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Nature of the serum proteins in the nephrotic syndrome

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THE NATURE OF THE SERUM PROTEINS IN THE NEPHROTIC SYNDROME

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INTRODUCTION

The role of the plasma proteins in disease processes has long been a source of conjecture, theorizing and experimentation which has not proved too fruitful. This has primarily been due to the fact that investigation of the blood proteins in disease is handicapped by lack of clinically applicable methods for resolving the complex system of proteins present in the blood stream into homogenous components. The only fractionation possible has been into groups of proteins having like solubility characteristics, or electrophoretic mobilities, or sedimentation constants, or other groupings based upon physical or chemical properties. Too little has been learned of the biological significance of these important constituents of the blood. However, the above methods, if rigidly standardized, give reproducible results, and in certain diseases, characteristic divergences from the normal patterns. This information, if properly correlated with clinical and pathological observations, may lead to important advances in the knowledge of the nature of disease processes.

In the case of Bright's disease the changes in the plasma proteins and their relationship to the

urinary proteins has long been of special interest. One has only to consider the long controversy over the etiology of edema in order to realize the major role played by the blood proteins in the study of this disease, or collection of diseases. Although, in the early part of the nineteenth century, Darwin (19) opposed "anyone who supposed this coagulable urine was separated from the blood by the kidneys", Blackall (9) a little later expressed the more popular state of mind when he said, "Whether the blood is presented for secretion in a vitiated state or the urinary organs themselves perform their office imperfectly is at present not well ascertained."

Before the time of Bright it was generally assumed that the cause of albuminuria could be found in a condition of the blood (hematogenous albuminuria). Just what this condition was, was not understood.

Richard Bright (13), however, did not lose sight of the problem of the blood in nephritis even though he formulated the idea of nephrogenous albuminuria, in which the kidney is thought to constitute the determining factor. He chose, rather, to leave the matter to further investigation. In certain cases which Bright examined he found that the albumin in

dropsical urine was precisely similar to the albumin in the serum of the blood in so far as coagulation by heat and reaction to certain chemical reagants was concerned. Upon investigating other samples of dropsical serum, he found that the serum was occasionally turbid, and upon standing for twenty-four hours a creamy substance rose to the surface, but he could not detect any oily matter in it. He felt that the serum in these cases contained less albumin than in health, although he was not able to state precisely the amount of this difference. In still other samples of dropsical serum he found that the specific gravity was almost exactly the same with that of the urine, being no more than 1.013. Upon heating it was converted into a mass having a consistency scarcely as dense as that of the coagulated urine. He concluded that this blood showed a marked deficiency of albumin.

The development of protein chemistry has been the signal for more exhaustive and conclusive research which has continued up to the present time. In the middle of the last century the "salting-out" methods of Hammarsten and Hofmeister (28, 30) led to a systematic attack upon the blood and urinary proteins. There have been various modifications

of this method and its use has been extensive, as will be seen later. Experimental work based upon osmotic pressure studies, immunological studies, variation in amino acid content of proteins, ultracentrifuge, electrophoresis, have contributed the knowledge of the blood proteins in normal and pathological states.

It will be the purpose of this thesis to present what is known at present about the nature and importance of the plasma proteins in the nephrotic syndrome, limiting this term to chronic glomerular nephritis and true lipoid nephrosis.

THE NEPHROTIC SYNDROME

Briefly the nephrotic syndrome may be defined as a pathological state, characterized by generalized edema, in which the low protein content of the edema fluid is its most characteristic single feature. With this definition as a basis, the following conditions may be included: the nephrotic phases of Bright's disease (including the nephrotic stage of chronic glomerular nephritis and lipoid nephrosis), nephropathies produced by poisons such as corrosive sublimate, uranium, and chromates, and amyloid disease of the

kidney. Berglund, Longcope, and Richards (8) also include the nutritional edemas, the anasarcas seen in cachectic states of various origins, in pernicious anemia, in pregnancy, beri-beri, in the complicated nutritional anemias common in India and China, in undernourished diabetics, in patients with severe diarrheas or short-circuited alimentary canal with impaired absorption, and in the nutritional mal-adjustments occurring during infancy and early childhood.

In this paper, only the findings in the cases of Bright's disease are to be considered.

The etiology of the edema in the nephrotic syndrome of Bright's disease has long been a source of controversy. Bright, (13), observing the quantitative significance of the proteinuria and the probable decrease in the plasma proteins assumed that the thin, watery or "hydremic" condition of the blood plasma made it more easily filtrable into the tissue spaces. He did not explain the fact that the return of fluid from the tissue spaces into the blood was apparently not related to the altered composition of the blood.

Stewart (60) and Bartels (5) supported Bright's theory of hydremic plethora, but promulgated the

idea that an increase in vascular pressure existed due to insufficient elimination of water by the kidneys.

Bright's ideas prevailed until 1895. At this time Senator (57) introduced the idea that capillary damage in renal disease extended to the cutaneous, peritoneal and other capillaries instead of to the glomerular capillaries alone. He assumed that the capillary damage was due to toxic agents. Rees , according to Berglund (8), attributed edema to toxic dyscrasia, with the kidney ceasing to excrete injurious substances.

Some sixty years after Bright, Starling (58) came forth with the theory that the escape of fluid from the vascular system into the tissues was due to changes in the colloid osmotic pressure of the blood. A few years previous to this, Csatasy (according to Kirk (34)) had noted a decrease in plasma proteins occuring in some patients with nephritis. He also noted the low albumin content of the serum.

In 1901 Achard (1) showed that production of edema and its severity varied with salt intake.

In 1910 Fischer (24) explained edema on the basis of colloidal chemical changes in the tissues. He thought the cause of the edema resided in the tissues

themselves with changes which increased swelling capacity of the tissues, probably due to an abnormal accumulation of acid products. He maintained that the edematous fluid in serous cavities was due to a squeezing off effect of proteins in the tissue.

Achard, Ribot and Leblanc (2) noted a change in the ability of the body fluids and tissues to hold water in relation to the lipoids. They based this on the observations of Mayer and Schaeffer, who found that the ability of the tissues to absorb water was proportional to their lipocytic index, expressed by the ratio of cholesterol to fatty acids.

About 1929 Elwyn (20) brought forth the hypothesis that edema formation was due to central nervous system control of water balance.

Today the issue is still unsettled. It is generally conceded that a multiplicity of factors contributes to the formation of nephrotic edema. The truth of this is probably best shown by the fact that therapeutic measures designed to correct but one of the factors have been unsuccessful.

Nevertheless it must be conceded that changes in the plasma proteins, both qualitative and quantitative, play an important role in the production of nephrotic

edema, and are deserving of further investigation.

NEPHROTIC GLOMERULAR NEPHRITIS AND LIPOID NEPHROSIS

Every student of Bright's disease constructs his own classification to meet his own individual needs. For this reason the nomenclature has been most confusing. In the case of the nephrotic stage of chronic glomerular nephritis and lipoid nephrosis we see two disease processes in which certain clinical and pathological differences have been established, but which exhibit so many similarities that their existence as clinical entities instead of variations in the manifestations of one process has long been debated.

The nephrotic stage of chronic glomerular nephritis is marked by the prominence of edema, accompanied by the other symptoms and signs of chronic glomerular nephritis--weakness, dyspnea on exertion, hypertension, and albuminuria and casts, with no marked impairment of renal function. The course of the disease is usually characterized by exacerbations and remissions. The exacerbations are brought on by colds, sore throat and other infections, and result in aggravation of all the symptoms. During remissions the patient improves, sometimes to the point of complete

disappearance of albuminuria. Renal function becomes progressively poorer during the course of the disease.

Hypertension is nearly always present, but may be absent in mild cases. The systolic blood-pressure usually ranges between 140 and 180 mm.Hg.. It is not often above 200 mm.Hg..

The kidneys in chronic glomerulonephritis are of two types, contracted and non-contracted. The latter are similar to those described in lipoid nephrosis. In general, the smaller kidneys are found in the cases of longer duration. The capsules are adherent firmly, and the external surfaces are finely pitted. On section the cortices are much thinner than normal and are indistinctly demarcated from the pyramids. The cortices usually have a yellowish tinge.

Microscopically, a large percentage of the glomeruli are hyaline, in some instances as high as ninety per cent. The tubules associated with such glomeruli have either disappeared entirely or they are represented by small epithelial cords. The areas previously occupied by tubules are shrunken and contracted, and the surface of the kidney over such areas is depressed, giving the pitted appearance. The increase of fibrous tissue in the atrophic areas is largely relative.

The tubules that have not undergone atrophy are associated with glomeruli that are enlarged, but not hyaline. These glomeruli show an increase of capillary endothelium, but the capillaries are not completely obstructed.

Only an occasional case of chronic glomerulonephritis begins as a typical acute clinical case, according to Berglund (8). The most convincing evidence that the disease is inflammatory in origin is the histological structure of the glomerular lesions. There is a definite relationship between the lesions of the acute and chronic stages.

The blood changes in the nephrotic stage of chronic glomerular nephritis are those commonly associated with nephrosis, namely high blood cholesterol, lowered serum albumin, and a reversal of the ordinary albumin-globulin ratio. There is no retention of urea or other nitrogenous substances. Boyd (12) says, "It is evident that this stage may be called the nephrotic phase of glomerulonephritis, for the clinical symptoms and the blood and urinary findings are identical with those of that somewhat misty entity."

According to Leiter (36) nephrosis began as

a point of view, which has since been transformed into a clinical entity. He states that Virchow first introduced nephrosis as a parenchymatous inflammation of the kidney. This explanation was inadequate and was replaced by concepts of exudation and proliferation in the vascular and supporting systems.

In 1905 Müller (45) suggested the term "nephrosis" for purely degenerative lesions of the kidney, meaning the tubular nephropathies and not the degenerative changes of arteriolar and arteriosclerosis. Müller, however, recognized that no clinical differentiation could be made.

Ten years later the term reached a clinical footing with Volhard and Fahr's (62) division of bilateral hematogenous renal diseases into genuine nephrosis, nephritis, and nephrosclerosis.

Bennett (7) summarized the views of Munk (46), a contemporary of Volhard and Fahr, as follows:

"According to the conception of Munk, all renal disease with oedema, apart from heart failure, should be classed as nephrosis, whilst cases with haematuria, uraemia, and cardio-vascular complications constitute true nephritis. When a water logged patient gets an attack of haematuria, and the blood-pressure rises

and nitrogen retention appears, it is nephritis complicating nephrosis; when, on the other hand, a patient after a febrile haematuria with transient hyperpiesis passes into a condition of anasarca, that is nephrosis supervening upon nephritis."

Epstein (21) introduced the term lipoid nephrosis into the United States in the early part of the present century.

Boyd (12) describes what he says the clinical picture of a pure nephrosis is "supposed to be", according to present day concepts. The patient as a rule is young, often only a child. The principal changes are edema, marked albuminuria, a urine with a high specific gravity, abundant casts, and doubly refractive lipoid bodies, but no red blood cells ("for these indicate an inflammatory lesion"), normal blood-pressure, a moderate but progressive anemia, marked salt retention, normal blood urea and total non-protein nitrogen, a decrease in the serum albumin with inversion of the normal ratio of albumin to globulin, a great increase in the blood cholesterol, and a low basal metabolic-rate. The three most striking positive features are the edema, the albuminuria, and the hypercholesterolemia, which may reach as

High as eight hundred mg. per one hundred cc. The important negative features are the normal blood pressure and the normal blood urea. The disease is subject to remissions and exacerbations, and Berglund (8) states that according to published reports, fifty per cent of the cases recover ultimately, with the usual cause of death, if it occurs, due to peritonitis and other infections. He states that uremia does not develop.

When a patient with nephrosis dies in the early stage from intercurrent infection, the kidneys are found to be large and pale, presenting the picture of the "large white kidney". On section the cortex is swollen and pale, and yellow spots or streaks are often discernible. In some cases the organ may present the peculiar appearance known as the "myelin kidney". This presents a naked-eye picture of great deposits of a meylin or lipoid substance. Boyd says it is not peculiar to the nephrotic kidney, but is also found in the second stage of glomerulo-nephritis.

Microscopic examination shows the condition to be essentially a tubular lesion. The convoluted tubules are commonly dilated, and the greatly swollen epithelium is seen to contain large numbers of droplets.

These droplets are considered by some to consist of the neutral fat seen in ordinary fatty degeneration, and by others to be lipoid or fat-like in nature. According to the supporters of the concept of lipoid nephrosis as a separate entity, the glomeruli should be unaffected. Boyd (12) and Berglund (8) maintain that if special techniques are used and high magnification, changes will be noted in the glomeruli of the kidneys of nephrosis. These lesions consist of proliferation of the endothelium lining the capillaries of the tuft and a thickening of the basement membrane.

On the other hand, Murphy and Warfield (48) have demonstrated cases of nephrosis in which no glomerular lesion could be found after careful study.

Thus we see that while Boyd is openly antagonistic to the concept of nephrosis as a clinical entity, Leiter presents a lengthy and convincing argument that the disease actually does exist and is not a part of glomerular nephritis.

Addis and Oliver (3) prefer to divide Bright's disease into hemorrhagic, degenerative, and arteriosclerotic divisions and let the matter rest. Achard (1) believes that pure lipoid nephrosis exists,

but he admits that cases which present all the features which have been described as characteristic of the disease are rare.

Bennett (7) accepts the possibility of a primary pure nephrosis, and suggests that the kidney in this disease may undergo secondary changes resembling those of glomerular nephritis. Elwyn (20), on the other hand, believes that in all cases there is an initial glomerulo-nephritis which often heals, leaving tubal damage which has resulted from the absorption of the pathological glomerular transudate. Likewise, Berglund et al (8) think that it appears probable that definite glomerular alterations could have been demonstrated with appropriate technique in the cases which have been described as free from glomerular involvement.

So the controversy rages. But regardless of whether the nephrotic syndrome in Bright's disease is represented by one disease or two, the changes in the blood and urine remain essentially the same.

OSMOTIC PRESSURE OF THE BLOOD COLLOIDS AND BLOOD VOLUME IN THE NEPHROTIC SYNDROME

The pathological variations in the osmotic

pressure of the proteins are amongst the best known of those colloidal changes which play a part in the causation of nephrotic edema.

Von Jaksch (64) in 1893, using serum obtained by cupping, showed a dilution of blood in acute illnesses and diseases of the heart, lungs and kidneys.

Starling (58) in 1895, observed that the osmotic pressure of the proteins of the serum was about thirty to forty mm. Hg., and stated that whereas capillary pressure determines transudation, the osmotic pressure of the proteins of the serum determines absorption. Epstein (21) later, commenting on the well known fact of the decrease in the blood proteins in cases of hydropigenous nephritis, assumed that this must lead to a low protein osmotic pressure, and would, according to Starling's theory, tend to produce edema.

Krogh (35) estimated the protein osmotic pressure of the oxalated serum in a patient of Hagedorn, Rassmussen and Rehberg, and obtained the very low figure of 10 cc. of water, whereas the normal is about 45 to 47.

Govaerts (26) in 1923 and again in 1924, using a different technique, found the tension in normal

subjects to be from 35-40 cm. of water, 1 gm. of protein per 100 cc. of serum corresponding to a pressure of 4.61 cm. In most of the edematous patients, with the exception of those with a purely mechanical edema, the pressure was less than 30, and it fell as low as 12 in parenchymatous nephritis. He postulated that the fall in osmotic pressure was due not solely to the diminution of the blood proteins, but was due also to the fact that, in equal concentrations, these proteins give a lower pressure than those in normal blood. Schade and Claussen (56), working with citrated plasma, obtained similar results, what they term the oncotic pressure being 25 mm. Hg., or 33.75 cm. of water in the normal, falling to 14 cm. of water in hydropigenous nephritis. This fall they said, is dependent on the specific nature of the proteins and in this case is associated with a particularly low specific oncotic pressure.

Rusznyak (55), using a collodion sac, also found that the protein pressure per gram, which he called the osmotic pressure, was lower in edema, and attributed this to an abundance of fibrinogen. In searching for the factors which determine this lowering of the protein pressure, Govaerts (26)

found that there was a relation between the relative proportions of albumins and globulins, i.e. between the A:G ratio and the protein osmotic pressure. One gram of albumin has a pressure of 7.54 cm. of water, a gram of globulin only 1.95 cm. This is based on a molecular weight for serum albumin of 45,000, of pseudo-globulin 80,000, and of euglobulin 140,000, with one gram of albumin therefore containing many more molecules than one gram of globulin. Moreover, as the isoelectric point of albumin (pH--4.7) is further removed from the neutral point than globulins (pH--5.4) they are more dissociated when the pH is about seven, which is the approximate figure for blood. These two factors result in albumins having a greater osmotic pressure than globulins.

Von Farkas (64) confirmed this relation between the albumin quotient and the protein osmotic pressure by means of Schade's oncometer. He also found that the protein pressure is at its maximum in the region of the concentration of normal saline; that neither the non-protein nitrogen content nor the diminution in the alkali reserve exerted any influence, but that the addition of lecithin and cholesterol tended perhaps to lower the osmotic pressure. The A:G ratio of the

serum he found reduced in nephritis with edema and in nephrosis.

Govaerts (26) compared the albumin quotient in various types of dropsy, and found it to be normal in cases of well compensated heart disease, but lowered in cardiac patients with failure of compensation, and in nephritis with edema. He concluded that the inversion of the albumin quotient, a disturbance of nutrition having no evident connection with an alteration in renal function, exists, as shown by the study of valvular cardiopathies, before the appearance of edema.

In 1926, Darrow (18) declared that there is a tendency to blood concentration during the edematous stages of nephritis in children, and Brown and Rowntree (14), at about the same time detected no abnormalities in the serum volume in glomerular nephritis and nephrosis with edema.

In 1933, Muntweiler, Way, Binns and Myers (47) made a rather extensive study of plasma colloid osmotic pressure in pathological conditions, in which they correlated the changes of the plasma colloid osmotic pressure in conjunction with the changes of the plasma protein concentration in the course of

disease processes. Their technique employed the use of colloidion sacs, and the plasma employed was obtained under oil from an arm vein, before breakfast and without stasis. The dialyzing fluid consisted of naCl, KCl, $NaHCO_3$ and Sorensen's phosphate. They used sixteen normal subjects and forty-two hospital patients. On the normal patients they found that the plasma osmotic pressure averaged 32.5 mm. Hg., the total proteins averages 6.86 gm. per 100cc., the albumin averages 4.84 gm. and the A:G ratio was 2.58.

They found that in cases of renal disease with edema there is generally a definite lowering of both the serum protein content and the colloid osmotic pressure. The lowest colloid osmotic pressure observed was 8.1 cm. of plasma, accompanied by a total serum protein content of 3.70 gm.--obtained in a case of nephrosis with marked pitting edema and slight ascites. In general the changes in the serum protein and albumin content were paralleled by changes in the colloid osmotic pressure. Further, in cases of renal disease (not complicated by cardiac disease) edema was generally absent when the colloid osmotic pressure attained a value of twenty cm. of plasma or more. Exceptions to these were observed in two cases of acute

glomerular nephritis. One had marked pitting edema and slight ascites in the presence of a colloid osmotic pressure of 29.5 cm. plasma, a total serum protein content of 6.43 per cent and an albumin content of 3.64 gm. One case of acute exacerbation of chronic glomerular nephritis was clinically free from edema with a colloid osmotic pressure of 14.1 cm. plasma, a total serum protein of 4.68 gm. and an albumin of 1.74 gm.

In six cases in which edema disappeared with high protein diet and low salt intake, the colloid osmotic pressure and the serum protein content showed a definite increase with improvement in the clinical condition. However, five cases were clinically free from edema when the colloid osmotic pressure was below 20 cm. plasma. The tendency toward edema still remains, however, until values increased above critical levels. With return to normal plasma proteins, there is a parallel increase in plasma colloid osmotic pressure.

Ten years after Muntweiler et al, Kagan (33) stated that since edema is the only clinical sign of hypoproteinemia, and is present in only three per cent of the cases of hypoproteinemia, it is necessary to use laboratory procedures to detect most of the cases.

He did not define the critical level of plasma proteins necessary for the production of edema.

Peters (49) states that because of its smaller molecular size and other properties as yet not entirely understood, albumin exerts a far greater osmotic pressure than globulin. In all other respects, also, including its origin and function, albumin appears so distinct from globulin that they should be considered as independent variables. He believes, that in consideration of these facts, that the expression, A:G ratio, has no significance and can lead only to confusion. Serum albumin depletion by leakage into the urine or plasmaphoresis or by protein deficiency in the diet serves as a measure of malnutrition or protein starvation. On the other hand, globulin is little affected by these disorders. In fact, he states that it is increased by most infactions and a variety of non-infectious diseases and disorders, as well as mal-nutrition. He considers a reduction of serum albumin important because it gives rise to edema and indicates a more serious loss of proteins from other sources.

Peters, Bruckman, Eisenman, Hald and Wakeman (50) ran a series of tests in which they determined the total serum protein one hundred-seventy-nine times

in twenty-one patients with nephrosis or nephrotic nephritis. On one-hundred-eighteen occasions, in fifteen patients, the albumin and globulin fractions were determined separately. They concluded that total protein deficiencies involved chiefly the albumin fraction. The globulin lay within normal limits or was slightly elevated. They related the hyperglobulinemia to the underlying infectious process. They maintain than it is not the proportion of albumin to globulin, but the absolute concentration of albumin, which is important in edematous nephritides. A low A:G ratio due to a reduction of albumin has a significance entirely different from that equally low due to an elevation of globulin.

Linder, Lundsgaard, Van Slyke, and Stillman (37) have not observed gross increases in plasma volume in glomerular nephritis, nephrosis or nephrosclerosis, even when the concentration of plasma proteins was much below normal. Their results indicate that "Hydremic plethora" does not occur.

According to Linder et al, a low plasma protein concentration is not due to an increase in plasma volume. Likewise, changes in plasma volume showed no constant relationship to changes in edema. In their

experiments the total globulin varies much less than total albumin, which is an important factor in the argument against the explanation of the fall in protein concentration on the hypothesis of simple dilution.

In 1945 Cohn (16) set forth the following as the values of the percentage of the total osmotic pressure of the plasma which is exerted by the various protein fractions:

PROTEIN	% OF TOTAL PROTEIN	% OF COLLOID OSMOTIC PRESSURE
Fibrinogen	4	1
Gamma Globulin	11	5
Lipoproteins (Alpha and beta G.)	20	7
Lipoid free alpha & beta globulins plus albumin	65	85

FRACTIONIZATION OF THE PLASMA PROTEINS BY SALTING-OUT METHODS AS RELATED TO STUDIES OF THE NEPHROTIC SYNDROME

Until the beginning of electrophoretic studies the question of the individuality of the proteins of the plasma was fundamentally one of the interpretation of the results which have accumulated by the classical methods of preparing the various protein fractions (salting-out and acidification) as evidence of the separation of an apparently homogenous fluid into chemical entities or the differential precipitation of colloidal

particles of various degrees of dispersion.

The early work on the proteins was based on the hypothesis that there were substances of different constitution in the blood. This was a natural assumption since the most characteristic protein of blood, fibrinogen, separated from the rest of the plasma in an insoluble form, fibrin, without the aid of special chenical manipulation. Fibrinogen had a definite property, that of coagulating. There was also a second protein, euglobulin, having properties in common with fibrinogen, but without that of coagulating spontaneously, such as insolubility in water, solubility in dilute salt solutions, and precipitable by the acidification of the plasma, particularly after dilution.

Other protein fractions with more or less definite properties have been obtained from plasma through the use of the temperature of coagulation and salting-out with salts of various concentrations. Thus we have, in addition to fibrinogen and euglobulin, pseudoglobulins, and albumin. The distinction between euglobulin and pseudoglobulin was the solubility of the latter in water, and the higher concentration of salt required for its precipitation. Thus euglobulin was precipitated by a saturated solution of sodium chloride, a one-half saturated solution of magnesium sulfate, or a one-third

saturated solution of ammonium sulfate, while pseudo-globulin was not precipitated by a saturated solution of sodium chloride and required a saturated solution of magnesium sulfate and a one-half saturated solution of ammonium sulfate for its precipitation. Albumin, on the other hand, was soluble in water and was precipitated only by saturation with ammonium sulfate, or upon acidification of a saturated solution of magnesium sulfate, or the addition of another salt, such as sodium sulfate.

The work of the Hofmeister (30) school extended the number of salts which could be used to fractionate protein mixtures, and showed the relation between various salts when used to precipitate euglobulin from serum. The limits between which various proteins were precipitated were defined in terms of the percentage of a saturated solution of ammonium sulfate. Howe (32) modified the technique by initiating the expression of the limits of precipitation of the various fractions in terms of fractional volume-molar concentrations of different salts. He has established that a definite increment of salt is always required to precipitate each of the fractions. In the opinion of Howe, the regularity of the increment, common to all salts, raises

the question of the significance of the protein fractions separated by salting-out. He thinks it is possible than we are not dealing with the precipitation of individual proteins but with a phenomenon of the periodic precipitation of protein which reflects the activity of the salt, or to a periodic reaction between colloidal particles and the added electrolyte. However, immunological studies, and electrophoretic analysis have substantiated the basic soundness of the salting-out methods.

The refractometer has been used to determine the concentration of total protein and of the protein fractions of plasma and serum. Robertson (51) has developed the most useful modification of this method.

In the latter part of the last century, Hammersten (28) and Hofmeister (30) laid the ground work for what was to follow. Hammersten precipitated the proteins with $MgSO_4$ and estimated the nitrogen by the Kjeldahl method. Hofmeister, and others, used ammonium sulfate in their work. The refractometer was introduced by Strubell in 1900.

The classic work of Albert Epstein (21, 22, 23) laid the groundwork for future advances in the study of the plasma proteins in Bright's disease. First of

all, Epstein stated: "Normally blood serum varies but little in its chemical composition, Its chief ingredients, the proteins (euglobulin, pseudoglobulin, and albumin), NaCl, urea, and the allied nitrogenous waste products, maintain definite proportions and may be regarded as physiological constants."

In his experimental work Epstein used as his normals Hammarsten's values for human blood sera:

Total Protein--5.5 to 8.4 gm., Mean--7.0 gm.

Globulin--37%-40%, Mean--38.5%--2.7 gm.

Albumin--6% to 63%--4.2 gm. to 4.4 gm., Mean--4.3 gm.

Globulin/Albumin ratio--1:1.6

In 1912 Epstein reported his studies upon twelve cases of chronic nephritis. He divided the cases into chronic parenchymatous nephritis, chronic interstitial nephritis, and chronic mixed nephritis. He described most cases as in uremia. In the parenchymatous nephritis he noted a marked relative increase in the albumin fraction. In these cases he also noted a greenish opalescent sera which he stated was lipoid in combination with protein. However, in cases of chronic interstitial nephritis he stated that there was a tendency for the globulins to be below normal.

A year later, Epstein (23) summarized his previous findings as follows:

"In the serum of cases of chronic parenchymatous nephritis the increase in the globulin content is most pronounced, and the globulin may constitute nearly all of the protein present. On the other hand, the protein fractions of sera from cases of chronic interstitial nephritis present in their quantitative relations, little or no change from the normal. In certain cases of the latter group, there appears to be a tendency for the globulin in the serum to fall below the normal percentage.--- -The author is of the opinion that the changes occurring in the protein composition of blood serum in different diseases are not accidental, but they are the result of well defined influences acting upon the blood and its serum. In contrasting the findings in the sera of patients with chronic interstitial nephritis and with chronic parenchymatous nephritis, the conclusion is reached that the two diseases are genetically different in respect to the blood, and that the change in protein content of the blood and serum plays a direct part in the production of some of the clinical manifestations of the two types of renal disease."

He further postulated that the composition of the

urine and the blood stream (the chlorides and nitrogenous waste products) in chronic interstitial nephritis depends upon the degree of impairment of the renal function; whereas in the chronic parenchymatous nephritis the chemistry of the urine, the retention of fluid and salt in the body, and in a large measure the character of the disease in the kidneys, are due to the changes occuring in the blood. It is thus seen that Epstein's conception of chronic interstitial nephritis was apparantly that of chronic Bright's disease which terminates in uremia, while parenchymatous nephritis leans toward the concept of nephrosis.

In 1917, Rowe (53) studied several cases of chronic nephritis with edema by means of the micro-refractrometric method of Robertson. He concluded that the most marked variation from the normal was a low total protein, which he said was due in a large part to the presence of hydremia, "though the toxic effect of retained waste products undoubtedly plays a role in reducing the available body proteins." He also found that the percentage of globulins and the non-protein substances was above normal. He considered the normal A/G ratio to be twenty-six per cent. Rowe also observed that in two cases with complete absence of clinical edema

the proteins were two per cent below normal values. He thought that this failure to return to normal was a manifestation of internal edema, which persists long after subcutaneous edema is demonstrable, and that it is also a manifestation of the chronic intoxication present in nephritis.--"That retained toxins can aid in breaking down serum proteins is certain." He cited as further proof of his theory the experience of Castaigne and Wi Chiray, who demonstrated by the refractometric method that subcutaneous injection of complex proteins causes a slight diminution of the serum protein, which is due to the toxic effect of these injurious substances, supposedly.

In 1921 Howe (32) introduced the use of sodium sulfate solutions at thirty-seven degrees centigrade for use in precipitating blood protein fractions. By this method he put forth evidence that there are two globulins in the blood serum in addition to euglobulin--pseudoglobulin I, and pseudoglobulin II. Gutman (27) lists normal values, according to the Howe method:

Total Serum Protein--6.5 gm.%--7.9 gm.%
Serum Albumin--4.7gm.%--5.7gm.%
Total Globulin--1.3gm.%--2.1gm.%
Euglobulin--0.4gm.%--0.1gm.%
Pseudoglobulin I--1.9gm%--0.8gm.%
Pseudoglobulin II--0.8gm.%--0.2gm.%

Berglund, Longcope and Richards (8), using Howe's method made a study of the plasmas of eighty-nine patients, predominantly with renal disease. They found that the mean total protein was 6.43 per cent, which figure did not vary significantly from the normal mean. The albumin was 2.96 per cent as versus 4.07 percent in the normals. The total globulin, including fibrinogen, showed significant increase, the mean being 3.47 per cent versus the normal 2.59 per cent. The pseudoglobulin II was 0.77 per cent as versus the normal 0.68 percent, and pseudoglobulin I was 1.13 per cent as versus the normal 0.05. Together the pseudoglobulins of the pathological plasmas were significantly higher than the normal, but they were found to vary less than any other protein fraction. The pathological albumins did not prove homogenous, showing two groups of slightly different size, one of approximanely of normal values, the other with lower values. The one with the lower values included the marked renal cases. The low albumin appeared to be due to heavy renal leakage, and not to other intrinsic plasma changes. Both fibrinogen and euglobulin under pathological conditions showed minimal values definitely smaller than the normal minimal values. These two fractions were marked by their great and

independent variability, both in negative and positive directions, particularly the latter.

The fact that the total protein both normally and pathologically varies less than the single fractions necessarily brings up the question whether the different fractions vary in a reciprocal and compensatory way. This sometimes seemed to be the case with the two pseudoglobulins. For the other fractions they believe the question is to be answered in the negative. They think that the relative constancy of the total protein depends on the fact that around the highly constant pseudoglobulins the euglobulin-fibrinogen and albumin vary usually in opposite directions. This variation is thought to be an independent one, however, the euglobulin-fibrinogen fraction frequently being increased independently of any alteration of albumin. These workers refrain from the use of the term albumin-globulin ratio.

Linder, Lundsgaard, and Van Slyke (38), using the micro-Kjeldahl method of Howe, analyzed sera from all types of Brights disease, including the nephrotic states. Their conclusion was that evidence is steadily accumulating to show that nephritis is a disease not merely of the kidneys, but that it includes widespead

changes in remote tissues and fundamental if still obscure changes in metabolism. They consider that the increase in globulin which is so often found may be a phase in this metabolic derangement. They surmise that possibly the formation of plasma globulin is the primitive form of plasma protein production and the organism in disease tends to return to this primitive form.

IMMUNOLOGICAL STUDIES ON PLASMA AND URINARY PROTEINS IN THE NEPHROTIC SYNDOME

A. NON-QUANTITATIVE METHODS. Several investigators have striven to prove whether or not abnormal proteins are present in the plasma and serum of patients who present the nephrotic syndrome. One of the first was Muller (44), who was unable to demonstrate the presence of a foreign protein in the urine of nephrotic patients, and concluded that the protein of urine and serum were identical in composition.

In 1929, Hewitt (29) obtained optical rotations on urine obtained with the Schmidt and Haentsch polarimeter, with a mercury vapor lamp. In his opinion also, the serum and urinary albumin was identical in nephrosis.

It is to be noted that neither of these workers ventured and opinion as to whether the serum and urinary proteins of the nephrotic compared favorably with the

normal.

Also in 1929, Andrews, Thomas, and Welker (4) came forth with the theory that the presence of serum proteins in the urine in nephritis is due to their combination with other nitrogenous factors of tissue origin, some of which are highly toxic. They believed that this combination acts as a detoxicating mechanism and that the elimination of this protein by the kidneys is due to alteration of normal serum proteins by their combinatiion with other split protein products whcih render them foreigh to the blood. They offer this theory as an explanation of albuminuria. It has not been substantiated by any other workers.

Widdowson (66) presents contradictory evidence. In her work, the albumins and globulins from the urine and serum of nephritic patients, a nephrotic patient, and normal human serum were separated by fractional precipitation with ammonium sulfate. The separated albumin and globulin were analyzed by a micro-modification of the Van Slyke nitrogen distribution method. Measurements of optical rotation and racemization, osmotic pressure and specific refraction of the proteins was also made. The results indicated that the proteins isolated from the urine of patients

suffering from nephritis or nephrosis are identical with corresponding serum proteins and there is no evidence of any alteration in the chemical or physical nature of the proteins during their passage through the kidney. Proteins isolated from the urine and serum of patients suffering from nephritis are identical in chemical and physical structure with those of normal human serum. However, the urine and serum proteins of the patient with nephrosis exhibited a slight difference in chemical structure from the normal. The percentage of ammonia nitrogen in the case of albumin is ten per cent lower than normal or nephritic serum or urine. The ammonia nitrogen for globulin is about two per cent higher than normal. The basic nitrogen was two per-cent higher for albumin and two per cent lower for globulin. Racemization curves, osmotic pressure, and specific refraction were identical with the normal, however. Widdowson concluded that it is probable in nephrosis that there is some alteration in the arrangement of the amino acids in the serum and urinary proteins, but this difference is not detectable by a measurement of optical rotations.

B. QUANTITATIVE METHODS--Use of quantitative methods in the study of plasma and urinary proteins

in nephrosis has begun only recently.

Goettsch and Reeves (25) recently developed antisera in rabbits with each fraction from pooled human sera. The fractions were separated by Howe's method or by the precipitin method .

The first patient was a twenty month old white girl in her initial attack of edema. The serum, urine, and edema fluid were analyzed by the precipitan method, and it was apparent at once from the small amount of protein thrown down that neither the albumin nor the globulin was reacting with antiserum as completely as normal serum proteins react. The estimated amount of albumin was only thirteen per cent of the value obtained by Howe's method, and the value for globulin was approximately one half that obtained on salting-out the proteins. A second sample yielded similar results. It was then decided that the presence of cholesterol might have changed the results. The serum was cleared with ehtylene chloride, and the results were the same as before. It was further determined that addition of nephrotic serum to normal serum caused no interference with the predicted precipitation of normal proteins.

Following diuresis and the disappearance of the

edema, the globulin fraction rapidly regained its ability to precipitate the antiserum completely. The albumin fraction recovered its normal serological response more slowly over a period of several months--long after the patient had been discharged from the hospital. Transfusions had little or no effect on the determinations by the precipitin method, provided the serum for analysis was withdrawn several days following the transfusion.

Similar findings were made on several other patients with nephrosis--all showed the same phenomenon of incomplete precipitation with specific antisera. In two patients during convalescence, a return to normal reaction was noted. A total of eight patients were studied. In patients with acute nephritis these changes did not occur.

Since both antigens used to prepare the antisera are composed of various subfractions which are antigenically distinct, and which vary in their ability to evoke antibody formation, it was expected that estimation by the precipitin method would fail where wide deviation from the normal distribution of these subfractions occur. A comparison of the analysis of serum from a patient who had recovered from nephritis

and from a patient in the edematous stage revealed practically the same subfractions in each, yet one precipitated completely, while the other showed a large deficit.

An antiserum was then prepared in a rabbit against nephrotic globulin. Albumin was not obtained in sufficient quantities for the experiment. The globulin could then be correctly estimated by the precipitin method, although the usual deficits occured when normal antiserum was used.

In urinary analysis with the precipitin method and normal antiserum, it showed a consisently larger fraction of the urinary proteins than of the serum proteins. It is thus suggested that protein excreted in the urine is not that which shows serological changes, as might be expected.

The experimental methods of Goettsch and Reeves seems sound, and their findings merit careful attention.

In 1947, McCarty (41) reported finding a protein not normally present in the blood, but which is present during acute infections. It was found to be not specific for any one disease. He found this protein to precipitate selectively with the C polysaccharide of

pneumoccuccus, but it is as I said before, not specific for pneumonia. He professed no knowledge of its source or function, but suggested that it may be related to the host reaction to infection. He has not isolated the protein in nephrosis, but he suspects its presence in many diseases. It is therefore, only of academic interest in the present discussion.

In view of the fact the experimental work in this phase of the study of proteins has been limited, it is difficult to evaluate the importance of the findings.

CHARACTERIZATION OF THE SERUM PROTEINS IN THE NEPHROTIC SYNDROME BY AMINO ACID CONTENT

Very little can be found in the literature concerning pathological changes in the amino acid content of the plasma proteins, and the findings can be reviewed briefly.

In 1932, Tuchmann and Sobatka (61) suggested that all cases of hypoproteinemia showed a change in the nature of the plasma proteins. They found that the serum albumin contained more tyrosine, than normal, and that the serum globulin contains less tyrosine than normal. No quantitative determinations were described.

Peters (40) suggests that in the resynthesis of serum albumin the albumin may wait upon the other proteins to be reformed. If the drains are serious the body therefore turns out imcomplete or inadequate albumin. According to Peters, this inadequacy is possible a lack of cystine--which is not detected in precipitation techniques, and does not alter the properties of the albumin.

Widdowson also suggests that there may be a change in the amino acid content of the plasma proteins, based upon her findings of the changes in nitrogen content of albumin and globulin, which have been previously described.

The importance of this type of work may be increased when more is known about the real function of the various amino acids in the human body.

As to the behavior of pathological sera in the ULTRACENTRIFUGE, it may be mentioned that McFarlane (42) found the serum normal in every respect when he studied one nephrotic patient. In another he showed a high globulin fraction and a low albumin fraction. More recent work, as described by Cohn (16) shows that results from ultracentrifuge can be correlated directly

with those of electrophoresis, which are to be described in detail later.

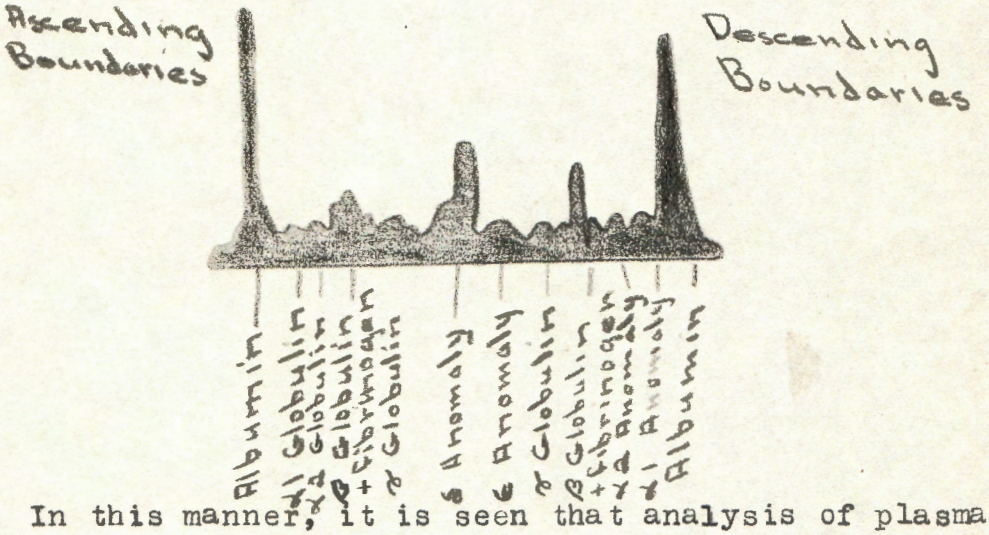
ELECTROPHORETIC STUDIES OF NEPHROTIC SERA

If an external electric field of force is applied to protein molecules dissolved in a suitable solvent, it is found that in acid solutions, proteins move in one direction, and in alkaline solutions, in the opposite direction. The significance of the phenomenon was recognized toward the end of the last century by the late Sir William Hardy. It remained for Tiselius (60) to design the modern electrophoretic apparatus used in today's studies.

Under a given field strength, the speed and direction of the movement of a protein will be determined by the pH, concentration and nature of the salts, and the viscosity of the solvent. Two or more different protein molecular species in the same solvent will usually move with different velocities. This makes it possible to use these velocities as a means of distinguishing protein molecules differing in kind; the velocity in a unit electric field is defined as the electrophoretic mobility. Longsworth (39), in his description of Teselius' moving boundary cell,

states that it iis ideal for the patterns for the two sides of the channel to be mirror images of each other. The actual patterns only approach this ideal as the protein concentration is decreased. However, at such low concentrations, the areas under the peaks are not of sufficient magnitude to be determined accurately.

The Schleiren pattern for normal human plasma is seen below (Taken from Cohn, Onclay, et al (17))



In this manner, it is seen that analysis of plasma as yielded at least six electrophoretic components. The fastest moving component in neutral or alkaline pH ranges is albumin. From the relative area of the peak which moves with this velocity, albumin is found to constitute about fifty-five per cent of the total plasma protein. Next in order of decreasing velocity comes the alpha-globulins, which under certain conditions

divide into two separate components, designated as alpha$_1$ and alpha$_2$. Moving more slowly than these are the beta-globulins, likewise sometimes separated into two components, beta$_1$ and beta$_2$. The total alpha- and beta-globulins, comprise about thirteen and fourteen per cent, respectively, of the total plasma proteins, as indicated by the areas of the separated peaks. Still more slowly moving is fibrinogen, which represents approximately seven per cent of the plasma proteins. Slowest of all are the gamma-globulins, estimated as representing eleven per cent of the plasma proteins.

The electrophoretic components into which the Teselius apparatus resolves the plasma proteins do not necessarily represent homogeneous molecular species, either with respect to size, shape, charge distribution, solubility characteristics, or physiological functions. The great advantage of the apparatus lies in its speed and simplicity of operation and in the reproducibility of the results. Teselius (60) believed that this method allows study of the proteins in states most nearly approaching the physiological.

Proteins of interrelated functions do not always possess comparable chemical properties and are thus not always found in the same fraction. The products

of plasma fractionation must also be considered, therefore, from the point of view of physiological function and clinical use.. According to Cohn, the largest categories are (1) the albumins, (2) the immune globulins, (3) the iso-hemagglutinins, (4) the hormones, enzymes, and related substances, and (5) the proteins concerned with the clotting of the blood. In electrophoretic studies the proteins are divided into fractions according to their increasing motility in an electric field, each possessing a specific function or functions connected with chemically discrete protein molecules.

The clotting of the blood depends upon fibrinogen, separated in Fraction I, plus thrombin and prothrombin, both separated in Fraction III.

The immune globulins are also found in Fraction II and Fraction III. These antibodies are largely concentrated in Fraction II, which consists in recent preparations of over ninety-eight per cent gamma-globulin. The principal impurities consist of small amounts of beta-globulin and albumin, of cholesterol and phospholipid. The isohemagglutinins are also separated quantitatively in Fractions II and III, and are further concentrated in Fraction III. These

include both anti-A and anti-B isohemagglutinins, and the anti-Rh globulin.

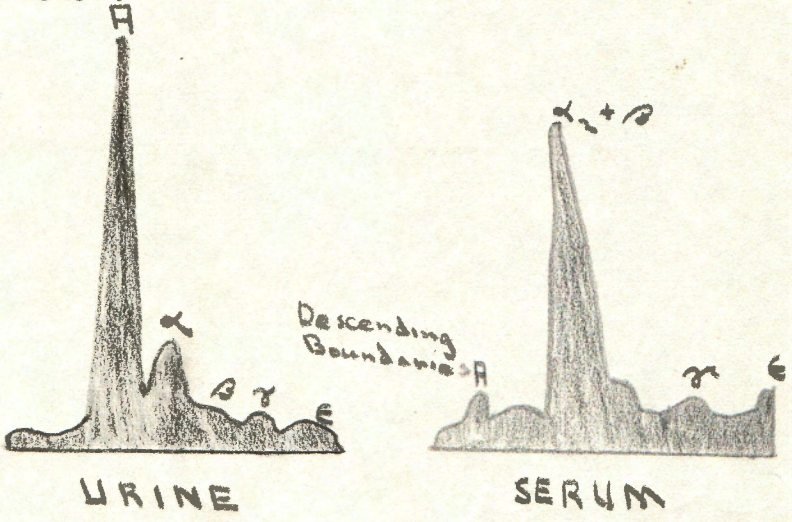
Enzymes and hormones are found in the several fractions, and for obvious reasons do not present constant findings.

The lipo-proteins are largely concentrated in fractions III and IV and appear to be associated with alpha- and beta-globulins. The lipid material seems to be cholesterol, carotene, and phospholipid.

The albumin is concentrated in the largest fraction, V. The component appears to be homogenous in the electrophoretic apparatus at neutral or slightly alkaline reactions.

One of the most promising problems in the field of elctrophoretic analysis of pathological states is that of renal disease. The first observation of the highly atypical serum pattern in nephrosis was made by Longsworth, Shedlovsky, and MacInnes (40). Their diagram showed an extremely low albumin peak and an electrophoretic component migrating with the velocity of alpha-globulin and having a concentration comparable to the albumin in normal serum. The beta-globulin component was also markedly elevated. Studies of the ufine of nephrotic patients was also made by these

workers. It was found that the urine of such patients gave an electrophoretic pattern closely resembling that of normal serum and in striking contrast to the highly abnormal diagram of the serum of the same patients. Schlieren patterns of their results are seen below.



These observations would seem to disprove the common notion that the urinary protein in nephrosis essentially represents albumin, and it also indicates that the excretion of urinary protein by the kidney is a highly selective process, rather than a simple filtration.

It has often been suggested that part of the pathological variation in the serum proteins in the nephrotic syndrome could be explained on the basis of malnutrition due to poor food intake. Zeldis and

Allen (68) investigated by electrophoresis the pattern of plasma protein regeneration which occurred in dogs that have been acutely depleted of their plasma protein within a few minutes--massive bleeding followed with simultaneous return of washed red cells. They found that in acute depletion the tissue protein reserves support the regeneration of all electrophoretic fractions of the plasma. The alpha and beta-globulin peak appear relatively and absolutely increased in the early portion of the recovery period. They believed that these components were derived from tissue reserves and rapid resynthesis from dietary proteins. Return of normal albumin values is found to proceed at a very slow rate even in the presence of adequate protein intake. An essential similarity between dog and human results was noted. In the first twenty-four hours appreciable quantities of all electrophoretic components enter the circulating blood stream, even without food. However, the relative proportions of the electrophoretic components may be disturbed from normal for as long as two to three weeks after total protein has returned to normal.

These studies suggest that the low albumin peaks found in the nephrotic syndrome are not due entirely to protein loss in the urine, but that some derangement of body metabolism is involved.

to protein loss in the urine, but that some derangement of body metabolism is involved.

Great interest has been shown recently in the effects of the lipids on the blood proteins--in lipo-protein combinations, and their effect upon the Schleiren patterns. In 1932, Bennhold (6) concluded that the cholesterol of the serum is bound not to the albumim, but to the globulin. He remarks, however, that on the positive side often a non-colored globulin zone appeared that did not contain cholesterol.

In 1935, Mellander (43), using Theorell's apparatus, concluded that the cholesterol, if atlall bound to proteins, probably is bound to sub-fractions of albumin and globulin.

Bruger (15) made a study of free and ester cholesterol in relation to the protein content of pathological body fluids, in which he found the cholesterol more or less parallels the protein concentration of both transudates and exudates. He further concluded that the total cholesterol in pathological effusions bears no direct relation to the total cholesterol content in plasma. The concentration of cholesterol in effusates may approach, but rarely exceeds that of plasma. Furthermore, the relation of free to ester

cholesterol is constant at times, and varies at other times.

Blix (10) performed extraction of lipids of the plasma with acetone and dry ether and removed them by centrifuging, The serum cholesterol was completely removed, but twenty-five per cent of the phospholipids was not extracted (lecithin was extracted, cephalin left). The electrophoretic apparatus of Tiselius was employed in Blix's further analysis. In lipid free sera the beta-globulin appeared clear, and he concluded that the opalescence of this component was due to lipid.

The only difference in the normal and lipid free serum was that no alpha-globulin appeared at pH 6.1-7.4. At pH 8.0 the alpha-globulin boundary became visible, but less so than normally. Whether this change was due to absence of lipids or an incipient denaturation of the protein connot be ascertained. He preferred to think that an incipient denaturation occured with the boundaries of alpha-globulin submerged in the bands of albumin or beta-globulin.

Blix regards the lipids of the alpha and gamma globulins as permanent elements in these proteins and suggests that beta-globulin handles lipids in a fat transportation function. In marked lipemic conditions

the turbidity appears between the alpha and beta boundaries, suggesting that the albumin and alpha-globulin can perform auxillary fat transport. Blix made the last conclusion after he studied the pattern resulting from the addition of mastic and cholesterol to lipid free serum.(11) Here then, is another possible explanation of the apparant increases in the globulin fractions, particularly the beta-globulin, in nephrotic sera. It will be remembered that many workers consider lipoid nephrosis to be primarily a disorder of fat metabolism.

Zeldis, Alling, McCoord, and Kulka (68) made similar studies. They concluded that a particularly rich content of lipid materials characterizes human beta-globulin. Abnormally large beta-globulin peaks occur regularly in the presence of elevated plasma lipids. Marked increases in the areas of other globulin components, particularly gamma-globulin, are also found to be due in large part to elevated plasma lipid levels in certain abnormal human plasmas.

Zeldis, et al, describe one case of a sixty-three year old white woman with nephrosis. Electrophoretic analysis of the native sample of serum showed a low albumin and markedly elevated globulin peak. Extraction of the lipid resulted in a striking decrease in the area

of the beta component and lesser changes in other components:

	Total	Alb.	alpha	beta	gamma
Native Plasma	31.2	6.5	8.7	11.3	1.4
Extracted plasma	36.3	5.7	7.3	2.5	1.6

(Figures are electrophoretic areas given in planimeter units)

Stern and Reiner (59) in a recent surgey of the importance of electrophoresis in medicine conclude that the protein spectrum in plasma and serum is the resultant of a host of factors concerned with the formaion, the interactions, and the destruction of the individual components. They believe that the blood, while often considered a tissue in itself, is perhaps more dependent on the physiological state of the organism as a whole than is any other tissue, and it is therefore rational to suppose that there exists a close correspondence between the blood, the protein system, and the physiological state of the individual as a whole, rather than to a given pathological condition.

SUMMARY AND CONCLUSIONS

The nature of the plasma proteins in the nephrotic syndrome has been the subject of a large amount of investigation, experimentation, and speculation during the past century. The findings, while not conclusive, have provided definite stepping stones toward a better understanding of this little understood disease process, and perhaps of disease in general.

Since the time of Richard Bright the role of the serum proteins in relation to the albuminuria and edema in Bright's disease has been recognized, and the ideas and theories which have been advanced are numerous. Since the most striking clinical characteristic of the nephrotic syndrome is a generalized edema, with a low protein content of the edema fluid, this factor has excited particular curiosity. Bright believed that a "hydremic" condition of the blood plasma made it more easily filtrable into the tissue spaces. Senator introduced the idea that generalized capillary damage due to toxic agents, resulting in increased permeability was the cause of the edema. Starling's brilliant work resulted in the theory that the escape of fluid from the vascular system into the tissues was due to changes in the colloid osmotic pressure

of the blood.

Other ideas included the theory that an increase in vascular pressure existed due to insufficient elimination of water by the kidneys, allowing for the release of fluid into the tissues, that the production of edema is related to salt intake, that colloid chemical changes in the tissues increased their swelling capacity, that there is a change in the ability of the body fluids and tissues to hold water in relation to the lipoids, and that edema formation was due to central nervous system control of water balance.

Today it is generally accepted that a multiplicity of factors account for the presence of edema, a fact which is evident when a survey is made of the many therapeutic measures which have failed, or provided only temporary relief because they served to alleviate only one of the possible causes.

Another factor which has led to confusion in the study of the nephrotic syndrome as related to Bright's disease has been the controversy about the existence of nephrotic glomerular nephritis and nephrosis as separate clinical entities. Nephrotic glomerular nephritis is usually defined as marked by the presence of edema, weakness, dyspnea on exertion, hypertension,

albuminuria and casts, with no marked impairment of renal function. The blood changes consist of a high blood cholesterol, lowered serum albumin, and a reversal of the ordinary albumin-globulin ratio. The course is described as chronic, with remissions and exacerbations, with renal function becoming progressively poorer. The pathology consists primarily of glomerular damage, presumably due to inflammatory change, which results in hyalinization of the glomeruli, with secondary degeneration of the renal tubules.

Nephrosis, to those who believe it exists, differs from nephrotic glomerular nephritis in that there is no hematuria, no elevated blood pressure, and although the disease is chronic, recovery can be expected. Pathologically the condition is essentially a tubular lesion, and the glomeruli are not affected. Opponents of the concept of lipoid nephrosis believe that glomerular and tubular damage are relative, and that if proper techniques are used, glomerular damage can always be demonstrated in nephrosis. Cases which fulfill all the criteria for lipoid nephrosis are rarely found. However the existence of the disease cannot be positively denied.

In surveying the methods which have been used in

the study of the serum proteins, it is probably best to begin with the effect of the osmotic pressure of blood colloids and of the blood volume upon the edema of the nephrotic syndrome. As well as shedding a great deal of light upon the mechanism of edema, these studies have also supplied evidence of abnormalities in character and relationship of the serum albumins and globulins in nephrosis.

In summary it may be said that changes in plasma volume probably have little relationship to edema, although Von Jaksch, claimed a dilution of the blood in kidney disease and Darrow declared that there is a tendency to blood concentration during the edematous stages of nephritis. Other workers have been able to discover no important changes in blood volume in the nephrotic syndrome. Simple dilution is an unlikely factor since the tendency toward a decrease in serum albumin is found in conjunction with both relative and absolute increases in the other proteins.

The protein osmotic pressure of the serum of nephrotic patients has been found by all workers to be consistently low. Findings for the osmotic pressure in normal subjects have varied in the region of twenty-five to thirty-five mm. of mercury. Findings in

nephrotic patients have varied from about ten to twenty-two mm. Hg. Edema free patients have been found with serum protein osmotic pressures far below the accepted normal, and in some patients edema has been present when the total proteins were within the normal range and the protein oncotic pressure likewise quite high. These cases, however, are the exception, but interesting in that they suggest that other factors are present in producing the tendency toward edema. It is generally accepted that this fall in pressure is due to a diminution in the serum proteins, chiefly of the albumin. One gram of albumin contains many more molecules than one gram of globulin since the molecular weight of the former is much less. Moreover it has been suggested that since the isoelectric point of the albumins is at a lower pH than that of the globulins, they are consequently more highly dissociated at the normal pH of the blood. In addition, the albumins comprise a greater percentage of the total protein than the other factors. Therefore, in the nephrotic patient, with extensive loss of albumin in the urine, or albumin deficit due to other causes, it is justifiable to believe that the above mentioned factors are important in the lowering of the colloid osmotic pressure

and the production of edema. However, other theories have been advanced to supplement the explanation just advanced. Govaerts believed that the fall in pressure is due to the specific nature of the proteins in nephrosis, and that in equal concentrations they give lower pressures. It has been suggested that an abundance of fibrinogen in nephrotic serum may account for the low pressures, while other workers attribute the same effect to the reversed albumin-globulin ratio in which there is a real and relative increase in globulins. Increased amounts of lecithin and cholesterol in the serum perhaps tends to lower the osmotic pressure, although this has not been proven.

Recent work indicates that albumin plus the lipid free alpha- and beta-globulins comprise about sixty-five per cent of the total serum protein and exert about eighty-five per cent of the total osmotic pressure. The lipoproteins comprise about twenty per cent of the total and exert seven percent of the total osmotic pressure. The gamma globulins and fibrinogen, comprising only fifteen per cent of the total, exert only six per cent of the total colloid osmotic pressure.

Until recently, the great majority of studies of the plasma proteins were based on fractionization of the proteins by the various salting out methods. This technique, based upon the fact that a definite increment of salt is required to precipitate each of various fractions of the plasma, does not assure us that we are dealing with the precipitation of individual proteins instead of a phenomenon of the periodic precipitation of protein, which reflects the activity of the salt. However, the findings resulting from this method have correlated well with the results of other methods and its soundness is accepted.

Based upon the salting out technique, the following constituents of the plasma have been determined: fibrinogen, euglobulin, pseudoglobulin, and albumin. The groundwork for this type of study was laid by Epstein. In his investigations of nephrosis he noted that there was generally a marked increase in the globulin fractions of nephrotic sera, although he made no distinction between pseudoglobulin and euglobulin. He also noted that there was a decrease in the albumin fraction. He concluded that the changes in the composition of the blood were due to well defined influences acting on the blood, but he did not postulate

what these influences might be.

Other workers have confirmed the work of Epstein and made additional contributions. Howe discovered that the most important change in nephrosis is a reduction in total protein, which he ascribed to hydremia and the toxic effect of retained waste products. He also noted an increase in the percentage of globulins in nephrotic sera. Howe brought forth the idea that pseudoglobulin existed in addition to euglobulin.

It is to be noted that the salting-out method, while it isolated well-defined fractions of human sera, did not shed much light upon the probable functions of these various fractions. The general conclusion was that the nephrotic syndrome is not primarily a disease of the kidneys, and that the changes seen are the result of metabolic derangements. For instance, it was suggested that the increase in globulins and decrease in albumins represented a return to primitive protein formation.

It has been the aim of several investigators to discover whether or not abnormal proteins are present in the plasma and serum of patients with the nephrotic syndrome. Results have been variable and hence inconclusive. Using non-quantitative methods, Muller,

Hewitt, and Widdowson concluded that the urine and serum of nephrotic patients contains proteins which are identical in chemical and physical structure with those of normal human serum and urine. However, Andrews, Thomas and Welker believed that the proteins in the urine in nephritis are in combination with other split protein products which render them foreign to the blood, and hence cause them to be excreted in the urine. These findings have never been duplicated be other workers.

By developing specific antisera to each fraction of pooled human sera and then causing the antisera to react with the serum and urine of nephrotic patients, Goettsch and Reeves found that neither the albumin nor the globulin of the nephrotic reacted with the antiserum as completely as normal serum proteins. The results were not changed by clearing the sera of cholesterol. In instances where a clinical return to normal was noted, it was found that the serological responses of globulin returned to normal rather quickly, while that of albumin returned slowly, over a period of several months. In addition, an antiserum was prepared against nephrotic sera, which confirmed the results found previously. In analyzing urine by the same technique,

it was found that a consistently larger fraction of the urinary proteins was precipitated by normal antiserum, suggesting that protein excreted in the urine is not that which shows serological changes.

It can be seen that these immunological studies have led to some interesting results, but further study is indicated in order to establish the validity of the findings.

Evidence that the serum proteins of the nephrotic are of a pathological nature has been gathered as a result of a few studies of the amino acid content of the serum. Changes in tyrosine and cystine content have been noted.

With the development of electrophoresis, new fields for the study of normal and pathological sera have been opened. This method has resulted in a new terminology in regard to the serum proteins, and consequently the findings are difficult to correlate with olderstudies.

The components of the serum proteins as separated by electrophoretic analysis are (1) albumin (2) alpha globulins (3) beta globulins (4) fibrinogen

and (5) gamma globulin. The alpha and beta globulins seem to correspond to the pseudoglobulins of the salting out methods, while the ga mma globulin can probably be compared to the euglobulin fraction.

Electrophoretic studies have also resulted in rather definite conclusions as to the functions of the various fractions. The clotting of blood, as is generally known, depends upon fibrinogen. The immune globulins are largely concentrated in the gamma-globulin fraction, and isohemagglutinins seem also to be at least partially in this fraction. Enzymes and hormones are distributed throughout the various fractions. The lipid of the serum seem to be largely associated with the beta-globulins, although the alpha-globulins and albumin may also assist in fat transport. The alpha globulin components are increased in all diseases accompanied by fever and tissue destruction.

Nephrotic serum shows a characteristic Schlieren pattern, with an extremely low albumin peak and markedly elevated alpha- and beta-globulin components. The gamma-globulin component is not greatly affected. It is generally accepted that a large amount of albumin is lost in the urine in nephrosis. However,

Longsworth and MacInnes have shown that the urine of nephrotic patients gives an electrophoretic pattern closely resembling that of normal serum, indicating that protein loss in the urine is not a simple filtration defect, but selective secretion. There is also some indication that in pathological states in which there is interference with normal protein resynthesis the production of albumin may lag behing that of the other serum proteins. In addition, albumin may serve a fat transportation function--of an auxiliary nature. This factor could possibly interfere with the area of albumin as seen in the Schlieren pattern in nephrosis.

Present knowledge indicates that the presence of abnormally large concentrations of beta-globulin (and possibly alpha- and gamma globulins) is due to a lipo-protein complex--beta-globulin plus cholesterol, caroteme, and phospholipid. This, in view of the marked lipemic condition of the serum which occurs in nephrosis, is a very logical explanation of the large beta-globulin peak in nephrosis.

Thus it is seen that during the relatively short period in which electrophoresis has been used to study pathological sera, there has been developed

a reliable system of classification of the serum proteins, plus some well founded ideas about the functions of the various components in normal and pathological states. This method has clarified many points which were suggested by earlier methods, and it is to be hoped that the not too distant future holds the knowledge that will clear the mystery of Bright's disease. At the moment it seems clear that the changes in the serum proteins in nephrosis depend on many other factors than these which depend directly upon changes in kidney function. It appears that a widespread metabolic disturbance occurs, which may be, at least in part, a manisfestation of the body reaction to chronic disease, and which may represent an specific reaction to the nephrotic state.

a great paper
Dr. M. Culp

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