

PLASMA ESTERASE ACTIVITY IN PATIENTS WITH LIVER DISEASE AND THE NEPHROTIC SYNDROME

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An increasing amount of evidence has been accumulated (1-3) which indicates that the albumin of the plasma is formed in the liver. The fact that the degree of albumin depression correlates well with the severity of liver involvement in patients with various types of liver disease (4) is strong evidence in favor of such a concept. In view of the significance of the metabolic and oncotic effects of the albumin level of the plasma in determining the course of patients with various types of hypoproteinemia, it would appear to be of special importance to obtain information regarding albumin synthesis in these patients in an attempt to discover possible remediable defects. Direct studies of albumin turnover have yielded some information, but the picture in cirrhosis and nephrosis is often obscured by continued loss of albumin in ascitic fluid or urine. The therapeutic use of concentrated solutions of human albumin has made it even more difficult to determine the rate of production of albumin and to evaluate the changes brought about by such therapy. The use of radioactive and isotope techniques has not as yet been feasible. Two other plasma proteins, fibrinogen and prothrombin, also have been shown to be formed by the liver (5, 6). To this list it is now possible to add a fourth protein appearing in the plasma and synthesized by the liver, namely plasma esterase.

The possibility arose that, through a study in various hypoproteinemic states of the other proteins synthesized by the liver and their changes during therapy, information could be obtained regarding the general formation of proteins by the liver which might be applicable to the problem of albumin synthesis.

Of the group of liver proteins the plasma esterase lends itself most readily to accurate estimation. In addition, the regeneration of this enzyme can be studied in various pathological states, following its destruction by a parenteral injection of diisopropyl fluorophosphate (DFP). This material is an extremely powerful and specific inhibitor of the esterase group of enzymes. Several observers (7, 8) have demonstrated conclusively that these enzymes are irreversibly destroyed both *in vitro* and *in vivo* by DFP. Comroe, Todd, and Koelle (8), in connection with their use of this material in patients with myasthenia gravis, studied the regeneration of plasma esterase following its destruction by DFP. Wescoe (9) and Grob (10) have independently applied this technique to the study of patients with liver disease.

A decreased rate of formation of the enzyme was noted. These results correlated with the fact that the level of this enzyme in the plasma is markedly depressed in patients with involvement of the liver (11, 12). Other workers (13, 14) have demonstrated a high concentration of a non-specific esterase in the liver, similar to that found in plasma. The amount of this enzyme in the liver is depressed following experimental liver injury (13). These observations and others (15, 16) demonstrate quite conclusively that this enzyme is formed in the liver.

A close correlation between the plasma albumin level and the esterase activity in various pathological states was pointed out by Faber (11). Such a correlation did not exist in various proteinurias in which he found the plasma albumin to be depressed considerably more than the esterase (17). He offered as explanation that the amount of esterase lost in the urine in such patients, relative to albumin, was small and out of proportion to the plasma albumin-esterase ratio.

The present report represents a further study of the relationship between plasma albumin and esterase in patients with liver disease, with special emphasis on the effects of albumin therapy on the formation of these plasma components. In addition, the regeneration of this enzyme under various conditions in patients with the nephrotic syndrome was investigated and a comparison was attempted with the findings in the presence of a damaged liver. An indication of a defect in esterase synthesis in certain patients with the nephrotic syndrome was observed which was quite different from the defect found in patients with liver disease.

Materials and Methods

Data reported in this communication were obtained from thirty patients with infectious hepatitis, twenty patients with cirrhosis of the liver, and ten patients with the nephrotic syndrome who were admitted to the Hospital or to the Out Patient Department of the Rockefeller Institute. The diagnosis of cirrhosis of the liver was clear from numerous liver function studies in addition to either the presence of ascites or direct visualization of biopsy sections of the liver. The ages of the patients with cirrhosis of the liver ranged from 12 to 60. The patients with the nephrotic syndrome all showed marked albuminuria, edema, ascites, and plasma albumin concentration below 2 gm. per cent. None of the patients showed a true lipid nephrosis. They all showed evidence of glomerulonephritis at some time in their course. Their ages were all below 14. The normal controls included fourteen adults and six children below 12 years of age.

Plasma esterase was estimated by manometric measurement of the CO_2 released from acetylcholine in bicarbonate buffer at pH 7.6. From 0.1 to 0.3 cc. plasma was diluted to 3 cc. with a buffer containing 0.03 M NaHCO_3 , 0.12 M NaCl , and 0.04 M MgCl_2 . This concentration of NaHCO_3 in the presence of 5 per cent CO_2 (in the gas phase) yields a pH of 7.6. The 3 cc. of diluted plasma was placed in the main compartment of Warburg flasks and acetylcholine chloride or bromide (0.5 cc. of 2.5 per cent solution in H_2O) was placed in the side arm. The flasks were gassed with 5 per cent CO_2 in O_2 or N_2 and allowed to equilibrate at 38°C . before mixing substrate

with plasma. After mixing, readings were taken every 5 minutes for 30 minutes and corrected for the change occurring in substrate and buffer alone. The results were expressed in terms of cubic millimeters of CO₂ released per 1 cc. of plasma per minute. Tributyrin also was used as substrate. Inhibition experiments demonstrated that the enzyme in the plasma splitting this substrate was inactivated by DFP and prostigmine in a manner completely similar to the enzyme-splitting acetylcholine. In addition, the curve of regeneration of enzyme activity following DFP inactivation *in vivo* was the same with the two different substrates. It appeared that the same enzyme was measured in each case. The non-specific nature of plasma esterase was clear. Acetylcholine was used as substrate in the following experiments because it was hydrolyzed somewhat more readily and more accurate results could be obtained.

The enzyme was found to be extremely stable, and sera which had been stored for 2 years at 0°C. showed the same activity that had been found initially. Either serum or plasma could be used. No evidence of inhibitors of the enzyme in the plasma was ever found. Bile salts and pigments did not inhibit the enzyme in concentrations that exist in plasma. Patients with cirrhosis of the liver with or without jaundice showed equally great depression of esterase activity.

A preparation of DFP in peanut oil was used for the *in vivo* destruction of plasma esterase. 0.04 mg. per kilo of body weight was administered intramuscularly. Plasma esterase was determined the day following administration and at periodic intervals thereafter. The concentrated human serum albumin used therapeutically showed no esterase activity.

EXPERIMENTAL

The regeneration of plasma esterase following administration of DFP was studied in five individuals, two with the nephrotic syndrome, two with cirrhosis, and one normal (Fig. 1). It was apparent that the rate of regeneration of the enzyme differed markedly in these patients. The patients with cirrhosis showed a very slow formation of the enzyme, while those with the nephrotic syndrome synthesized the enzyme very rapidly. In each case the plasma esterase level approached the original concentration in approximately 30 days.

A close relationship appeared to exist between the rate of regeneration and the initial level of the enzyme in the plasma. It seemed as if the level of the enzyme in the plasma reflected its rate of formation. To test this hypothesis the absolute values used in constructing the widely divergent regeneration curves of Fig. 1 were expressed in terms of percentage of the initial concentration of the enzyme. When these figures were plotted against time (Fig. 2), very similar curves were obtained for each of the five patients. In other words, the percentage regeneration of the enzyme was the same; the wide difference in the absolute rate of regeneration in the patients was evident from the initial level of the enzyme in the plasma. The conclusion that the concentration of enzyme appearing in the plasma at any one time is a reflection

of the rate of formation of the enzyme seems justified. The major information regarding the synthesis of the enzyme can be obtained from its concentration

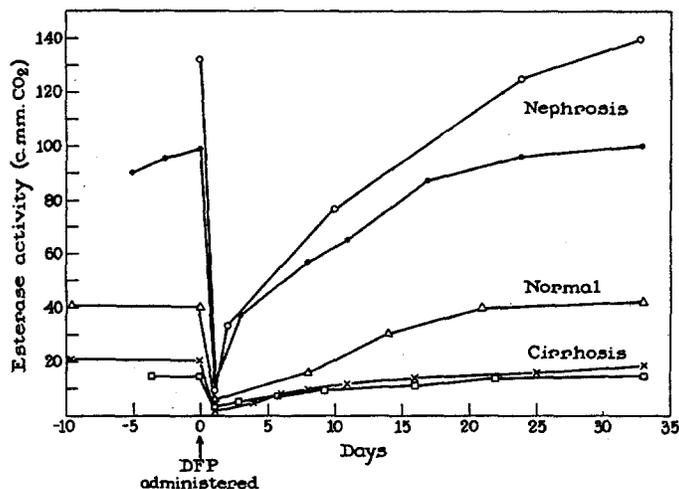


FIG. 1. Curves showing the regeneration of plasma esterase following the administration of DFP in five individuals, two with the nephrotic syndrome, two with cirrhosis of the liver, and one normal.

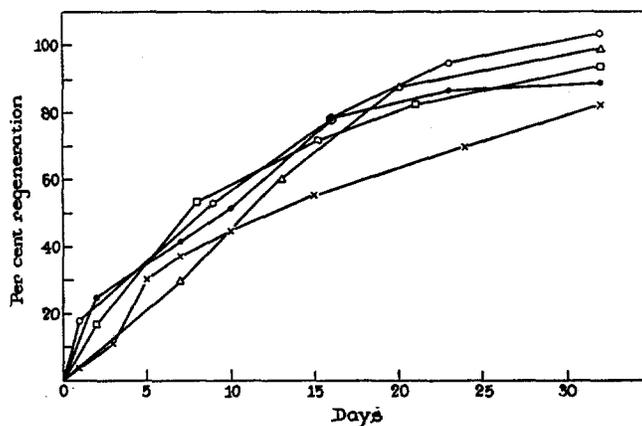


FIG. 2. Curves showing the plasma esterase regeneration following DFP with values expressed in terms of percentage of the initial esterase concentration in the same five individuals shown in Fig. 1.

in the plasma, and the difficult procedure of observing regeneration following DFP is unnecessary.

Plasma Esterase and Albumin Concentrations in Patients with Acute In-

fectious Hepatitis.—The relation of the esterase level to the albumin concentration of the plasma was studied in thirty patients with acute infectious hepatitis. This disease offered the ideal opportunity for investigating the aberrations following acute liver damage. Forty-four per cent of this group showed an albumin level below 4 gm. per cent which returned to normal late in convalescence. All of the patients showing abnormalities in the amount of plasma albumin also showed lowered esterase values (Table I). The degree of lowering was somewhat difficult to determine because the esterase values tended to rise above normal during convalescence, and in order to determine the normal for each individual it was necessary to obtain values several months after the illness had terminated. The wide variation of esterase concentration in healthy individuals (40 to 90 c.mm. CO₂) made it essential to obtain the normal for each person. A depression of less than 10 c.mm. CO₂ was not considered significant. Seventeen patients or 57 per cent of the thirty cases

TABLE I
Comparison of Plasma Esterase Depression in Infectious Hepatitis Patients with and without Abnormalities in Plasma Albumin

| | No. of patients | Per cent | Per cent showing esterase depression > 10 | Average maximal esterase depression c.mm. CO ₂ |
|---|-----------------|----------|---|--|
| Plasma albumin < 4 gm. per cent. | 13 | 43 | 100 | 28 |
| Plasma albumin > 4 gm. per cent (normal) | 17 | 57 | 46 | 18 |

showed no change in albumin concentration during the course of the disease. The esterase depression was considerably less in this group as shown in Table I.

These observations demonstrate that the plasma esterase is more readily depressed in the presence of liver damage than the albumin level but that some relationship does exist. The fact that the patients with a depression of plasma albumin were the most severely ill generally should be considered in evaluating this relationship. Of greater significance was the finding that the depression of albumin and esterase occurred at approximately the same period of the disease. Both showed a delayed fall at a time when the symptoms of the illness were subsiding but prior to the rise in the globulin level (18). Table II illustrates this relation in two typical cases.

Plasma Esterase Activity in Patients with Cirrhosis of the Liver and the Changes Occurring with Therapy.—In patients with cirrhosis of the liver the esterase depression was much more marked than in those with infectious hepatitis and the concentration was always below the normal range of 40 to 90 c.mm. CO₂. Table III illustrates the results in eighteen patients with cirrhosis of the liver with edema and ascites. It is apparent that the patients with the lowest

albumin levels also had the most marked depression of the esterase concentration. Serial determinations of plasma esterase over long periods of time in these patients demonstrated that the esterase levels remained very constant. The patients often improved markedly on various types of therapy without any significant change in esterase activity. It was only in those few patients who regained the ability to synthesize normal amounts of plasma albumin

TABLE II
Comparison of Serial Determinations of Plasma Esterase and Albumin in Two Patients with Infectious Hepatitis

| Day of disease | Plasma esterase | Plasma albumin | Day of disease | Plasma esterase | Plasma albumin |
|----------------|-----------------------------|---------------------|----------------|-----------------------------|---------------------|
| | <i>c.mm. CO₂</i> | <i>gm. per cent</i> | | <i>c.mm. CO₂</i> | <i>gm. per cent</i> |
| 10 days before | 68 | 4.1 | 2 | 60 | 4.0 |
| 2 | 70 | 4.3 | 3 | 63 | 4.2 |
| 5 | 52 | 3.6 | 6 | 41 | 3.5 |
| 7 | 50 | 3.7 | 10 | 32 | 3.5 |
| 12 | 68 | 4.0 | 18 | 54 | 4.0 |
| 19 | 76 | 4.2 | 25 | 74 | 4.2 |
| 27 | 75 | 4.1 | 90 | 64 | 4.1 |
| 34 | 80 | 4.1 | | | |
| 56 | 72 | 4.3 | | | |

TABLE III
Comparative Results of Plasma Esterase Determinations in Patients with Cirrhosis of the Liver with Different Concentrations of Serum Albumin (All Had Ascites and Edema)

| | No. of patients | Albumin level | Average esterase level |
|--------------|-----------------|---------------------|-----------------------------|
| | | <i>gm. per cent</i> | <i>c.mm. CO₂</i> |
| Group A..... | 13 | 2-3 | 22 |
| Group B..... | 5 | 1-2 | 13 |

that a rise in esterase occurred. Fig. 3 illustrates the results of serial determinations of serum albumin and esterase over a period of 18 months in a patient with cirrhosis of the liver. The patient received large amounts of albumin intravenously, which brought about marked clinical improvement with loss of ascites and edema after approximately 4 months of therapy. However, he was unable to synthesize sufficient albumin to preserve permanently the normal serum albumin level provided by the injections. The plasma esterase concentration remained constant throughout this period. After approximately 12 months of continued general improvement, during which time he put on considerable body weight, he finally regained the capacity to maintain a

normal plasma albumin level. At precisely the same time he demonstrated the capacity to synthesize larger amounts of plasma esterase. Following prolonged supportive therapy, the function of the liver finally improved sufficiently to enable it to synthesize significant amounts of these two proteins. Two other patients with cirrhosis of the liver who were treated with intravenous liver extract for prolonged periods also regained their ability to preserve a normal plasma albumin level as well as a normal esterase level. Ten patients with cirrhosis of the liver who have not regained the ability to form albumin in a normal manner have shown no rise in plasma esterase although considerable clinical improvement was evident in the majority. In every case the serum esterase remained abnormal as long as a defect in albumin synthesis persisted.

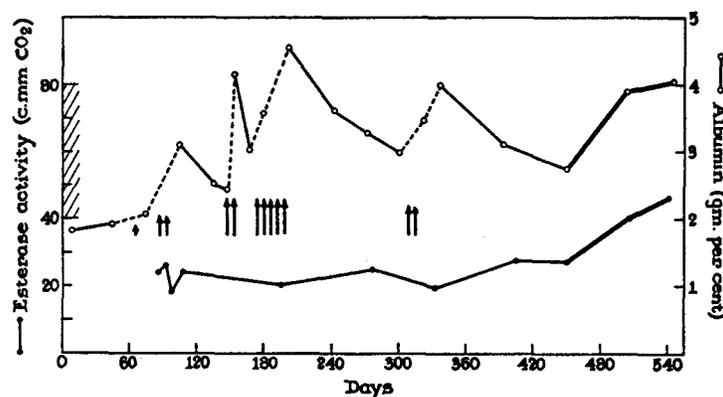


FIG. 3. Curves showing the influence of long continued intravenous administrations of concentrated human serum albumin on the plasma albumin and esterase levels in a patient with cirrhosis of the liver.

Plasma Esterase Activity in Patients with the Nephrotic Syndrome.—In direct contrast to the findings in patients with liver disease, the esterase levels in patients with the nephrotic syndrome usually are higher than normal. Table IV shows the average results from ten patients. For purposes of comparison the results from normal individuals and patients with cirrhosis of the liver are added. Fig. 1 illustrates the rapid regeneration of this enzyme in two patients with the nephrotic syndrome.

In order to obtain information as to the reasons for the high values for plasma esterase in the nephrotic syndrome, serial determinations were carried out over prolonged periods of time in such patients and variations during different phases of the disease were studied. Fig. 4 illustrates the results of serial determinations of plasma esterase over a period of 3 months in two children with severe nephrosis.

Both patients had marked edema and ascites with severe albuminuria and a serum albumin below 1 gm. per cent. Neither patient received any specific therapy. In each case the esterase values persisted above normal as long as the patients were followed. Patient 1 showed no evidence of improvement in his general condition

TABLE IV
Comparative Amounts of Plasma Esterase in Patients with the Nephrotic Syndrome, Cirrhosis of the Liver, and in Normal Controls

| | No. of cases | Highest value | Lowest value | Average value |
|----------------------|--------------|---------------|--------------|---------------|
| Nephrosis..... | 10 | 180 | 45 | 110 |
| Cirrhosis..... | 18 | 30 | 6 | 19 |
| Normal controls..... | 20 | 91 | 42 | 65 |

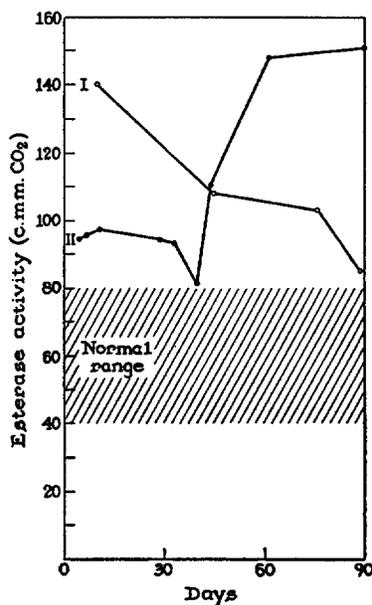


FIG. 4. Curves showing the results of serial determinations of plasma esterase in two children with the nephrotic syndrome. Patient 1 died, while Patient 2 developed a spontaneous remission.

and following a series of secondary infections finally died. His esterase values showed a gradual decline reaching a minimum at the time of death. Patient 2 also showed little change at first but suddenly, after approximately 3 months in the hospital, he developed a diuresis with loss of 10 kilos of body weight and showed a gradual rise in his plasma albumin level. This improvement was accompanied by a rise in his plasma esterase which closely paralleled the rise in plasma albumin.

Similar increases of the enzyme also were observed in two other patients with nephrosis who had a spontaneous improvement in their general condition. The rise in esterase was associated with an increase in plasma albumin despite persistent albuminuria.

Effect of Albumin Therapy on the Esterase Level of the Plasma of Patients with the Nephrotic Syndrome.—In view of the rise of plasma esterase following a spontaneous remission in patients with nephrosis, it seemed advisable to ob-

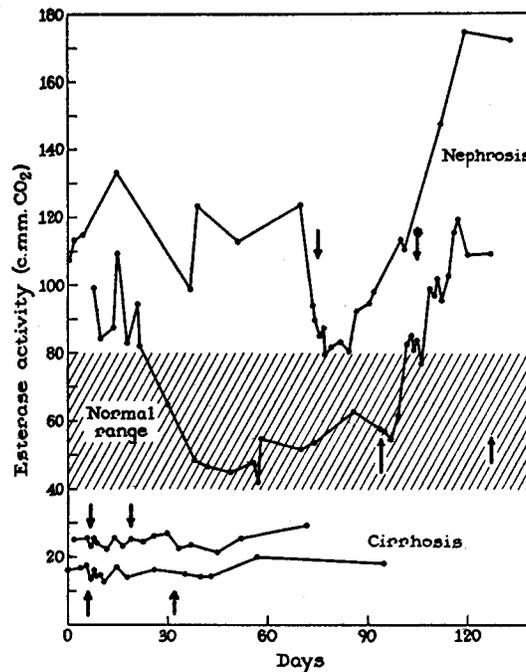


FIG. 5. Curves showing the effect of intravenous albumin administration on the plasma esterase level in two patients with the nephrotic syndrome and two patients with cirrhosis of the liver. The arrows indicate the beginning and end of therapy in each case.

serve the changes in the enzyme level produced by various types of therapy. Determinations were carried out serially in two patients with the nephrotic syndrome who had very low plasma albumin levels and who were treated intravenously for approximately 30 days with an average of twenty-four 25 gm. units of concentrated human albumin (Fig. 5). For purposes of comparison, the results in two patients with cirrhosis of the liver who were similarly treated are charted. The patients with the nephrotic syndrome had a striking rise in the concentration of esterase which began approximately 10 days after the onset of therapy. This was of special interest because the patients im-

proved considerably symptomatically with little other laboratory evidence of improvement. In contrast to the patients with the nephrotic syndrome, the patients with cirrhosis of the liver showed very little variation in the plasma esterase level despite clinical improvement.

DISCUSSION

The chief value of the technique of observing the regeneration of plasma esterase following destruction by DFP was to demonstrate that the level of the enzyme in the plasma reflects its rate of formation. Added significance may now be given to the concentration of the enzyme in the plasma in various disease states. The rapid regeneration of plasma esterase in patients with the nephrotic syndrome as compared with the very slow regeneration in patients with cirrhosis of the liver could be estimated as well by the determinations of the level of the enzyme in the plasma as by the time-consuming technique using DFP.

The low levels of plasma esterase in patients with severe liver disease appeared to reflect the inability of the liver to form normal amounts of this enzyme. The liver's ability to form another protein, albumin, was similarly affected. The delayed fall in these two proteins following acute liver damage also suggested a formation defect. The degree of depression was proportional to the severity of the liver damage. In patients with cirrhosis of the liver long sustained therapy is necessary before the liver regains the ability to synthesize normal amounts of albumin and esterase. The fact that the time necessary for this to occur was approximately the same for the two proteins is perhaps the best evidence of a similar formation. In these patients there was little doubt about the site of the defect in protein synthesis. The damaged liver is unable to perform this function adequately regardless of the availability of the essential materials necessary for protein production.

Numerous investigators have postulated a possible defect in protein synthesis by the liver in patients with the nephrotic syndrome (19-22). The frequent observation of low plasma albumin levels in certain patients losing an amount of albumin that the normal person could readily replace has led to the belief that the primary defect may lie in the formation of albumin. The application of the usual liver function tests, however, has not revealed any abnormality of the liver in these patients (23). Moreover, since the formation of plasma esterase is dependent on the condition of the liver, the high values found in patients with the nephrotic syndrome suggest that the liver is performing this function in a hypernormal fashion. Although this evidence argues against a primary hepatic defect in protein formation in nephrosis, it is of interest that albumin replacement therapy leads to rises in esterase values to even higher levels, as is shown also during spontaneous remissions.

It seems possible that the hypernormal levels of plasma esterase in nephrosis

reflect a general response on the part of the liver to regenerate protein more rapidly as a result of the loss of albumin in the urine. Further evidence for this hypothesis can be found in the high concentrations of fibrinogen found in the plasma of these patients (24). As with plasma esterase, fibrinogen is synthesized by the liver and is depressed in patients with severe liver involvement. Electrophoretic studies have shown that certain of the alpha and beta globulins may rise in patients with nephrosis (25). It is not known whether all of these proteins are synthesized in the liver. Immunological studies have also revealed high concentrations of unusual proteins precipitating with the albumin fraction of the serum of these patients. On the other hand, the gamma globulins which are generally considered to be formed independently of the liver are actually depressed (25); in liver disease they are increased (26). It would appear, therefore, that in patients with nephrosis the liver is functioning well and is stimulated by the albumin loss to form unusual amounts of the proteins which it synthesizes. The question whether some of the stimulus comes as a response to furnish proteins for the maintenance of the oncotic pressure of the blood in the absence of albumin cannot be answered from the data at hand.

The rapid rise in plasma esterase concentration following spontaneous remissions of the nephrotic syndrome or following therapy with albumin, when contrasted with the difficult alterability of the enzyme concentration in patients with cirrhosis, demonstrates quite strikingly the extreme difference between these two disease states. The fact that a rise in esterase concentration does occur in certain patients with the nephrotic syndrome demonstrates that a defect in esterase synthesis may be present. This defect would appear to be very different from that existing in patients with cirrhosis of the liver. The rapidity of the esterase change following albumin therapy in patients with the nephrotic syndrome strongly suggests that the albumin supplies some factor which in the presence of an adequately functioning liver brings about increased synthesis of the enzyme. The essential factor may very well be albumin itself.

The defect in esterase formation may be similar to that which is thought to exist in the synthesis of albumin in the severely ill nephrotic patient. Since the synthesis of albumin is obscured by the constant loss of albumin in the urine, study of the plasma esterase system yields information concerning a similar protein, not lost in the urine to a similar extent, but the synthesis of which is altered by the conditions underlying the nephrotic syndrome. It may be possible by this means to define the exact defect in protein formation that exists in these patients.

The studies here reported appear to indicate that the liver is not primarily at fault but that certain essential materials which the liver needs for the synthesis of proteins in adequate amounts are lacking in certain patients with the

nephrotic syndrome. If these are supplied, the liver can readily form the necessary protein. Evidence was presented that albumin administration aids in the synthesis of esterase by the liver. It may well be that a certain amount of albumin must also be present in the blood and tissues for proper synthesis of albumin by the liver.

SUMMARY

By a study of plasma esterase in various hypoproteinemic states information was gained concerning the synthesis of a protein by the liver, which may be applicable to the problem of albumin synthesis. Patients with infectious hepatitis and cirrhosis showed defective formation of plasma esterase that paralleled the defect in albumin formation. The defect could only be altered in patients with cirrhosis by very prolonged therapy indicating that liver function itself had to improve before the proteins could be formed in a normal manner. Patients with the nephrotic syndrome showed a normal or hyper-normal formation of plasma esterase. Following spontaneous remissions or the administration of albumin the esterase level showed a marked rise which was in direct contrast to the difficult alterability of the enzyme level in patients with severe liver involvement. It is suggested that the defect in protein synthesis by patients with the nephrotic syndrome may be due to the lack of certain essential materials, one of which may be albumin itself, rather than to any abnormality in the liver.

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