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TISSUE PROTEIN STUDIES IN NORMAL AND UNDERNOURISHED MALES: THE CHANGES IN TOTAL CIRCULATING PROTEIN AFTER AN INTRAVENOUS SALINE INFUSION AS AN INDEX OF PROTEIN STORES¹

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In 1934 Whipple *et al.* (1) suggested that protein stores existed in body tissues and postulated that a "dynamic equilibrium" between plasma proteins and these tissue stores was an integral part of protein metabolism. This concept has gradually been accepted by most investigators in the field of nutrition (2-4). Shearburn in 1942 (5), following the lead of Calvin (6, 7), presented data obtained in normal and in hypoproteinemic dogs showing that the presence or absence of protein stores could be demonstrated after an intravenous saline infusion. These fundamental observations in regard to tissue proteins are important in explaining the equilibrium state as set forth by Whipple and to our knowledge have not been included in any of the recent discussions on this subject.

At present, the clinical signs which suggest protein deficiency, except in extreme cases, are totally inadequate to give an exact evaluation of the state of nutrition of a given subject. Also, it is generally accepted that the serum protein concentration does not reflect the true nutritional state. Ravdin (8) has stated, "If the means to determine it were available, the first effect of protein undernutrition is a reduction in the amount of protein stores in the tissues of the body since every attempt is made to maintain the serum protein concentration at a nearly normal level."

Localio *et al.* (9) in an attempt accurately to evaluate protein reserves, determined the nitrogen

content of the rectus abdominis fascia, at the time of surgery, in patients suspected of having a protein deficiency and in patients who were considered to be in a state of normal nutrition. This method of evaluating tissue proteins is of no value before surgery and, in addition, is arduous and time consuming. Furthermore, one must assume that all tissues react in a manner similar to the rectus abdominis fascia. It has long been known that the amount of nitrogen excreted by animals or man, during a period on a protein deficient diet, decreases as the experiment is continued until a "minimal" level is reached. Allison (2) has stated: "The amount of nitrogen excreted when the animal is eating a protein-free diet is a measure of the magnitude of the protein stores of the body, being high when the labile cytoplasmic proteins are in abundance and being low when these stores are depleted." Boothby *et al.* (10) and Deuel *et al.* (11) did similar work in humans, using intravenous thyroxin to increase metabolism in an attempt to evaluate protein stores in man. Such a procedure is not practical for routine evaluation of the state of protein nutrition in clinical patients.

Realizing the need for a means of evaluating the status of protein nutrition in clinical patients, studies were undertaken to develop a method of quantitatively assaying the nutritional state of a patient with respect to proteins. This report presents the results of these observations; it provides a practical method for demonstrating the existence or absence of adequate tissue protein.

METHODS

The nine undernourished subjects in this study were chosen on the basis of the following criteria: a definite weight loss over a variable period up to six months preceding the study (estimated at 20 pounds or more in severe undernutrition); a history of inadequate food intake;

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signs of vitamin deficiencies (*e.g.*, poor night vision, red tongue, cheilitis or peripheral neuritis) which in general would denote poor dietary habits. Some of these subjects had a low serum protein value which was accepted as evidence of poor intake or faulty utilization of protein; others had normal levels of total serum protein, but this was not considered necessarily to indicate good protein nutrition. A low serum albumin value, with or without a rise of globulin, was also accepted as evidence of poor protein intake or faulty utilization. The six normal subjects used in the study manifested none of the above signs and had had no recent illness.

In each of the normal subjects the plasma volume, total serum proteins and fractions (albumin and globulin) were performed before and again 30 minutes after the infusion of 1 liter of physiological saline. The infusion was given at a rate of approximately 20 ml. per minute. In one subject, J. Ca., these studies were conducted twice. In the undernourished subjects, these same determinations were made once in all instances and in four cases once or more following periods of refeeding as described below.

The plasma volume was determined using the dye T-1824 according to the method of Gibson and Evans (12) as modified for the photoelectric colorimeter (13). Dyed-blood specimens were obtained without venostasis at intervals of 10, 15, 20 and 25 or 10, 13, 16 and 19 minutes following the injection of the dye. The same needle was used to withdraw all of the dyed-blood specimens; a 0.4 per cent solution of sodium citrate was used to keep the needle "open." To accomplish this a 2-ml. syringe containing 1 ml. of the citrate was attached to the needle and ½ ml. of citrate was injected slowly; to avoid dilution, ½ ml. of blood was withdrawn into the 2-ml. syringe and discarded just prior to withdrawing each sample to be analyzed. Each patient was thus subjected to a minimum of venipunctures, usually only three, during each complete study involving two plasma volume determinations.

The concentration of protein in the serum was estimated by nitrogen determination and converting nitrogen to protein by multiplying by the factor 6.25. Serum samples were subjected to Kjeldahl digestion using sulfuric acid and hydrogen peroxide; the ammonia formed was determined by means of Conway cells (14).

Fractionation of the serum proteins into albumin and globulin was accomplished by using the methanol precipitation procedure of Pillemer and Hutchinson (15). Albumin was determined by estimating the nitrogen in the methanol solution after removing the precipitated globulin; nitrogen was determined by the same method given above for serum proteins. The difference between total protein and albumin in the serum was considered to be globulin.

After the above procedure, four of the malnourished patients were encouraged to consume a high caloric and high protein diet. The amount consumed varied from between 3000 to 4500 calories per day. This diet provided from 2.0 to 4.5 gms. of protein per kg. of body weight per 24 hours. These high values were obtained using as interval feeding through the day and at bedtime,

1 to 2 quarts of the Varco-type formula,⁴ to which was added 2 ounces of Protenum.⁵ One and one-fourth quarts of this formula contained 120 gms. protein, 410 gms. carbohydrate and 36 gms. fat with a caloric value of 2444. The subjects were followed for from 32 to 64 days during which they approached or completely returned to a normal nutritional status.

While on this regimen it was found that the less severely malnourished subjects rapidly gained weight, and reported a feeling of well being with a marked increase in appetite. The more severely emaciated subjects tolerated the forced feedings less well and required a longer period of readjustment. If too vigorous therapy was attempted, gastrointestinal disturbances such as anorexia, vomiting or diarrhea occurred and the emaciated patient would actually lose more weight.

RESULTS

The changes in plasma volume and total circulating protein following the 1000 ml. intravenous saline infusion in the six normal subjects are presented in Table I. It will be seen that plasma

TABLE I
Changes in plasma volume and total circulating protein after saline infusion
Normal Subjects

Patients	Plasma volume ml.		Change in plasma volume ml.	Total circulating protein gms.		Change in total circulating protein gms.
	Before	After		Before	After	
S. K.	2580	3000	+420	184.2	195.6	+11.4
I. T.	2450	2927	+477	181.7	194.2	+12.5
A. R.	2710	3120	+410	169.0	180.0	+11.0
L. H.	3221	3878	+657	225.5	244.1	+18.6
J. Ca	2869	3240	+371	177.1	188.5	+11.4
J. Ca	2939	3239	+300	174.8	189.6	+14.8
W. S.	2409	2859	+450	152.6	170.2	+17.6

volumes increased an average of 440.7 ml. The total circulating proteins, expressed in gms., following saline infusion were increased in every instance. The degree of this change, also expressed in gms., may be seen in the column to the far right of this chart and ranged from 11.0 to 18.6 gms. or an average of 13.9 gms.

Changes in plasma volume and total circulating protein following saline infusion in eight of the nine undernourished subjects are summarized in Table II. It will be seen that, in general, a lower de-

⁴ Varco-type formula: a high caloric, low fat mixture which consists of 1000 ml. skim milk, 4 ounces milk powder, six eggs, two egg whites and 300 gms. lactose.

⁵ Kindly supplied by Dr. Warren Cox of the Mead Johnson and Company.

TABLE II
Changes in plasma volume and total circulating protein
after saline infusion
Undernourished Subjects

Patients	Plasma volume ml.		Change in plasma volume ml.	Total circulating protein gms.		Change in total circulating protein gms.
	Before	After		Before	After	
J. Ch	2988	3355	+367	244.8	218.1	-26.7
H. O.	3564	3941	+377	225.8	215.0	-10.8
J. H.	3172	3236	+64	213.9	165.9	-48.0
H. L.	2050	1975	-75	116.6	93.4	-23.2
E. G.	3076	3455	+379	227.6	223.8	-3.8
D. F.	2873	2815	-58	179.2	176.0	-3.2
L. M.	2568	2730	+162	176.4	174.5	-1.9
W. J.	2446	2581	+135	170.8	171.2	+0.4

gree of change in the plasma volumes occurred in these subjects as compared with the normal group; there being a decrease in two subjects and the maximum increase in the remaining six being 377 ml. The total circulating proteins decreased in all except one (W. J.) of the eight subjects. The amount of this change, expressed in gms., can be readily seen in the right hand column of this chart.

The initial plasma volumes recorded in the normal and undernourished subjects are listed in Table III as ml. (total volume), as ml. per square meter of body surface and as ml. per kg. of body weight. The range from 39.5 to 47.8 ml. per kg. with a mean of 44.6 ± 1.1 ml. per kg. in the normal subjects is within normal limits.⁶ Initial

⁶ Reported normal limits for plasma volume determined by the T-1824 dye method are: Gibson and Evans (12) 41.3 ml. per kg., Gregersen (16) 45 ml. per kg., Henschel *et al.* (17) 45.01 ± 4.0 ml. per kg.

TABLE III
Plasma volume in normal and undernourished subjects

Normal			Undernourished				
Subject	Plasma volume ml.	Plasma volume ml./sq. m.	Plasma volume ml./kg.	Subject	Plasma volume ml.	Plasma volume ml./sq. m.	Plasma volume ml./kg.
S. K.	2580	1480	43.6	J. Ch	2988	1726	47.6
I. T.	2450	1570	46.9	J. Ch	2903	1730	49.3
A. R.	2710	1540	43.6	J. Ch	3375	1985	56.2
L. H.	3221	1653	43.0	H. O.	3564	2072	61.7
J. Ca	2869	1639	47.6	H. O.	3641	2035	57.6
J. Ca	2939	1660	47.8	J. H.	3172	1740	48.8
W. S.	2409	1384	39.5	E. G.	3076	1700	45.1
				L. M.	2568	1526	43.8
				W. J.	2446	1664	54.4

Average plus standard error of the mean 44.6 ± 1.1

51.6 ± 6.4

plasma volumes in the undernourished subjects ranged from 43.8 to 61.7 ml. per kg. with a mean of 51.6 ± 6.4 ml. per kg. These values are similar to those reported by Henschel *et al.* (17) for subjects at the end of the first 12 weeks of their classical starvation studies.

Table IV shows the amount that albumin and globulin contributed to the change in total circulating protein. Albumin made up more of the increase in total circulating protein than did globulin in four of the normal subjects while in one the reverse was true. In five of the six undernourished subjects, however, albumin contributed more to the decrease in total circulating protein than did globulin. In either case the migration of protein is mainly due to the albumin fraction though the globulin portion also takes part to a smaller degree.

In Table V are summarized data on one subject (J. Ch.) who was studied through two different refeeding courses. This subject, a chronic alcoholic, first entered the hospital in severe undernutrition with marked liver damage and ascites. The initial observations were made at that time (Period I, day 1). It will be seen that following the saline infusion, the plasma volume increased by 367 ml. and the total circulating protein decreased by 26.7 gms. After 29 days on the high caloric diet the same studies were repeated (Day 29). During this time he had gained ten pounds in body weight with loss of ascites and edema. When the saline infusion test was repeated the plasma volume was found to increase by 348 ml.; the total circulating protein did not change. The patient refused further hospitalization, left the institution and began to drink heavily and eat an inadequate diet. He re-entered the hospital 90 days later in severe undernutrition, manifesting ascites, dependent edema, marked dehydration and by roentgen examination, miliary and cavernous tuberculosis of the lung. The study using intravenous saline was performed immediately (Period II, day 1) and before any fluid and electrolyte disturbances were corrected. In this instance the plasma volume decreased 98 ml. and the total circulating protein decreased 18.4 gms. He was again started on the refeeding program in addition to streptomycin. During the succeeding 21 days he gained only 2 pounds in weight in spite of strict adherence to the refeeding program. Apart from hydration there was little clinical change in the subject. The

TABLE IV
Change in total circulating protein, total circulating albumin and total circulating globulin

Normal			Undernourished				
Patient	△ Total circulating protein	△ Total circulating albumin	△ Total circulating globulin	Patient	△ Total circulating protein	△ Total circulating albumin	△ Total circulating globulin
	<i>gms.</i>	<i>gms.</i>	<i>gms.</i>		<i>gms.</i>	<i>gms.</i>	<i>gms.</i>
S. K.	+11.4	+ 6.8	+ 4.6	H. O.	-10.8	+12.0	-22.8
I. T.	+12.5	-12.5	+25.0	J. H.	-48.0	-25.5	-22.5
A. R.	+11.0	+ 8.3	+ 2.7	H. L.	-23.2	-18.3	- 4.9
J. Ca	+11.4	+11.4	0.0	E. G.	- 3.8	- 7.4	+ 3.6
W. S.	+17.6	+ 9.4	+ 8.2	D. F.	- 3.2	-18.7	+15.5
				L. M.	- 1.9	-13.3	+11.4

tests were repeated (Day 21, period II) at which time the plasma volume was decreased, following saline infusion, by 30 ml. and the total circulating protein decreased by 27.8 gms. Just prior to discharge to a tuberculosis sanatorium the tests were again repeated (Day 64, period II). The subject had gained 14 pounds in weight with loss of ascites and edema and felt much improved; there had been no demonstrable change in the pulmonary tu-

berculosis. It will be seen that the plasma volume was increased by 400 ml. and the total circulating protein was increased by 5.7 gms. The increase in total circulating protein after the saline infusion is interpreted as showing partial repletion of body tissue protein.

Data on three subjects in various degrees of undernutrition studied through the refeeding program are presented in Table VI. Subject H. O.

TABLE V
The influence of refeeding on the total circulating protein

Date	Weight	Plasma volume ml.		Total serum protein gms.%		Total circulating protein gms.		Serum albumin gms.%		Total circulating albumin gms.	
		Before	After	Before	After	Before	After	Before	After	Before	After
Period I											
Day 1	138	2988	3355	8.20	6.50	244.8	218.1	4.51	—	134.7	—
Day 29	148	3652	4000	7.31	6.68	267.1	267.1	4.67	3.86	170.6	154.4
Period II											
Day 1	130	2903	2805	5.89	5.45	171.0	152.6	1.69	1.57	49.2	44.0
Day 21	132	3375	3345	8.09	7.36	273.0	245.2	2.33	2.30	78.6	76.8
Day 64	144	2855	3255	8.00	7.18	228.2	233.9	2.51	2.41	71.7	78.4

TABLE VI
The influence of refeeding on the total circulating protein

Subject	Date	Weight	Plasma volume ml.		Total serum protein gms.%		Total circulating protein gms.		Serum albumin gms.%		Total circulating albumin gms.	
			Before	After	Before	After	Before	After	Before	After	Before	After
H. O.	Day 1	127	3564	3941	6.34	5.46	225.8	215.0	2.72	2.77	97.0	109.0
	Day 30	139	3641	3880	7.00	6.39	254.8	248.0	3.31	3.08	120.5	119.5
	Day 47	145	3310	3460	6.96	7.00	231.0	242.0	3.75	3.71	121.1	128.4
J. Co	Day 1	—	2588	2816	6.26	5.91	161.8	166.4	3.39	3.17	87.8	89.0
	Day 52	—	2575	3380	6.60	6.30	171.5	213.0	3.85	3.47	99.2	117.3
J. H.	Day 1	143	3172	3236	6.74	5.12	213.9	165.9	3.03	2.18	96.0	70.5
	Day 34	158	2522	2679	6.64	6.53	167.5	174.9	4.16	4.03	105.0	109.5

had a pulmonary abscess which responded sluggishly to penicillin and which evacuated spontaneously, after bronchoscopy, 25 days after the initial determinations. Tests on Day 1 showed an increase in plasma volume of 377 ml. after saline infusion and the total circulating protein decreased 10.8 gms. The same tests were repeated on Day 30, during which time the subject had gained 12 pounds in weight, and showed an increase in plasma volume of 239 ml. and a decrease in total circulating protein of 6.8 gms. By Day 47 the subject had increased 18 pounds in weight and the tests revealed an increase in plasma volume of 150 ml. and an increase in total circulating protein of 11 gms.; this increase in total circulating protein is interpreted as a normal response. Subject J. Co. was an aged patient with rheumatoid arthritis. The tests accomplished on Day 1 show that following the saline infusion, the plasma volume was increased by 228 ml. and the total circulating protein was increased by 4.6 gms. Fifty-two days later (Day 52) it will be seen the plasma volume increased 805 ml. and the total circulating protein increased 41.5 gms. following the infusion. This increase in total circulating protein is interpreted as a normal response and probably confirms the over-compensation phenomenon as demonstrated by Madden *et al.* (18) in protein repleted dogs. Subject J. H. had a severe pneumonia which responded only to combined penicillin and streptomycin therapy. The initial studies, Day 1, were accomplished nine days from the onset of illness and revealed an increase in plasma volume of 64 ml. and a decrease in total circulating protein of 48 gms. Repeat studies on Day 34, after an increase of 15 pounds in weight, showed that, following the saline infusion, the plasma volume was increased by 157 ml. and the total circulating protein was increased by 7.4 gms.

DISCUSSION

The data presented in this report are best explained by the mechanism of protein exchange, or the dynamic equilibrium state, as presented by Whipple, Schoenheimer and others (19, 20).

It was noted, in Table I, that normal subjects responded to the saline infusion by increasing plasma volume, an average of 440.7 ml., and increasing total circulating protein, an average of 13.9 gms. The increase in plasma volume in these

subjects is comparable to that obtained by Jacobson and Smyth (21) following intravenous saline infusions in normal subjects. The response to the saline infusion observed in the undernourished subjects, Table II, was characterized by an increase in plasma volume, an average of 169.6 ml., and a decrease in total circulating protein, an average of 14.7 gms. Although the increases in plasma volume demonstrated by the undernourished subjects were, generally, less than those increases shown by the normal subjects, more data of this type are needed to ascertain whether or not there is a correlation between change in total circulating protein and the amount of change in plasma volume. The changes in total circulating protein manifested by both groups of subjects were opposite in direction (*i.e.*, increased 11 gms. or more in normal subjects and decreased in undernourished subjects). Since the degree of normality is generally more constant than is the degree of abnormality, in a given situation, it is not surprising that the total circulating protein in the normal subjects was increased within a rather narrow range (+ 11 to + 18.6 gms.). The malnourished patients, however, were so far as could be determined clinically, in various degrees of undernutrition and it is presumed for that reason they showed a more variable response (+ 0.4 to - 48.0 gms.).

These data show that under the influence of intravenous saline there is a migration of protein either into or out of the circulatory system. The direction of migration depends on the presence or absence of tissue protein stores. In the well-nourished individual where there are adequate protein stores the direction of flow is into the blood stream. On the other hand, where the protein stores are depleted as in the undernourished patient, proteins leave the circulation following the injection of saline. It may be that the liver cells are chiefly responsible for the different patterns demonstrated by the normal and abnormal subjects. These protein interchanges depend upon the passage of intact proteins through cellular membranes. This concept has already been suggested by Whipple (19).

It is an accepted fact that a normal serum protein concentration may mask an undernutritional state (22). Total circulating protein determinations, alone, may also convey an erroneous impression as to the subject's nutrition. We believe an

intravenous saline infusion of at least 1000 ml. is a sufficient stimulus to either increase or decrease total circulating protein and so differentiate a normal state of protein nutrition, in the first instance, from a state of protein depletion in the second case.

SUMMARY

1. A relatively short and rapid method of demonstrating the presence or absence of labile protein stores in man is presented.

2. Plasma volume, total circulating proteins, and fractions (albumin and globulin) were determined before and after an intravenous infusion of 1 liter of physiological saline in six normal and in nine undernourished subjects.

3. The response to the saline infusion demonstrated by the normal subjects was characterized by an increase in plasma volume, an average of 440.7 ml. and an *increase* in total circulating protein, an average of 13.9 gms.

4. The response to the saline infusion demonstrated by the undernourished subjects was characterized by an increase in plasma volume, an average of 169.9 ml., and a *decrease* in total circulating protein, an average of 14.7 gms.

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