of complement (Hobbs 1966).

Table 1

Fresh serum electrophoresis on cellulose acetate in the differential diagnosis of cirrhosis in 188 patients

Final diagnosis	*Pattern 5*	*Equivocal*	*Pattern 6*
Juvenile cirrhosis	2	4	67
Other cirrhoses (excluding biliary)	103	5	7

In fresh serum β_1C has β_2 mobility, but within 4 hours or so this changes to β_1A-globulin which has β_1 mobility; thus only cellulose acetate results obtained within 4 hours of sampling are reliable. Complement depletion is typical of systemic lupus erythematosis (Seligman 1964) and is now known to occur in many autoimmune diseases. Indeed, absence of β-γ fusion is a typical finding during activity of these conditions, for example, as already mentioned, in autoimmune hæmolytic anæmia (Pattern 2).

Recently the healthy scepticism of my colleagues over the definition of cirrhoses has taught me to proceed with caution. The immunoglobulin results which follow are based on three years' work, accepting only the most critically investigated patients: a complete follow-up with continuous clinical assessment, full serial biochemical tests, in particular enzymes, proteins, immunoglobulins and bromsulphalein tests, and various auto-antibody tests (Dr E J Holborow or Dr D Doniach). This type of survey would have been impossible without the excellent care and investigation undertaken by my clinical colleagues, mainly of Hammersmith Hospital. In all 114 patients the liver has been examined histologically, and one-third have come to post-mortem. I am most grateful to our morbid anatomists, without whose help the classification could not have been made.

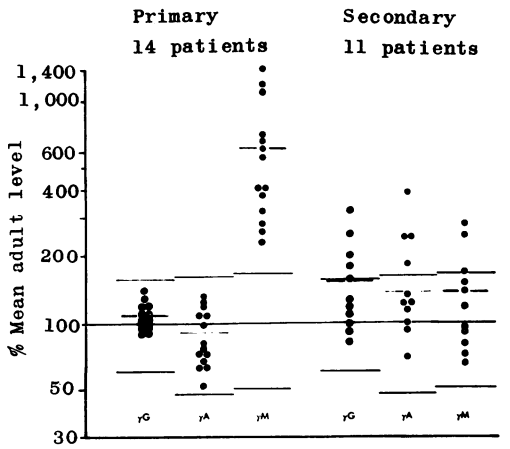

Fig 2 *Serum immunoglobulin levels in 25 patients with biliary cirrhosis, shown as % mean of levels of 107 normal subjects on a logarithmic scale. (±2 SD limits shown) Those secondary to recurrent cholangitis may show increased levels to about the same degree in all three classes. In primary biliary cirrhosis the increase is mainly in the γM class*

Serum immunoglobulin levels have been measured against a reference serum calibrated with fresh pure solutions of γG, γA and γM globulins using a modified Mancini method (Hobbs & Maatela, in preparation). In every case the total of the serum immunoglobulin results was further checked against the electrophoretic estimation of γ-globulin, agreement usually being within ±0·2 g/100 ml. This is an important safeguard, for many commercial standards deteriorate and lead to overestimation of immunoglobulin levels. The results of the γD-globulin estimations also done are not included, as they added so little to the picture. The log-normal ranges shown in Figs 2–5 are derived from the skew distributions found in 107 normal adult subjects and for this reason all results are compared on logarithmic scales.

Biliary Cirrhosis (Fig 2)

In 11 patients with histologically confirmed fibrosis there was clearly some lesion of the bile ducts, predisposing to classical clinical attacks of recurrent cholangitis eventually leading to biliary cirrhosis which was therefore termed secondary. In most, cholangitis was quiescent at the time of assay and these showed immunoglobulin levels within the normal range. In 4 patients, recently febrile, increases were found to about the same degree in each class and this has been the usual response measured in over 100 other patients following infections. None of the above 11 patients showed any auto-antibodies. In contrast, all the 14 patients with the typical features of primary biliary cirrhosis had mitochondrial antibodies (Walker *et al.* 1965), and all showed marked isolated increases in γM-globulin. This confirms the reports of 2 patients each by Heremans (1960) and McKelvey & Fahey (1965). Paronetto *et al.* (1964), using specific fluorescent antibody, showed that the plasma cells around the liver lesions were largely producing γM-globulin. They found raised serum titres of γM-globulin both in primary and in secondary cases. Indeed, mitochondrial antibodies and raised γM-globulin levels can be found in many types of cirrhosis with jaundice, but in most of these measurement reveals high levels of γG and γA-globulin also, in contrast to the isolated increase in γM-globulin found in primary biliary cirrhosis.

Infective Hepatitis (Fig 3)

From some 30 patients suspected of having virus hepatitis, only 8 are included here, for only these

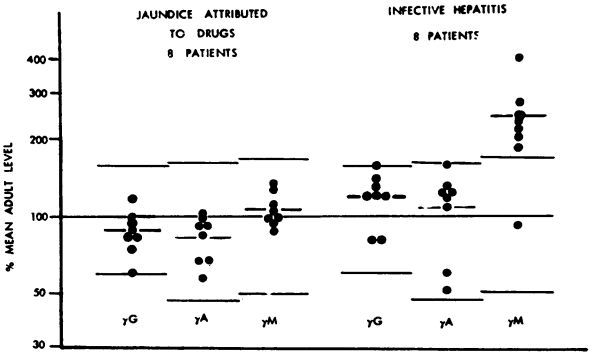

Fig 3 *Immunoglobulin levels. In sera collected about ten days after the onset of symptoms in 8 patients with probable virus hepatitis, the increase is mainly in γM (av. × 2·4) with some in γG (av. × 1·2) which may later rise further. In sera from 8 patients with jaundice following chlorpromazine the immunoglobulin levels remain within normal limits*

had a clear history of contact followed 22–34 days later by the onset of symptoms. These 8 had negative Paul-Bunnell tests, all made a complete clinical and biochemical recovery and 3 appeared to give hepatitis to contacts. About ten days after the onset of symptoms they showed mainly increased γM-globulin (×2·4) as described by Heremans (1960), Tomasi & Tisdale (1964) and Lee (1965) who found that γG-globulin increases (×1·5) followed.

Jaundice Attributed to Drugs

Fig 3 also shows that normal immunoglobulin levels were found in 8 patients with jaundice attributed to chlorpromazine after exhaustive elimination of other causes. This finding can thus have good negative value.

Laennec's Cirrhosis (Fig 4)

These 44 patients were at one fairly well-defined end of a spectrum of cirrhosis. Their livers were uniformly affected, with fine nodules separated by regular thin fibrous trabeculæ. Clinically and biochemically their course was insidious in onset and slowly and steadily progressive, with serum isocitrate dehydrogenase only showing elevation terminally or during acute alcoholism. In 12 patients there was undoubtedly alcoholism and this was probable in another 4. Six patients had very severe and long-standing congestive heart failure with cirrhosis confirmed at post-morten. Two others had hæmochromatosis and one had Wilson's disease. In the other 19 the ætiology is completely unknown. Almost without exception they showed a dominant increase in γA-globulin; those with raised γG (av. ×1·8) and γM levels (av. ×1·2) had higher γA levels (av. ×2·9) than the normal mean.

There were 12 patients with severe chronic alcoholism with no evidence of liver disease and with normal bromsulphalein clearance. It is of interest that, if anything, they show depression of their immunoglobulins. These cases were selected by Dr H I Coombs of St Bernard's Hospital. The increased γA level of 'alcoholic' cirrhosis thus seems secondary to the cirrhosis rather than attributable to alcohol. Furthermore, the levels were highest in the long-standing cases.

Crabbe *et al.* (1965) showed that 85% of the plasma cells lining the gut produce γA-globulin. Gowans & Knight (1964) showed how the lymphocytes from the thoracic duct tend largely to return to the gut where some can differentiate into plasma cells. Since the liver is of endodermal origin, its natural plasma cell differentiation might be expected to be to γA-producing types, and this is what we have found in biopsies of 2 of the above cases using specific fluorescent anti-

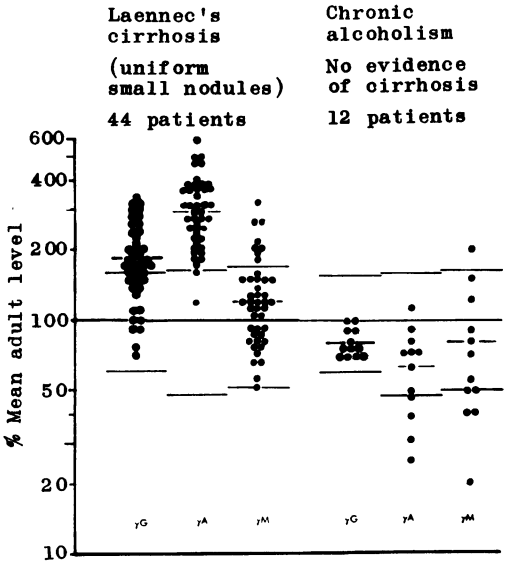

Fig 4 *Serum immunoglobulin levels. In 44 patients classified on clinicopathological evidence as at the Laennec end of the spectrum of cirrhosis, while γG (av × 1·8) and γM (av. × 1·2) are raised the main increase is in γA (av. × 2·9). In 12 chronic alcoholics without evidence of liver disease levels are at the lower side of normal. Thus γA increases seem secondary to cirrhosis rather than due to alcohol* per se

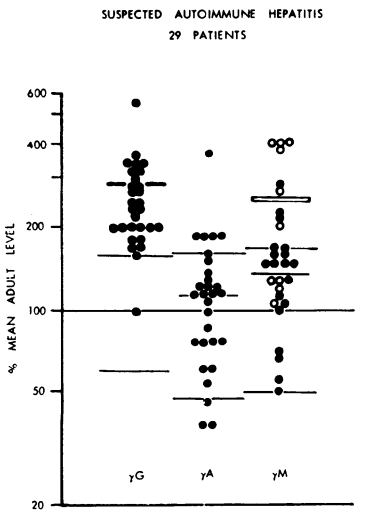

Fig 5 *Serum immunoglobulin levels in 29 patients classified on clinicopathological evidence as at the 'juvenile' end of the spectrum of cirrhosis. The increase is mainly in γG (av. × 2·8). The open circles refer to 10 patients with positive antinuclear factor fluorescence, in 8 of whom hepatitis was part of the general picture of systemic lupus erythematosis. They show higher γM (av. × 2·5) and had been ill for longer than the other 19 patients (γM av. × 1·4) who had presented with hepatitis only. In 17 of the latter smooth muscle fluorescence was positive*

γA-antibody. Thus increase in serum γA-globulin may result from the usual inflammatory response to any exogenous harmful influence within the liver. In this light, the endogenous infiltrations of the liver by mainly γM-producing cells in primary biliary cirrhosis, or by mainly γG-producing cells in 'juvenile cirrhosis' might reflect an autoimmune attack on the liver.

Suspected Autoimmune Hepatitis (Fig 5)

At the other end of the spectrum were these 29 patients. They eventually all showed non-uniform cirrhosis with marked variation in the size of the nodules and in the thickness of the fibrous trabeculæ. There was a pronounced infiltration with lymphocytes and plasma cells, and in some cases this was all that was found initially. Clinically and biochemically they largely had an acute onset, frequently labelled as infective hepatitis yet without known contact or any transmission. Their course was erratic, in and out of jaundice with intermittent high serum levels of isocitrate dehydrogenase. In 27 of 29 patients, serum electrophoresis showed Pattern 6, with reduced β_1C, and in Fig 5 they show an increase largely in γG-globulin. Of these patients, 8, mainly from the large clinic of Professor E G L Bywaters, had other manifestations of systemic lupus erythematosus and hepatitis was an incidental and unusual complication. The others all presented with jaundice. The sera were examined, 'blind' by Dr Holborow. He found antinuclear factor in the 8 with SLE and in 2 others, in whom rheumatoid arthritis was present. None of these 10 showed any smooth muscle antibody, and as a group they showed a higher γM level than the rest. In 17 of the remaining 19, smooth muscle antibody was found together with ANF in only 3. In the Lænnec group none has so far shown smooth muscle antibody though ANF is present in about a quarter. This group, selected on clinico-pathological grounds, thus show electrophoretic Pattern 6, increased γG-globulin mainly (× 2·8), decreased complement and the presence of antibodies not frequently detected in other varieties of cirrhosis (Johnson *et al.* 1965). In 11 patients the γG-globulin was markedly raised on admission within a few days after their first onset of jaundice and the γM only increased a year or so later. This is unlike infective hepatitis where the rise is not seen for about ten days and is then largely in γM-globulin. Such is the evidence that the γG-globulin-producing plasma cells may play a primary autoimmune role in this group.

Between this and the Laennec end of the cirrhotic spectrum there were still, after two years' observation, 8 patients with undoubted cirrhosis where the histology and biochemistry were equivocal. They had no antibodies and, mainly, increases in γA. These have not been included in Fig 4 or 5. In ordinary practice, without intensive investigation over two years, this would probably be the largest group.

To conclude, none of the patterns mentioned is absolutely specific. Other diseases may complicate the picture and cases should always be assessed in the light of all the findings. With these reservations, serum electrophoresis and immunoglobulin estimations can be helpful in the diagnosis and follow up of liver disease.

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