FOOD PROTEIN EFFECT ON PLASMA SPECIFIC GRAVITY, PLASMA PROTEIN, AND HEMATOCRIT VALUE*

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(Received for publication, December 25, 1947)

We shall show that the amount of protein eaten by normal individuals alters the specific gravity of their plasma and the hematocrit value of their blood. A point of particular interest is that these two changes move in opposite directions. The specific gravity rises but the hematocrit value falls as the protein intake increases from low but adequate, through moderate, to high but not excessive levels.

For clinical purposes a rise in specific gravity is taken as an index of a rise in the protein concentration of the plasma. It might be supposed, therefore, that in these experiments, an increase in protein consumption had been followed by a rise in plasma protein concentration. In that case a plausible explanation for the opposite movement of the hematocrit value is available. Since a rise in plasma protein concentration is frequently followed by an increase in blood volume, the fall in the hematocrit value may be regarded as a secondary phenomenon consequent to a dilution of the blood in respect of its red cell concentration.

The analysis of variance was applied to the data. The factors postulated in these experiments were thus defined in regard to their relative importance and in regard to some of their interrelations. Finally it was shown that the rise in specific gravity and the fall in hematocrit value, as protein consumption increased, could not reasonably be ascribed to chance.

The hypothesis that the changes in specific gravity and hematocrit values are causally related takes it for granted that the rise in specific gravity was due to an increase in protein concentration. This is, perhaps, a doubtful assumption, particularly when protein consumption is increased, for the observed degree of change in specific gravity might conceivably arise from changes in other constituents of the plasma than protein. Direct plasma protein measurements during a reiteration of the experiment would have answered the question. But since, for various reasons, this repetition was not feasible, we had resource to a similar experiment on rats. It was found that the protein concentration of the rats' serum rose, much as the plasma specific gravity rose in the human experiments. However in the rats there was no corresponding fall in the hematocrit readings. Thus the suggested explanation of the

* This work was aided by a grant from the Nutrition Foundation, Inc.

inverse relation between the specific gravity and hematocrit measurements in man is left as an, at best, unconfirmed hypothesis. This explanation became still less plausible when it was found that both serum protein concentration and packed cell volume may rise when the food contains no protein. The facts, however, remain and have, in themselves their own limited and empirical value[1].

Conditions and Methods

The first observations were made in 1940 when 10 residents and internes collaborated with us in trying to answer a question relating to renal function. During 3 successive weeks they took 0.5, 1.5, and 2.5 gm. of protein per day per kilo of body weight. The food in which these quantities of protein were given was calorically adequate. There was no limitation of water or salt consumption. During each week the diets were taken from Monday to Friday inclusive, while on Saturdays and Sundays no restrictions of any sort were imposed.

In 1940 we were beginning, in our out-patient clinical work, to use an approximate and rapid hematocrit method based on centrifuging blood at a constant speed for a constant time. We needed, from normal individuals, a measure of the variability of the measurements obtained with this method, and we used the 60 samples of blood obtained from our subjects for this purpose. The collections were made at 11:45 a.m. on the 4th and 5th days of each of the three diets. The blood was drawn into a vaselined syringe. Six ml. of well mixed blood was transferred to a cylindrical graduated tube containing 12 mg. of dry potassium oxalate. After inversion to ensure solution of the oxalate, the tube was centrifuged for exactly 5 minutes in a centrifuge driven by an induction motor that ran at 3500 R.P.M. The percentage of the total volume occupied by red cells was taken as the hematocrit value. For the specific gravity measurements we used Kagan's method (1) which is an adaptation of the falling drop technique introduced by Barbour and Hamilton (2).

The second series of observations was made in 1945, on a group of 10 medical students (9 men and 1 girl) under the same dietary conditions. After an interval of 2 months, the results were checked by repeating the observations on the 0.5 and the 2.5 gm. protein per kilo body weight diets. The uniformity of the increase in urea excretion as the protein consumption rose indicated that there was no significant difference between the dietary conditions to which the 1940 and the 1945 groups were subjected. The observations were made at different times than in 1940, namely on the 5th day of each diet, and 4 collections of blood were taken at 7:15 a.m., 11:45 a.m., and 4:45 p.m., just before breakfast, lunch, and dinner; and at 9:45 p.m., just before going to bed.

The standard Wintrobe method (3) was used in 1945 with the hematocrit, and for the specific gravity the copper sulfate method introduced by Phillips and his associates (4). In the subsequent experiments on the rats, the hematocrit determinations were made on the first blood obtained by severing the abdominal aorta. Potassium oxalate, 2 mg. per 1 ml. of blood, was used as an anticoagulant, but, thereafter, Wintrobe's technique was followed. The protein concentration of the serum was measured by a rather tedious and time-consuming gravimetric method, the details of which have been summarized by Barrett in a paper on another procedure (5). This is the method we used in determining the significance of the colors developed in the biuret measurements employed in the experiments we report on the effect of very low protein diets in man. In a considerable experience over many years with

[1] The work was divided so that one author (T. A.) measured the specific gravity and hematocrit values, another (H. G.) was responsible for the statistical analysis, while the third (E. B.) made the gravimetric and biuret determinations of the protein content of the serum or plasma.

many devices for the determination of protein, we have been forced to the conclusion that the ancient and laborious gravimetric method is still the most reliable and the only one we can depend on as a standard.

RESULTS

The 59 specific gravity and hematocrit measurements made in 1940 are too small in number for an adequate analysis of the various factors involved, and this precludes a complete comparison with the 1945 data, wherein we have 189 specific gravity and 198 hematocrit determinations. In any case, however, the existence of a pronounced diurnal variation restricts us to a comparison of observations made at the same time of day. Thus only the data obtained at 11:45 a.m. can be compared, and of these only those obtained on the 5th day of the diet are strictly comparable.

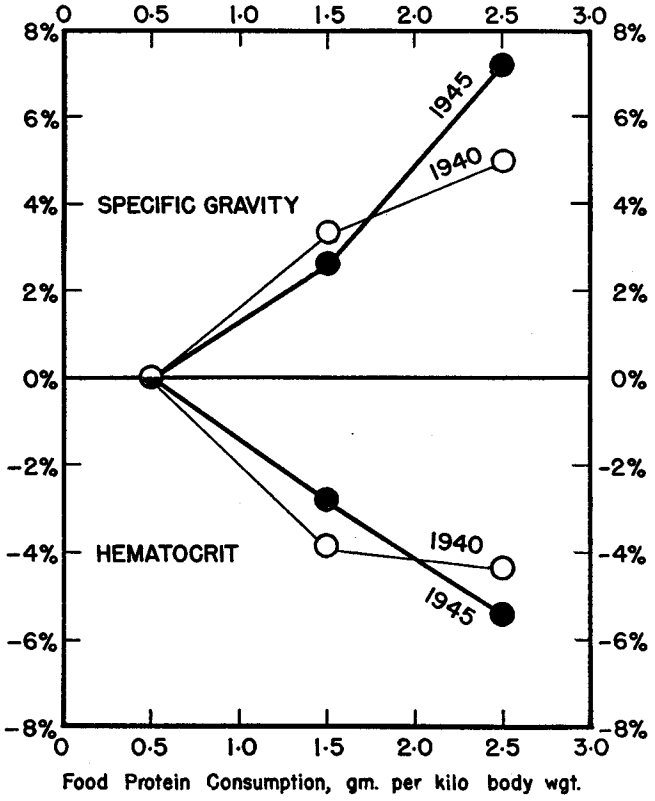

FIG. 1. Comparison of the relative changes in specific gravity observed at 11:45 a.m. on the 5th day of each diet in 1940 and in 1945. Expressed as percentage changes from the means on the 0.5 gm. protein per kilo body weight diets.

Fig. 1 compares these few data from 1940 and 1945 expressed in percentage changes from their mean values on the 0.5 gm. diet. In both experiments,

though the methods were different and the subjects were not the same, the specific gravity rises and the hematocrit falls as the protein intake is increased.

TABLE I

Mean Specific Gravity and Hematocrit Values

1940 experiment		
Protein intake	Specific gravity (Kagan) as protein	Hematocrit value 5 min. at 3500 R.P.M.
gm. per kilo body weight	*gm. per 100 cc.*	*per cent*
0.5	6.8	50.0
1.5	6.9	47.9
2.5	7.2	47.1
1945 experiment		
Protein intake	Specific gravity ($CuSO_4$) as protein	Hematocrit value 35 min. at over 3000 R.P.M.
gm. per kilo body weight	*gm. per 100 cc.*	*per cent*
0.5	7.4	46.6
1.5	7.6	45.5
2.5	8.0	43.6

In Table I, we give the means of all the observations made in 1940 and 1945. The absolute values in the two experiments are not comparable because of time relation differences and because the hematocrit specimens in 1940 were not centrifuged to complete packing. Relatively however, the change in both cases is similar, for as the protein intake is increased the specific gravity rises and the hematocrit value falls.

Interpretation of the Results According to the Analysis of Variance

The data given in the first part of this paper have been tested by the method known as the analysis of variance. Apart from the intrinsic value of the conclusions thus validated, it may be hoped that other clinical investigators, whose results are often obtained under conditions similar to those observed in the present experiments, may find a use for this method in the treatment of their own data. The analysis is presented as an illustration of the fact that more information as to the meaning of a series of measurements can be obtained from the analysis of variance than is possible by the use of the older methods that are still almost exclusively used in the treatment of the results obtained in clinical investigation.

The idea underlying the continued use of the older methods may be described as the "crucial experiment" doctrine. For long this has been insisted on as the invariable scientific approach. It purports to test putative causes

one at a time, on the assumption that laboratory workers can control all other factors than the hypothetical one with assurance, even with certainty. Historically, this notion derives from the 19th century, when it was thought proper to extend the assumptions underlying work in Newtonian physics to cover the treatment of biological measurements.

This doctrine, so long held an indisputable though difficult ideal, has in time been undermined by two forces. The first is the frequency of controversy when a reported crucial experiment is repeated under supposedly identical conditions but a different result is obtained. Each expert is then inclined to believe that he controlled the essential factors and even to assert that the other failed to do so. This attitude involves the assumption of a single factor law of cause and effect, although this visionary simplicity is non-existent. On the contrary, biological problems even more than physical, involve multiple factors which are much less easy to control or even exclude than is supposed by crucial experimenters. For many years it has been evident that the crucial experiment is an ideal trouble-maker, though no other principle of experimental approach has received general acceptance in clinical experiments. The crux still exerts its dead hand.

The second undermining force is the variability of nearly all human factors, even under standardized conditions. Of recent years, this difficulty has been solved for many situations by the work of mathematicians, for they have increasingly simplified and extended the rules of probability to small samples, say 30 repetitions of an experiment, or even less. So in biological and medical papers the age-old average and range of variation are now supplemented by some measure of variability. This has permitted precise statements as to whether the difference between observed means was attributable to chance or was significant. All this has led to a weakening of the doctrine of the crucial experiment.

The further advance with which we want to deal here, permits a working hypothesis that a postulated factor is primary, but it provides evidence which will confirm that postulate or alternatively deny it in favor of some other factor in the original hypothesis, visualized as secondary or possibly one hardly considered. Furthermore the method affords evidence as to the degree to which the factor which proves primary is not only accompanied but itself influenced by other factors.

Fisher's analysis of variance, designed originally for the simultaneous testing of multiple factors during experiments in agriculture, is sometimes appropriate to experiments in man. This method involves the splitting of the total variability and the assignment of the several fractions to the factors responsible for their production.

The classical exposition is found in Fisher (6) and useful practical guides to the computations and interpretations in Snedecor (7). Here it is impractical

to do more than give a concrete illustration of the use of the method in the analysis of the data given in this paper.

The working hypothesis in our experiment was the supposition that in normal individuals a change in protein consumption might be followed by a change in specific gravity and hematocrit measurements. As far as averages can go, this hypothesis was supported (Table I). In 1940, as in 1945, an increase in protein consumption was associated with an increase in specific gravity and a decrease in hematocrit readings, in different subjects and in measurements made by different methods. Furthermore, in both experiments the use of the conventional methods indicates that the changes are "significant;" that is, that there is only a remote possibility that they were a consequence of chance variation.

It would be an error, however, to assume that the variation in protein intake was the only factor involved. The results are influenced significantly in both 1940 and 1945 by variation amongst the subjects of the experiments, and in the 1945 experiment by the time of day at which the measurements were made. In addition it is shown that in both experiments the measurements were altered by a synergistic combination of the effect of the protein intake and the effect of the individual differences amongst the subjects. This is known technically as an "interaction." It is indicated by the fact that the protein variation was followed by a greater effect in some subjects than in others. These interactions are multiple. Thus for the 1945 experiment we have estimated the effect of the interactions between the protein and the times at which the observations were made, and between the protein and the subject, and have measured the effect of a triple interaction between the protein, the subject, and the time. That, in addition to these reactions, other factors than those we have examined were operative is certain. These, however, will suffice to indicate the scope of the method.

Tabulations of the condensed analysis of variance are put on record for those interested in such documentation, in Tables II and III. With reference to Table II it may be noted that discrepance = subclass discrepance, or between days within subjects, or, in less technical language, between replications on each patient on different days. This discrepance is the residual error which we have been unable to analyze into smaller parts; it is therefore to be taken as the basic unavoidable variability or error. With it we compare the variability due to each factor stated; for example, protein intake. The variability is examined in terms of the mean square, and most conveniently by the F ratio; *i.e.*, the MS to be tested, divided by the error-MS. In this example, we have $46.5/2.3 = 20$, and entering Snedecor's table with $F = 20$, and with the proper degrees of freedom here 2 and 30, we read the probability; it turns out less than 0.01; *i.e.*, the 1 per cent level: this means that the chance of finding a variability (mean square) as large as here, would be less than one in a hundred repetitions of this experiment; a chance so small that it is customary to ignore it and say the result is significant.

With reference to Table III, the computations revealed the operation of several 2-factor interactions. This indicated the necessity of tabulating the complete analysis, instead of

reporting only the simple form which covered the fractions of variance found significant in the 1940 experiment. Then the non-significance of the interaction Times × Subjects and of Proteins × Subjects × Times, warranted the combination of these (lines 6 and 7) into a pooled error variance (line 8). Finally this last item was used as the basis of reference to demonstrate the significance of items 1 to 5, as already stated in general terms. The computations to demonstrate significance, by the F test, are not reproduced; the method is the same as illustrated for Table IV; here the protein intake variability is tested; 28.3/0.63 = 45; and entering Snedecor's table, we again find this value statistically significant.

What proportion of the total variability is attributable to each of these three main factors? This simplification is not customary, but as a rough estimate the following items in the tables may be pointed to. In the 1945 experiment, for the hematocrit readings, the subjects contributed by far the largest proportion of the total sum of squares: 1581/1924 = 82 per cent, the time factor contributed 181/1924 = 9 per cent, but the protein level contributed only 57/ 1924 = 3 per cent; with minor contributions by the various interactions.

Similarly in 1945, for specific gravity, the subjects contributed 10.25/21.03 = 49 per cent of the total sums of squares, the protein level contributed 2.70/21.03 = 13 per cent, and the time element contributed 1.21/21.03 = 6 per cent; with minor contributions by the interactions.

The subjects made much the most notable contributions; but for the other two factors the order was not the same. Hematocrit readings seemed influenced by time factor more than by protein intake, whereas for specific gravity the protein was the more important factor.

The essence of the analysis distils out as follows:—

Subject Factor.—This was markedly significant by the statistical test, and in general was the most potent factor.

Protein Factor.—This was also markedly significant.

Time Factor.—This in the 1940 experiment was days, *i.e.* blood samples on 2 successive days; it caused no variability, either in hematocrit readings or specific gravity; and therefore the calculations are not reproduced in Table II. On the other hand, time in the 1945 experiment was hours, *i.e.* samples at 4

TABLE II

1940 Experiment

Source of variation	Degrees of freedom, *df*	Hematocrit value		Specific gravity	
		Sum of squares, *SS*	Mean square, *MS*	Sum of squares, *SS*	Mean square, *MS*
Protein, *P*	2	93.1	46.5	1.289	0.645
Subjects, *S*	9	549.8	61.1	1.140	0.127
Interaction *P* × *S*	18	65.7	3.6	0.524	0.029
Discrepance, for error	30	69.3	2.3	1.240	0.041
Total	59	777.9		4.193	

different hours on the same day; this factor was a real source of variability and therefore the analysis is shown in the appropriate detail in Table III.

TABLE III

1945 Experiment

Analysis of Variance

Line	Source of variation	Degrees of freedom, *df*	Hematocrit value		Specific gravity	
			Sum of squares, *SS*	Mean square, *MS*	Sum of squares, *SS*	Mean square, *MS*
	Main effects:					
1	Protein, *P*	2	56.6	28.3	2.70	1.349
2	Subjects, *S*	9	1581.2	175.7	10.25	1.139
3	Time, *T*	3	181.0	60.3	1.21	0.403
	2-Factor interactions:					
4	$P \times T$	6	32.8	5.46	0.93	0.155
5	$P \times S$	18	21.8	1.21	2.23	0.124
6	$T \times S$	27	22.1	0.82	1.36	0.050
	Triple interaction:					
7	*P S T*	54	29.0	0.54	2.36	0.044
8	Pool 6–7, for error	81	51.0	0.63	3.71	0.046
9	Total	119	1924.5		21.03	

Does the Protein Concentration as Well as the Specific Gravity of the Plasma Increase When Protein Consumption Increases?

By virtue of the analysis of variance it is possible to derive from the data many well founded conclusions, of which the most important for our immediate purpose are the rise in specific gravity and the fall in hematocrit value. Why may we not suppose than an increase in plasma protein concentration is followed by an increase in plasma water, not enough to prevent some rise in protein concentration but sufficient to account for the fall in the hematocrit value? The advantage would be that we could then view both of these changes as a consequence of a single underlying metabolic alteration such as might well follow an increase in protein consumption.

The difficulty is that the statistical analysis supports only one of the two facts that the hypothesis purports to explain. We now know that an increase in protein intake is associated with a decrease in the hematocrit value, but it is not proved that there is any increase in plasma protein concentration. What we measured was specific gravity. Unquestionably that rises as the hematocrit value falls. But when, following clinical usage, we expressed these specific gravity changes in terms of protein, we were making an assumption

which may not be valid under the conditions of our experiments. It is this assumption that warrants a brief discussion.

We are not concerned here with the general question as to whether, for most clinical purposes, it may or may not be justifiable to predict plasma protein concentration from specific gravity measurements. We think that question has already been answered in the affirmative by the careful work of many investigators between 1930 (8) and the present day.

What we should most of all like to know is whether the increase in the specific gravity in our subjects can be accounted for by a parallel increase in protein. That knowledge we cannot get because we did not measure the protein while the experiments were under way, and various circumstances prevent us from reduplicating the conditions we once had. All that can be said now is that there are two general considerations which make us hesitate to assume a parallelism between specific gravity and protein concentration under the particular circumstances of our experiments. First, the rise of specific gravity acquires most of its statistical significance from the number of observations from which the averages were derived. The absolute change in specific gravity as we pass from 0.5 to 2.5 gm. of protein is represented in terms of protein by a rise from 6.8 to 7.2 gm. protein per 100 cc. in 1940, and from 7.4 to 8.0 gm. protein per 100 cc. in 1945. Now the general relation between specific gravity and protein amount that we find so useful in clinical work, where we rarely attempt to draw inferences unless the differences from our normal standards are larger than those we are here considering, depends mainly on the fact that the electrolyte concentration in the plasma remains relatively constant under a wide variety of conditions. Major changes in specific gravity can thus be ascribed and have, in fact, been shown to be due, in the main, to shifts in protein concentration. However in this particular experiment, we have no assurance that the non-protein organic constituents remain as constant as the inorganic. Thus, both in 1940 and in 1945, we found that the serum urea concentration more than doubled as we went from 0.5 to 2.5 gm. of protein per kilo (9) and though, in itself, this could have had no appreciable effect on our measurements, it is possible that the summation of parallel increases in other organic constituents may have been a factor in the rise of specific gravity as the protein intake was increased. Second, plasma is a mixture of substances, and we cannot, as yet, assign to each substance its part in the production of the specific gravity we measure. For that we should need a knowledge of the concentrations of all of the substances in the plasma. Each has its own specific effect and while, for most of them, an increase in their concentration will increase the specific gravity, there are others, fats for instance, that decrease specific gravity as they accumulate in the plasma. Thus a decrease in fat concentration as the protein intake rose is at least a possible reason for the observed rise in specific gravity.

Since we could not reiterate our experiments on human subjects, we had resource to a parallel experiment on rats.

We have compared the protein concentration predictions from specific gravities as determined with the copper sulfate method with actual protein measurements by the gravimetric method. The variation in protein consumption was obtained by replacing corn starch with casein so that we got three diets with the same caloric, vitamin, and mineral content, in the first of which there was no casein, in the second 17 per cent, and in the third 74 per cent of the total food weight.

There were 10 rats on each of these three diets, 5 male and 5 female, chosen so that they were of similar weight (200 gm. for the male and 180 gm. for the female), all selected from a colony living on a stock diet that contained 17 per cent of protein. The blood was obtained by serving the abdominal aorta under ether anesthesia. The observations were made 48 hours after the diets were given because we have found that at this time on these diets the major changes had occurred in the total and organ protein content of the animals (10). The results are given in Table IV.

TABLE IV

Protein consumption	Protein concentration in serum		
	Gravimetric	Biuret	Specific gravity ($CuSO_4$)
	gm. per 100 cc.	*gm. per 100 cc.*	*gm. per 100 cc.*
Low (1 per cent protein in diet)	5.72	5.91	5.58
Medium (16 per cent protein in diet)	6.23	6.07	5.76
High (74 per cent protein in diet)	6.57	6.25	5.94

The results given in Table IV give us some reason to suppose that the specific gravity changes we observe in man when the consumption of protein is increased may be a consequence of changes in protein concentration and that they were probably not due to alterations in other constituents of the plasma. Certainly, in the rat, increase in protein consumption is followed by an increase in serum protein concentration and the direction of the change is given by specific gravity measurements.

The Mechanism of the Relation between Protein Consumption and Serum Concentration

In the experiment whose results are given in Table IV we have an example of the fact that an increase in protein consumption is associated with an increase in serum protein concentration. But this same experiment denies our original supposition that there should be a concomitant fall in the packed cell volume. The averages of closely agreeing values were 41.7 for the low, 41.5 for the moderate, and 42.5 per cent for the high protein intake. An explanation that was plausible for man is found to be inapplicable to the rat and is thus shown to be devoid of any general physiological significance. There

remains the question as to why the serum protein concentration rises and falls with change in protein consumption.

The question as to why the serum protein concentration falls when the protein consumption is sharply reduced to levels that are inadequate for nutrition has been investigated by Bloomfield (11). Using the gravimetric method, and working with large numbers of rats, he showed that all of the decrease in serum protein concentration occurred within 48 hours of the change in diet. Thereafter, over very long periods of time, there was no further significant decrease in concentration, in spite of a pronounced and continuing loss of body weight. After 147 days on the low protein diet, the serum protein concentration promptly returned to its original level when an adequate amount of protein was given. It is reasonable to assume that continued observation would have shown that it stayed thereafter at about that level. Certainly it is a fact we could support by many observations, that, after the initial increase, there is no further change in concentration, no matter how long we go on feeding adequate or even excessive amounts of protein.

Bloomfield noticed that the rapid shifts in total concentration levels associated with pronounced changes in protein consumption were not due to a change in all of the proteins of the plasma. They could be accounted for by change in albumin alone, an observation which, in general, is in consonance with the original work of Govaerts (12). In theory, this reduces the problem of the mechanism of the food protein effect to an inquiry into the reasons for change in only one of the components of the plasma protein complex. In practice, however, we have not been able to take advantage of this simplification because we could not satisfy ourselves as to the reliability of our serum albumin measurements, and thus have had to be content with total protein determinations.

In experiments which we shall publish later, we have found that serum albumins given parenterally to rats are digested. Part of the injected albumin is used for anabolic purposes and part of its nitrogen is excreted as urea. This circumstance makes it worth while to study the time relations between the change in serum protein concentration and the change in protein metabolism that occur when the protein content of the diet is suddenly reduced to very low levels. If they occur together we shall have some reason to suspect that the serum albumin shift may be one of a series of interrelated events that accompany any pronounced reorganization in the relative magnitude of the rates of protein anabolism and catabolism.

We therefore studied the time relation between the decrease in protein metabolism that follows a change from a high to a low protein diet and the decrease in serum protein concentration which we quite confidently expected would follow the diet change. Four medical students were asked to make a sudden and pronounced alteration in their protein consumption. On Monday

and Tuesday they ate weighed diets that contained 2.5 gm. of protein per kilo body weight. On Wednesday and on Thursday up to 12 noon they were given only 10 gm. of protein a day with adequate calories. For their body weight this approximated a consumption of 0.13 gm. protein per kilo. Throughout Tuesday and Wednesday, and during the forenoon of Thursday, they collected urine at 5 hourly intervals during the day, between 7 a.m. and 10 p.m., with night collections between 10 p.m. and 7 a.m. In each individual the serum protein concentration was determined by the biuret method and the average results were confirmed by gravimetric determinations on pooled sera. In Table V the average results are given.

TABLE V

Failure of Any Significant Change in Serum Protein Concentration to Occur during the First 29 Hours after Change from a High to an Inadequate Protein Consumption. Averages from Four Subjects

Day	Protein consumption	Serum protein concentration			
		7:15 a.m.	11:45 a.m.	4:45 p.m.	9:45 p.m.
	gm. per kg.	*gm. per 100 cc.*	*gm. per 100 cc.*	*gm. per 100 cc.*	*gm. per 100 cc.*
Tuesday	2.5	8.45	8.23	8.65	8.42
Wednesday	0.1	8.37	8.49	8.75	8.74
Thursday	0.1	8.50	8.77		

It is apparent that there was no decrease in concentration during the first 29 hours following a change from high to a very low protein consumption. The hematocrit values also remained unaltered. Yet during this period the rate of urea excretion fell from 46.6 gm. per 24 hours on Tuesday, when they were on the high protein diet, to 17.5 gm. per 24 hours during the last collection on the inadequate protein ration. Over this time, also, the serum urea concentration declined from 49.2 to 23.1 mg. per 100 cc. Thus during the 29 hours in which the major part of the metabolic readjustments reflected by the excretion and concentration of urea was accomplished, the serum protein concentration did not fall.

Although the results given in Table V show that a great reduction in protein catabolism may occur without any fall in serum protein concentration, we still thought that we had only misjudged the time at which the fall occurred and we supposed we should observe it if we extended the period of observation. But when another group of four students took for 5 days a diet that was theoretically adequate in all respects except that it contained no more than traces of protein, we got the results given in Table VI.

The measurements given in Table VI were obtained by the biuret method in serum drawn every morning at 9:30 a.m. Instead of a fall in concentration

we find a steady daily rise over the whole 5 day period. This was accompanied by a slight increase in the hematocrit values. When the same subjects took diets containing 5, 10, or 15 gm. of protein there was no fall in serum protein concentration but rather, on the average, a slight and dubious increase without any definite change in packed cell volume over 5 or 6 day periods. Only when no protein at all was given were the results consistent and definite. Here the conjunction of an increase in both serum protein concentration and hematocrit reading made it seem possible that both phenomena might be a result of a decrease in total plasma volume. Dr. J. Hopper of the University of California Medical School was good enough to test this hypothesis for us. Comparing the results at the end of the period of zero protein consumption with those he obtained when the subjects were on an unrestricted diet, he found

TABLE VI

Increase in Serum Protein Concentration during the 5 Days Following Change from Unrestricted Food Consumption to a Diet That Contained No Protein

Subject	Days on a diet with no protein				
	1st	2nd	3rd	4th	5th
	gm. per 100 cc.	*gm. per 100 cc.*	*gm. per 100 cc.*	*gm. per 100 cc.*	*gm. per 100 cc.*
F.	7.48	7.63	7.68	8.29	8.71
G.	7.78	7.48	8.14	8.03	8.05
C.	6.17	6.83	7.08	7.55	8.31
L.	6.73	6.91	7.74	7.65	8.07
Averages.....	7.04	7.21	7.66	7.88	8.28

no consistent differences with the CO and the Evans' blue methods, and concluded that there was no evidence that the diet had had any effect on either total blood or plasma volume.

On all these diets there was a steady loss of body weight in spite of what appeared to be a more than sufficient consumption of calories. The readjustment of protein metabolism had been completed before the 5th day. On the zero protein diet the minimum urea excretion of 7.17 gm. per 24 hours came on the 3rd day and the serum urea concentration fell to 13.3 mg. per 100 cc. But the question with which we started, the relation in time between the change in protein metabolism and the expected fall in serum protein concentration, remains, of course, unanswered because instead of a fall we found a rise in concentration which was still continuing after all the metabolic change we can measure by urea excretion had been accomplished.

DISCUSSION

The subsidiary experiments, which were planned to throw light on the mechanism of the protein effect, have served only to show that the inverse

changes in serum protein concentration and packed cell volume which were our starting point, are not necessarily related as cause and effect. They have also demonstrated that we cannot derive from our results any general rule about the effect of change in protein consumption. The divergent direction of movement in protein concentration and hematocrit reading was found only in man. This phenomenon is further restricted to the particular conditions that obtain when the variations in the amounts of protein consumed are within the zone of what is defined as adequate for maintenance. When inadequate amounts are given, quite different or even contradictory effects are found. For this reason we have not been able to learn anything about the mechanism of the changes by pushing the dietary alterations to their extreme limit. From a negative point of view, however, it is of interest that a maximal and rapid decrease in protein metabolism may be associated with an increase in serum protein concentration not due to diminution of plasma volume. Nevertheless we must admit that the methods we used have been shown to be entirely inadequate for the purpose of answering the questions we have raised. Nor is it to be expected that they might have been solved if we had measured blood and plasma volumes and given the results in terms of total circulating plasma protein and red cell mass. Certainly much more would have been learned, for it is inadmissible to assume that blood volume remains constant when protein consumption is changed. In the work of Metcoff, Favour, and Stare (13) volume measurements were made but they were still not able to reach an unequivocal definition of the mechanisms involved in the total changes they measured. The excellently controlled and precise study of the effects of prolonged undernutrition in man reported by Keys and his collaborators (14) reveals how complex are the relations between the body as a whole and the volume and concentration changes in plasma protein and red cells, and to what a high degree any real understanding of the significance of these changes is dependent on a quantitative knowledge of total body composition in respect of the proportions of water in the cells, in the interstitial tissues, and in the circulating blood. The difficulty in reaching definitive conclusions about protein effects seems to lie in the circumstance that changes in protein consumption induce change in the system as a whole, as well as in all the parts that compose it. We thus have no fixed, unchanging, common reference value in terms of which we can express our measurements in order to compare them and learn their relative and absolute meaning in relation to the whole organism. At least we now know that body weight or any function of body weight does not give us what we need. Even if, admitting our incapacity to draw precise general inferences, we restrict our attention to the circulating blood whose volume we can at least approximately measure and in which relatively precise determinations of the concentration of its constituents can be made, we still do not have the information required for a full comprehension of the mechanism

of the effects of change in protein consumption. For this circulating blood is not a closed system, isolated from the body as a whole. In many respects it is like a reservoir into which newly formed protein molecules and red cells are always running, and out of which protein molecules and red cells as continuously are passing as they are metabolized or phagocytosed. What we need, in addition to the volume and concentration, are simultaneous measurements of the total inflow and total outflow of protein and red cells.

From an empirical point of view, however, it is of interest that in man a change from a low to a moderate, and then to a fairly high protein consumption, should be associated with an increase in specific gravity and a decrease in the packed cell volume. This protein effect is one of the minor factors which contribute to the total variability of these measurements in normal individuals.

SUMMARY

When the protein consumption of normal human individuals is increased from 0.5, to 1.5, to 2.5 gm. of protein per kilo body weight, the specific gravity of the plasma rises and the hematocrit value falls.

The analysis of variance demonstrates that the change in protein consumption is a significant but minor factor in determining the total variability of the observations.

When albino rats were given diets containing a small, a moderate, and a large amount of protein, there was an increase in serum protein concentration but no change in hematocrit value.

During the period over which the most rapid changes in rate of urea excretion and serum urea concentration occurred as normal human individuals passed from a 2.5 to an 0.1 gm. of protein per kilo body weight consumption, there was no change in serum protein concentration.

Over a 5 day period during which a diet that was adequate in calories but almost wholly devoid of protein was taken, the serum protein concentration of normal individuals steadily rose. This was associated with a slight increase in hematocrit value but no change in blood or plasma volume.

The protein effect is one of the minor factors that contribute to the variability of serum protein and hematocrit measurements in normal individuals.

The general conclusion is reached that we shall have to measure the rate at which red cells and protein enter and leave the circulating blood stream before we can hope to comprehend the mechanism of the protein effect.

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