

FRACTIONATION OF THE SERUM AND PLASMA PROTEINS BY SALT PRECIPITATION IN INFANTS AND CHILDREN.¹ 1. THE CHANGES WITH MATURITY AND AGE. 2. THE CHANGES IN GLOMERULONEPHRITIS. 3. THE CHANGES IN NEPHROSIS

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The various components constituting the plasma protein complex have been characterized by many different chemical methods. Perhaps the most extensively used method of defining the component proteins has been by describing their solubility behavior in salt solutions. Thus, the terms fibrinogen, euglobulin, pseudoglobulin, and albumin have been applied, not to molecular species or chemical entities, but to the fractional parts of the total plasma protein complex, separated by precipitation at specified salt concentrations.

In this study, the plasma protein fractions "salted out" by increasing concentrations of salt solution were quantitated in normal infants and children. The influence of maturity and age upon the concentration of the individual fractions precipitated was studied by examining and comparing the bloods of premature infants, full-term newborn infants, older infants, and children. The quantitative changes occurring during the course of various diseases, especially nephritis and nephrosis, were measured.

METHODS

The phosphate salt mixture used by Butler *et al.* (1, 2) in their study of the solubility curves of human plasma proteins was employed. These authors, using an equation derived by Cohn (3), obtained a discontinuous curve when the solubility of the protein expressed logarithmically was plotted against increasing concentrations of the phosphate precipitant. They stated that the breaks in the discontinuous curve were caused by successive precipitation of the progressively more soluble protein fractions.

Briefly, the method entailed precipitation of the proteins at room temperature with increasing concentrations of a phosphate solution. The phosphate precipitant was made of equal parts of monobasic and dibasic potassium phosphate, so that the pH of the different concentrations employed was constant at 6.5. Blood was collected without

stasis and allowed to clot. After centrifugation, the serum was separated. One volume of serum was added to 30 volumes of phosphate precipitant. (The different concentrations of phosphate precipitant were prepared from a stock 3 molar solution.) In actual practice, 0.2 cc. of serum was added to 6.0 cc. of phosphate solution; instead of filtrations as practiced by Butler *et al.* (1, 2), the precipitated protein was removed by centrifugation at 2,000 R.P.M. for 15 minutes in the angle centrifuge. Supernatant fluids were analyzed by the micro-Kjeldahl method. The concentrations of phosphate precipitant used with each serum ranged from 0.8 molar to 3.0 molar.

Because there is, as indicated by Butler *et al.* (2), a lack of sharp breaks in the solubility curve of the serum proteins obtained by salt precipitation, we have recorded the absolute amount of protein (grams per 100 cc.) precipitated by each successive increase in salt concentrations of precipitant. In the interest of consistency, we have also designated in our tables the fractions precipitated by the Na_2SO_4 method of Howe (4) as the fractions precipitated and soluble in 22 per cent sodium sulphate. The fraction precipitated by this concentration of Na_2SO_4 is, in conformity with custom, referred to in the text as total globulin and the protein remaining in solution, total albumin. It is to be emphasized that the protein fractions precipitated by either method employed are not chemical entities and that the terms globulin and albumin are used merely for convenience.

Fibrin was determined by the method of Cullen and Van Slyke (5) on oxalated plasma obtained at the same time as the serum sample.

I. NORMAL INFANTS AND CHILDREN

Ten healthy children, ranging in age from 5 to 10 years, were studied. Their serums and plasmas were examined on 2 or more occasions. Each determination was done in duplicate. The values for the protein fractions determined by the phosphate precipitant and the Howe sodium sulphate methods were found to be fairly constant for a given child, on determinations done at different times. Table I contains the values for the protein fractions for 2 of the normal children, and is presented as an example of the relatively constant

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TABLE I

Grams of protein per 100 cc. of serum obtained by single fractional precipitation of serum proteins of the normal child on different occasions

Subject	Date	Fibrin*	1.5 Molar Na ₂ SO ₄ †		Molar concentrations of phosphate precipitant					
			Insoluble ("Globulin")	Soluble ("Albumin")	1.6 Molar	2.0 Molar	2.4 Molar	2.6 Molar	2.8 Molar	3.0 Molar
D. L. Female 8 years old	May 4, 1939	0.240	1.54	5.14	1.22	1.10	1.30	0.95	1.28	0.80
	May 10, 1939	0.252	1.50	5.07	1.16	0.95	1.06	1.09	1.24	0.80
	May 17, 1939	0.248	1.56	5.15	1.16	1.07	1.29	0.96	1.44	0.60
S. G. Male 6 years old	January 23, 1939	0.347	2.21	4.44	0.95	1.35	0.98	0.83	1.77	0.68
	February 1, 1939	0.334	2.37	4.57	1.02	1.46	0.93	0.83	1.83	0.84

* Fibrin determined on oxalated plasma by method of Cullen and Van Slyke.

† By method of Howe. The fraction of the serum proteins insoluble in 1.5 Molar Na₂SO₄ is commonly referred to as globulin, the fraction remaining in solution, as albumin.

values of the protein fractions in the same child. The duplicate determinations done on the same blood sample checked closely. We feel that the validity and reproducibility of our analytical technique were established by these checks.

In Tables II and III are summarized the data obtained from the examination of the serum and plasma proteins of normal infants and children. In these tables and all subsequent tables, the values obtained for the group of 10 children from 5 to 10

years of age are used as the standard of reference. Between these ages, it is known that the plasma proteins have attained adult values (6). Seventeen premature infants, ranging in age from one to 68 days, were studied. All weighed less than 5 pounds at the time of study. Seventeen full-term newborn infants, weighing from 7 to 8½ pounds, were studied during the first 48 hours of life. Sixteen healthy infants, ranging in age from 2 to 11 months, constituted the group of older infants examined. In addition, the serums and plasmas of 6 mothers of the premature infants were analyzed. In the premature and newborn infants and in some of the older infants, blood samples were obtained from the superior longitudinal sinus. When blood was drawn from a vein, it was obtained without stasis.

The concentrations of the protein fractions are expressed as grams per 100 cc. of serum or plasma. The standard deviation of each average value is included. Using the average obtained from the 10 older children as normal standards, the "t" test of Fisher (7) was applied to determine significant difference for the values obtained with the other subjects studied.

Examination of Table II shows that the blood fibrin levels are remarkably constant and similar at all age periods studied. These values are in the normal range for adults (8). The high fibrin values for the mothers of the premature infants is

TABLE II

Influence of age and maturity on serum proteins in grams per 100 cc.

	Fibrin	Total serum protein	1.5 Molar Na ₂ SO ₄	
			Insoluble†	Soluble†
Normal children (10 children)	0.28±0.04	7.30±0.59	2.4±0.74	5.0±0.78
Premature infants (17 infants) Ages: 1 to 68 days	0.27±0.15	4.55±0.59*	1.01±0.45*	3.55±0.66*
Full-term newborn infants (17 infants)	0.24±0.04	5.11±0.76*	1.34±0.41*	3.76±0.43*
Older infants (16 infants) Ages: 2 to 11 months	0.28±0.06	6.10±0.29*	1.38±0.68*	4.97±0.73
Mothers of premature infants (6 mothers)	0.56±0.08*	7.20±0.59	2.20±0.76	4.83±0.44

* Significant difference from value for normal children (Fisher's "t" test).

† The fraction in grams per 100 cc. of serum protein precipitated by 1.5 Molar Na₂SO₄ is commonly referred to as globulin, the amount of serum protein remaining in solution, as albumin.

a characteristic finding in pregnant and postpartum women (9).

The total serum protein concentration rises with increasing maturity, starting with a level of 4.55 ± 0.59 grams per 100 cc. of serum in the premature infant and reaching a value of 7.30 ± 0.59 grams in the normal child. This value for the child from 5 to 10 years is the same as that of the adult. The values for older infants (2 to 11 months) are still significantly below the adult figure. We have no data covering the age period from 1 to 5 years, so that we cannot state at which time the normal adult level of total serum protein is attained.

As is obvious in Table II, the increase of total serum protein concentration with age is dependent upon increases in the 2 fractions commonly designated as globulin and albumin. However, in the attainment of the adult level of total serum protein, there is a proportionately greater increase in globulin concentration than in albumin. The low value of the globulin fraction in the premature infant (1.01 ± 0.45 grams) is in good agreement with the values obtained by Darrow and Cary (10). It is interesting to note that the albumin concentration reaches the adult level in the infant at a time when the globulin concentration is still low. Achard and his co-workers (11) reported that the serum globulin of the newborn infant was lower than that of the maternal serum, but that the serum albumin was of the same order of magnitude in both infant and mother. However, our data indicate that the serum albumin of pre-

mature and full-term newborn infants is significantly lower than the maternal serum albumin.

Table III summarizes the data obtained by fractionation of the serums of the same individuals represented in Table II, with the phosphate precipitant. We have charted our data as increments of protein (in grams per 100 cc. of serum) precipitated by successive increases of 0.4 molarity of phosphate precipitant from 0.8 to 2.4 molarity. From 2.4 to 3.0 molar concentration, the protein fractions were separated by successive increases of 0.2 molarity.

Because of technical difficulty in obtaining clear supernatant fluids after centrifugation of the protein precipitates with the 0.8 and 1.2 molar phosphate solutions, we do not feel justified in drawing any conclusions concerning any existing differences in the amounts of protein salted out by these concentrations of precipitant. From 1.2 to 3.0 molar concentration of phosphate precipitant, the supernatant fluids were always clear and subject to accurate analysis. It appears that some of the fraction termed albumin by Howe is present in the precipitate below 2.0 molar phosphate solution, since the summation of the protein increments precipitated below this level is greater than the total globulin value obtained by the Na_2SO_4 method. Also, from the nature of the precipitation curves (1), it would appear that some of the fraction termed globulin may be in solution above 2.0 molar concentration. Thus it may be repeated that, in reporting these increments of protein precipitated by the phosphate or Na_2SO_4 precipitants,

TABLE III
Effect of age and maturity on precipitation pattern of serum proteins

Molar concentration of phosphate precipitant	Grams of protein precipitated per 100 cc.							
	0.8	1.2	1.6	2.0	2.4	2.6	2.8	3.0
Normal children (10 children)	0.12 ± 0.06	0.30 ± 0.13	1.11 ± 0.22	1.21 ± 0.19	1.16 ± 0.25	0.88 ± 0.17	1.68 ± 0.43	0.71 ± 0.28
Premature infants (17 infants) Ages: 1 to 68 days	0.05 ± 0.07	0.19 ± 0.15	$0.53 \pm 0.23^*$	$0.85 \pm 0.09^*$	$0.83 \pm 0.16^*$	$0.59 \pm 0.13^*$	$0.89 \pm 0.17^*$	0.49 ± 0.18
Full-term newborn infants (17 infants)	0.06 ± 0.12	0.20 ± 0.10	$0.68 \pm 0.17^*$	$0.99 \pm 0.17^*$	0.99 ± 0.19	$0.66 \pm 0.12^*$	$0.87 \pm 0.24^*$	0.50 ± 0.18
Older infants (16 infants) Ages: 2 to 11 mos.	$0.02 \pm 0.08^*$	$0.06 \pm 0.07^*$	$0.52 \pm 0.09^*$	1.18 ± 0.16	$1.46 \pm 0.20^*$	$1.02 \pm 0.16^*$	1.29 ± 0.24	$0.32 \pm 0.12^*$
Mothers of premature infants (6 mothers)	0.13 ± 0.12	0.26 ± 0.08	1.03 ± 0.17	1.28 ± 0.30	1.15 ± 0.10	0.94 ± 0.17	1.23 ± 0.18	0.45 ± 0.41

* Significant difference from value for normal children (Fisher's "t" test).

we are not implying chemical specificity for the fractions. However, we feel that the fractions in a sense may be designated as entities for purposes of comparison because of their reproducibility.

In the present state of our knowledge, there appear to be at least 4 electrochemical protein components in the serum protein complex, *viz.*, gamma, beta, and alpha globulin and albumin, named in order of increasing mobility in the electrophoretic cell (12, 13). While the globulin fractions obtained by the Howe method have been correlated with the globulins identified by electrophoretic analysis (14), we have no data comparing the fractions obtained by the phosphate precipitation to electrophoretic fractions. However, there is evidence to suggest that the protein with the lowest mobility (gamma globulin) is salted out by the lowest concentration of precipitant, and that with increasing concentration of precipitant, the proteins of increasing electrophoretic mobility are salted out progressively (14, 15, 16).

From Table III, it is apparent that the values for each of the protein fractions precipitated by concentrations of phosphate precipitant from 1.2 to 2.8 molar are significantly low in the premature infant. Similar low values are encountered in the full-term newborn infant, except that the protein fraction precipitating between 2.0 and 2.4 molar concentration is not significantly low. In the older infant, the low values are only in the range below 1.6 molar concentration. At the higher concentrations of phosphate precipitant, from 2.0 to 3.0 molar concentration, the changes in the amounts of protein precipitated exhibit no constant trend, 2 of the fractions being high and 2, low in value. The reason for these variations is not apparent, but it is possible that the varying proportions of albumin to globulin with increasing age may in some way be responsible for these alterations in precipitation pattern.

Even though there is a progressive increase in total globulin with increasing maturity (Tables II and III), it is apparent that the protein fraction precipitated below 1.6 molar phosphate is lowered throughout the entire period of infancy, having the same value in the older infant as in the premature. This fraction has been said to be chiefly the euglobulin fraction (1, 2). The rise in total globulin occurring with increasing maturity of the

infant is reflected, at least in part, in the progressive increase in value of the fraction precipitated between 1.6 and 2.0 molar phosphate. Since it seems that the globulins with the lowest electrophoretic mobility are salted out first, it is likely that the serum protein fraction precipitated below 1.6 molarity is chiefly gamma globulin, the fraction which has been shown to contain many immune bodies. One is tempted to correlate the low concentration of this fraction in the infant with his well-known poor immune responses (17).

There is no apparent difference in the protein fractions of the older normal child and the adult woman, as is obvious from the values obtained for the 6 mothers of premature infants.

II. CHILDREN WITH GLOMERULONEPHRITIS

The plasmas and serums of a group of children in different stages of glomerulonephritis were examined and the results are summarized in Tables IV and V. In Table IV, the values for the pro-

TABLE IV
Serum proteins in nephritis as grams per 100 cc.

	Fibrin	Total serum protein	1.5 Molar Na_2SO_4 precipitant	
			Insoluble†	Soluble†
Normal children (10 children)	0.28±0.04	7.30±0.59	2.4±0.74	5.0±0.78
Nephritis: active stage (20 children)	0.42±0.11*	7.04±0.85	2.8±0.95	4.24±0.97
Nephritis: chronic stage (4 children)	0.43±0.20*	5.03±0.94	1.28±0.20*	3.75±0.83*
Nephritis: healed stage (7 children)	0.24±0.06	7.11±0.25	1.73±0.42	5.39±0.38

* Significant difference from value for normal children (Fisher's "t" test).

† The fraction in grams per 100 cc. of serum protein precipitated by 1.5 Molar Na_2SO_4 is commonly referred to as globulin, the amount of serum protein remaining in solution, as albumin.

tein fractions usually termed fibrin, albumin, and globulin are tabulated. The normal reference values are again the group of 10 healthy children, aged 6 to 10 years. In the active stage of acute glomerulonephritis, there is an increase in blood fibrin which returns to normal with healing. In patients in the chronic stage of glomerulonephritis, this elevation of fibrin persists. The total serum protein is reduced during the active stage of acute

TABLE V
Precipitation pattern of serum proteins in nephritis

Molar concentration of phosphate precipitant	Grams of protein precipitated per 100 cc.							
	0.8	1.2	1.6	2.0	2.4	2.6	2.8	3.0
Normal children (10 children)	0.12 ± 0.06	0.30 ± 0.13	1.11 ± 0.22	1.21 ± 0.19	1.16 ± 0.25	0.88 ± 0.17	1.68 ± 0.43	0.71 ± 0.28
Nephritis: active stage (20 children)	0.12 ± 0.12	0.29 ± 0.19	1.57 ± 0.42*	1.38 ± 0.30	1.25 ± 0.36	0.90 ± 0.32	0.98 ± 0.30*	0.45 ± 0.21*
Nephritis: chronic stage (4 children)	0.04 ± 0.09	0.11 ± 0.09*	0.65 ± 0.16*	1.35 ± 0.34	0.94 ± 0.28	0.74 ± 0.27	0.82 ± 0.23*	0.32 ± 0.21*
Nephritis: healed stage (7 children)	0.03 ± 0.07	0.20 ± 0.07	1.02 ± 0.17	1.23 ± 0.15	1.43 ± 0.15	1.48 ± 0.29		0.82 ± 0.05

* Significant difference from value for normal children (Fisher's "t" test).

glomerulonephritis, the reduction being caused by a lowering of the sodium sulphate albumin fraction. These reductions were not statistically significant. In the chronic stage of glomerulonephritis, there is a persistently significant reduction in both the sodium sulphate globulin and albumin fractions.

Table V summarizes the values obtained with the phosphate precipitant. The protein fraction precipitating below 1.6 molar phosphate is significantly increased in amount in patients in the acute stage of glomerulonephritis. This significant increase in a sub-fraction of the total globulin (probably gamma globulin) is obscured on determination of the total globulin by the Howe method (Table IV). With healing, this fraction assumes its normal value. In contrast to the acute nephritic, the patients in the chronic stage of glomerulonephritis have a reduction in this fraction. This is in keeping with the observation of Kendall (18) who has demonstrated, by an immunological method, a decrease in a globulin fraction in chronic nephritis. The decrease in the protein fractions precipitated from 2.6 to 3.0 molar concentration, in both the acute and chronic nephritic, corresponds to a decrease in what Butler *et al.* term Albumin II (2). The finding of this decrease is especially interesting in the active stage of acute nephritis where it is not apparent when total albumin is determined alone. In the patients with chronic nephritis, the reduction in the fractions precipitated between 2.6 and 3.0 molar concentration accounts for the total reduction in the albumin fraction as determined by the Howe method.

As criteria of healing in glomerulonephritis, we have utilized the blood sedimentation rate and the Addis count. In another publication (19), we have pointed out that the blood sedimentation rate is usually rapid as long as activity of the nephritis continues, and becomes normal as the process heals. Ham and Curtis (20) have pointed out a direct correlation between the rapidity of blood sedimentation and the plasma fibrin level. Our data would seem to confirm this observation. In the patient with acute glomerulonephritis, the plasma fibrin is increased, as is the sedimentation rate, both returning to normal with healing. Furthermore, in chronic nephritis, there is a sustained increase in both plasma fibrin and blood sedimentation rate. This correlation is pointed out with the usual reservation that a positive correlation does not necessarily imply cause and effect. It has been stated also that an elevation of the serum globulin was correlated with a rapid blood sedimentation rate (20). This is not in keeping with our data. Since, while the patient with active acute glomerulonephritis had an elevated globulin at a time when his blood sedimentation was rapid, the chronic nephritic showed the reverse relationship, *viz.*, a lowered total globulin and a rapid blood sedimentation rate.

We have already suggested that the protein fraction precipitated below 1.6 molar phosphate is probably largely so-called gamma globulin and a carrier of antibodies. The increase in amount of this fraction in the acute stage of glomerulonephritis may be correlated with an increase in the antistreptolysin titer found during the acute phase of this disease (21), and the return of this frac-

tion to a normal level with healing of the disease coincides with the fall in antistreptolysin titer occurring during recovery. It is also pertinent that the chronic nephritic, in the absence of active infection, exhibits a decrease in this fraction and does not have a very high antistreptolysin titer (22).

III. CHILDREN WITH NEPHROSIS

The plasmas and serums of 11 children with the nephrotic syndrome were examined 2 or more times during periods when they were in an active phase of their disease, as evidenced by edema,

TABLE VI
Serum proteins in nephrosis as grams per 100 cc.

	Fibrin	Total serum protein	1.5 Molar Na_2SO_4 precipitant	
			Insoluble†	Soluble†
Normal children (10 children)	0.28 ± 0.04	7.30 ± 0.59	2.40 ± 0.74	5.0 ± 0.78
Nephrosis: active stage (11 children) Observed in 26 active periods	$0.65 \pm 0.17^*$	$3.88 \pm 0.77^*$	2.70 ± 0.70	$1.19 \pm 0.88^*$
Nephrosis: inactive stage (6 children)	$0.67 \pm 0.30^*$	$5.89 \pm 1.30^*$	2.23 ± 0.29	$3.52 \pm 1.54^*$

* Significant difference from value for normal children (Fisher's "t" test).

† The fraction in grams per 100 cc. of serum protein precipitated by 1.5 Molar Na_2SO_4 is commonly referred to as globulin, the amount of serum protein remaining in solution, as albumin.

massive albuminuria, and hypercholesterolemia. Fractionation by the Howe technic (Table VI) shows the well-known changes of this disease: normal or slightly elevated sodium sulphate globulin values, low sodium sulphate albumin values.

Six of these children were re-examined during an inactive phase, when they had been free from edema and apparently "clinically well" for a 3-month period. It is apparent that the fibrinogen value was still elevated, perhaps related to the still rapid sedimentation rate. Similarly, evidence of incomplete recovery was also apparent from the reduced serum albumin.

In the fractions obtained by the phosphate precipitation (Table VII), the striking finding is the reduction in the globulin fraction precipitated between 1.2 and 1.6 molar concentrations, which became normal when the disease was apparently inactive clinically (Table VIII).

This reduction is similar to the lowered value found in chronic nephritis. However, there is this difference; in the chronic glomerulonephritic, the total globulin value by Na_2SO_4 precipitation is low, while in the nephrotic the total globulin value by the same method is normal. One may thus interpret this finding as an indication that in both diseases there is a change in a specific globulin moiety (gamma globulin).

Since the total sodium sulphate globulin value for the active nephrotic is normal or slightly elevated (2.7 grams per 100 cc. of serum) and since all the proteins, precipitating from 1.6 molar phosphate concentration and below, total only 0.87 grams, a large part of the globulin (1.83 grams) appears to reside in the fraction precipitating between 1.6 and 2.0 molarity and in fractions above this concentration of phosphate precipitant.

This apparent altered dispersion of the globulin fractions, obtained by phosphate precipitation in the nephrotic patient, was subjected to further study. Because of the well-known elevated lipid

TABLE VII
Precipitation pattern of serum proteins in nephrosis

Molar concentration of phosphate precipitant	Grams of protein precipitated per 100 cc.							
	0.8	1.2	1.6	2.0	2.4	2.6	2.8	3.0
Normal children (10 children)	0.12 ± 0.06	0.30 ± 0.13	1.11 ± 0.22	1.21 ± 0.19	1.16 ± 0.25	0.88 ± 0.17	1.68 ± 0.43	0.71 ± 0.28
Nephrosis: active stage (11 children) 26 periods of observation	0.23 ± 0.16	0.20 ± 0.14	$0.44 \pm 0.20^*$	1.43 ± 0.46	1.00 ± 0.40	$0.23 \pm 0.13^*$	$0.15 \pm 0.17^*$	$0.03 \pm 0.04^*$
Nephrosis: inactive stage (6 children)	0.23 ± 0.22	0.19 ± 0.06	1.25 ± 0.23	$1.50 \pm 0.19^*$	1.04 ± 0.07	0.62 ± 0.33	$0.65 \pm 0.47^*$	0.42 ± 0.14

* Significant difference from value for normal children (Fisher's "t" test).

TABLE VIII

Changes in serum and plasma protein fractions, in grams per 100 cc., of a patient during subsidence of nephrotic syndrome

Date	Clinical condition of patient	Fibrin	Total serum protein	1.5 Molar Na ₂ SO ₄ precipitant		Precipitated by increasing concentrations of phosphate precipitant						
				In-soluble	Soluble	0 to 1.2 Molar	1.2 to 1.6	1.6 to 2.0	2.0 to 2.4	2.4 to 2.6	2.6 to 2.8	2.8 to 3.0
February 3, 1939	Edematous (clinically active)	0.87	4.28	3.91	0.37	0.17	0.36	2.05	0.98	0.58		
February 6, 1939	Edematous (clinically active)	0.78	4.47	4.08	0.39	0.46	0.38	2.05	1.42			
February 21, 1939	Edema almost gone	0.55	5.70	4.57	1.13	0.54	0.89	2.17	1.14	0.92		
March 27, 1939	No edema (clinically inactive)	0.42	6.65	2.19	4.46	0.56	1.08	1.56	1.08	0.74	1.11	0.44
April 12, 1939	No edema (clinically inactive)	0.38	7.16	1.92	5.24	0.37	1.47	1.46	0.97	0.91	1.11	0.64

content of the plasma of nephrotic individuals, the effect of removal of serum and plasma fat on the globulin dispersion pattern was investigated.

Samples of plasma and serum of 7 nephrotic patients were obtained during an active stage of the disease and divided into 2 aliquots. One serum and plasma aliquot was subjected to the analytic procedures described before. The second aliquot was shaken with 2 volumes of ether, allowed to stand, and the supernatant ether transferred to weighing bottles. This extraction was done 7 times with each sample. After the last extraction, the residual ether was allowed to evaporate at room temperature, under a gentle stream of air. The residual serum sample was thus brought back to its original volume.² Following the removal of fat, the serum was subjected to the same analytical procedures as its non-ether extracted aliquot.

Table IX contains the data showing the effect of the removal of fat on the protein dispersion. It will be noted that in all but one instance, the values for the protein fractions precipitating at 1.6 molar concentration and below are increased significantly, following the removal of fat. Con-

versely, the fractions precipitating at 2.0 molar concentration were usually reduced.

In order further to determine whether hyperlipemia was responsible for the peculiarities of precipitation in the nephrotic patient, the sera of premature infants, who also have a low 1.6 protein fraction, were similarly studied. Ether extraction of 3 serum samples resulted in no significant alteration. It is pertinent that the amount of fat obtained by ether extraction of the premature infant's serum was much lower than that of the nephrotic child's. The fat removed from the premature infant's serum varied from 0.1 to 0.21 gram per 100 cc., while the nephrotic serum samples yielded from 0.77 to 2.04 grams. The hyperlipemic plasma of a child with cretinism yielding 0.612 grams of fat per 100 cc. with ether extraction was also studied. Following ether extraction, the protein precipitation pattern changed in the same directions as that of the nephrotic patient, the 1.6 protein fraction increasing significantly. Fat extraction of a recovered nephritic patient's serum produced no change in the concentration of the 1.6 fraction. This patient's serum yielded a relatively small amount of fat (0.23 gram). Similar treatment of a sample of pooled adult serum resulted in no change in precipitation, again with a small fat yield.

From these considerations, it appears that an increased amount of plasma or serum fat alters the precipitation pattern of the proteins with the phosphate precipitant. Thus, hyperlipemia in the

² There is little difficulty in the ether extraction of fat from the serum of normal children or premature infants. However, fatty nephrotic sera, when ether extracted, are likely to form an emulsion of much greater volume than the original serum. These emulsions may be resolved by partial evaporation of the ether and then allowing the sample to stand in the ice-box overnight. The residual supernatant ether which separates out is very fatty.

TABLE IX

Effect of removal of fat by cold ether extraction on precipitation of protein fractions (grams per 100 cc.) of nephrotic plasmas and serums

Subject	Condition of serum and plasma	Grams per 100 cc. of serum protein precipitated by phosphate precipitant of increasing molar strength						Fibrin	Total serum protein	1.5 Molar Na ₂ SO ₄ precipitant	
		1.6 Molar	2.0 Molar	2.4 Molar	2.6 Molar	2.8 Molar	3.0 Molar			In-soluble†	Soluble†
N. B.	Untreated	0.61	1.66	1.40	0.34	0.00	0.01	0.74	4.53	2.19	2.44
	Ether extracted	1.41	1.16	1.14	0.15	0.39		0.74	4.74*	2.76	1.98
N. B.	Untreated	0.64	1.79	1.21	0.57	0.72		0.50	5.49	2.09	3.40
	Ether extracted	1.41	1.28	1.16	0.71	0.81		0.50	6.30*	2.74	3.56
D. B.	Untreated	0.33	0.94	0.54	0.11	0.05			2.31	1.90	0.41
	Ether extracted	0.45	0.92	0.46	0.12	0.00			2.29	1.91	0.37
J. B.	Untreated	0.81	1.42					0.65	3.53	2.65	0.88
	Ether extracted	1.33	1.13					0.65	3.57	2.72	0.85
S. K.	Untreated	0.78	1.77	0.87	0.06	0.07	0.00	0.75	3.59	3.08	0.51
	Ether extracted	1.26	1.31	0.80	0.14	0.06		0.75	3.60	2.93	0.67
L. W.	Untreated	0.61	1.77					0.74	4.68	2.20	2.47
	Ether extracted	2.82						0.74	4.72		
V. D.	Untreated	0.66	1.58					0.48	3.95	2.08	1.87
	Ether extracted	0.99						0.48	3.80		

† The fraction in grams per 100 cc. of serum protein precipitated by 1.5 Molar Na₂SO₄ is commonly referred to as globulin, the amount of serum protein remaining in solution, as albumin.

* The apparent increase in total serum protein concentration following ether extraction of these 2 serums was due to evaporation of water from the serums during the evaporation of ether. However, these changes do not alter the significance of the changes in the fractions precipitated by 1.6 and 2.0 molar phosphate precipitant.

nephrotic appears to have some causal relationship to the low protein values at 1.6 (and lower) molar concentrations of precipitant. An increase in serum fat apparently changes the dispersion of globulins, causing them to be precipitated in the range in which the albumins begin to precipitate.

An experiment performed on a normal 8-year-old girl would seem to lend support to this statement. The serum of this child was fractionated following an 18-hour fast. As is apparent from Table X, ether extraction of this serum yielded 230 mgm. of fat per 100 cc. of serum and resulted in no apparent change in the 1.6 and 2.0 fractions. A serum sample taken from the same child, 2 hours after a high fat meal, yielded 530 mgm. of fat on extraction with ether. Following ether extraction, there were shifts in the 1.6 and 2.0 fractions, similar to those encountered in the nephrotic patient.

Obviously, the low 1.6 protein fraction in the premature infant, the full-term newborn infant, and the normal older infant are not dependent

TABLE X

Effect of increased blood fat on the precipitation pattern of serum proteins with phosphate precipitant

		Amount of fat removed by ether extraction	Grams per 100 cc. of protein precipitated by phosphate precipitant	
		mgm. per 100 cc.	1.2 to 1.6 Molar	1.6 to 2.0 Molar
C. L. Serum from normal patient after 18 hour fast	Untreated serum		1.05	1.06
	Same serum extracted with cold ether	230	1.02	0.96
C. L. Serum from same patient 2 hours after high fat meal	Untreated serum		0.94	1.22
	Same serum extracted with cold ether	530	1.32	1.03

upon this mechanism, but represent real low values, which reflect the low value for total globulin in these subjects.

Analysis of nephrotic serum by electrophoresis by Longworth and MacInnes (23), and Luetscher (24) showed a reduction in gamma globulin and high values for alpha and beta globulins. Our analytical data with phosphate precipitation of nephrotic sera would seem to fit in with these findings, in that the 1.6 fraction is below the normal value and the 2.0 fraction tends to be above normal. Following extraction of serum fat with ether, Longworth and MacInnes found a reduction in both alpha and beta globulin fractions, with no change in concentration of gamma globulin. This is not in keeping with our findings, in that an increase in the 1.6 fraction (gamma globulin) accompanied the decrease in the 2.0 fraction after removal of serum fat with ether. Longworth and MacInnes regard the decrease in alpha and beta globulins as being due to refractometric changes resulting from the removal of fat with a refractive index similar to that of the serum globulins. Obviously, this explanation does not hold for our data, in that actual changes in nitrogen content of the fractions precipitated were measured. We are inclined to believe that an altered distribution of globulins occurs in the nephrotic patient.

Longcope (21) has noted that the antistreptolysin titer of nephrotic patients was usually low; Earle and his co-workers (22) have observed that the basal antistreptolysin titer of the patient with chronic nephritis falls to a lower level with the onset of a nephrotic stage, returning to a normal titer with the subsidence of edema. It is possible that the elevated blood fat of the nephrotic, through an altered distribution of globulins, may have some bearing on these lowered antistreptolysin values.

While all the protein fractions at the albumin end of the phosphate precipitation have lowered values in the nephrotic patient during the active stage of his disease, the reduction is more marked in the protein fractions precipitating between 2.6 and 3.0 molar concentration of precipitant, the so-called Albumin II. In the inactive stage of the disease, the values for the albumin fractions tend to return toward normal levels. It is apparent that these inactive patients are still abnormal,

since the total albumin as measured by the Howe method was still reduced. Our data suggest the possibility of the existence of a labile albumin component, which fluctuates in concentration with the clinical state of the nephrotic patient, being low during the edematous stages and rising with the disappearance of edema (Table VIII). A similar change of lesser degree is to be noted in the patient with acute nephritis. There is evidence available to show that serum albumin is composed of more than one moiety. Hewitt (25) was able to isolate two albumins of different chemical composition. Luetscher (24) found, by electrophoretic analysis, that there was comparatively greater reduction of one component of the albumin complex in the serum of the nephrotic patient. It is interesting too, that Bourdillon (26) found that the urine and plasma albumins of nephrotic patients had different molecular weights, an observation confirmed by Longworth and MacInnes (23). It is possible that this urinary albumin of low molecular weight may correspond to the labile serum albumin fraction (Albumin II), so strikingly reduced in the nephrotic patient.

Fat extraction of nephrotic serum did not produce significant changes in the albumin fractions precipitated with the phosphate precipitant.

SUMMARY

I. The serum and plasma protein fractions, separated by sodium sulphate and phosphate precipitants, were studied in premature infants, full-term newborn infants, older infants, and young children. Blood fibrin was also determined.

(a) The blood fibrin levels were found to be constant at all ages, and equal to adult values.

(b) The total serum protein values rise with increasing maturity. Both the albumin and globulin fractions are involved in the increase, but there is a proportionately greater increase in the globulin fraction.

(c) Throughout all of infancy, there is a reduction in certain of the globulin fractions (probably gamma globulin).

II. The serum and plasma protein fractions were determined in children during the different clinical phases of glomerulonephritis.

(a) Plasma fibrin was elevated during the acute stage of the disease and returned to normal

with healing. In the chronic phase of the disease, there is persistent elevation of the fibrin.

(b) In acute glomerulonephritis, there is a slight lowering of serum albumin. In the chronic stage of the disease, both serum albumin and globulin are reduced.

(c) In acute glomerulonephritis, there is an increase in a globulin subfraction (gamma globulin), which returns to its normal level with healing. This globulin subfraction is reduced in value in chronic nephritis.

(d) The relationships between the changes in plasma protein fractions and the alterations in rate of blood sedimentation in glomerulonephritis are discussed.

III. The serum and plasma protein fractions were determined during the different clinical stages of lipoid nephrosis.

(a) Plasma fibrin was elevated during the active phase of the disease.

(b) During the active phase of the disease, the well-known reductions in total protein and serum albumin, and the normal or slightly elevated serum globulin, were encountered.

(c) A reduction in the globulin fraction, presumed to be gamma globulin, was found during the active disease, returning to a normal value with the subsidence of the acute edematous stage. This reduction in the globulin fraction is not of the same nature as that found in young infants. Evidence is presented indicating that the low value for this globulin fraction in the nephrotic, in a large measure results from an altered dispersion of the globulins, caused by the hyperlipemia of nephrosis. Other conditions in which hyperlipemia was present showed similar changes in globulin dispersion.

(d) The reduction of total serum albumin in the nephrotic and in the nephritic patient is largely due to a decrease in a labile subfraction of the albumin.

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