METABOLIC CHANGES ASSOCIATED WITH THE ADMINISTRATION OF SALT-POOR HUMAN SERUM ALBUMIN IN TWO CASES OF INFECTIOUS HEPATITIS[1,2]

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Human serum albumin for therapeutic use was made available during the war and was used chiefly in the treatment of shock because of its high osmotic properties (1–3). Its use in kidney and liver disease as a nutrient material was also investigated (1, 4, 5). These and later studies (6, 7) have raised questions as to the availability and utilization of this substance for metabolic needs in man. In connection with a general investigation of protein metabolism in disease, we have had the opportunity to make a detailed metabolic study of two patients with severe liver disease to whom salt-poor human serum albumin was administered. While the problem is complex and data on two patients obviously limits the drawing of broad conclusions, the material is being presented in the hope that it will supplement that of other investigators engaged in gathering similar data.

MATERIALS AND METHODS

The first patient, age 64, was admitted to the Foundation Hospital with the chief complaint of liver disease of two and a half months' duration. An exploratory laparotomy was performed on October 13, 1947, and a severely damaged liver was visualized. A diagnosis of infectious hepatitis was confirmed microscopically from biopsy sections. He was transferred to the metabolic ward three days later, placed on a standard diet and the studies begun. At this time, the patient was still gravely ill, and showed a severe anorexia which had been present for the duration of the disease. His weight had decreased during his illness from his usual weight of 142 pounds to 127 pounds.

Patient 2, age 47, was admitted to the hospital with a history of liver disease of three months' duration. In contrast to the first patient, the appetite of Patient 2 had returned and there had been no significant weight loss. Needle biopsy revealed a severe infectious hepatitis and beginning cirrhosis. On admission to the metabolic ward, he was placed on a high protein diet and studies were begun.

Careful records were kept of the dietary intake of calories, protein, carbohydrate, fat, and fluid. At times, an effort was made to provide the patients with an excess of food; calculations of the intake were made from the amount offered and refused. At other times, the food intake was maintained constant by the use of three standard comparable diets which were rotated successively. The diets were analyzed for N, Ca, and P at intervals during the experiments. The analyses agreed to within ± 5 p.c. of the values in the standard tables (8).

The balance experiments were divided into six-day periods by the use of carmine markers. Daily urines were collected in bottles containing 5 ml. of concentrated HCl and 5 ml. of toluene as preservatives. The stools were prepared for analysis by the usual "wet method" (9). Aliquots of the daily urine and six-day stool specimens were analyzed for N by the Kjehldahl method, for Ca by the method of McCrudden (10), and for P by the method of Fiske and Subbarow (11). Total protein, serum albumin, and globulin were estimated by the method of Howe (12). Electrophoretic analyses of the plasma were made at the beginning of each period in the experiments on the first patient using a barbiturate buffer system. Plasma and red cell volumes were measured by the dye (T-1824) and the radioactive phosphorus (P^{32}) methods respectively (13, 14) and the total "circulating" amounts of the various blood constituents were calculated from these values. A study of the use of these methods in disease states and the relationship of these values to the hematocrit have been published elsewhere (15). Hematocrits were determined in Wintrobe tubes, and hemoglobin was determined as oxyhemoglobin. The salt-poor albumin was obtained from the American National Red Cross Society in the form of 25 gm. ampoules made up in 100 ml. buffer solution. An equivalent of 75 gms. was infused daily for a period of six days

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on two occasions in Patient 1 and for one six-day period in Patient 2. There were no untoward reactions, and albuminuria was not encountered. Patient 2 was also fed a food supplement (Protenum) during one metabolic period.

RESULTS

The balance studies for Patient 1 are shown in Figure 1. At the beginning of the study, the patient was in negative balance with respect to N, P,

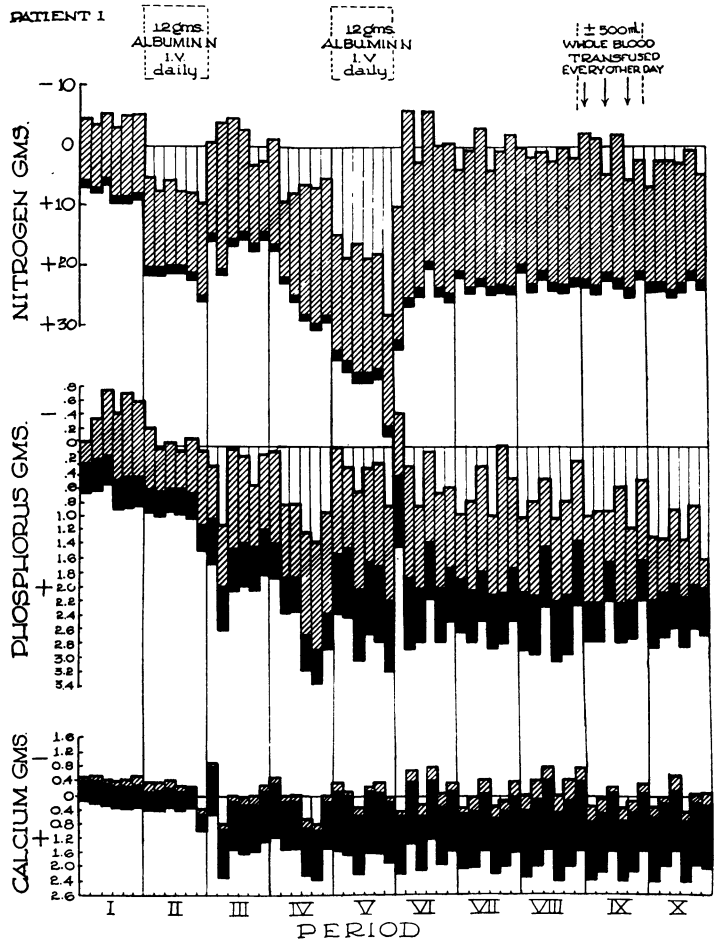

FIG. 1. BALANCE DATA FOR PATIENT 1

The ordinates represent intakes and balances in gms./24 hrs. The abscissa denote time in days. The horizontal line at 0 of the ordinate is the base-line to which intake and balance refer. The intake is plotted as an area from the base-line toward the bottom of the diagram. The urinary excretion is plotted as a hatched area and the fecal excretion as a solid area. When the total excretion does not reach the base-line, the white area left between the excretion line and the base-line represents a positive balance. When the excretion reaches the base-line, the balance is in equilibrium. A negative balance is indicated when the hatched area goes above the base-line. The data for fecal excretion are plotted in amounts per 24 hours although the measurements were made on pools of six-day periods.

The ordinate for the phosphorus data is constructed on the basis of a N : P ratio of 15 : 1 The ordinate for the calcium data is constructed on the basis of a Ca : P ratio of 2 : 1.

The nitrogen contained in the blood transfused in Period IX is not included in the balance data.

TABLE I

Electrophoretic analyses in Patient 1

Percentage of each component
(Calculations based on six-day periods)

Period	Day	Albumin	Globulins				
			Alpha$_1$	*Alpha*$_2$	*Beta*	*Fibrinogen*	*Gamma*
I	4	32.0	5.5	9.3	21.6	7.9	23.7
II*	2	48.3	4.0	6.5	17.3	7.7	16.2
III	1	72.0	2.2	2.5	8.4	4.7	10.2
IV	1	55.4	2.8	5.5	12.8	7.1	17.4
V*	1	53.5	5.3	7.8	12.5	6.9	13.9
V	3	64.6	2.8	1.9	13.2	6.3	10.4
VI	3	72.8	2.3	1.4	10.4	5.8	7.4
VII	1	64.0	4.0	6.4	7.0	5.2	14.0
IX†	1	54.4	4.9	7.5	11.0	8.6	13.5
X	1	52.3	3.2	13.3	7.5	9.2	14.6
X	6	50.4	6.4	4.4	13.2	5.4	20.0
Average of 32 analyses on 18 "normal" individuals		55.8	5.8	9.1	11.2	7.5	10.5

* 12 gms. albumin N I.V. daily during this period.
† Transfusions of ±500 ml. of whole blood given on the first, third, and fifth days of this period.

and Ca. This may be the reflection of a catabolic response to the infectious disease as well as evidence of an insufficient intake of protein and/or calories during this period (see Table II). The N balance became and remained positive during the period in which albumin was being administered (Period II), the Ca balance appeared slightly less negative and there was approximate equilibrium with respect to P. During the succeeding period (Period III), the N balance reverted toward the original level of Period I while the P and Ca balances became positive as the Ca and P intake increased. The increased food intake during Period IV is reflected in definite positive balances in the three constituents. The second series of albumin administrations (in Period V) again reflects the added N retention while the positive P balance decreased and Ca showed no significant change. Except for a temporary rebound during the first part of the succeeding period (Period VI)

TABLE II

Analysis of N retention in terms of N:P ratio in Patient 1

(Calculations based on six-day periods)

Period	Average daily caloric intake	I N Balance*	II N moving into (+) or out of (−) "metabolic pool" from circulating N	III N available for "metabolic pool"; Col. I − Col. II	IV Additive N available to "metabolic pool"	V P Balance	VI P moving with Ca into (+) or out of (−) bone	VII P available for "metabolic pool" Col. V − Col. VI	VIII Additive P available for "metabolic pool"	IX N:P ratio Col. III: Col. VII	X Additive N:P ratio Col. IV: Col. VIII
		gms.	*gms.*	*gms.*	*gms.*	*gms.*	*gms.*	*gms.*	*gms.*		
I	1715	− 27.1		−27.1		−3.02	−1.32	−1.70		15.9:1	
II†	1998	+ 70.7	−16.1	+ 54.6		−2.85	−0.71	−2.14		25.5:1	
III	2232	+ 20.4	+ 1.8	+ 22.2	76.8	+5.22	−1.07	+5.15	7.29	4.3:1	10.5:1
IV	2821	+ 60.7	+ 1.8	+ 62.5	139.3	+8.21	−1.65	+6.56	13.85	9.5:1	10.1:1
V†	3747	+140.1	−15.4	+124.7	264.0	+5.40	− .86	+4.54	18.39	27.5:1	14.3:1
VI	3628	+ 25.7		+ 25.7	289.7	+5.79	− .61	+5.18	23.57	5.0:1	12.3:1
VII	3837	+ 30.1	+ 5.8	+ 35.9	325.6	+6.58	−1.34	+5.24	28.81	6.9:1	11.3:1
VIII	3612	+ 35.2	+ 8.2	+ 43.4	369.0	+7.09	−0.22	+6.87	35.68	6.3:1	10.0:1
IX‡	3906	+ 33.6	− 5.2	+ 28.4	397.4	+7.62	−1.42	+6.2	41.88	4.6:1	9.5:1
X	3773	+ 40.5	+10.0	+ 50.5	447.9	+9.12	−1.13	+7.99	49.87	6.3:1	9.0:1

* Balance based on Period I as base-line.
† 72 gms. albumin N I.V. during six-day period.
‡ Three transfusions of ±500 ml. each of whole blood on days 1, 3, and 5 of this period.

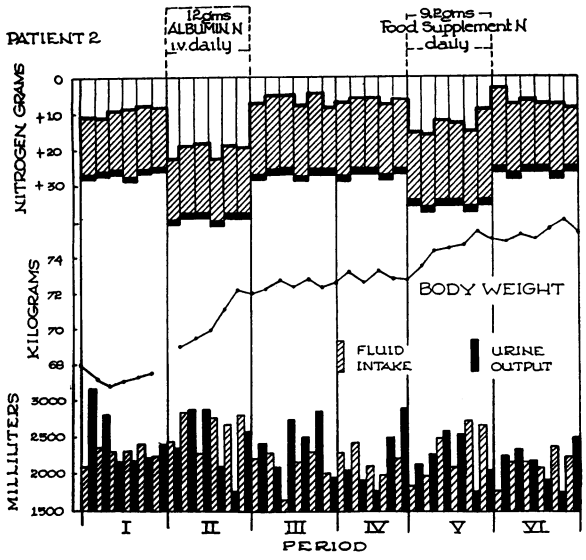

FIG. 2. NITROGEN BALANCE DATA FOR PATIENT 2

The nitrogen balance data are charted as in Figure 1. For discussion, see text.

the N and P balances may be considered as remaining positive during the remainder of the experiment. Ca balance remained in equilibrium.

The N balance data for Patient 2 are given in Figure 2. This patient, in contrast to Patient 1, was in positive balance at the start of the study. This experiment also differs from the first in that the basal N intake was kept constant throughout. The administration of albumin (Period II) and the addition of a food supplement (Protenum) (Period V) are reflected in increases in N balance, almost quantitative in the former instance and less so with the food supplement.

The values for fluid intake, urinary output and body weight are charted in Figures 2 and 3. The administration of albumin resulted in variable changes in fluid balance. Patient 1 was in approximate fluid balance at the beginning of the experiment but showed an increased urine output during the two days prior to albumin administration in Period II. This increased output persisted for the first part of this period, gave way to a urine retention during the latter part, and fluctuated considerably for the next two periods (III and IV). The second period of albumin administration (V), however, was accompanied by a definite and persistent deficit in urinary output, followed during the next period by a marked diuresis which persisted for about a week. The second patient failed to show any diuresis during the period of albumin administration (Period II); there was

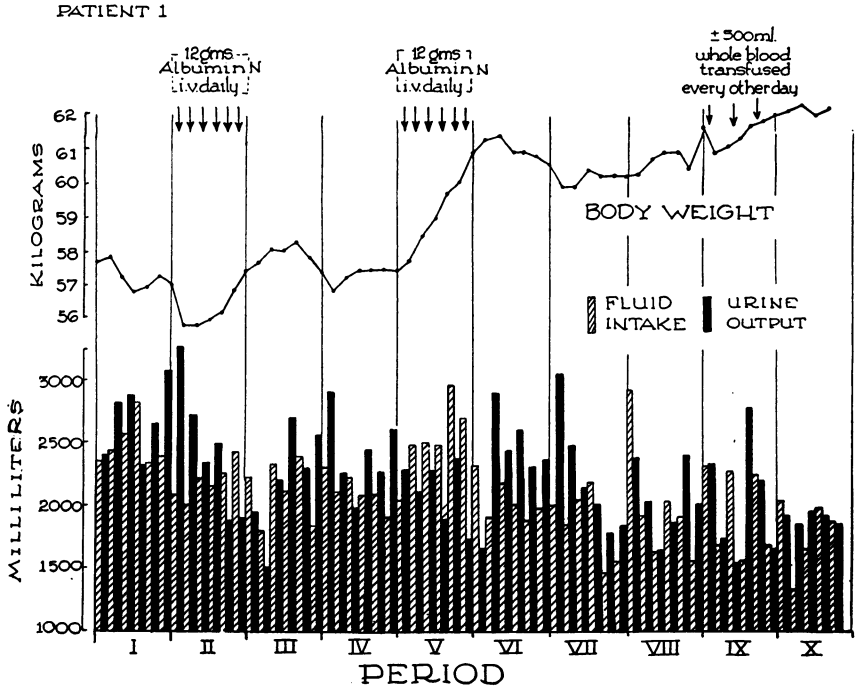

FIG. 3. WEIGHT CHANGES AS RELATED TO FLUID INTAKE AND OUTPUT IN PATIENT 1

again a tendency toward a decreased urine formation at the end of the period. This was also true during Period V when the food supplement was fed.

Analysis of the weight data indicates that the patients gained 4.9 Kg. and 7.3 Kg. respectively during the entire course of the metabolic studies. Patient 1 shows a deficit of 5.1 Kg. of water, while Patient 2 lost 3.6 Kg. of water. The real weight gains of these patients were, therefore, 10.0 Kg. and 10.9 Kg. respectively. Patient 1 retained a total of 450 gms. of nitrogen, which amount may be considered equivalent to 13.5 Kg. of tissue on the assumption that 1 gm. of nitrogen is equivalent to 30 gms. of tissue. The second patient retained 370.6 gms. of nitrogen equivalent to 11.1 Kg. of tissue.

The changes in the electrophoretic pattern of the proteins in Patient 1 are given in Table I. At the beginning of the experiment, the percentage of albumin was low while the alpha globulin and fibrinogen was almost double the usual value. The relative value of gamma globulin was more than double that found in our "normal" series. Similar changes have been described by previous investigators (16). The changes observed in Period II and on the first day of Period III unquestionably reflect the dilution occasioned by the increase in plasma volume as a result of the retention of albumin in the circulation. A similar dilution effect is also apparent during and after the second period of albumin administration. The subsequent persistence of relatively low values for the beta and gamma globulins in Periods VII, VIII, and IX are of interest as a possible reflection of improved liver function. The values for alpha globulin and beta globulin for Period VII, day 1 and Period X, day 1 appear to be aberrent due to technical errors, but it was not possible to repeat these determinations. The rise in beta and in gamma globulins at the end of Period X may be indicative of a loss of albumin greater than that of globulins from the circulation after the transfusions.

The changes in total circulating proteins are shown in Figures 4 and 5. In Patient 1, the total protein was low at the beginning of the experiment due to the marked deficit in total circulating albumin. The albumin level was also low in the second patient, but, in this case, the total protein level was within the normal range because of the large amounts of globulins in circulation. Despite the marked retention of N which apparently went to build body tissues, the total circulating albumin was still below normal levels at the end of the experiment in Patient 1. In the second patient, however, the circulating albumin level was within the normal range at the end of the experiment.

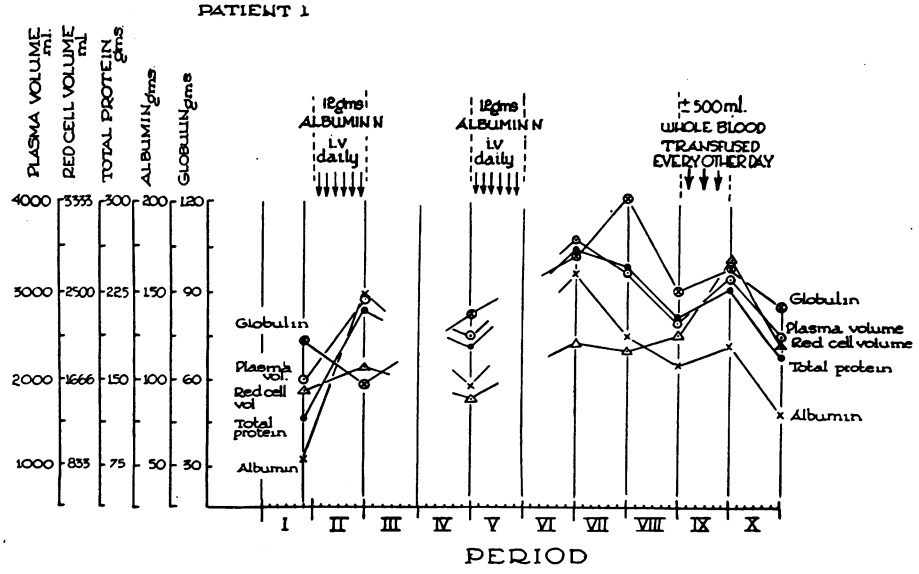

FIG. 4.

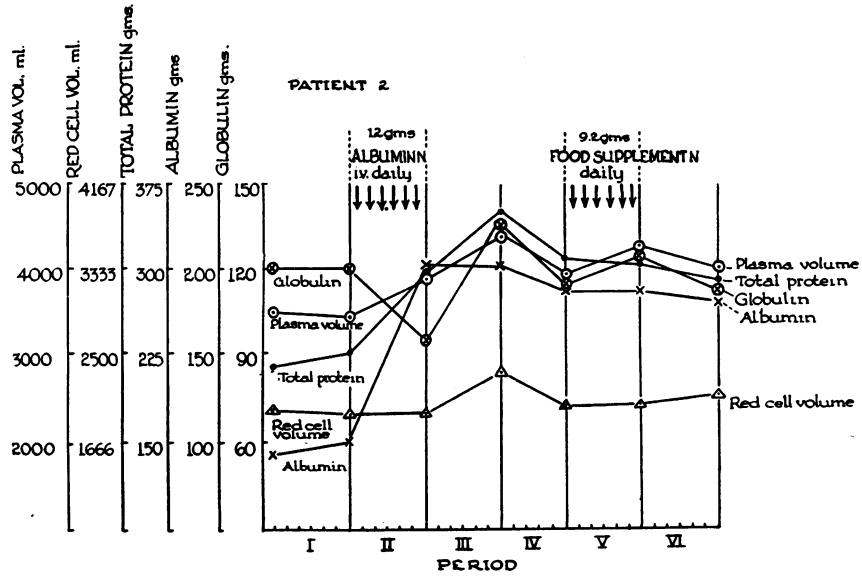

FIG. 5.

The total circulating globulins in both patients remained high throughout the experiment except for a fall during the periods of albumin administration (Period II in each experiment). The reason for this is not obvious.

The changes in plasma volume and red cell volume in Patient 1 are shown in Figure 4. This patient had a large initial deficit in plasma volume and in red cell volume. As was anticipated, the administration of albumin was associated with an increase in plasma volume, and as the albumin left the circulation, there was a concomitant fall in the plasma volume. The total red cell volume showed a slight but definite increase during the experiment up to the time of the transfusions. The transfusions resulted in the expected rise of red cell volume. The subsequent drop to the pre-transfusion level suggests rapid destruction of red cells but there was no gross clinical evidence of a major hemolytic episode. The persistence of a high globulin level and the return of albumin toward the original low level suggest, as did the electrophoretic analyses, that albumin was leaving the circulation faster than globulin.

Patient 2 (Figure 5) had a plasma volume which was slightly above normal level at the beginning of the experiment, and which fluctuated in the expected fashion with the experimental procedures. The red cell volume was low at the beginning and failed to rise.

DISCUSSION

Analysis of the changes in nitrogen balance in Patient 1 is, of course, complicated by the facts that the patient was permitted to select his diet and that his appetite and consequent food intake increased during the first part of the study (see Table II). It is also difficult, in a variable disease state, to establish a definite base-line for reference. However, it seems permissible to conclude that this patient retained, for the most part, a quantity of nitrogen equal to the nitrogen in the administered albumin. This, likewise, was true of the second patient.

In order to evaluate the significance of the retained nitrogen, an analysis has been made of the data with reference to the phosphorus and calcium balances, following the procedures described by Albright and his colleagues (6). This concept is based on the fact that the body P is almost entirely contained either in bone (Ca : P ratio = 2.23) or as an integral part of protoplasm (N : P ratio of muscle = approximately 14.7). Hence by allowing for the P which moves with Ca into and out of bone, one can derive an N : P ratio based on balance studies which will aid in the interpretation of retained N or P.

Table II shows the result of the analysis of the data of Patient 1. This indicates that during the control period (Period I) the body was losing N

and P at approximately a 16:1 ratio. This figure fits fairly well with the ratio ascribed to body tissues by Benedict (17). The retention of considerably more N than P during the period of albumin administration (Period II) suggests that the N was not being built into body tissues directly. In the period following albumin administration (Period III) there was a marked change in the N:P ratio, which was now due primarily to the increased retention of P. This would suggest that the retained albumin N was being converted to "body tissues." This low N:P ratio persisted during the next period. The overall N:P ratio for the 24 days of the experiment up to this time was 10:1. The same pattern of events occurred during and after the second period of albumin administration (Periods V through X).

The N:P ratio for the entire experiment was only 9:1. This apparent discrepancy may be explained, at least in part, by the alteration in the state of the liver during the course of the experiment. It is reasonable to assume that the marked clinical improvement was associated with glycogen storage and liver cell regeneration. Both factors would account for increased P retention (18). It should be noted that the N:P ratio of liver is approximately 10:1 (9). Finally, it should be emphasized that these relationships represent only approximations since, for example, no allowance has been made for P shifts related to alterations in extracellular or intracellular fluids.

Table II (column II) also shows that the shift of N into and out of the circulation is relatively small as compared with the large retention of N by the "tissues."

Because of the variable calcium intakes, these studies do not lend themselves to a precise appraisal of the effects of albumin administration on calcium metabolism. The data do suggest, however, that there was a decrease of urinary calcium associated with albumin administration. Albright (6) has noted similar findings and discussed their possible significance with relation to osteoporosis.

The limited amount of data does not permit of any conclusions with respect to the comparative values of oral food supplement and intravenous serum albumin. Patient 1 seemed to utilize nitrogen furnished by either supplement with equal facility. Thus in Period II, approximately one-half of the nitrogen intake was in the form of ingested food protein. In Period IV, essentially the same degree of positive balance obtained when the entire diet was in the form of ingested food protein. As previously mentioned, Patient 2 showed a somewhat poorer retention of N ($\pm$ 60 per cent) when it was given in the form of a food supplement than when given intravenously as albumin. It should be borne in mind, however, that the capacity for "increased anabolism" may have varied with improvement in clinical condition and so influenced the results. It should also be emphasized that the patients were on a high protein diet throughout the periods of observation and that their responses may have been conditioned by this fact. Finally, it is probable that no single factor is responsible for the maintained N balance in these experiments and that the increased food intake, change in body response, and albumin therapy represent only some of the factors which are important in this complex situation.

It is notable that neither patient significantly replenished red cells or hemoglobin, and that Patient 1 failed to augment appreciably his deficient plasma albumin despite the marked N retention in both cases which apparently went to rebuild body tissues. This type of separation of effect has been reported by many observers as being present in liver disease, nephroses, anemia of infection, etc.

SUMMARY AND CONCLUSIONS

Two patients suffering from infectious hepatitis were studied on a metabolic regime for periods of 60 and 35 days, respectively. Large doses (75 gms.) of salt-poor human serum albumin were administered daily during two separate six-day periods to the first patient and during one six-day period to the second patient. The latter was subsequently fed a protein supplement during one period and the results compared with those obtained after albumin administration. Daily nitrogen, calcium, and phosphorus balances were obtained as well as frequent determinations of the plasma proteins, red cell and plasma volumes. The results indicate that intravenously administered salt-poor human serum albumin is well retained and appears to be incorporated into body tissues after several days' delay. The striking retention of the albumin in the experimental situations described may be related to the marked anabolism which was present. Some of its usefulness clinically was undoubtedly

due to its stimulating effect on the appetite. Thus both patients were able to maintain a total nitrogen intake of approximately 40 gms. a day for six days when the intravenous albumin was added to a large protein intake by mouth. Under the conditions of our experiments, there was a failure to replenish red cells and hemoglobin in both patients and plasma albumin in one patient in spite of the marked retention of nitrogen and the apparent building of tissue proteins. This discrepancy is apparently an inherent defect due to the disease state.

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