JCI The Journal of Clinical Investigation

CHEMICAL, CLINICAL, AND IMMUNOLOGICAL STUDIES ON THE PRODUCTS OF HUMAN PLASMA FRACTIONATION XXX. THE USE OF SALT-POOR CONCENTRATED HUMAN SERUM ALBUMIN SOLUTION IN THE TREATMENT OF CHRONIC BRIGHT'S DISEASE

G. W. Thorn, S. H. Armstrong Jr., V. D. Davenport, L. M. Woodruff, F. H. Tyler

J Clin Invest. 1945;24(6):802-828. https://doi.org/10.1172/JCI101666.

CHEMICAL, CLINICAL, AND IMMUNOLOGICAL STUDIES ON THE PRODUCTS OF HUMAN PLASMA FRACTIONATION

XXX. THE USE OF SALT-POOR CONCENTRATED HUMAN SERUM ALBUMIN SOLUTION IN THE TREATMENT OF CHRONIC BRIGHT'S DISEASE [1, 2, 3]

By G. W. THORN, S. H. ARMSTRONG, Jr.,[4] V. D. DAVENPORT, L. M. WOODRUFF,[5] and F. H. TYLER[6]

(From the Departments of Medicine and Physical Chemistry, Harvard Medical School, and the Medical Clinic, Peter Bent Brigham Hospital)

(Received for publication May 1, 1945)

Much of the disability induced by chronic Bright's disease, particularly during the nephrotic stage, is attributable to thte coexistent disturbances in protein metabolism. The latter may be characterized as follows: *1,* proteinuria; *2,* hypoalbuminemia; and *3,* depletion of tissue protein stores. During the past few years, numerous attempts have been made to correct the hypoalbuminemia, depletion of body protein stores, and attendant edema in patients with nephrotic syndromes by administering human plasma intravenously (1, 2, 3). This form of therapy obviously had as its objectives the use of protein solution as a diuretic agent by virtue of its high colloid osmotic pressure, the restoration of serum albumin level, and the replenishment of body protein stores. The last two objectives were rarely, if ever, attained because of the limited quantity and high cost of human plasma solutions. The former objective was frequently not accomplished, even with concentrated solutions of human plasma, possibly, as has been suggested (1), because of the relatively high content of sodium chloride in these preparations.

Recently it has been shown (4) that a single dose of 25 grams of concentrated high-salt human serum albumin proved inadequate to induce any prolonged changes in serum protein level or clinical condition although small but consistent increases in urine volume were observed in all three nephrotic patients following therapy. In another study (5) the administration of considerably larger amounts over longer periods led to inconstant results. As in the case of plasma, the salt content of the albumin used in these studies was relatively high, as considerations of stability under field conditions for which the concentrated normal human serum albumin was designed led to the adoption of a concentration of 0.3 M. sodium chloride (6).

Recently there has been developed a series of preparations of normal human serum albumin whose stability characteristics are even better than those of the high-salt albumin, but whose salt content is extremely low (7). In the following table is presented a comparison of the amount of sodium (expressed as sodium chloride) contained in the protein dosage sufficient to hold 1 liter of plasma water in the blood stream in the normal range of plasma colloid osmotic pressure.

Citrated plasma	High-salt albumin	Salt-poor albumin
11.7 gm.	4.8 gm.	1.6 gm.

Though limitation of these preparations has made direct comparison impossible in the studies reported here, we have attempted to answer the following questions:

[1] The albumin used in this work has been prepared under contract, recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and Harvard University.

[2] This is paper No. 41 in the series, "Studies on Plasma Proteins" from Harvard Medical School, Boston, Massachusetts, on products developed by the Department of Physical Chemistry from blood collected by the American Red Cross.

[3] This article has been released for publication by the Division of Publications of the Bureau of Medicine and Surgery of the United States Navy. The opinions or assertions contained herein are the private ones of the writers and are not to be construed as official or reflecting views of the Navy Department or the Naval Service at large.

[4] Welch Fellow in Internal Medicine of the National Research Council.

[5] Lieutenant Commander, Medical Corps, United States Naval Reserve.

[6] Lieutenant (junior grade), Medical Corps, United States Naval Reserve.

1. Is salt-poor concentrated human albumin a safe and effective diuretic agent in edema of renal origin?

2. What is the optimum dose and method of administration?

3. What effect does the administration of salt-poor concentrated human albumin have on the serum protein level of patients with chronic nephritis?

4. What effect does the intravenous administration of a concentrated albumin solution have on nitrogen balance in the presence of adequate dietary protein?

METHODS

The salt-poor albumin used in these investigations was prepared in the form of a 25 per cent solution without preservative by the Plasma Fractionation Laboratory of the Department of Physical Chemistry.[7] The physical chemical characterization of normal human albumin has been presented in previous papers of this series (8 to 11). The albumin was diluted with sterile dextrose solution so that the resulting fluid contained 10 per cent albumin and 6 per cent dextrose.[8]

During these studies, patients were restricted to bed rest and were maintained on a constant regimen.[9] Each patient received a diet of constant composition containing at least 1 gram of protein per kilogram of body weight per day and adequate in carbohydrate and fat to meet caloric requirements. The diet was salt-poor in all cases except that of K. N. who received a "restricted salt" diet.[10] The daily fluid intake of each patient was also maintained at a constant level. On those days when albumin solution was administered, an equivalent volume of fluid was subtracted from the patient's drinking water.[11]

All blood samples were drawn in the morning while the patients were fasting. The urine of each patient was collected quantitatively, pooled in 12- or 24-hourly amounts, and preserved with toluol and refrigeration. Stool specimens were collected for 3- or 5-day periods and were dried at once on a steam-bath. In calculating nitrogen balance, a value of 15 per cent of nitrogen intake was used for the value of nitrogen excretion in the stool in all instances since stool analyses on several occasions indicated that the fecal nitrogen excretion in these patients did not exceed this value.

Blood sera were analyzed for total nitrogen by micro-Kjeldahl digestion followed by direct Nesslerization. Nonprotein nitrogen of blood and urine was determined by Nesslerization following micro-Kjeldahl digestion of a trichloracetic acid filtrate.[12] Serum albumin and globulin were determined chemically by the method of Howe (13) using the arbitrary nitrogen factor 6.25, urea nitrogen by a modification of the method of Karr (14), and cholesterol was estimated colorimetrically by direct application of the Liebermann-Burchard (15) reaction on an alcohol-ether extract without hydrolysis of esters.

In electrophoretic analyses[13] carried out in the apparatus of Tiselius (16), the long cell described by Longsworth (17) was used. Schlieren diagrams were obtained by the cylindrical lens method (18). Sodium diethyl barbiturate buffer at pH 8.6 and ionic strength 0.1 permitted, in most cases, adequate separation of albumin from alpha-1 globulin when the run was carried out at a field strength of approximately 5 volts per centimeter for 2 or 3 hours at 2° to 4° C.

Because of the abnormally large amount of lipoids bound to the non-dialyzable protein nitrogen and migrating principally with the beta (19, 20) and alpha globulins, the application of electrophoretic distribution of compo-

[7] We are indebted to Dr. L. E.. Strong and the Staff of the Plasma Fractionation Laboratory for the carrying out of the preparation, and to Dr. Geoffrey Edsall and the Staff of the Antitoxin and Vaccine Laboratory of the Massachusetts Department of Public Health for control of sterility and safety.

[8] Pyrogen reactions were observed rarely; in one instance this was traced to the albumin itself, in the other to the dextrose solution used in diluting.

[9] We are indebted to Dr. Thomas J. Kennedy for his aid in the supervision of this regimen and of many of the experiments.

[10] The salt-poor diet on the Metabolism Service of the Peter Bent Brigham Hospital contains 1 to 2 grams of salt per day; the "restricted salt" diet contains 5 to 6 grams of salt per day.

[11] In the initial studies the pulse, respiration, blood pressure, and venous pressure of the patients showed little variation; these observations were later discontinued. In one case only, that of R. S., did the intravenous administration of albumin cause a significant rise in blood pressure.

[12] Beckman and associates (12) have reported considerable amounts of a trichloracetic acid soluble protein in the urine of patients exhibiting proteinuria of etiology unspecified. In electrophoretic mobility at pH 8.6 this protein appeared intermediate between alpha-1 and alpha-2 globulins. In that both blood and urine of patients with chronic Bright's disease contain abnormally high amounts of protein of similar electrophoretic characteristics, it is possible that the use of trichloracetic acid as a precipitant introduces a systematic error in nonprotein nitrogen and, thus, by difference, in protein nitrogen determinations.

The fact that the error is small in the instance of serum protein values is indicated by N.P.N. values within normal range in these experiments. Whereas in the case of urine the error in partition between protein and nonprotein nitrogen may be larger, it will not affect either the overall nitrogen balance studies nor, indeed, the data obtained on changes in protein excretion following albumin administration in that albumin is insoluble in trichloracetic acid and the administration of albumin appeared not to affect significantly the daily output either of nonprotein nitrogen or of electrophoretically determined globulins.

[13] We are indebted to Mr. Metchie J. E. Budka for carrying out most of these analyses.

nents to total protein nitrogen figures in order to determine the plasma concentration of a given component is frequently subject to extraordinary error.[14] Likewise, difficulty in extracting dye T-1825 from highly lipemic sera rendered grossly inaccurate several attempts at blood volume determinations.[15] Therefore, in the studies here reported, no attempt has been made to calculate from electrophoretic schlieren distribution, protein nitrogen, and plasma volume the total circulating amount of any given component. The electrophoretic distributions have in the main been used as a check on the direction of change of serum albumin as shown by the Howe (13) method and to indicate any gross shifts in individual globulin components as a result of therapy. Although approximations in change of plasma volume have occasionally been calculated from hematocrit changes, the limitations of this method are clearly recognized, particularly the error introduced by change in red cell volume during albumin infusion.

With the variations in the clinical course and physiologic factors known to occur in chronic Bright's disease, it is apparent that a therapeutic agent which might be helpful at one stage of the disease might be of no help at another time or might even be contraindicated. Thus, it is essential to study the same patients in various stages of the disease throughout their long course of illness, or else one must include in the group of cases under observation representatives of the several stages of the disease. Fully aware of the changes in the clinical course and inconstancy of effectiveness of therapeutic agents, we have studied a small group of patients with chronic nephritis in which certain of the classical stages are represented. Detailed clinical summaries are recorded at the end of the paper.

Distributions of components in the electrophoretic schlieren diagrams of the plasmas of these untreated patients are presented in Table I and illustrated together with certain clinical data in Figure 1. It is of interest to correlate the range of these distributions with the clinical stages. At one extreme is patient J. G., whose massive edema, severe hypoproteinemia, albuminuria, and extreme hypercholesterinemia constituted the nephrotic picture in its most severe form. The schlieren diagram at the top of Figure 1 likewise shows striking deviations from the normal that have been often noted in nephrosis (4, 19, 20). Albumin constitutes less than 10 per cent of the diagram in contrast to a normal value of 55 per cent. Likewise, the gamma globulins comprise less than half their normal area. The principal components are alpha-2 globulins and beta globulins, of which alpha-2 are present in excess of beta. The fibrinogen is elevated.

Patients L. I., W. H., D. S., and K. N., who clinically present the nephrotic picture in progressively less marked degree, show in the elec-

[14] This error, far greater than that encountered in normal plasma (21), applies to a much less degree in the instance of nephrotic urine owing to the rather small amount of lipoid bearing protein which passes through the kidney. In the following table are presented the refractive index increments per gram of nondialyzable plasma, and urinary protein nitrogen of patient J. G., during a control period and following the injection of albumin. The plasma albumin content corrected on the basis of the refractive index increment differs markedly from the uncorrected value obtained directly from the schlieren diagram, the urinary albumin but slightly. The magnitude of correction will vary with the degree of lipemia. The careful addition experiments of Luetscher (4) demonstrated that in his studies such errors were negligible.

	Refractive index increment	Uncorrected albumin content	Corrected albumin content *
	per gram protein N	*per cent*	*per cent*
	Plasma		
Control period	2.07×10^{-4}	9	17
Following intravenous albumin	1.63×10^{-4}	32	45
	Urine		
Control period	1.20×10^{-4}	64	66
Following intravenous albumin	1.17×10^{-4}	88	89

* The refractive index increment per gram of normal human serum albumin nitrogen has been found in this laboratory to be approximately 1.16×10^{-4}. The refractive index correction is based on this value although the actual value of nephrotic serum albumin may be somewhat higher owing to migration of some lipoid with it. The correction for the effect of protein concentration and ionic strength on apparent albumin concentration, which is less in amount and opposite in sign, has been neglected in this table.

[15] We are indebted to Dr. John G. Gibson, 2nd, for both carrying out and providing the following critical comment on the determination of the plasma volume of certain of the patients by the Evans blue technique.

"The turbidity of these patients' sera is such as to cause a great deal of deflection of incident light in the absorption cup. Even when each individual dyed plasma sample is read at a wave length of 620 against dye-free plasma, the results obtained do not reflect the light absorption due to the presence of dye alone. Extraction with tri-ethyl-phosphate, which is ordinarily adequate in dealing with moderate lipemia, failed in the cases of these milky plasmas. No method of dye extraction is to my knowledge available which will overcome this difficulty."

The practice of Longsworth and co-workers (19) of brief centrifugation at 30,000 RPM was not attempted.

TABLE I

Distributions of components in electrophoretic schlieren diagrams of plasma proteins of patients before albumin administration*

Patient	Albumins	α_1 Globulins	α_2 Globulins	β Globulins	Fibrinogen	γ Globulins
	per cent					
J. G.	7	4	42	28	16	3
L. I.	17	5	36	22	16	4
W. H.	17	5	21	38	15	4
D. S.	26	8	22	30	9	5
K. N.	37	6	15	22	12	8
E. B.	46	4	34**		13	3
R. S.	32	5	20	23	(clotted)	20
Normal pooled human plasma	55	5	9	13	7	11

* Sodium diethylbarbiturate buffer, pH 8.6.
** Not resolved.

trophoretic diagrams a progressively greater contribution of albumin to the total area and a trend toward the restoration of the normal beta–alpha-2 ratio wherein the beta peak is predominant.

At the other extreme of the group is patient R. S. He alone presents a gamma globulin which, in contrast to the subnormal value in the other patients, comprises almost twice the normal contribution of this component to the total picture. In this respect, the electrophoretic diagram resembles that of acute rheumatic fever (22, 23), of disseminated lupus, (23, 24) and of periarteritis nodosa (23).

In view of the fact that in these latter diseases an immunological component in the mechanism has long been suspected, that in certain forms of experimental nephritis such a component has been demonstrated (25), and also in view of the fact that the gamma globulin fraction in normal human plasma contains the majority of the antibodies (26), it is of particular interest that this patient alone gave evidence of recent acute exacerbation of his renal disease in the form of considerable oliguria and hematuria.[16]

In Figure 2 are presented electrophoretic schlieren diagrams with the plasma of patient J. G. before and after a remission in her disease. It will be noted that her plasma protein components appear to progress through the same changes as are illustrated in passing from severe to mild nephrosis in the group of patients detailed in Table I and Figure 1.

OBSERVATIONS

1. *Dosage and method of administration*

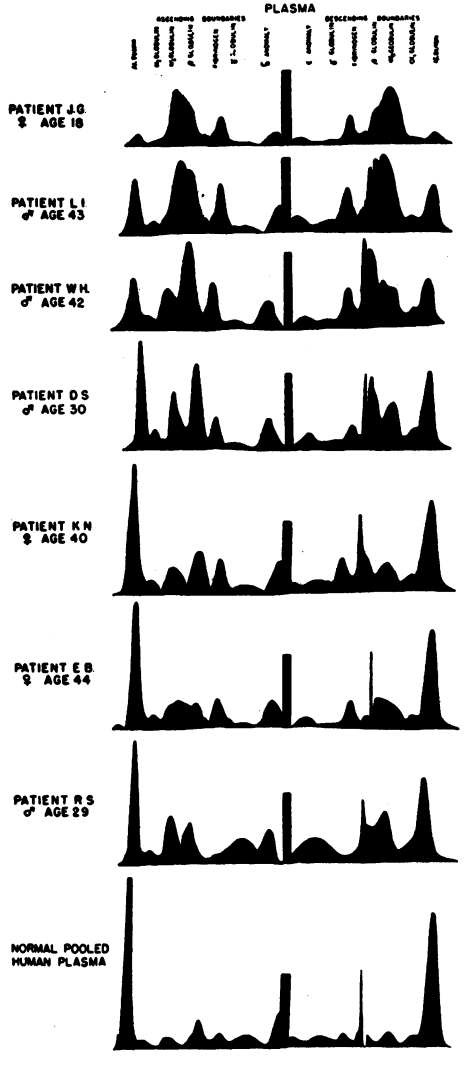
In the treatment of shock in war casualties, for which concentrated serum albumin was originally developed, rapid injection was desirable to effect an immediate increase in plasma volume. In patients with chronic nephritis and edema, however, it was obvious that a slower rate of injection might be required if circulatory failure were to be avoided. In exploratory experiments the intravenous administration of 10 grams of salt-poor albumin per hour occasioned small rises in pulse rate, blood pressure, and venous pressure. This rate appeared well within limits of safety for initial injection in all patients studied. Faster administration proved uneconomical owing to increased urinary protein loss in patients with marked proteinuria.

In order to facilitate the slow intravenous administration of such a small quantity of protein solution, the concentrated (25 per cent) solution of human albumin was added to 10 per cent dextrose solution in such proportions that 500 ml. of final solution contained 50 grams of albumin (10 per cent) and 30 grams of glucose (6 per cent). This was administered at a rate of 100 ml. per hour.[17] Control studies were made using 6 per cent dextrose without albumin.

[16] Studies by Dr. Clement Finch on the complement level on 4 patients of this group showed normal values for all but R. S. whose level was strikingly and consistently depressed.

[17] It was apparent early in these studies that the time-consuming slow administration of albumin seriously compromised dietary intake unless such care were taken not

Summary of Findings on Untreated Patients



	CLINICAL			BLOOD			URINE	
	ONSET	B.P. RANGE	EDEMA	N.P.N. (MGM./100 ML.)	CHOLESTEROL (MGM./100 ML.)	TOT. PROT. (GM./100 ML.)	PROTEIN (GM./24 HRS.)	R.B.C.
PATIENT J.G. ♀ AGE 18	5 MO. INSIDIOUS EDEMA FOLLOWING RESPIRATORY INFECTION	100/60-145/110	+++	43	1100	2.8	22	++
PATIENT L.I. ♂ AGE 43	6 MO. INSIDIOUS ALBUMINURIA, 2 MO. EDEMA	140/90-175/120	++	47	1170	3.8	18	+
PATIENT W.H. ♂ AGE 42	1 MO. EDEMA OLIGURIA SIMULTANEOUS WITH RESPIRATORY INFECTION	120/70-165/100	++	30	755	4.1	19	+
PATIENT D.S. ♂ AGE 30	18 MO. INSIDIOUS ALBUMINURIA, 12 MO. EDEMA	110/60-150/95	++	27	1000	5.3	11	±
PATIENT K.N. ♀ AGE 40	5 WKS. INSIDIOUS ALBUMINURIA EDEMA	90/60-130/70	+	23	430	5.6	7	+
PATIENT E.B. ♀ AGE 44	12 MO. INSIDIOUS HYPERTENSION PROTEINURIA, 3 MO. EDEMA	125/80-170/100	+	49	880	4.1	5	±
PATIENT R.S. ♂ AGE 29	4 MO. INSIDIOUS EDEMA WITH IMPROVEMENT, 3 WKS. OLIGURIA SMOKY URINE	140/90-175/115	±	61	340	5.6	12	+++

Fig. 1

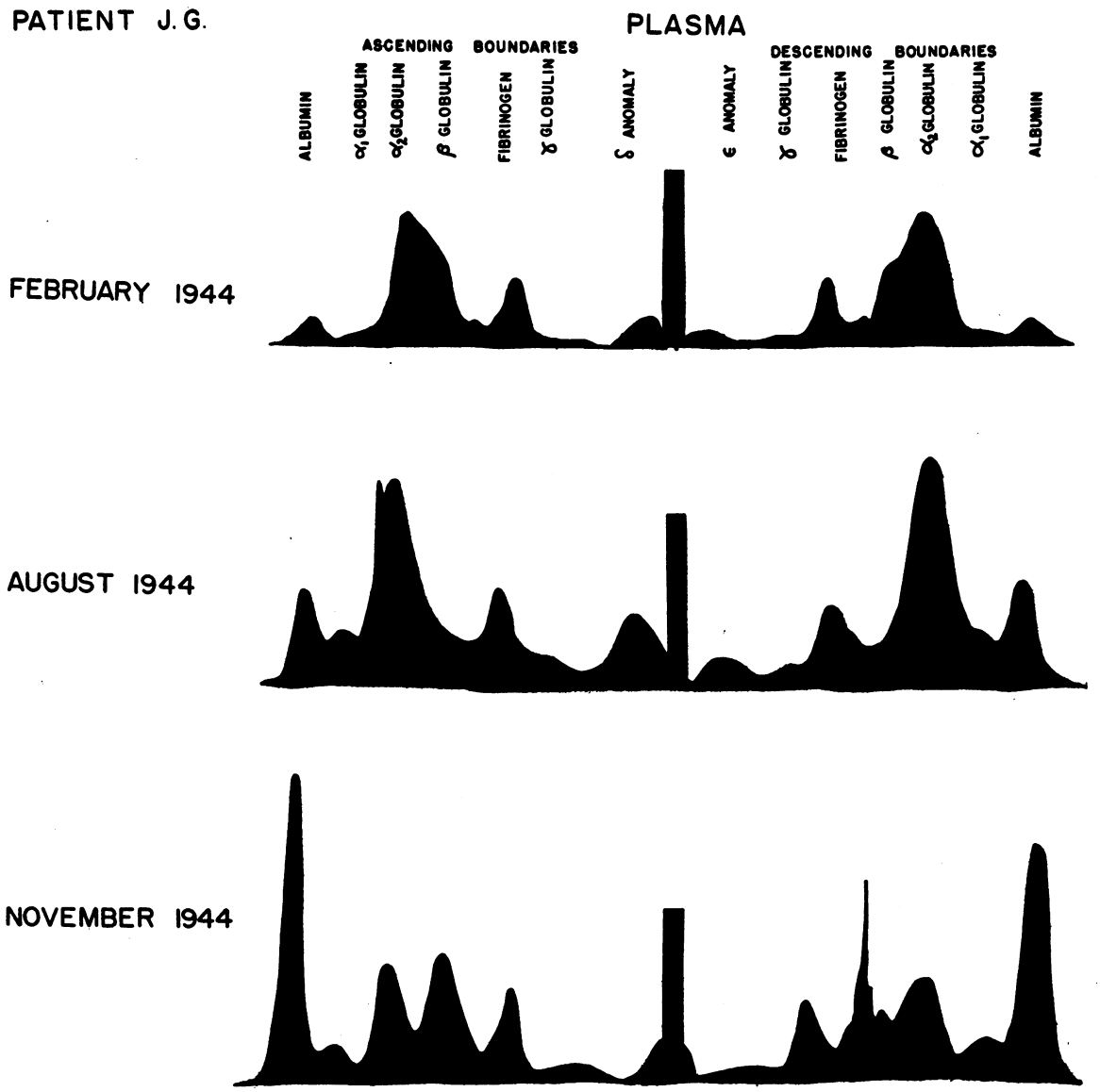


FIG. 2

In 1 patient, R. S., with moderate hypertension, azotemia, and *no edema,* the administration of 50 grams of albumin at 10 grams per hour on 3 successive days resulted in a rise in serum albumin level from 2.5 grams to 3.5 grams per 100 ml. This change was associated with a gain in body weight, no appreciable diuresis, and a definite increase in blood pressure accompanied by headache, slight nausea, and some cardiac enlargement by x-ray, which receded in the next 5 days. Further treatment was deemed inadvisable.

to arrange infusions at times when they would interfere with meals. It was found practical to give the patient an early lunch at 11:30 a.m. and then to give the albumin from 1:00 p.m. to 6:00 p.m. after which dinner was served. It was also convenient to give the evening meal at 5:30 p.m. and administer the albumin from 7:00 p.m. to midnight.

2. *Preliminary experiments*

Studies on the effect of a single large dose of albumin in patient J. G. with massive anasarca are illustrated in Figure 3. It is apparent that immediately following the beginning of administration there was a striking increase in urine volume

associated with, though not preceded by, a rise in the rate of excretion of protein, chloride, and phosphorus and to a lesser extent of non-protein nitrogen.

Such experiments showed that albumin in quantities greater than 50 grams daily was not proportionately more effective as a diuretic agent. In adults, quantities of 25 grams or less daily could not be depended upon to induce a diuresis and a positive nitrogen balance. Hence, with a limited supply of albumin available, a standard dose of 50 grams daily was adopted. It is possible that a quantity of albumin which was insufficient to induce a diuresis in an edematous patient might have been adequate to maintain a diuresis once it had been established with a larger dose.

3. *Diuretic effect of salt-poor concentrated human albumin administered intravenously*

A. *A single infusion of 50 grams.* In 18 experiments carried out on 7 patients, an average increase in urine volume of 480 ml. and an average loss of body weight of 0.5 kgm. were observed on the day of infusion. Details of these experiments are presented in Table II. The amount of diuresis, varying from 140 to 1,240 ml., appeared to be roughly correlated with the severity of edema.

In these experiments 500 ml. of fluid were deducted from the patient's constant daily water intake on the day of infusion. That 500 ml. of 6 per cent glucose when infused over a period of 5 hours did not ordinarily induce the diuresis when substituted for an equivalent quantity of water in the diet is indicated by the following experiment made on the patient whose average increase in urine volume on 50 grams of albumin approximated 1,000 ml.

Patient	Urine volume control	Urine volume Rx. glucose intravenously
J. G.	910 ml.	875 ml.

B. *Fifty grams daily for 3 days* (total 150 grams). An increase in urine volume occurred

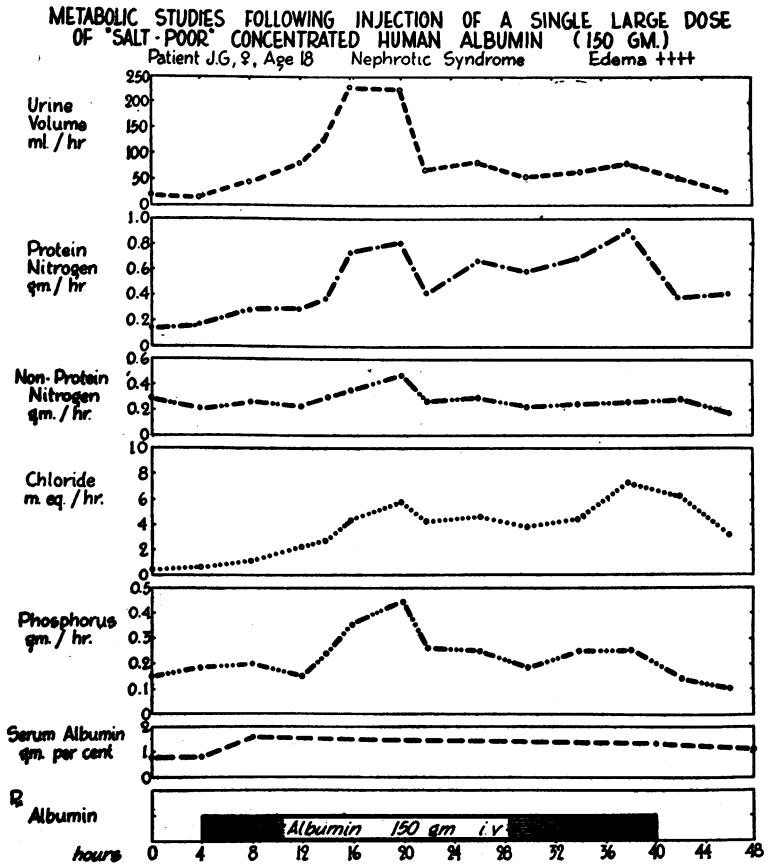


FIG. 3

TABLE II

Effect of a single dose of 50 grams of salt-poor albumin on urine volume in chronic nephritis, constant fluid intake

Patient	Edema	Control urine volume	Urine volume on day of treatment
		ml.	*ml.*
J. G.	4+	820	1720
	4+		1920
	4+		1630
	4+		2060
L. I.	4+	780	1980
W. H.	2+	2010	2620
	2+		3130
	2+		2630
	1+		2300
	1+		2790
D. S.	2+	1600	1850
	2+		2480
	1+		1220
	1+		1820
K. N.	1+	1920	2150
E. B.	1+	2160	1850
R. S.	0	2960	3410
	0		3100

during the period of albumin administration in 13 of the 15 experiments carried out on 7 patients. During the control 3-day period, the daily urine volume was 1,750 ml., whereas during the period of albumin therapy the average daily volume was 2,280 ml., yielding an average daily increase of 530 ml. The average daily weight loss was 0.5 kgm. per day or a total of 1.5 kgm. for the 3-day period. Details of such an experiment on patient L. I. are presented in Figure 4. Thus, the average daily change in urine volume and body weight during the 3-day experiments was comparable to that observed during the 1-day experiment. Again the amount of diuresis appeared roughly correlated with severity of edema; the 2 patients who failed to have an increase in urine volume were R. S., who had no edema clinically, and D. S., who had only barely detectable edema at the time of this particular experiment.

C. *Fifty grams daily for 10 days* (total 500 grams). Five experiments were carried out on 3 patients of which 1, J. G., had massive anasarca, whereas the other 2, D. S. and W. H., had minimal edema during this period. In the first patient, the striking and continued diuresis with average daily weight loss of 0.8 kgm. is illustrated in detail in Figure 5. The courses of the latter 2 patients in whom only slight increases in urine volume over the control periods were observed are presented in Figures 6 and 7.

D. *Fifty grams daily for 22 days and 30 days* (total 1,100 and 1,500 grams respectively). More prolonged courses of albumin therapy were given to 2 patients, J. G. with massive edema, and W. H. at a time when edema was barely detectable. The contrast between their responses is illustrated in Figures 8 and 9.

Patient W. H. showed no diuresis and lost no weight during therapy. However, at the termination of therapy, there occurred a gain of weight of approximately 3 kgm. in 10 days.

Patient J. G., who for months before this experiment had gained weight inexorably (Figure

TABLE III

*Summary of effects of salt-poor concentrated human albumin on urine volume in chronic nephritis**

Dosage of albumin Duration of therapy	Number of experiments	Number of experiments with increased urine volume	Average		
			Urine volume in control period equal to that of therapeutic period	Urine volume in period of therapy	Change in weight in period of therapy
			ml.	*ml.*	*kgm.*
50 grams × 1 day = 50 grams	18	16	1750	2230	− 0.5
50 grams × 3 days = 150 grams	15	13	5250	6840	− 1.5
50 grams × 10 days = 500 grams	5	5	14800	20300	− 2.7
50 grams × 22 days = (edema +) 1100 grams	1	0	44220	45760	0
50 grams × 30 days = (edema ++++) 1500 grams	1	1	24600	57900	−22.9
Totals	40	35			

* Five instances in which urine volume was not increased occurred in patients with edema either absent or minimal.

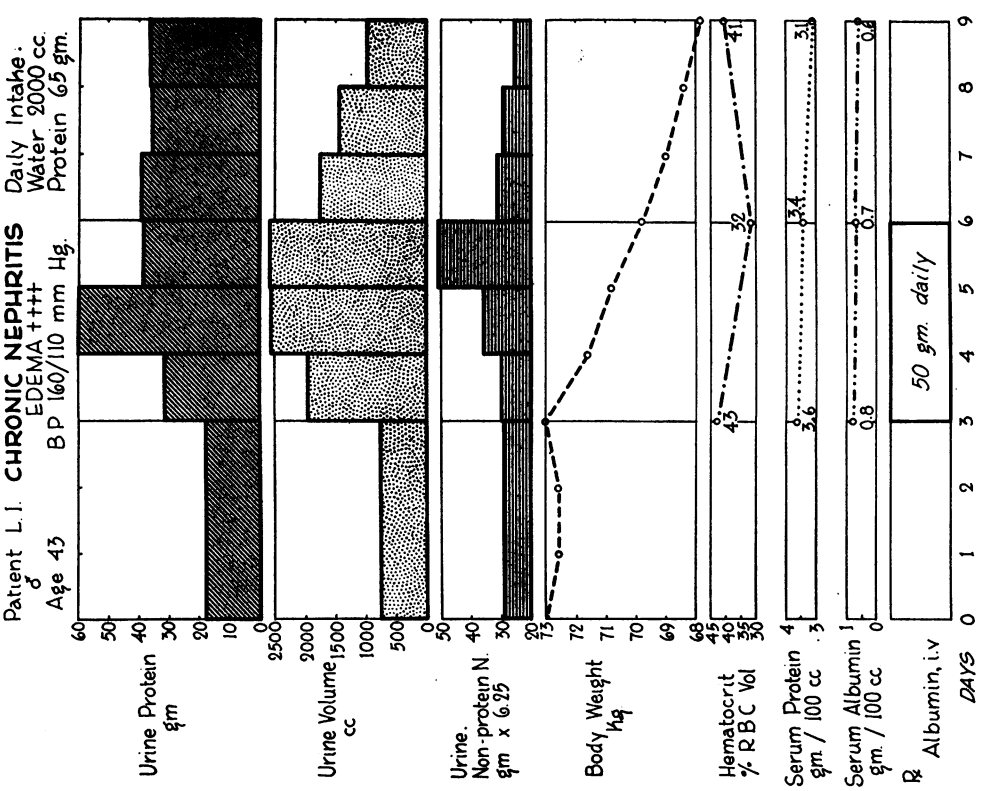

Patient L.I. ♂ Age 43
CHRONIC NEPHRITIS
EDEMA ++++
BP 160/110 mm Hg.
Daily Intake:
Water 2000 cc.
Protein 65 gm.
Urine Protein gm
Urine Volume cc
Urine Non-protein N gm x 6.25
Body Weight kg
Hematocrit % R B C Vol
Serum Protein gm / 100 cc
Serum Albumin gm / 100 cc
℞ Albumin, i.v
50 gm. daily
DAYS

FIG. 4

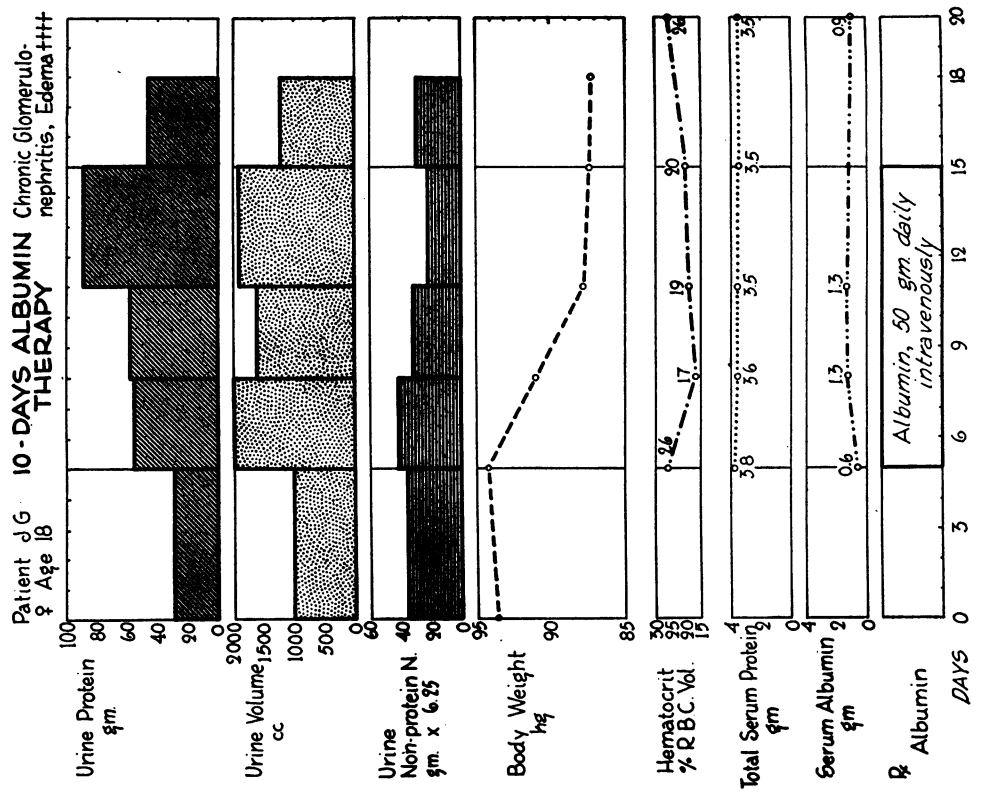

Patient J.G. ♀ Age 18
10-DAYS ALBUMIN THERAPY
Chronic Glomerulo-nephritis, Edema ++++
Urine Protein gm.
Urine Volume cc
Urine Non-protein N. gm. x 6.25
Body Weight kg
Hematocrit % R B C Vol.
Total Serum Protein gm
Serum Albumin gm
℞ Albumin
Albumin, 50 gm. daily intravenously
DAYS

FIG. 5

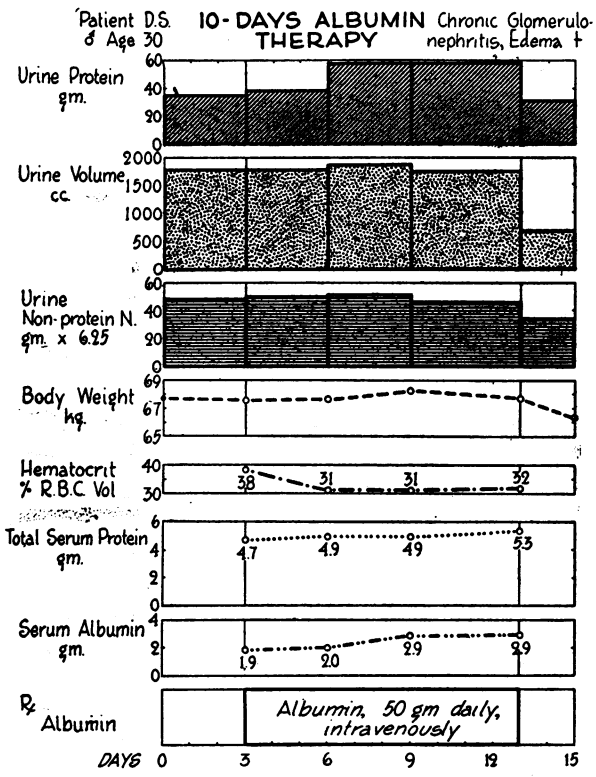


FIG. 6

10), showed an average increase in urinary output over that of her control figure of 1,110 ml. for 30 days and lost 0.76 kgm. daily or a total of 22.9 kgm. during the experiment.

A summary of the observations in the 40 experiments is presented in Table III. It is of interest that the 5 failures to increase urine volume during the administration of salt-poor albumin occurred either in patients with no visible edema or at most minimal edema.

E. *Maintenance of weight loss following albumin therapy.* In the edematous patients, the maintenance of weight loss appeared as a rough approximation to be related to 2 factors; first, the long-term trend of the patient's weight curve during control periods, and secondly, duration of therapy.

Thus patient J. G. showed the beginning of a weight gain within 24 hours after the diuresis of 3-day albumin administration. After the diuresis of 10-day albumin administration, her weight remained constant for 4 days. After the extensive diuresis during the 30-day administration, her weight remained constant for a control period of 20 days.[18]

In contrast to patient J. G., patient L. I., also edematous, not only maintained his weight loss, but continued to diurese at an accelerated rate following 3-day albumin administration (Figure 4). It is of interest that his weight curve for some weeks previous to therapy had tended slowly downward at the rate of 1 to 2 kgm. a week. Further study will be needed to determine whether, in a patient undergoing slow diuresis under routine therapy, relatively small doses of albumin may precipitate acceleration of weight loss.

4. *Effect of salt-poor concentrated human albumin on serum proteins*

The serum protein values here presented were obtained by the Howe method occasionally supplemented by electrophoretic analyses[19] on serum taken at 8:00 a.m. following the last albumin treatment, *i.e.* 8 hours after the end of the infusion.

A. *Single injection of 50 grams.* Serum protein values were followed in 4 patients who received a single injection of 50 grams of albumin. On the morning after the injection, there appeared to be a slight rise in serum albumin level, a slight decrease in serum globulin level, with little change in total protein concentration (Table IV). The reduction in hematocrit from 33 per cent volume packed cells to 29 per cent suggests that the total circulating albumin was increased appreciably, and that total content of globulin in the circulation was neither greatly increased or decreased and may have been merely diluted. These changes were very transient, moreover, lasting only 24 to 48 hours at most.

[18] At the end of this period, administration of penicillin and removal of an abscessed tooth were followed by a slow spontaneous diuresis.

[19] Electrophoretic analyses (uncorrected, for refractive indices) yielded, as has been observed by previous authors (27, 28), albumin-globulin ratios consistently lower than those obtained by the Howe method. Where simultaneous measurements have been made following albumin administration, the direction and, indeed, the degree of change have as a rule agreed quite closely by the two methods. Notable exceptions have comprised instances where little or no albumin increase was revealed by the Howe method following albumin administration, whereas considerable increase was indicated by electrophoresis. Such discrepancies are noted in the tables.

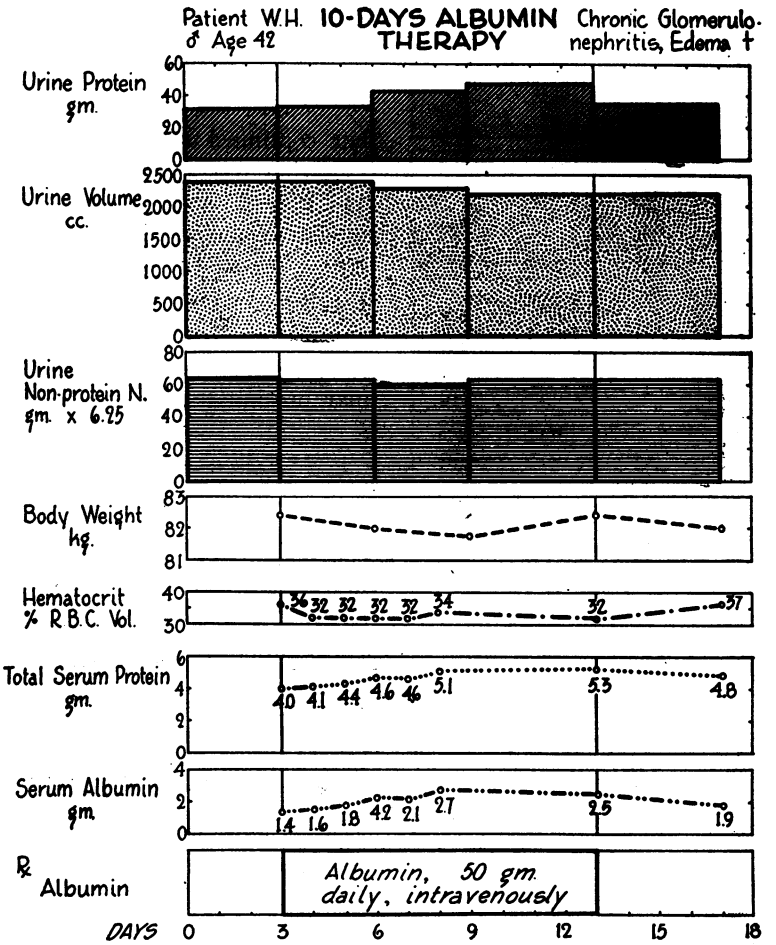


FIG. 7

B. *Three daily injections of albumin* (total 150 grams). In all 7 patients who received a total of 150 grams of albumin, serum albumin level increased. The average change for the entire group was 0.4 gram per 100 ml. (Table V). In 4 of these patients, some elevation of albumin level persisted for at least 3 weeks following cessation of therapy.

TABLE IV

Changes in serum proteins noted 8 hours after a single infusion of 50 grams of salt-poor concentrated human albumin (Howe method)

Patient	Serum albumin		Serum globulin		Hematocrit (cell volume)	
	Control	Treatment	Control	Treatment	Control	Treatment
	grams per 100 ml.		*grams per 100 ml.*		*per cent*	
J. G.	0.6	0.9	3.1	2.5	26	23
W. H.	0.6	1.4	2.6	2.3	31	31
D. S.	1.4	1.6	3.0	2.5	43	33
R. S.	2.4	2.5*	4.1	4.1*	31	28
Average	1.3	1.6	3.2	2.9	33	29

* Electrophoretic evidence indicated a much larger increase in albumin and decrease in globulin in this instance.

C. *Ten daily injections of albumin* (total 500 grams). In 5 experiments on 3 patients (detailed in Table VI), it was obvious that 10 days of therapy were more effective in raising serum albumin level than either the 1- or 3-day periods. All patients showed increased albumin levels in all experiments, the smallest increase being 0.7 gram per cent, the maximum 1.3 gram per cent, and the average 1.0 gram per cent. In 3 experiments, serum albumin level fell rapidly following the last injection of albumin, although after 3 or 4 days, the levels were still slightly elevated over control figures.

TABLE V

Effect of 3 daily injections of albumin (total 150 grams) on serum proteins (Howe method)

Patient	Serum albumin		Serum globulin		Hematocrit (cell volume)	
	Control	Treatment	Control	Treatment	Control	Treatment
	grams per 100 ml.		*grams per 100 ml.*		*per cent*	
J. G.	0.8	1.1*	2.4	2.3*	23	16
L. I.	0.8	0.7	2.6	2.7	43	32
W. H.	0.9	1.4	2.7	2.8	33	29
D. S.	2.2	1.7**	2.0	2.9**	32	30
K. N.	2.8	3.5	3.2	3.1	40	36
E. B.	1.7	2.6	2.4	2.6	40	35
R. S.	2.5	3.5	4.1	3.4	28	26
Average	1.7	2.1	2.8	2.8	34	29

* Electrophoretic analysis, uncorrected, indicated an albumin increase from 0.3 to 1.2 grams per 100 ml., with a corresponding reduction in globulins.

** Electrophoretic analysis, uncorrected, indicated an albumin increase from 1.4 to 2.2 grams per 100 ml., with a corresponding reduction in globulins.

TABLE VI

Effect of 10 daily injections of albumin (total 500 grams) on serum proteins (Howe method)

Patient	Serum albumin		Serum globulin		Hematocrit (cell volume)	
	Control	Treatment	Control	Treatment	Control	Treatment
	grams per 100 ml.		*grams per 100 ml.*		*per cent*	
J. G.	0.6	1.3	3.2	2.3	26	19
	0.9	2.2	2.6	2.4	26	26
W. H.	1.4	2.5	2.6	2.6	36	32
	1.9	3.0	2.9	2.5	37	35
D. S.	1.9	2.9	2.8	2.4	36	32
Average	1.4	2.4	2.8	2.5	34	29

D. *Albumin injected for 22 and 30 days* (total 1,100 and 1,500 grams respectively). Following the long-term administration to patient J. G. with edema and patient W. H. without edema, it was apparent that the increase in albumin level was not appreciably greater than that observed in the 10-day experiments, nor was the elevated level of albumin maintained following therapy for a longer period of time. Within 10 days after cessation of administration, levels had attained the

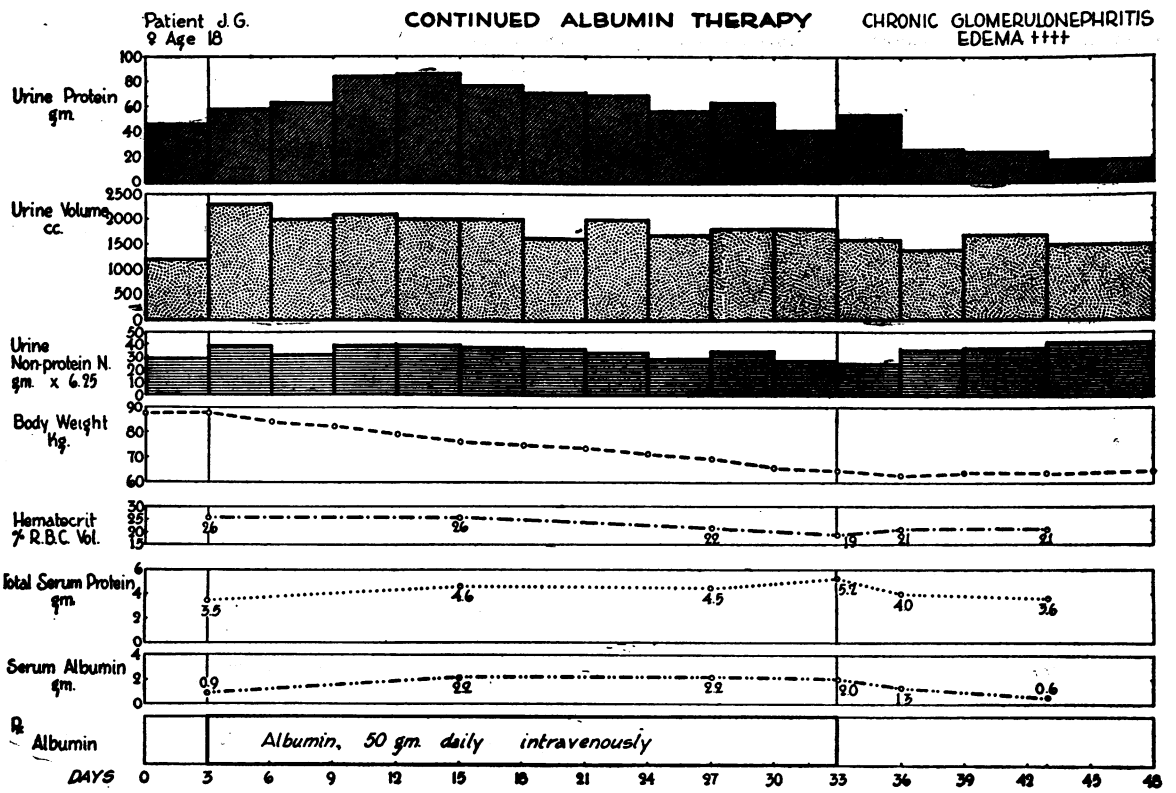


FIG. 8

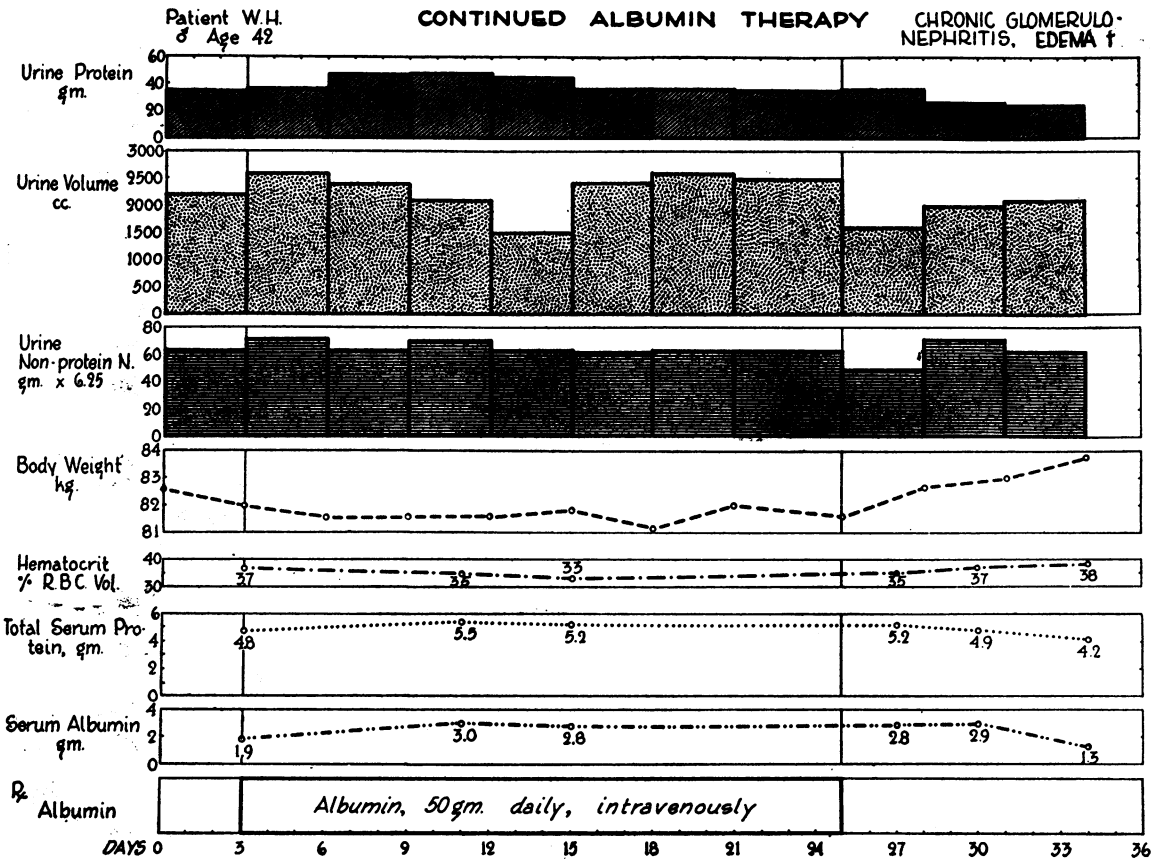

Patient W.H.
♂ Age 42
CONTINUED ALBUMIN THERAPY
CHRONIC GLOMERULO-NEPHRITIS. EDEMA ↑
Urine Protein gm.
Urine Volume cc.
Urine Non-protein N. gm. x 6.25
Body Weight kg.
Hematocrit % R.B.C. Vol.
Total Serum Protein, gm.
Serum Albumin gm.
℞ Albumin
Albumin, 50 gm. daily, intravenously
DAYS 0 3 6 9 12 15 18 21 24 27 30 33 36

FIG. 9

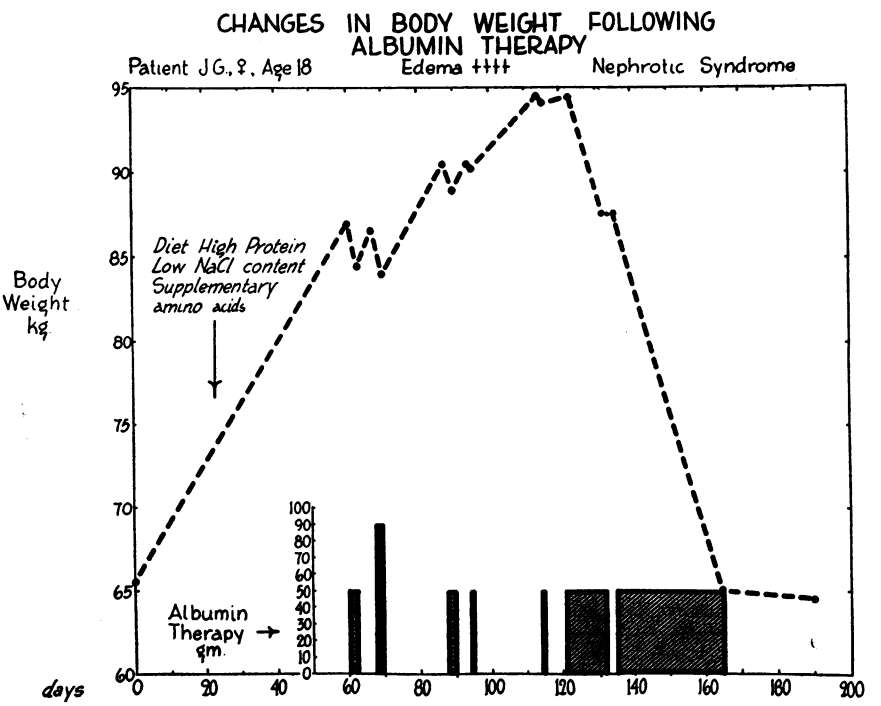

CHANGES IN BODY WEIGHT FOLLOWING ALBUMIN THERAPY
Patient J.G., ♀, Age 18
Edema ++++
Nephrotic Syndrome
Body Weight kg
Diet High Protein
Low NaCl content
Supplementary amino acids
Albumin Therapy → gm.
days 0 20 40 60 80 100 120 140 160 180 200

FIG. 10

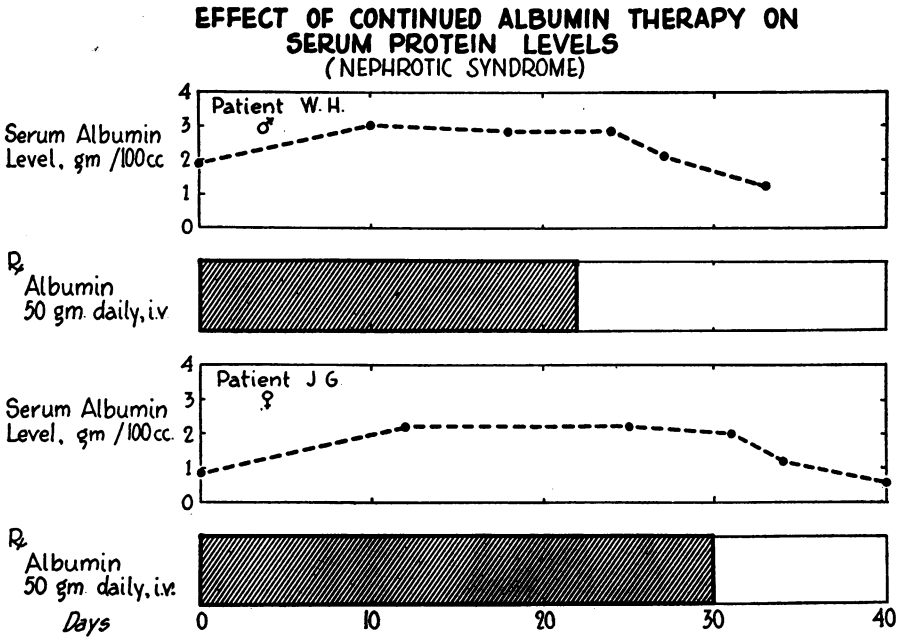


FIG. 11

control values (Figure 11). Details of these experiments are presented in Figures 8 and 9. A summary of the effect of various dosages on serum albumin level is shown in Figure 12.

E. *Effect of albumin administration on distribution of electrophoretic components.* Beyond an inconstant decrease in ratio of the elevated beta globulin to the total globulins, no significant change in ratios between the various electrophoretic globulin components, either in plasma or urine, could be demonstrated as an immediate result of albumin therapy over the periods previously discussed. This may be seen in Figures 13 and 14 in which are presented electrophoretic schlieren diagrams of patient W. H., whose initial low

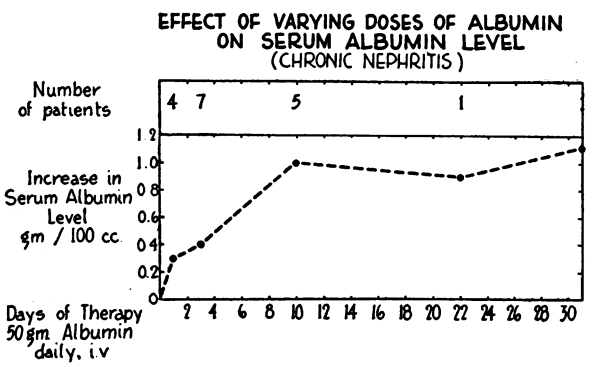


FIG. 12

gamma globulin and high beta and alpha-2 globulin are characteristic of the nephrotic syndrome, and by contrast, patient R. S., whose high gamma globulin suggested a more acute process. Whereas the relative areas of the albumin peaks increase, the globulin peaks bear approximately the same relation to one another. Moreover, in the patients studied following short-term albumin injections, reversion to control pattern had occurred within 2 to 3 weeks.

Four months after treatment had been discontinued, W. H. presented the identical electrophoretic diagram as that done during the control period. Furthermore, no change in clinical condition or urine sediment was noted during this period. The gradual reversion in the electrophoretic pattern of patient J. G. toward normal, although it took place following prolonged albumin administration, cannot unequivocally be ascribed thereto. It is of interest that the reversion in electrophoretic pattern was associated with striking clinical improvement and a decrease in proteinuria.

F. *The effect of albumin administration on total circulating globulin.* Estimations of changes of total circulating globulin calculated from changes in hematocrit and plasma protein distribution by both the Howe and the electrophoretic methods

were inconstant in direction and well within the considerable limits of error which the methods involve (Table VII). As in the instance of the 3-day injection, the fact that the total globulin content was neither greatly increased nor decreased suggests that the effect of albumin administration was merely a dilution of the globulins. It should be pointed out that the methods employed are far too insensitive to detect such small increases of total circulating globulin as have been described immediately following a single injection of 25 grams (15).

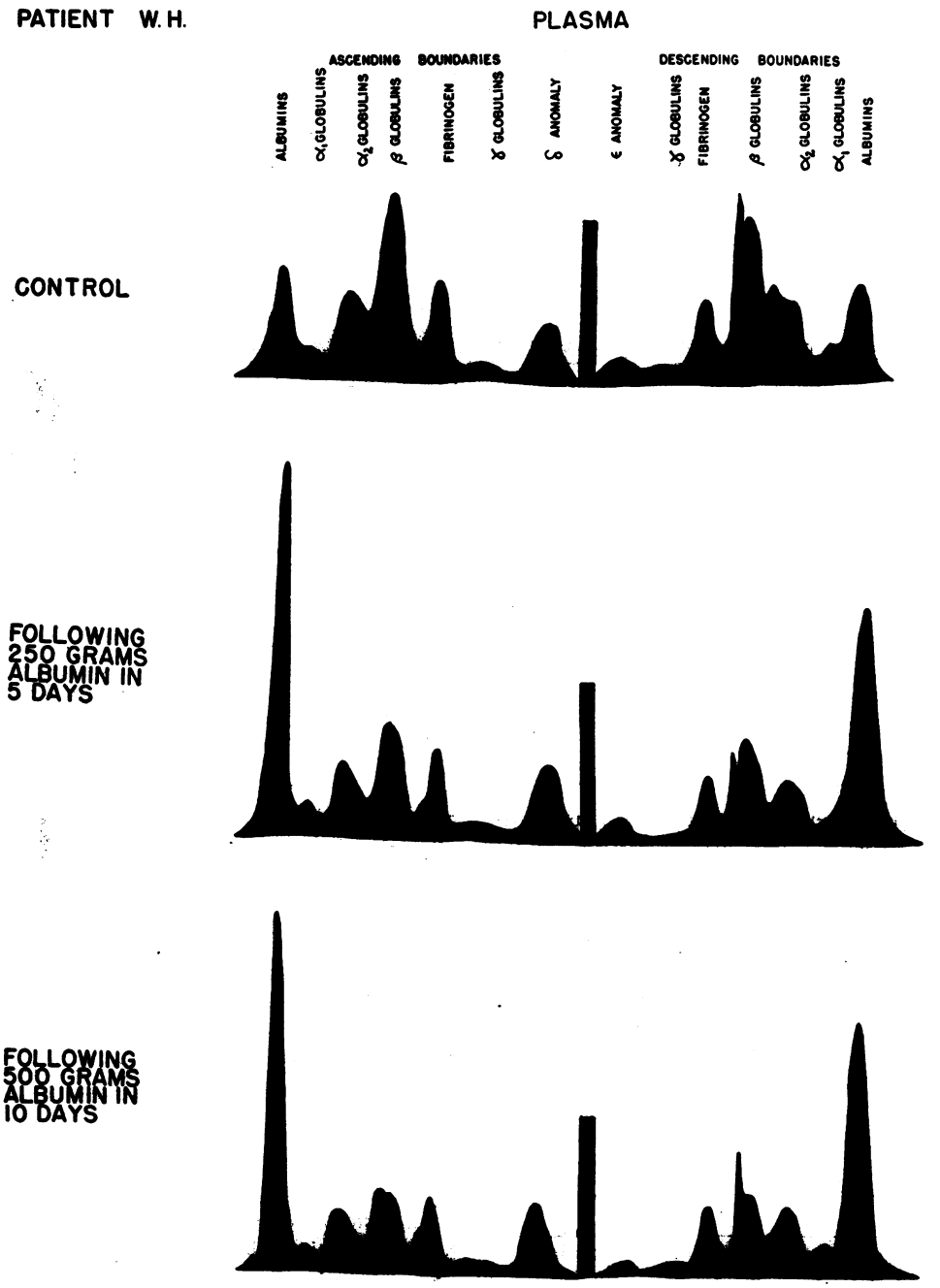


FIG. 13

TABLE VII

Comparison of estimates of effect of albumin administration on amount of circulating albumin and globulin by uncorrected electrophoresis and by the Howe method

Patient	Experiment		Uncorrected electrophoresis	Howe salting out	Uncorrected electrophoresis	Howe salting out
			grams of albumin per 100 ml. RBC		*grams of globulin per 100 ml. RBC*	
W. H.	500 grams in 10 days	Control	1.7	2.5	5.3	4.7
		Following albumin	4.7	5.3	5.6	5.7
	1100 grams in 22 days	Control	2.7	3.2	5.4	4.9
		Following albumin	5.2	5.7	5.4	4.9
D. S.	150 grams in 3 days	Control	3.0	4.7	6.0	4.2
		Following albumin	5.0	3.9	5.6	6.7
	500 grams in 10 days	Control	2.6	3.2	5.0	4.5
		Following albumin	6.0	6.0	5.3	5.0
R. S.	150 grams in 3 days	Control	5.3	6.4	11.4	10.3
		Following albumin	8.6	10.0	11.0	9.7

5. *Effect of albumin administration on plasma volume*

Whereas estimated increases in plasma volume per gram increase in circulating albumin showed wide spread (coefficient of variation 28 per cent) owing to the errors inherent in the nature of the data available for calculation, it is of interest that the average value was fairly close to the value predicted from osmotic pressure measurements. (11).[20] It is of the same order of magnitude as that derived from experiments on injection of concentrated albumin following acute blood loss. (29).

Average plasma protein concentration	Average estimated increase in plasma volume (ml.) per gram increase circulating albumin	Predicted increase in plasma volume (ml.) per gram increase circulating albumin
grams per 100 ml.		
4.9	19	20

[20] In that the value of Scatchard and his associates (Figure 3 of their paper (6)), is based on iso-osmotic addition of protein to a system, it is not strictly comparable to measurements on these patients where albumin administration in all instances yielded increases in albumin concentration in addition to increases in plasma volume. Correction of Scatchard's figure for this different situation would involve a small revision downward, the size of which is insignificant in comparison with the size of the errors in computing plasma volume changes from hematocrit and hemoglobulin data.

It also is of interest that patient J. G., whose plasma proteins were significantly lower than the average appeared to show with some consistency, as would be predicted from osmotic pressure data, larger increases in plasma volume than the average.

6. *Studies on nitrogen balance*

A. *A single injection of albumin* (50 grams). Protein excretion increased on the day of albumin therapy by approximately 7 grams in the 7 patients studied. Studies in 4 patients (J. G., D. S., W. H., and R. S.) for 3 successive days revealed that an additional 21 grams was excreted before equilibrium was attained. Striking differences in protein excretion were observed in different patients (Table VIII). The largest loss of protein occurred in the patient with the greatest initial

TABLE VIII

Urinary protein excretion following a single injection of 50 grams of albumin

Patient	Control	Day of treatment
	grams per 24 hours	*grams per 24 hours*
J. G.	24	39
L. I.	18	32
W. H.	19	26
D. S.	12	18
K. N.	8	6
E. B.	7	13
R. S.	15	18
Average	15	22

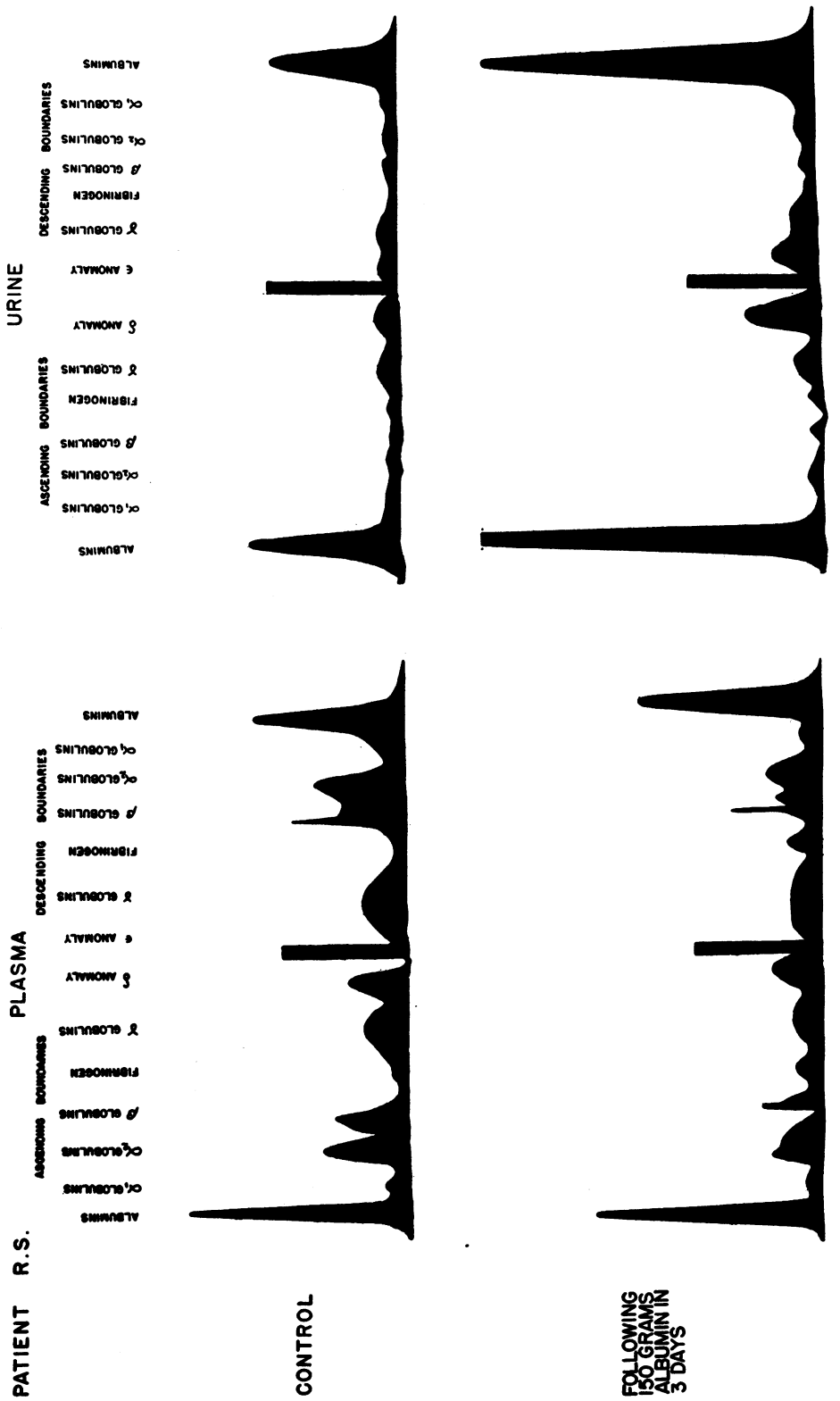

PATIENT R.S.
PLASMA
URINE
ALBUMINS
α1 GLOBULINS
α2 GLOBULINS
β GLOBULINS
FIBRINOGEN
γ GLOBULINS
δ ANOMALY
ε ANOMALY
ASCENDING BOUNDARIES
DESCENDING BOUNDARIES
CONTROL
FOLLOWING 150 GRAMS ALBUMIN IN 3 DAYS

FIG. 14

proteinuria (J. G.). Such a correlation did not appear to hold for the rest of the patients. Although proteinuria increased, nonprotein nitrogen decreased; and balance studies indicated a total net gain of nitrogen of 4.9 grams, equivalent to 31 grams of protein or approximately 60 per cent of the injected dose of albumin.

B. *Three daily injections of albumin* (total 150 grams). Studies on 6 patients indicated an increase in proteinuria during the 3 days of treatment and for at least 3 days after treatment was discontinued in 5 of the group (Table IX). The increase in protein excretion during the period of treatment amounted to approximately 16 grams daily (32 per cent of injected albumin), and an additional 12 grams daily was excreted during the subsequent 3 days. Thus, approximately 56 per cent of the albumin injected was excreted during the period of injection. Nitrogen balance studies indicated a slight overall reduction in nonprotein nitrogen excretion with a resulting positive balance during the entire experiment of 12.9 grams of nitrogen equivalent to 81 grams of protein or approximately 54 per cent of the injected protein.

TABLE IX

Urinary protein excretion following 3 days of albumin therapy (total 150 grams)

Patient	Control	Treatment			Following treatment		
		Day 1	Day 2	Day 3	First day	Second day	Third day
	grams per 24 hours						
J. G.	24	39	59	56	48	33	27
L. I.	18	32	60	39	40	36	36
W. H.	19	24	38	38	40	39	24
D. S.	12	17	44	52	53	16	19
K. N.	8	6	6	6	8	7	
R. S.	15	16	18	28	19	29	23
Average	16	22	37	37	35	27	23

In 3 experiments on 2 patients no increase in average daily globulin output was observed on albumin dosage of this magnitude (Table X). This both confirms and extends the identical finding following injection of a single 25-gram dose (15).

C. *Ten daily injections of albumin* (total 500 grams). Three patients were studied during and following 10 days of albumin therapy. Albumin excretion increased by an average of 36 grams daily during the 10-day period of therapy (Table XI). During the 3 days following cessation of treatment an increased excretion of 18 grams (average) per day was noted. Approximately 80 per cent of the injected albumin was thus excreted during the experiment. Nitrogen balance studies, however, indicated a slight overall reduction in nonprotein nitrogen excretion with a consequent average nitrogen retention of 20 grams of nitrogen equivalent to 124 grams of protein during the experiment. This represented a retention of approximately 25 per cent of the injected protein.

TABLE X

Excretion of globulins following albumin administration

Patient	Amount of albumin	Number of days	Average 24-hour urine output		Average 24-hour globulin output	
			Control	Treated	Control	Treated
	grams		ml.		grams	
J. G.	150	2	820	1840	8.7	7.1
	178	3	820	1700	7.5	8.4
R. S.	150	3	2960	2930	5.7	5.2

D. *Longer continued studies—22 and 30 daily injections of albumin* (total 1,100 and 1,500 grams respectively). The results of these experiments are summarized in Figures 7 and 8. Patient W. H. excreted an excess of 3.4 grams nitrogen daily (22 grams of protein) during the 22-day experiment. During a 9-day period following withdrawal of therapy, excess proteinuria amounted to 11 grams daily. This total increase in protein excretion amounted to 580 grams. Nitrogen balance indicated a total positive balance of 52 grams of nitrogen or 327 grams of protein equivalent to 30 per cent of injected protein.

Patient J. G. excreted 42.3 grams of protein daily above her control level of excretion during

TABLE XI

Urinary protein excretion following 10 days of albumin therapy (total 500 grams)

Patient	Control	Treatment			Following treatment 3 days
	3-day period	1 to 3 days	3 to 6 days	6 to 10 days	
	grams per 24 hours				
J. G.	24	56	58	89	46
W. H.	19	33	43	47	35
D. S.	12	39	58	58	31
Average	19	43	53	65	37

the 30-day period of treatment, and 10.3 grams daily during the 10-day period following cessation of therapy. This represented a total loss of approximately 1,370 grams of protein or 90 per cent of the quantity of injected albumin. Nitrogen balance studies, however, indicated a retention of 1.6 grams of nitrogen daily, or 10 grams of protein, with a negative balance of 2.7 grams daily for 3 days after treatment was discontinued, balance being established after the third day. Summary of this indicates a total overall retention of approximately 40 grams of nitrogen or 250 grams of protein during the experiment, or approximately 16 per cent of injected protein.

A summary of all nitrogen balance studies is presented in Table XII.

TABLE XII

Summary of nitrogen balance studies in patients with chronic nephritis treated with salt-poor concentrated human albumin intravenously

Number of patients	Group of experiments (albumin)	Average protein retention	Total injected protein retained
	grams	*grams*	*per cent*
4	50	30	60
6	150	81	54
3	500	124	25
1	1100	327	30
1	1500	250	16

7. *Follow-up studies*

The response of 2 patients to the injection of 150 grams of albumin over 3 days was checked some 6 months after the original studies. The first patient, J. G., had lost her massive anasarca, her serum albumin level had risen from 0.8 to 1.9 grams per cent, and her daily spontaneous proteinuria had decreased from 24 to 13 grams. In this improved condition 150 grams of albumin occasioned a rise of serum albumin level to 3.7 grams per cent as against the rise to 1.1 gram per cent noted during the initial study. Sixty-two per cent of the injected nitrogen was retained as against an initial 21 per cent.

Patient K. N., originally in a far less severe nephrotic state, showed no essential change in clinical condition at the time of follow-up injection. Her response to 150 grams of albumin was almost identical to her initial response both from the standpoint of change of serum albumin level and retention of protein nitrogen.

8. *Effect of other forms of therapy on this group of patients*

A. *Mercupurin.* Over a period of 14 days, patient J. G. was given 4 injections (totaling 7.5 ml.) of mercupurin, in 3 instances preceded by 8 grams of ammonium chloride. At this time, her weight curve was showing a gain of 2.5 kgm. a week (Figure 10). Although the urine volume increased some 300 ml. per 24 hours over the average control value following each injection, during the period of therapy the patient continued to gain weight at a rate identical with the control period.

B. *Amino acids.*[21] *Patient J. G.* During the period when this patient's already massive edema was increasing at a rate of 2.5 kgm. a week, she received approximately 70 grams of amino acids daily by mouth for 10 days. Although the average daily urine volume increased by approximately 300 ml. over that of the control period, weight gain continued at the same rate. There was no significant change in total protein or albumin levels.

Patient R. S. At a time when his edema was barely detectable and his basal weight curve was falling 1 kgm. a week, the patient received approximately 60 grams of amino acids a day orally for 8 days. During this period, he lost 3 kgm. He gained 1 kgm. in the week following cessation of therapy after which his weight curve remained essentially flat. There was no change in the plasma proteins.

Patient W. H. In contrast to patient R. S., on essentially the same daily dosage of amino acids over a period of 30 days, W. H. gained 1.5 kgm. per week, although during the preceding control period he had lost 3 kgm. a week. His moderate edema did not decrease.

Patient D. S. A similar increase in weight, although slightly less in amount, occurred in patient

[21] The oral and intravenous preparations of Frederick Stearns and Company were used. The oral preparation contains 2.8 per cent nitrogen; 280 ml. contain the same amount of nitrogen as 50 grams of albumin. The intravenous preparation contains 2 per cent nitrogen; 400 ml. are equivalent in nitrogen to 50 grams of albumin. The use of these preparations increased only slightly the sodium intake of the basal regimen, for they contain approximately 0.130 gram sodium (expressed as sodium chloride) per 100 ml.

D. S. during both 15 days of oral amino acids therapy at this identical dosage, and 9 days of intravenous amino acids administration at a nitrogen dosage equivalent to 37.5 grams of albumin a day. During control periods for both these experiments, the patient's basal weight curve decreased at a rate of approximately 1 kgm. a week. During both periods of therapy the average daily urinary volume decreased by approximately 300 ml. over that of the control periods. In neither experiment was there a significant change in serum total protein nor albumin. During the intravenous therapy, N.P.N. excretion increased from a control average of 12.5 to 16.2 grams daily, and protein excretion from 7.5 to 12.5 grams daily. Less than 0.5 per cent of amino acids was excreted in the urine as such. During the 10-day period of therapy and the ensuing 3 days, however, approximately 70 per cent of the injected nitrogen was excreted, yielding a retention of 30 per cent, a figure comparable to that encountered with a dose of albumin of similar magnitude in this patient.

C. *Urea.* The anorexia which so frequently attends the nephrotic syndrome made the oral administration of this substance impossible in the instance of patient J. G. and limited the tolerated dosage in certain other patients.

Of the group, patients L. I., W. H., and D. S., whose edema ranged from minimal to moderate, were able to ingest a dose of approximately 22 grams a day, in the first instance for 9 days, in the latter instances for 22 days. In no case was there an increase in urine output over the control values. In the first 2 patients the weight curves remained flat; in the third patient there was a weight loss totaling 2 kgm. There was no significant effect on the plasma proteins.

A second period of urea administration at a dosage of 90 grams a day was instituted in patient D. S. shortly after his 10-day period of albumin therapy. Whereas during the periods of control, albumin therapy, and urea at a rate of 20 grams a day his weight curve had remained flat, on the larger dosage, he lost weight at the rate of 3 kgm. a week, and his urine output increased over that of the control period by approximately 400 ml. a day. In the 3 days following cessation of urea, the patient regained 3 kgm. Weight loss again occurred at the same rate on the resumption of 90 grams of urea a day. A comparable rate of diuresis was observed over a shorter period in patient R. S. at a time when he had no detectable edema.

This small number of observations suggests that in patients with minimal edema in whom albumin has been a relatively ineffectual diuretic, urea in moderate dosage is likewise without effect, but in large amounts may induce diuresis and maintain it during the period of administration.

DISCUSSION

This study of the use of salt-poor human serum albumin in glomerulonephritis represents a step in a systematic program aimed at the study of the effect of the administration of purified plasma protein fractions, characterized both with respect to physical-chemical properties and in so far as possible to physiological function, in those conditions in which the normal equilibrium state of these proteins is known to be altered.

Whereas the use of a molecule of different characteristics (for example, of similar diameter but increased length yielding increased retention in the blood stream (30) may ultimately prove more effective, salt-poor albumin has several advantages over the various substances of high molecular weight that have been used in attempts to effect diuresis and positive nitrogen balance in patients with low total proteins and a high degree of albuminuria. It is a native protein. Although low in sodium, it shares with normal plasma or serum the fact that its amino acid composition gives it a nutritional capacity approximately equal to that of whole plasma (31) and superior to that of such incomplete proteins as gelatin (32). The reaction rate following injection of normal human serum albumin in our experience is even less than that of banked plasma. Its use in large quantities does not carry the same risk of liver deposition and subsequent impairment of albumin synthesis such as has been reported both experimentally and clinically with acacia (33).

It has been a frequent finding of many workers endeavoring to treat nephrotic edema with solutions of high molecular weight substances that administration of acacia resulted in no prolonged rise in plasma colloid osmotic pressure, nor did administration of plasma or serum yield prolonged rises in serum protein levels (2 to 4, 34),

Such observations have led to many interpretations of the mechanism of the irregularly occurring diureses encountered with these substances. Following a suggestion by Peters(35), evidence was presented for a correlation between diuresis and increase in plasma volume rather than an increase in colloid osmotic pressure (36, 2). Although the possibility of this mechanism found corroboration in studies of urine output following plasma infusion into normal dogs (37), increases in blood volume were observed in nephrotics, following plasma therapy where no diuresis occurred (3). Again, whereas an increased chloride output has been described (34), the chloride loss during diuresis following acacia infusion was roughly equal to the amount of chloride in the injected acacia solution plus that of the excreted edema fluid (38).

Finally, the unchanging serum protein level at the onset of the diureses produced by plasma was reminiscent of the spontaneous diureses of nephrosis and suggested that in addition to osmotic action "serum . . . supplies some . . . substances which set off the patient's own mechanism of diuresis" (1). Hence, appraisal of therapeutic efficacy was based on an increased incidence over the expected of a diuresis of this type.

Indeed, a study of the course of diureses reported following administration of serum, plasma, and acacia reveals that in many instances it further resembles the spontaneous diuresis in that it persists long after the period of administration of colloid substances (39).[22]

The diureses following salt-poor albumin appear to differ from those reported with other substances in the time relation between administration and weight loss. In no instance did administration of albumin provoke the type of spontaneous diuresis which long outlasts the period of therapy. The average daily weight loss on uniform daily dosage was approximately the same for periods of therapy varying between 1 and 30 days. This weight loss appeared to be superimposed on the patient's basal weight curve. Thus, in patient J. G. whose basal weight curve over 2 months before therapy showed an average gain of 10 kgm. a month, short periods of therapy with attendant diureses ultimately were followed by continued weight gain. After the loss of 20 kgm. during the 30-day administration, the patient, although still strikingly edematous, maintained a level weight for 3 weeks. The significance of this level weight curve is emphasized by the subsequent spontaneous diureses in the absence of any albumin which began immediately following removal of an abscessed tooth.

In this group of patients the correlation between extent of diuresis and the extent of the patient's edema was striking. The data set forth here do not elucidate the mechanism. Extent of diuresis showed no constant correlation with either increase in serum albumin level or in total circulating albumin. The estimated increases in plasma volume per gram increase in circulating albumin following treatment were but little larger in the edematous patients who showed good diureses than in those with little edema whose weight curves remained flat. The fact that the average daily weight loss per gram albumin was as great on the first as on the third day of a course of treatment, although the average quantity of excreted protein was far less on the first day, excluded any constant correlation with total protein excretion.[23] Nor could diuresis be related to average urinary protein concentration.[24]

From the correlation between the extent of edema and the diuresis produced by salt-poor al-

[22] The diuresis illustrated by Landis (38) taking place during the administration of about 20 grams of acacia a day for 8 days resembles more the diuresis obtained with albumin.

[23] This is shown by examination of the charts of J. G. and D. S. between the third or sixth day of their 10-day period of therapy (Figures 5 and 6). Although patient J. G. showed a typical diuresis and D. S. did not, they both excreted an average of 56 grams of protein per day. Moreover, the fact that no temporary increase in urinary protein concentration preceded diuresis following the injection of a single dose of salt-poor albumin in patient J. G. (Figure 3) fails to provide evidence that the presence of high protein concentration in the tubule initiates diuresis. In this connection, it is of interest that in cirrhotic patients (40) who have no proteinuria, the same correlation between occurrence of diuresis and extent of edema would appear to hold.

[24] The average urinary protein concentration of patient J. G. during the initial 10 days of her 30-day period was 2.8 grams per cent. At this time she was losing weight at a rate of 0.76 kgm. per day. During the last 6 days in patient D. S.' 10-day period of treatment, his average urinary protein concentration was 3.3 grams per cent at which time the patient having little edema had no change in weight.

bumin treatment, both the values and the limitations of this form of therapy in the various stages of chronic Bright's disease emerge.

The greatest use would appear to be in the extreme nephrotic stage. In the edema-free patient, tending toward a fixation of specific gravity, nitrogen retention and hypertension, its value is less and occasionally its use may be contraindicated from the cardiovascular standpoint by reason of its ability to produce and maintain striking rises in blood volume.

Indeed, it is in extreme nephrosis, the edema of which is notoriously quite resistant to mercurial diuretics, in which administration of amino acids has no constant effect, and in which anorexia often prohibits oral urea in large doses, that an agent which will induce and maintain a diuresis may occasionally give dramatic symptomatic relief to a desperately sick patient.

It is important to emphasize the basal dietary regimen of these patients in evaluating the ability of albumin to maintain diuresis and positive nitrogen balance. Had not these patients been very close to positive nitrogen balance on the dietary intake alone, larger quantities of albumin may well have been required to induce the significantly positive nitrogen balance attained and the feeling of well-being that appeared to accompany that state. Likewise, it is obvious that the rigid restriction of salt is important in the maintenance of diuresis with salt-poor albumin, particularly in view of the possibility that salt-poor albumin might have succeeded in the previously reported instances (5) where high-salt albumin has failed in attainment of diuresis.

No evidence emerges from this study that the diuresis or positive nitrogen balance induced by salt-poor albumin results in any change in the natural history of the disease process. Clinical follow-up studies have shown no deleterious changes in renal function ascribable to the relatively large doses of albumin.[25] This is in agreement with other findings which revealed an instance of complete recovery with absence of proteinuria in an 8-year-old nephrotic boy who had received over 600 grams of high-salt normal human serum albumin in a period of 30 days (5).

SUMMARY AND CONCLUSIONS

Salt-poor concentrated human serum albumin has been administered intravenously for periods varying between 1 and 30 days at a dosage of 50 grams a day and a rate of 10 grams an hour to a group of 7 patients in several stages of chronic nephritis maintained on a diet adequate in calories, containing 80 to 125 grams of protein daily and low in sodium chloride.

The therapeutic value of salt-poor albumin varied with the stage of the disease. In patients with edema, low serum proteins, and absent to moderate hypertension and nitrogen retention, albumin in this dosage was a safe agent in increasing the serum albumin level and in inducing positive nitrogen balance.

The diuretic effect of salt-poor albumin administration was most striking in the severe nephrotic state with massive edema. Control studies both with amino acid mixtures administered by mouth and intravenously in comparable quantities and with mercupurin yielded no diuresis under these circumstances.

Unlike the diureses that have been reported as initiated by a wide variety of therapies and which resemble the spontaneous diureses of the nephrotic state, the diureses following albumin proceeded at a constant rate during the period of administration only and stopped at the end of it.

Following short periods of therapy (10 days or less), the ability to maintain weight loss was roughly related to the slope and direction of the patient's weight curve on basal regimen. In the one instance of prolonged (30-day) therapy in nephrosis, a previously rapidly ascending weight curve became flat following diuresis of 20 kgm. although the patient was still edematous. In that

[25] The work of Bailey and Hawn (41) on bovine albumin, together with the correlation noted by Blackman and Davis (42) of severe globulinuria with progressive renal damage, would not lead to the expectation of such tubular changes following normal human serum albumin administration as have been described by Hueper (43) following the administration of certain forms of purified gelatin and of egg albumin in dogs, and by Smetana (44) following absorption and storage of homologous, heterologous and conjugated proteins in the open nephrons of urodeles. The only direct evidence on this point is provided in the histological examination of the kidneys of L. I. in this series of patients, who died of uremia some 6 months after his last albumin treatment. No changes specifically referable to therapy were found.

such a change in basal weight curve is consistent with the spontaneous course of the disease, the significance of this observation as related to salt-poor albumin therapy can only emerge after far wider clinical use.

In contrast to its effect on nephrotic anasarca, the diuretic effect of salt-poor albumin in the presence of minimal edema was slight. Although control studies showed that both amino acid mixtures in comparable quantities and urea (20 grams daily) were likewise ineffective, massive doses of urea (90 grams a day), when tolerated, appeared to induce further diuresis and weight loss of a transient character.

Albumin administration in the presence of severe hypertension, nitrogen retention, and in the absence of edema appeared contraindicated owing to its efficacy in rapidly increasing blood volume beyond the tolerance of the cardiovascular system.

Although in this study salt-poor albumin appeared to have no influence on the natural history of the disease process, in a condition as variable as chronic glomerulonephritis, observations far more extensive, both in duration and in range of clinical material, are necessary to determine this point. The symptomatic benefits here reported in the nephrotic state well warrant such observations.

CASE HISTORIES AND INITIAL CLINICAL FINDINGS

1. J. G. (M–65757), an 18-year-old white school girl, was admitted to the Peter Bent Brigham Hospital on January 24, 1944, because of generalized edema which had progressed to massive anasarca over the past 5 months.

Her health had been excellent with the exception of measles, mumps, and scarlet fever without known complications.

The present illness began in August, 1943, with the onset of ankle edema associated with a head cold. Following this, she felt well and was active, but the edema increased, and she began to note progressive swelling of her abdomen. There were no urinary symptoms beyond the fact that during her rapid weight gain, she noted her urine volume to be unusually small. In the month preceding admission she had anorexia, flatulence, and occasional abdominal cramps.

Physical examination: Temperature 98.6° F., pulse 80, respirations 18, blood pressure 120/76 mm. Hg. A well-developed and -nourished young girl showed marked general pallor and puffiness of the face together with striking soft pitting edema beginning at the mid-abdomen and extending down the lower extremities.

Fundi were not remarkable. Pharynx was normal. There was evidence of fluid in both pleural cavities and the abdomen showed shifting dullness and an easily demonstrable fluid wave. The heart was not remarkable. The remainder of the examination was noncontributory.

Laboratory data: Blood Hinton negative. Urine, serial specimens: Specific gravity varied between 1.020 and 1.036, protein 4+ (22 grams in 24 hours), sugar negative, centrifuged sediment—red cells varied between 2 and 45 per high power field, white cells varied between 1 and 10 per high power field, casts varied between 1 and 10 per low power field. Blood: Red cells 4,000,000 per c.mm., hematocrit 35, sedimentation rate 61 mm. per hour, white cells 12,000 per c.mm. with 78 per cent neutrophiles. Blood chemistry: Urea nitrogen 28 mgm. per cent, total protein 2.8 grams per cent, cholesterol 1,100 mgm. per cent, chlorides 107 m.eq. per liter, carbon dioxide combining power 28 m.M. per liter. Vital capacity 1,500 m.l. Electrocardiogram: Low EMF with a P–R interval of 0.20 seconds.

X-ray examinations: Chest: Fluid at both bases and a heart normal in size and shape. Sinuses: negative.

2. L. I. (M–66634), a 43-year-old white male watchman, was admitted to the Peter Bent Brigham Hospital on June 23, 1944, because of generalized edema of 2 months' duration.

The patient had had uncomplicated scarlet fever at the age of 7 without any nephritic sequellae. For at least 6 years before entry, he had suffered from mild bronchial asthma.

Although asymptomatic, a routine urine examination 6 months prior to admission disclosed massive albuminuria. Two months prior to admission, the onset of generalized edema, unassociated with any noticed infection, caused him to consult his family doctor who advised bed rest, thyroid, and a high protein diet. Despite a period of transitory improvement, edema progressed and nausea, anorexia, and constipation appeared.

Physical examination: Temperature 98.0° F., pulse 84, respirations 20, blood pressure 160/110 mm. Hg. A pale moderately obese man presented generalized edema of his legs, genitalia, and to a lesser degree of his abdomen. Fundi showed irregularities in the calibre of the vessels and one small area of exudate above the right disc. The pharynx was red. The tonsils were large and red; no pus was observed. Many asthmatic squeaks and fine basal rales were heard over both lung fields. The heart was not enlarged; rhythm was regular. There was a grade I apical systolic murmur. Beyond these findings the examination was noncontributory.

Laboratory data: Blood Hinton negative. Urine, serial specimens: Specific gravity varied between 1.008 and 1.032, protein 4+ (18 grams in 24 hours), sugar negative, centrifuged sediment—red cells varied between 0 to 4 per high power field, white cells varied between 2 to 30 per high power field, many hyaline and granular casts per low power field. Blood: Red cells 5,200,000 per c.mm., hematocrit 48, sedimentation rate 32 mm. per hour, white count 8,600 with 77 per cent neutrophiles and 3 per cent eosinophiles. Blood chemistry: Urea nitrogen 25 mgm. per cent, total protein 3.8 grams per cent, cholesterol 1,170 mgm. per cent, chlorides 108 m.eq. per liter, carbon dioxide combining power 24 m.M. per liter. Vital capacity

1,800 m.l. Venous pressure 90 mm. of saline. Circulation time (Decholin) 10 seconds. Congo red test negative. P.S.P. excretion: 15 minutes 10 per cent; total in 2 hours, 45 per cent.

X-ray examinations: Chest: Negative beyond clouding at right base and minimal fluid in the right costophrenic angle; heart normal in size and shape. Sinuses: Clouding of ethmoid cells and right frontal sinus, evidence of thickening in the sphenoid sinuses.

3. W. H. (M–65128), a 42-year-old male shipyard worker, was admitted to the Peter Bent Brigham Hospital on October 13, 1943, because of edema of 1 month's duration.

An anterior urethritis, in 1919, was followed by development of a stricture which was dilated, in 1927, with sounds. In 1942, an attack of prostatitis accompanied by frequency and nocturia was treated by his family doctor. The bacteriology of these infections is not known. In recent years, he had been subject to many sore throats.

His present illness began early in September, 1943, when, simultaneous with an acute upper respiratory infection characterized by chills, fever, and sore throat, puffiness was noted about his eyes. He had some soreness in the calves of his legs and in his feet. He subsequently developed ankle edema. Later, the edema became more generalized. He also had some malaise, headaches, and dizzy spells and had noted that his urine had been dark orange in color and decreased in amount. Ten days before admission he was put at bed rest on a low-salt, low-protein diet. During this time, he had had 2 episodes of paroxysmal nocturnal dyspnea.

Physical examination: Temperature 98.0° F., pulse 88, respirations 20, blood pressure 120/70 mm. Hg. A well-developed colored man exhibited marked puffiness about the eyes and moderate pitting edema of the ankles, sacrum and genitalia. Fundi showed no abnormalities. Teeth were carious and moderate pyorrhea was present. Although the throat was not red, the left tonsil was abnormally large and free of pus. Heart was normal. Lungs were clear. The prostate was not obviously enlarged. The remainder of the examination was noncontributory.

Laboratory data: Blood Hinton negative. Urine, serial specimens: Specific gravity varied between 1.005 and 1.014, protein 4+ (19 grams in 24 hours), sugar negative, centrifuged sediment—red cells varied between 1 and 8 per high power field, white cells varied between 3 and 15 per cent high power field, many hyaline and granular casts per low power field. Blood: Red cells, 3,400,000 per c.mm., hematocrit 32, sedimentation rate 15 mm. per hour, white cells 9,400 per c.mm., with 63 per cent neutrophiles and 3 per cent eosinophiles. Blood chemistry: Nonprotein nitrogen 30 mgm. per cent, total protein 4.1 grams per cent, cholesterol 755 mgm. per cent, chlorides 113 m.eq. per liter, carbon dioxide combining power 27 m.M. per liter. Vital capacity 2,800 m.l.. P.S.P. excretion—15 minutes 40 per cent, total 70 per cent in 2 hours. Multiple urine cultures showed staphylococcus albus. Electrocardiogram: Low voltage, P–R interval 0.20 seconds, otherwise normal.

X-ray examinations: Lungs: Moderate pulmonary congestion. Heart: Twelve per cent above average by height-weight ratio. Sinuses: All sinuses showed thickening of the membranes but no fluid level. Teeth: Small areas of absorption along the roots of 2 molars.

4. D. S. (M–65112), a 30-year-old white male, was admitted to the Peter Bent Brigham Hospital on October 11, 1943, because of ankle edema of about 1 year's duration.

One and a half years before this admission, he was found to have marked albuminuria unassociated with other signs or symptoms. In the fall of 1942 he developed ankle edema. This gradually became more severe and spread to involve the entire body. He gained 15 pounds during the year preceding admission.

Physical examination: Temperature 98.4° F., pulse 84, respirations 20, blood pressure 140/90 mm. Hg. A well-developed and -nourished but pale middle-aged male showed pitting edema extending from his legs to his lower abdominal wall and involving his hands. The fundi were negative. The pharynx was normal. The lungs were clear. The heart was negative except for a soft systolic bruit at the apex. The remainder of the examination was noncontributory.

Laboratory data: Blood Hinton negative. Urine, serial specimens: Specific gravity varied from 1.009 to 1.018, protein 3+ (11 grams in 24 hours), sugar negative, centrifuged sediment—red cells varied between 0 to 4 per high power field, white cells varied between 0 to 5 per high power field, many granular casts and rare hyaline casts per low power field. Blood: Red cells 4,400,000 per c.mm., hematocrit 37, sedimentation rate 38 mm. per hour, white cells 7,000 per c.mm., with 62 per cent neutrophiles and 3 per cent eosinophiles. Blood chemistry: Nonprotein nitrogen 27 mgm. per cent, total protein 4.8 grams per cent, cholesterol 1,000 mgm. per cent, chlorides 108 m.eq. per liter, carbon dioxide combining power 29 m.M. per liter. Vital capacity 4,200 m.l. P.S.P. excretion—15 minutes 20 per cent and a total of 75 per cent in 2 hours. Electrocardiogram normal.

X-ray examinations: Lungs: Clear. Heart: Five per cent below average size by height-weight ratio. Sinuses: Negative. Teeth: Negative.

5. K. N. (M–66677), a 40-year-old divorced stenographer, entered the Peter Bent Brigham Hospital on July 3, 1944, complaining of mild generalized edema of 5 weeks' duration.

At the age of 8, she had had an episode of generalized malaise and aching for which she was confined to bed for 2 weeks with the diagnosis of rheumatic fever, although no history of acute arthritis could be obtained, and there has never been any evidence of cardiac damage. In 1938, her urine was found to be entirely negative. For some 10 years, the patient had nocturia once a night and urinated approximately 5 times during each day. For many years, the patient had suffered from biannual attacks of tonsillitis, the last of which occurred in February, 1944, and lasted for 3 weeks. Patient had also had symptoms referable to the right maxillary sinus for a period of 2

weeks in 1942. No recrudescence of respiratory symptoms could be correlated with the present illness.

Physical examination: Temperature 99.0° F., pulse 84, respirations 20, blood pressure 130/80 mm. Hg. A well-developed and -nourished white female, without significant pallor or facial edema, presented minimal pitting edema of her ankles. Fundi were within normal limits. The pharynx was slightly injected. Both tonsils were present but not inflamed. There were numerous nontender, discrete, small cervical lymph nodes. Lungs were clear. The heart was normal. The remainder of the physical examination was negative except for a creamy white vaginal discharge.

Laboratory data: Blood Hinton negative. Urine, serial specimens: Specific gravity 1.010, protein 3+ (7 gm. in 24 hours), sugar negative, centrifuged sediment—red cells varied between 1 to 12 per high power field, white cells varied between 0 to 5 per high power field, occasional hyaline and fine granular casts. Blood: Hemoglobin 12.5 gm. per cent, hematocrit 39, sedimentation rate 45 mm. per hour, white cells 9,500 per c.mm., with 58 per cent neutrophiles and 3 per cent eosinophiles. Blood chemistry: Urea nitrogen 9 mgm. per cent, total protein 5.6 gm. per cent, cholesterol 430 mgm. per cent. Throat cultures showed normal throat flora. Serial urine cultures were sterile. Culture of tooth root following extraction showed alpha streptococci and staphylococcus albus. P.S.P. excretion—15 minutes 45 per cent and a total of 75 per cent in 2 hours. Congo red test negative. Basal metabolic rate +3 per cent. Vital capacity 3,600 m.l. Electrocardiogram normal.

X-ray examinations: Chest: Clear. Heart: Six per cent below average in size by height-weight ratio. Sinuses: Negative. Teeth: Areas of absorption around the roots of the right lower first molar. Retrograde pyelograms: Negative.

6. E. B. (M-67041), a 44-year-old white housewife, was readmitted to the Peter Bent Brigham Hospital on September 11, 1944, because of increasing lassitude, exertional dyspnea, and generalized edema over the past 3 months.

In the fall of 1943, she was found to have hypertension with blood pressures as high as 160/110 mm. Hg and moderate proteinuria. She felt relatively well until a few months prior to the present admission at which time she began to complain of loss of vigor, mild exertional dyspnea, slight generalized edema, and lack of mental acuity.

Physical examination: Temperature 98.4° F., pulse 82, respirations 20, blood pressure 175/105 mm. Hg. A well-developed and well-nourished middle-aged woman, clear in mind, showed a slightly pale skin and generalized puffiness which graded in the arms, sacrum and legs into mild but pitting edema. Beyond abnormally narrow retinal arterioles, examination of the fundi was negative. The pharynx showed no evidence of infection. The lungs were clear except for an occasional crackling rale at the right base. Beyond soft nontransmitted apical and basal systolic murmurs, no further cardiac abnormalities were found. The remainder of the physical examination was noncontributory.

Laboratory data: Urine, serial specimens: Specific gravity varied between 1.010 and 1.026, protein 4+ (5 grams in 24 hours), sugar—initially 4+, with regulation of diabetes 0, centrifuged sediment—red cells varied between 2 to 4 per high power field, white cells varied between 0 to 1 per high power field, occasional hyaline and granular casts. Blood: Red cells 4,000,000 per c.mm., hematocrit 40, sedimentation rate 50 mm. per hour, white cells 10,300 per c.mm. with 73 per cent neutrophiles and 4 per cent eosinophiles. Blood chemistry: Urea nitrogen 20 mgm. per cent, total protein 4.1 grams per cent, cholesterol 880 mgm. per cent, chlorides 104 m.eq. per liter. Basal metabolic rate —21. Bromsulphalein test—2 per cent retention after 40 minutes. Venous pressure 115 mm. saline. Circulation time (Decholin) 28 seconds. Electrocardiogram, low voltage, with occasional premature ventricular beats.

X-ray examinations: Lungs: Clear. Heart: Twenty per cent above average size by height-weight ratio.

7. R. S. (M-65187), a 29-year-old white married welder, was admitted to the Peter Bent Brigham Hospital on October 25, 1943, with the complaint of "kidney disease" of 6 months' duration.

Patient had had a chronic cough with numerous exacerbations in the winters during the previous 2 decades. Clubbing of the fingers had been present for many years.

In May, 1943, following a period of generalized malaise, he developed swelling of his ankles and was found to have both albuminuria and marked microscopic hematuria. He was treated at home by his local physician with bed rest, low-protein and low-salt diet. There had been no previous acute infection. His edema gradually subsided, but he developed hypertension with levels as high as 170 mm. Hg systolic. Albumin, red and white cells persisted in the urine. He returned to work, however, and did well until October, 1943, when he again developed edema of his legs, and he noted that his urine was scant and smoky. He went to bed 2 weeks before admission and his ankle edema disappeared. There had been intermittent nocturia during the 8 months preceding admission, but there had been no other symptoms suggesting genito-urinary disease. His only cardiovascular manifestation was minimal exertional dyspnea developing since his first acute episode.

Physical examination: Temperature 97.8° F., pulse 78, respirations 18, blood pressure 150/90 mm. Hg. A well-developed and nourished man in early middle age, presented a sallow complexion. Although his face was not puffy, there was minimal pitting edema of both ankles and sacrum. Fundi were within normal limits. Although the patient's tonsils were present, neither they nor the pharynx appeared infected. The trachea was deviated slightly to the left. The lungs were negative. To percussion the heart appeared moderately enlarged to the left, but beyond a soft apical systolic murmur no other abnormalities were found. Prostate was normal. The remainder of the examination was noncontributory.

Laboratory data: Blood Hinton negative. Urine, serial specimens: Specific gravity varied between 1.006 and 1.012, protein 3+ (12 grams in 24 hours), sugar negative, centrifuged sediment—red cells varied between 1 to

2 per high power field to a sediment loaded with red cells such that other elements were obscured, white cells usually between 3 to 10 per high power field, on 2 occasions many more white cells were seen among large quantities of red cells, casts varied between 1 and 20 per low power field. Blood: Red cells 4,400,000 per c.mm., hematocrit 30, sedimentation rate 61 mm. per hour, white cells 10,000 per c.mm., with 70 per cent neutrophiles. Blood chemistry: Urea nitrogen 46 mgm. per cent, total protein 5.6 grams per cent, cholesterol 340 mgm. per cent, chlorides 116 m.eq. per liter, carbon dioxide combining power 20 m.M. per liter. Vital capacity 4,500 m.l. Overnight urine concentration test—maximum specific gravity 1.010. Pituitrin concentration test—maximum specific gravity 1.008. P.S.P. excretion—15 minutes 10 per cent, and a total of 65 per cent in 2 hours. Electrocardiogram normal. Serial urine cultures showed either no growth or an occasional staphylococcus albus or alpha streptococcus.

X-ray examinations: Chest: Clear. Heart: Slightly enlarged to right and left. Sinuses: Negative beyond thickened membranes in left antrum. Lipiodol examination of bronchi: No obvious bronchiectasis although bronchus to right lower lobe filled poorly. Teeth: Negative.

BIBLIOGRAPHY

1. Aldrich, C. A., Stokes, J., Jr., Killingsworth, W. P., and McGuiness, A. C., Concentrated human blood serum as a diuretic in the treatment of nephrosis. J. A. M. A., 1938, **111**, 129.
2. Weech, A. A., Goettsch, E., and Lyttle, J. D., Intravenous injection of serum and acacia in the treatment of edema. M. Clin. North America, 1940, **24**, 807.
3. Brown, H., Gray, C. H., and Mollison, P. L., Treatment of the nephrotic syndrome with serum transfusions. Brit. Med. J., 1942, **1**, 515.
4. Luetscher, J. A., Jr., The effect of a single injection of concentrated human serum albumin on circulating proteins and proteinuria in nephrosis. J. Clin. Invest., 1944, **23**, 365.
5. Janeway, C. A., Gibson, S. T., Woodruff, L. M., Heyl, J. T., Bailey, O. T., and Newhouser, L. R., Chemical, clinical, and immunological studies on the products of human plasma fractionation. VII. Concentrated human serum albumin. J. Clin. Invest., 1944, **23**, 465.
6. Scatchard, G., Gibson, S. T., Woodruff, L. M., Batchelder, A. C., and Brown, A., Chemical, clinical, and immunological studies on the products of human plasma fractionation. IV. A study of the thermal stability of human serum albumin. J. Clin. Invest., 1944, **23**, 445.
7. Scatchard, G., Strong, L. E., Hughes, W. L., Jr., Ashworth, J. N., and Sparrow, A. H., Chemical, clinical, and immunological studies on the products of human plasma fractionation. XXVI. The properties of solutions of human serum albumin of low salt content. J. Clin. Invest., 1945, **24**, 671.
8. Cohn, E. J., Oncley, J. L., Strong, L. E., Hughes, W. L., Jr., and Armstrong, S. H., Jr., Chemical, clinical, and immunological studies on the products of human plasma fractionation I. The characterization of the protein fractions of human plasma. J. Clin. Invest., 1944, **23**, 417.
9. Williams, J. W., Petermann, M. L., Colovos, G. C., Goodloe, M. B., Oncley, J. L., and Armstrong, S. H., Jr., Chemical, clinical, and immunological studies on the products of human plasma fractionation. II. Electrophoretic and ultracentrifugal studies of solutions of human serum albumin and immune serum globulins. J. Clin. Invest., 1944, **23**, 433.
10. Brand, E., Kassell, B., and Saidel, L. J.: Chemical, clinical, and immunological studies on the products of human plasma fractionation. III. Amino acid composition of plasma proteins. J. Clin. Invest., 1944, **23**, 437.
11. Scatchard, G., Batchelder, A. C., and Brown, A., Chemical, clinical, and immunological studies on the products of human plasma fractionation. VI. The osmotic pressure of plasma and of serum albumin. J. Clin. Invest., 1944, **23**, 458.
12. Beckman, W. W., Hiller, A., Shedlovsky, T., and Archibald, R. M., The occurrence in urine of a protein soluble in trichloroacetic acid. J. Biol. Chem., 1943, **148**, 247.
13. Howe, P. E., The use of sodium sulfate as the globulin precipitant in the determination of the proteins in blood. J. Biol. Chem., 1921, **49**, 93.
14. Karr, W. G., A method for the determination of blood urea nitrogen. J. Lab and Clin. Med., 1924, **9**, 329.
15. Snell, F. D., and Snell, C. T., Colorimetric methods of analysis. Vol. 2, p. 35, D. Van Nostrand and Company, New York. 1937.
16. Tiselius, A., Dissertation. Nova Acta Reg. Soc. Sci. Upsala, 1930, **7**, 4.
17. Longsworth, L. G., Recent advances in the study of proteins by electrophoresis. Chem. Rev., 1942, **30**, 323.
18. Philpot, J. St. L., Direct photography of ultracentrifuge sedimentation curves. Nature, 1938, **141**, 283.
19. Longsworth, L. G., Shedlovsky, T., and MacInnes, D. A., Electrophoretic patterns of normal and pathological human blood serum and plasma. J. Exper. Med., 1939, **70**, 399.
20. Luetscher, J. A., Jr., Electrophoretic analysis of plasma and urinary proteins. J. Clin. Invest., 1940, **19**, 313.
21. Armstrong, S. H., Jr., Budka, M. J. E., Morrison, K. McD., and Hasson, M., The refractive index increments and nitrogen factors of normal pooled human plasma proteins and normal human serum albumin. To be published.
22. Rutstein, D. L., personal communication.
23. Armstrong, S. H., Jr., unpublished observations.
24. Coburn, A. F., and Moore, D. H., The plasma proteins in disseminated lupus erythematosus. Bull. Johns Hopkins Hosp., 1943, **73**, 196.

25. Kay, C. F., The mechanism by which experimental nephritis is produced in rabbits injected with nephrotoxic duck serum. J. Exper. Med., 1940, **72**, 559.
26. Enders, J. F., Chemical, clinical, and immunological studies on the products of human plasma fractionation. X. The concentrations of certain antibodies in globulin fractions derived from human blood plasma. J. Clin. Invest., 1944, **23**, 510.
27. Gutman, A. B., Moore, D. H., Gutman, E. B., McClellan, V., and Kabat, E. A., Fractionation of serum proteins in hyperproteinemia, with special reference to multiple myeloma. J. Clin. Invest., 1941, **20**, 765.
28. Dole, V. P., The electrophoretic patterns of normal plasma. J. Clin. Invest., 1944, **23**, 708.
29. Heyl, J. T., Gibson, J. G., 2nd, and Janeway, C. A., Studies on the plasma proteins. V. The effect of concentrated solutions of human and bovine serum albumin on blood volume after acute blood loss in man. J. Clin. Invest., 1943, **22**, 763.
30. Cohn, E. J., The plasma proteins: their properties and functions. Tr. and Stud., Coll. Physicians, Philadelphia, 1942, **10**, 149.
31. Hegsted, D. M., Hay, A. L., and Stare, F. J., Chemical, clinical, and immunological studies on the products of human plasma fractionation. XXIV. Studies on the nutritive value of human plasma fractions. J. Clin. Invest., 1945, **24**, 657.
32. Cannon, P. R., Humphreys, E. M., Wissler, R. W., and Frazier, L. E., Chemical, clinical, and immunological studies on the products of human plasma fractionation. XXIII. The effects of feeding possible blood substitutes on serum protein regeneration and weight recovery in the hypoproteinemic rat. J. Clin. Invest., 1944, **23**, 601.
33. Dick, M. W., Warweg, E., and Andersch, M., Acacia in the treatment of nephrosis. J. A. M. A., 1935, **105**, 654.
34. Goudsmit, A., Jr., Binger, M. W., and Power, M. H., Acacia in the treatment of the nephrotic syndrome. Influence of acacia, injected intravenously, on concentration of proteins and on colloid osmotic pressure of the serum. Arch. Int. Med., 1941, **68**, 701.
35. Peters, J. P., Body water. The exchange of fluids in man. p. 287, Charles C. Thomas, Springfield, Illinois, 1935.
36. Lepore, M. J., Acacia therapy in nephrotic edema. Ann. Int. Med., 1937, **11**, 285.
37. Metcalf, W., The fate and effects of transfused serum or plasma in normal dogs. J. Clin. Invest., 1944, **23**, 403.
38. Landis, E. M., Observations on acacia therapy in nephrosis. J. A. M. A., 1937, **109**, 2030.
39. Smalley, R. E., and Binger, M. W., Chronic glomerulonephritis and the nephrotic syndrome. A follow-up investigation of patients treated with acacia. J. A. M. A., 1944, **126**, 532.
40. Thorn, G. W., Armstrong, S. H., Jr., and Davenport, V. D., Chemical, clinical, and immunological studies on the products of human plasma fractionation. XXXI. The use of salt-poor concentrated human albumin solution in the treatment of hepatic cirrhosis. J. Clin. Invest., To be published.
41. Bailey, O T., and Hawn, C. v. Z., The effect of crystallized bovine serum albumin on the tissues of normal animals. I. Morphologic changes in normal rabbits induced by intravenous injection of crystallized bovine serum albumin. Am. J. Path., 1943, **19**, 267.
42. Blackman, S. S., Jr., and Davis, B. D., Electrophoretic and chemical analysis of protein in nephritic urine. J. Clin. Invest., 1943, **22**, 545.
43. Hueper, W. C., Experimental studies in cardiovascular pathology. V. Effects of intravenous injection of solutions of gum arabic, egg albumin, and gelatin upon the blood and organs of dogs and rabbits. Am. J. Path., 1942, **18**, 895.
44. Smetana, H., and Johnson, F. R., The origin of colloid and lipoid droplets in the epithelial cells of renal tubules. Am. J. Path., 1942, **18**, 1029.