ELECTROPHORETIC STUDIES IN LIVER DISEASE. II. GAMMA$_1$ GLOBULIN IN CHRONIC LIVER DISEASE [1,2]

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The electrophoretic method of analysis has proved to be a most valuable aid in the study of protein alteration in hepatic diseases. Characteristic changes consisting of reduced albumin and increased gamma globulin components have been reported by many observers (1–3). The depressed albumin levels frequently observed in cirrhosis have been attributed to several factors, such as defective synthesis by the liver, inadequate dietary protein and the loss of albumin in ascitic fluid. There has been much speculation concerning the nature and cause of the globulin elevation as well as the fibrinogen changes in hepatic cirrhosis. At present the problem is still unsolved. Several observers have reported elevated electrophoretic fibrinogen values in cirrhosis (3, 4). One author (5) attributed some of this elevation to the migration of a gamma$_1$ globulin fraction in the mobility range of the "fibrinogen" peak. Deutsch and his co-workers (6) have described such a gamma$_1$ globulin component with a mobility similar to fibrinogen in normal serum. It constituted 2–3% of the total proteins. There are reports (7–9), of single cases of cirrhosis with a component in the electrophoretic pattern between the gamma and beta peaks which has been called the "X" component.

In a recent study (10) of paired serum and plasma from 66 persons having hepatic or miscellaneous diseases, and normal subjects, frequently

great elevations of the "fibrinogen" peak were present in the plasma electrophoretic pattern. The serum patterns from the same persons did not show complete elimination of the "fibrinogen" peak but there was a component with a mobility between that of gamma and beta globulin. This component was included in the "fibrinogen" peak and accounted for a large portion of the elevation. It was present in normal sera, very high in cirrhotic sera, and there were smaller elevations in obstructive jaundice and acute hepatitis. The miscellaneous group had no increase of this globulin fraction. This component seems to be identical with the gamma$_1$ globulin component described by Deutsch in normals and the "X" component described in cirrhosis by others (7–9). It apparently is analogous to a new protein fraction found by many workers (11–15) in the serum of hyperimmunized animals and it, too, migrates between the gamma and beta globulin electrophoretic peaks. The present report deals with electrophoretic studies of the gamma$_1$ globulin alterations and their possible significance.

MATERIAL AND METHODS

The subjects and methods were the same as those described in Part I of these studies, the preceding article (10), except for additional gamma$_1$ globulin determinations in serum alone in eight more cases of cirrhosis, four of infectious hepatitis, one of toxic hepatitis, five of obstructive jaundice, two of hemolytic jaundice, 73 of diabetes mellitus, 23 of poliomyelitis, 16 of rheumatoid arthritis and 21 of hypertrophic arthritis. Chemical globulin determinations were performed according to the method of Wolfson and Cohn (16). The method of Chaney (17) was used for the cholesterol determinations. In addition total lipids (18), zinc sulphate turbidity (18), cephalin flocculation (19) and thymol turbidity (20) were determined.

RESULTS

The relation of gamma$_1$ globulin to the total gamma globulin in the serum and to the "fibrino-

TABLE I

Comparison of electrophoretic protein partition in a case of cirrhosis using different electrophoresed material

Electrophoresed material	Per cent of total proteins										
	Alb.	α_1	α_2	β	ϕ	ϕ (corr.)	γ_1	γ_2	Tot. γ_s	Tot. γ_p	Tot. γ_p (corr.)
Serum	45.6	2.5	2.8	22.0	—	—	4.0	23.1	27.1	—	—
Serum and thrombin	45.4	4.3	3.3	20.2	—	—	4.3	22.5	26.8	—	—
Serum and oxalate	44.4	4.7	5.2	17.7	—	—	4.4	23.6	28.0	—	—
Plasma	42.5	4.0	3.0	17.4	10.0	6.0	—	—	—	23.1	27.1

gen" peak in the plasma patterns is illustrated in Figures 1, 3, 4, and Tables I and III of the preceding article (10). The average normal value of the gamma$_1$ globulin in the group with paired serum and plasma samples was 2.5% of the total protein with a range between 1.7 and 3.4%. The cirrhotic patients had the most marked increase in this component, having an average gamma$_1$ globulin value of 7.1% with a range between 2.1 and 11.5% (Tables I and III of the preceding article [10]). In the group of cirrhotic cases in which only serum determinations were performed, the average gamma$_1$ was slightly higher (7.6%) with one case showing 19% gamma$_1$ globulin (Figure 1). The cases of obstructive jaundice and acute hepatitis revealed less gamma$_1$ elevation. In interpreting the results in these groups, particu-

larly in the obstructive jaundice group, the limited number of cases must be considered. The average gamma$_1$ globulin values for the miscellaneous disease group was within normal limits. The total serum gamma globulin increases present in the cases of liver disease and rheumatoid arthritis are in agreement with previously reported observations (21–26). No relation existed between the gamma$_1$ globulin and the total serum proteins (Figure 2a). This is to be expected since the total serum proteins in the pathologic cases and the normal individuals are similar, whereas the gamma$_1$ fractions in these two groups are dissimilar. A relatively greater increase in the gamma$_1$ fraction was found in cirrhosis than in other liver diseases (Figure 2b). This increase is proportionately greater than the increase in total gamma globulin. The average gamma$_1$ globulin values for the miscellaneous group were normal despite the increase in total gamma globulin. Only two cases of rheumatoid arthritis in this group exhibited values beyond the normal range. The single case of multiple myeloma, despite the highest total gamma globulin percentage (60%), had a gamma$_1$ value of 4%, only slightly above the normal limit. The non-liver disease cases have average values of gamma$_1$ globulin within the normal range although in each group there were cases with elevated values (Figure 1). The gamma$_1$ increase is not merely a reflection of elevated total gamma globulin but apparently bears some relation to chronic liver disease although not necessarily confined to that pathologic state.

To determine whether the gamma$_1$ globulin component was related to beta rather than gamma globulin, this component was compared with the electrophoretic beta globulin and the chemically-determined cholesterol and the total lipids which are said to be intimately related to the beta globu-
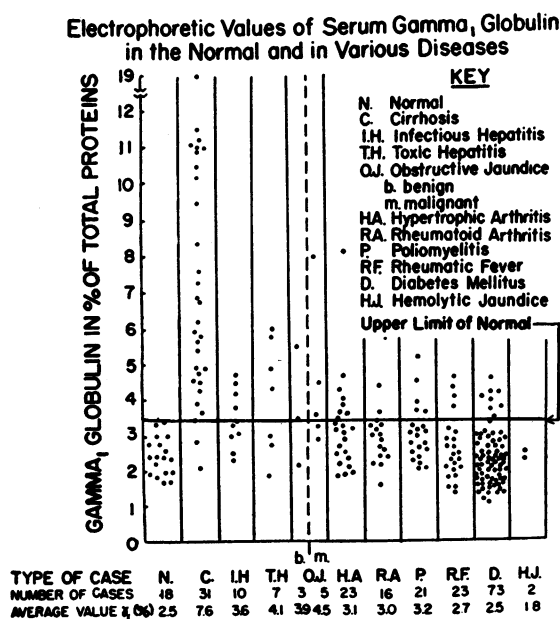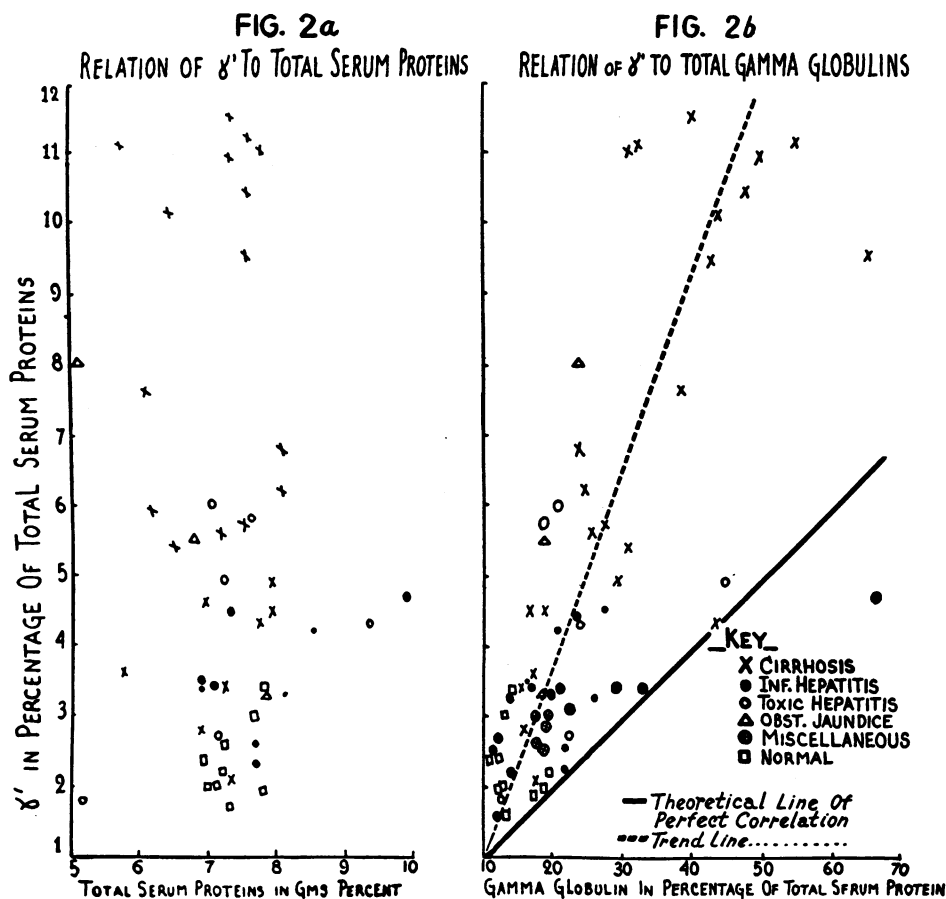

FIG. 1. ELECTROPHORETIC VALUES OF SERUM GAMMA GLOBULIN IN THE NORMAL AND IN VARIOUS DISEASES

FIG. 2a
RELATION OF γ' TO TOTAL SERUM PROTEINS

FIG. 2b
RELATION OF γ' TO TOTAL GAMMA GLOBULINS

FIG. 2a. RELATION OF GAMMA₁ GLOBULIN TO TOTAL SERUM PROTEINS

FIG. 2b. RELATION OF GAMMA₁ GLOBULIN TO TOTAL SERUM GAMMA GLOBULINS

lins (27, 28). There is no relation between the gamma₁ component and the beta globulins, total cholesterol or total lipids (Figure 3). This is in keeping with Deutsch's contention that gamma₁ globulin is actually a gamma rather than a beta globulin. The similarity of all three scattergraphs, although showing no relation between gamma₁ globulin and beta globulin, cholesterol and total lipids, suggests that all but gamma₁ are related to each other.

Gamma₁ globulin does not appear to be related to results of the cephalin flocculation, thymol turbidity and zinc sulphate turbidity tests (Figure 4). It is probably not responsible for the abnormal results of these tests in liver disease.

There is no essential difference between the gamma₁ value as determined in serum, serum plus thrombin and serum plus oxalate (Table I). Therefore the gamma₁ component in the electropho-

retic pattern was probably not residual fibrinogen, nor could the serum-plasma electrophoretic difference be attributed to the anticoagulant and denaturation of protein fractions by it. The relation of the gamma₁ component to the fibrinogen in the plasma pattern and to the serum-plasma gamma globulin difference is illustrated in Figures 1, 3, and 5 of the preceding article (10). The similar electrophoretic mobilities of gamma₁ and fibrinogen are illustrated in Table II of the preceding article (10). This similarity accounts for the fact that a large portion of the area underneath the "fibrinogen" peak is due to the gamma₁ globulin. The elevation of this component in cirrhosis and its presence in the fibrinogen range is largely responsible for the elevated electrophoretic "fibrinogen" values frequently encountered in this disease. The inclusion of the gamma₁ component in the fibrinogen peak also accounts for the total

gamma globulin values of plasma being less than those of serum. The comparison of the gamma$_1$ globulin values in normal subjects, cirrhotics and other disease states is illustrated in Figure 1. Elevations of gamma$_1$ globulin in other diseases, although present, are infrequent and are usually of mild degree only. In contrast the great majority of cirrhotics have gamma$_1$ globulin elevations and these are much higher than those found in other diseases.

DISCUSSION

The characteristic alterations in the serum proteins in chronic liver disease, namely, hypoalbuminemia and hyperglobulinemia chiefly of the gamma fraction, have been reported by numerous observers (5, 21–23). Although failure of albumin synthesis by the liver is generally regarded as the cause of the marked hypoalbuminemia, other factors undoubtedly play a role. Inadequate protein intake, absorption or utilization and loss in ascitic fluid and urine may increase the albumin depletion in cirrhosis.

The cause of gamma globulin increase in chronic liver disease is obscure and has been the basis of much conjecture. The explanation frequently advanced that the hyperglobulinemia is compensatory for the lowered serum albumin seems improbable. This would not explain why the elevation is usually in the gamma fraction and why it frequently is sustained after the serum albumin has returned to normal. Albumin has a much higher osmotic activity than globulin and a rise in the latter probably would be of little significance in elevating a depressed colloid osmotic pressure resulting from hypoalbuminemia (5).

The perplexing problem of hyperglobulinemia in many diseases including cirrhosis is now further complicated by a necessity to explain the gamma$_1$ globulin fraction elevations characteristic of cir-
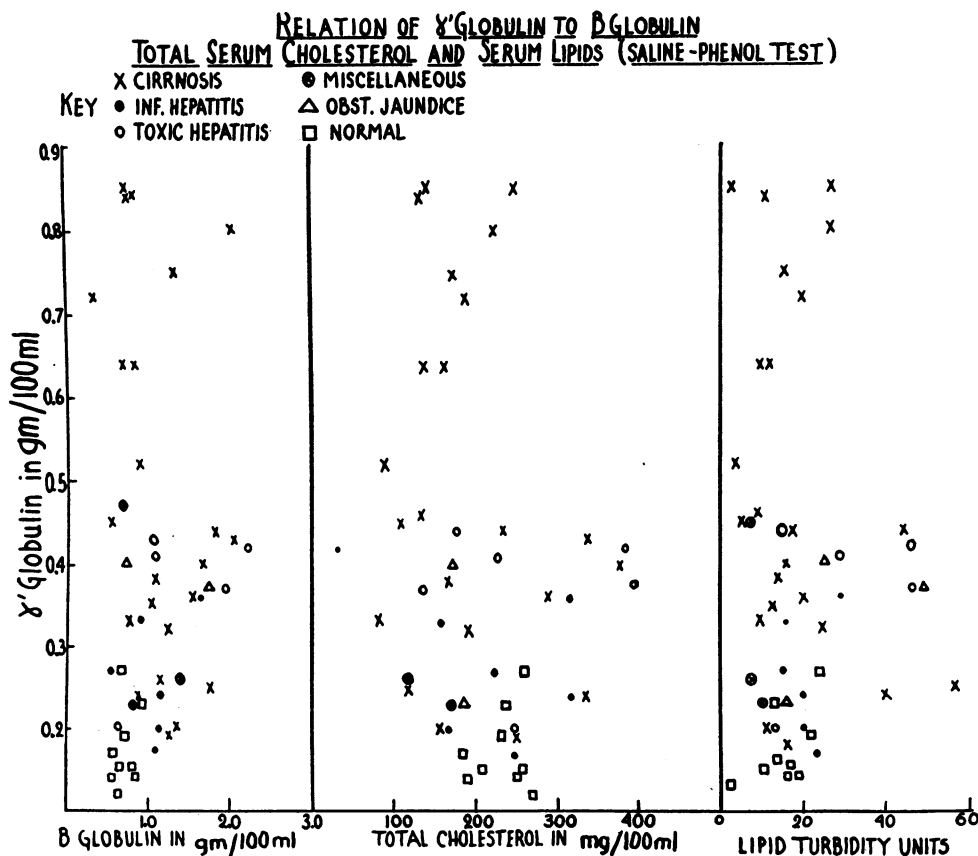

FIG. 3. RELATION OF GAMMA$_1$ GLOBULIN TO BETA GLOBULIN, TOTAL SERUM CHOLESTEROL AND TOTAL SERUM LIPIDS
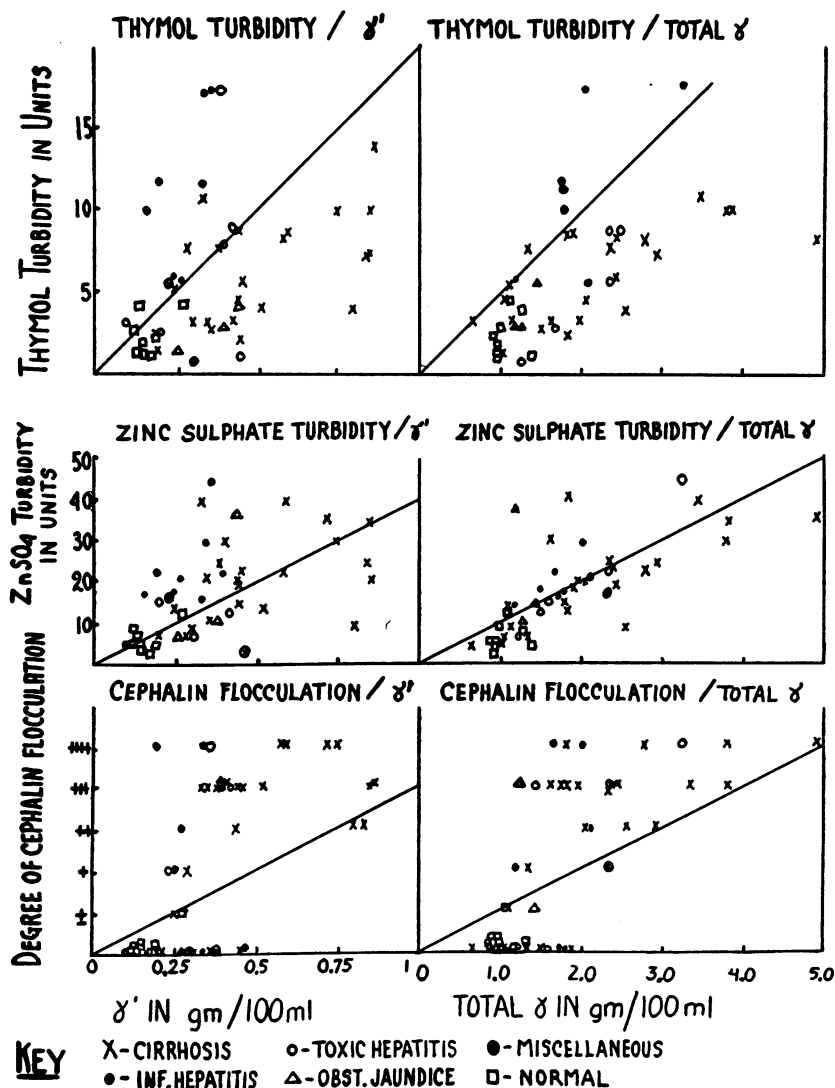
FIG. 4. RELATION OF GAMMA₁ GLOBULIN AND TOTAL SERUM GLOBULINS TO CEPHA-
LIN FLOCCULATION, ZINC SULPHATE AND THYMOL TURBIDITY TESTS

rhosis. Before hypothesizing as to its origin, several questions concerning its nature must be answered. Is this component, found in the serum, merely residual fibrinogen which has not been completely removed in the clotting process? Is gamma₁ globulin denatured serum protein produced by the anticoagulant? If it is not a residual or denatured protein, is it a gamma or beta globulin? What relation has it to other protein complexes situated between the gamma and beta components in the electrophoretic pattern in other diseases? When does it appear in human serum and what is its relation to similar electrophoretic components found in the serum of animals?

The similarity of the electrophoretic gamma₁ values of the different electrophoresed materials in the same patient (Table I) indicates that this component was not residual fibrinogen nor was the anticoagulant responsible for the serum-plasma electrophoretic differences. It is a protein component of normal human serum, designated gamma₁ by Deutsch who separated it from the bulk of the gamma fraction by means of ethanol fractionation (6). He found it to be present in normal human serum to the extent of 2–3% of the total serum proteins and that it was free of lipids and cholesterol. Our results are in agreement. Gamma₁ globulin is apparently the same protein fraction de-

scribed in cirrhosis as the "X" component by some observers (7–9).

Gutman and his co-workers (29) and others (8, 30) have found in multiple myeloma an abnormal serum protein component with electrophoretic mobility between that of gamma and beta globulins. This abnormal component does not appear to be the gamma₁ globulin in the electrophoretic pattern of the cirrhotic. The former appears as a very tall narrow peak whereas the gamma₁ globulin component is not as narrow and tall and is in the form of a plateau rather than a distinct peak.

Purified Wassermann antibody also migrates with a mobility intermediate between beta and gamma globulins (31). However, the sera of syphilitic patients show no electrophoretic changes referable to this specific antibody. There is also no correlation between the titer of the quantitative Kahn reaction and increase in gamma globulin (32, 33). The concentrations of antibody are too low for detection by electrophoresis.

Our data reveal gamma₁ elevations infrequently in pathologic states other than liver disease. Moreover when they occur they are usually of slight degree only. In contrast the great majority of cirrhotics in our series had gamma₁ elevations and they were much higher than those found in sporadic cases of other types of disease showing elevations. Wallis (34) described a component with an electrophoretic mobility between that of gamma and beta globulins in three out of four cases of rheumatoid arthritis. He called this a "T" component and found that it comprised from 4.9 to 8.9% of the total serum proteins in these cases of rheumatoid arthritis. This "T" component, similar to the "X" component described in cirrhosis (7–9), is identical with the gamma₁ globulin in our cases. However, such elevations were present in only two of our 27 cases of rheumatoid arthritis and their elevations were only of very meager degree. The elevation of the gamma₁ component in human serum, although not pathognomonic, is highly characteristic of chronic liver disease.

What causes the gamma₁ elevation in cirrhosis? Has it any relation to the increase in total gamma globulin in this disease, the cause of which is conjectural? The intimate relation of antibodies and gamma globulin has long been known (11–15, 35–37). The increase in globulin in many infec-

tions consists not only in an elevation of specific antibodies identified by immunochemical methods but also in an increase that has been called "nonspecific antibody" or "reaction" globulin (38). Are these analogous to the gamma₁ and gamma₂ globulins? Animal experimentation has formed the basis for most of our knowledge of antibody formation. The antibodies of animals from various species immunized with different antigens are present in the globulin fraction having an electrophoretic mobility either of gamma globulin or between that of gamma and beta globulin. This latter component, not present normally in animals (6, 11–15), has been designated beta₂ by Kekwick and Record (15) and as "T" component by Van der Scheer and Wyckoff (12, 14). It has different physical and chemical properties from the classical gamma globulin and apparently is identical with the globulin fraction in normal human serum designated gamma₁ by Deutsch and associates (6) who showed that in normal persons this fraction was associated with a variety of antibodies. This gamma₁ globulin which was similarly found present in our normal subjects was elevated in most of our cirrhotic cases. Can the gamma₁ elevation in chronic liver disease be attributed to antibody formation?

The gamma₁ elevations in our subjects were most marked in the chronic liver cases. Elevations in the obstructive jaundice group may have been due to the secondary parenchymal damage revealed by liver function studies in these cases. It is impossible to draw any conclusions from only three cases. The elevation of the gamma₁ globulin component in chronic liver disease and the slight elevation in acute types of hepatic damage might appear to suggest typical antibody response such as is seen in infections in which antibodies increase as the condition becomes chronic. However, previous studies (39) have shown that scarring following viral hepatitis may occur with decreasing concentrations of electrophoretically separated gamma globulin. Viral hepatitis, the only hepatic condition in this study definitely known to be infectious, strangely enough showed little rise in the gamma₁ fraction. On the other hand, the cases of cirrhosis of the Laennec's type in which an infectious agent is not usually implicated showed the greatest elevation of gamma₁ globulin. We compared the gamma₁ globulin in cases of alcoholic cirrhosis (in which malnutrition was a major factor)

with that found in several cases of cirrhosis of the Hanot and cholangiolitic types presumed to be secondary to some form of infection such as viral hepatitis. There was essentially no difference in gamma₁ alteration in the "malnutrition" and "infectious" types of cirrhosis. Malnutrition experiments in humans (40, 41) and animals (42) have shown that malnutrition is not responsible for any rise in gamma globulin. We must therefore look to some other cause than infection or malnutrition for the elevation of the gamma globulin and particularly the gamma₁ portion which in hyperimmunized animals (11–15) and normal humans (6) is rich in antibodies.

Certain features of the cirrhotic state itself lend support to a possible relation between gamma globulin increase and antibody formation in cirrhosis. Cirrhosis is frequently featured by an increased mesenchymal reaction. Increase in lymphocytes and plasma cell elements (43) may be striking in the bone marrow (44). The degree of plasma cell and lymphocyte hyperplasia correlates roughly with the degree of hyperglobulinemia (44, 45). Berlin and his co-workers (46) could not confirm these findings. They state, however, that where hyperglobulinemia exists without plasmacytosis in the bone marrow, other sites of proliferation of plasma cells must be considered, especially the liver and spleen or specific sites of chronic infection. They state that it is possible that globulins may be formed at several different sites, namely, in plasma cells, lymphocytes and reticulo-endothelial cells. Histiocytic proliferation in the liver likewise has been reported to be related to a reversal in the albumin-globulin ratio (47). Pulp hyperplasia, consisting largely of proliferation of active histiocytes in the lumen of the sinuses (48), is frequently a factor in the splenomegaly of cirrhosis. Bjørneboe and Gormsen (49) immunized rabbits with polyvalent vaccine which resulted in plasma cell proliferation in many organs, especially in the spleen and liver; the degree of this plasma cell proliferation closely corresponded to the antibody concentrations. They believed that the antibodies were formed by plasma cells and the increase in splenic weight after intensive immunization was due to the plasma cell proliferation in the spleen. The relation between hyperglobulinemia and plasma cell proliferation and the possible role of the histiocytes as globulin producers has been mentioned by other authors

(50–53). The hyperglobulinemia may well be related to the histiocytosis and plasma cell activity frequently found in the liver, spleen and bone marrow in cirrhosis.

Since infections have not been proved as a causative factor in most cases of Laennec's cirrhosis, we must look for some other cause of the increased mesenchymal and globulin reactions in the cirrhotic state. Marked increases in plasma gamma globulin occur frequently in heterogenous diseases having in common extensive tissue breakdown such as in chronic suppurative infections (5). Gutman believes that the mechanism here may be homologous tissue antibody formation. It is conceivable that liver cell breakdown products of a protein nature act as an autogenous antigen causing a mesenchymal proliferation in the liver, spleen and bone marrow with a consequent or concomitant rise in gamma globulin. Necrosis of parenchymal cells may lead to fibrous tissue proliferations seen in cirrhosis (54).

The reason for the more frequent and greater elevation of gamma₁ globulin in chronic liver disease as compared with other diseases may be the damaged liver's inability to metabolize protein-breakdown products. This allows them to circulate and possibly act as autogenous antigens.

We have described the marked increase of gamma₁ globulin in cirrhosis and have hypothesized its nature and the reason for its presence in chronic liver disease. The gamma₁ globulin component is normally present in human blood and has been shown by Deutsch to have antibody activity (6). In addition, many workers have definitely demonstrated in animals an analogous protein component which is not present normally, but arises only after hyperimmunization with various bacterial antigens. Thus gamma₁ globulin in normal man and in hyperimmunized animals has been shown to have antibody properties. There is a possibility that circulating protein breakdown products, not metabolized by the diseased liver, act as an autogenous antigen and are responsible for the increased histiocytic plasma cell activity and increased gamma₁ globulin content frequently found in the cirrhotic state.

Realizing that this report has raised more questions than it has solved, we nevertheless believe that the approach to the complex problem of gamma globulin alterations in liver disease is promising

and warrants further investigation using electrophoretic and immunochemical methods.

SUMMARY

1. Electrophoretic determinations were performed on the sera of 241 persons, including 18 normal, 56 patients with various liver diseases and 167 patients with miscellaneous diseases.

2. There were marked elevations of a gamma$_1$ component between the commonly described gamma and beta globulin in the electrophoretic pattern in cirrhosis, and smaller elevations in other liver disease.

3. Average values in conditions other than liver disease were within the normal range.

4. The significance of this gamma$_1$ elevation in chronic liver disease and its possible relation to antibody formation are discussed.

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