PLASMA PROTEIN TURNOVER AND TISSUE EXCHANGE

Influence of Dietary Protein and Protein Depletion*

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Chemical nitrogen balance studies leave much to be desired in supplying information about internal protein metabolism, relying, as they do, on net differences between intake and output. By isotopically labeling plasma and tissue proteins valuable information concerning the turnover, transport, and equilibria of these proteins under normal basic conditions has been obtained. The present studies deal with some of these aspects of internal metabolism under conditions of altered nitrogen balance.

The protein metabolic status of a group of normal dogs was modified by varying the protein content of the diet from 0 to 6 gm. of protein per kilogram of body weight per day. Carbon-14–labeled homologous plasma proteins were then injected intravenously. The daily plasma protein turnover rate in these animals was accelerated from 0.65 to 1.00 gm. per kilogram by increasing the oral protein intake from 0 to 2.0 gm. Further increases were not observed with oral protein increments up to 6.0 gm. per kilogram of body weight. Turnover of both albumin and globulins was increased and was associated with a greater exchange of C^{14} between plasma and tissue proteins and a greater excretion of C^{14} in the expired air.

In protein-depleted animals receiving a very low protein diet the rate of turnover as indicated by a shorter half-life, was also increased. However, owing to a marked reduction in the total plasma protein pool the amount metabolized per day was reduced.

In a small group of animals with both plasma and tissue proteins simultaneously labeled by feeding DL-lysine-6-C^{14} a significant recycling of C^{14} from

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tissue to plasma protein, even in the face of adequate oral nitrogen intake was indicated by a marked slowing of the apparent turnover rates.

In 1954 Steinbock and Tarver (6) reported an increasing turnover rate of total plasma protein in rats receiving increasing amounts of dietary protein. More recently Jeffay and Winzler (3) showed in the same species a relationship between the level of dietary protein and the rate of turnover of serum albumin but not of serum globulins. In

TABLE I

Summary of Intravenous C^{14}-Labeled Plasma Protein Experiments

Dog No.	Average weight	Experimental conditions	Protein intake	Average plasma protein	Albumin globulin/ ratio	Labeled plasma protein injection		
						Volume	Protein	C^{14} activity
	kg.		*gm./kg./day*	*gm./100 ml.*		*ml.*	*gm.*	*μc.*
51-171	7.4	Depletion 56 days	LPD*	4.6	0.35	80	4.0	1.6
54-36	8.0	" 53 "	"	4.3	0.54	125	5.2	1.1
52-132‡	9.3	" 36 "	"	4.3	0.54	142	6.7	1.3
53-154	9.3	" 26 "	"	4.4	0.83	133	7.3	1.3
50-37	8.9	Normal	LPD*	5.6	0.87	133	6.2	3.8
P. B.	9.1	"	"	6.6	0.61	144	6.9	2.4
53-243	7.2	"	"	6.0	0.94	122	7.5	1.4
54-313§	10.2	"	"	6.8	0.70	105	5.0	0.8
54-233	7.5	Normal	0.6	6.0	0.77	109	6.6	1.2
54-354§	9.0	"	1.0	6.1	0.74	127	8.6	1.2
54-224	14.3	"	1.5	5.7	0.78	118	6.5	1.4
51-135	8.0	"	2.0	6.3	0.62	100	5.6	1.1
55-1§	7.4	"	3.0	6.3	1.10	132	6.2	1.2
54-9	10.2	Normal	3.9	6.3	0.97	136	8.5	1.2
53-244	8.3	"	5.7	6.5	0.86	118	7.4	1.2
53-250	9.8	"	6.1	6.5	1.10	155	8.9	1.4

* Very low protein diet.

‡ Male dog.

§ Not killed.

both these studies S^{35}-labeled plasma proteins were used. Blahd *et al.* (1), using iodinated albumin in patients with the nephrotic syndrome, found that albumin turnover was rapid with high protein diets and low when dietary protein was reduced.

Methods

Labeled plasma proteins, obtained from donor dogs fed DL-lysine-6-C^{14}, were injected intravenously into 16 recipient dogs. Details of this procedure, sampling schedules, and methods dealing with the collection and analysis of blood, urine, expired CO_2, and tissues were essentially similar to those described in previous publications (5, 10, 11).

The protein content of the diet was varied by substituting weighed amounts of cooked pig liver for calorically equivalent portions of the very low protein diet. This contained su-

crose, lard, bone ash, salts, and vitamins (8), and the protein additions supplied from 3 to 30 per cent of the total calories.

EXPERIMENTAL OBSERVATIONS

Table I summarizes the experimental conditions prevailing during the 7 day period after C^{14} labeling in the 16 dogs given tagged plasma proteins intravenously.

TABLE II

Excretion of Nitrogen and Carbon-14

Dog No.	Protein intake	Net nitrogen balance average	Urine C^{14}		Expired $C^{14}O_2$	
			Total per cent dose excreted	Plasma protein equivalent	Total per cent dose excreted	Plasma protein equivalent
	gm./kg./day	*gm./kg./day*		*gm./kg./day*		*gm./kg./day*
51-171	LPD and depletion	−0.11	2.1	0.01	10.6	0.08
54-36	" " "	−0.10	4.2	0.04	9.4	0.08
52-132	" " "	−0.07	0.3	0.01	5.9	0.04
53-154	" " "	−0.08	0.4	0.01	13.4	0.10
50-37	LPD	−0.17	2.5	0.02	7.8	0.10
P. B.	"	−0.15	0.9	0.01	8.3	0.10
53-243	"	−0.18	2.3	0.03	16.6	0.25
54-313	"	−0.17	2.4	0.03	11.5	0.14
54-233	0.6	−0.03	1.4	0.01	21.6	0.20
54-354	1.0	0	0.9	0.01	11.3	0.13
54-224	1.5	0	0.8	0.01	11.3	0.14
51-135	2.0	0	1.7	0.02	14.5	0.20
55-1	3.0	+0.02	1.3	0.02	19.5	0.30
54-9	3.9	+0.03	2.6	0.04	20.4	0.26
53-244	5.7	+0.12	3.7	0.04	25.2	0.45
53-250	6.1	+0.09	1.6	0.03	23.9	0.40

With the exception of 1 male (dog 52-132) in the depleted group, all the experimental animals were adult females, in a state of apparent good health and nutrition. In all but 3 instances, as indicated in Table I, the experiments were terminated by viviperfusion of the animals under light ether anesthesia with complete autopsy and tissue C^{14} analysis.

All animals received approximately 100 calories per kilogram per day in the form of the basic protein free diet (LPD) alone or with various amounts of protein substituted as indicated. Except in the depleted group (see accompanying paper for details) (12), the special diet was fed for a period of 4 days prior to labeled plasma injection during which time the urinary nitrogen output became relatively stable.

Total plasma protein levels and albumin/globulin ratios remained relatively constant and little change in weight was noted during the 11 day experimental periods. Maximum loss was 400 gm. by 2 dogs receiving the very low protein diet while a maximum gain of 400 gm. was observed in dog 53-244 which

received 5.7 gm. of protein per kilogram of body weight per day. Food consumption was 100 per cent in each instance.

Injection of rather large amounts of donor plasma protein were necessitated by the relatively low specific activity obtainable and a high dilution factor. As pointed out by Wasserman and Mayerson (9), injection of these quantities will result in more rapid equilibration between intra- and extravascular fluid proteins than after injections of trace amounts. However metabolic turnover of labeled protein would not be affected by the size of increments to the pool.

Fig. A graphically illustrates, by means of composite curves, the more rapid turnover of plasma proteins in animals fed 2 or more gm. of protein per kilogram of body weight per day compared to those on a protein-free diet. The dietary protein additions did not alter the early equilibration phase of the curves but accelerated the slower metabolic portion. Turnover rate of albumin increased by 49 per cent, that of combined globulins 38 per cent.

Data pertaining to the excretion of nitrogen and C^{14} are given in Table II. Net nitrogen balance figures (column 3) are expressed as grams per kilogram of body weight per day. Corrections were made for the injected protein, and fecal nitrogen excretion was estimated to be 0.2 gm. per day.

The depleted dogs lost about half as much nitrogen as those on the low protein diet despite an identical nitrogen intake averaging a total of 0.3 gm. per day. The animals receiving protein supplements of from 0.6 to 3.9 gm. per kilogram remained essentially in nitrogen balance throughout the experimental period and total urinary nitrogen output did not rise until the 1.5 gm. protein intake level was reached. Significant though slight nitrogen retention was observed only in the last two dogs. These were fed the largest amounts of protein and each showed some weight gain.

Little loss of C^{14} in the urine following intravenous labeled plasma protein was observed. The major variations in total urinary C^{14} excretions were related to unexplained differences in output during the first 24 hours.

Loss of $C^{14}O_2$ in the expired air was low in the depleted group and showed some tendency to parallel protein intake in the other animals, with major discrepancies again being related to variations in the first 24 hour output.

If the amounts of C^{14} eliminated daily in both urine and expired CO_2 are equated to the average C^{14} activity per gram of plasma protein on the same day, an estimate of the amount of plasma protein degradation needed to supply the C^{14} excreted will be obtained. Such plasma protein equivalents, expressed as grams of plasma protein per kilogram of body weight per day, are shown for urinary C^{14} in column 5 and for $C^{14}O_2$ in column 7 of Table II. Values for urine C^{14} are not significantly altered by the various experimental modifications while those for $C^{14}O_2$ tend to be lowest in the depleted animals and highest in those receiving protein in excess of 1.5 gm. per kilogram.

Table III lists, for each experiment, the half-life of the total plasma protein

as well as of the albumin and globulin fractions. The size of the total plasma protein pool, expressed as grams of protein per kilogram of body weight is also indicated. Values for pool size are derived by extrapolating the metabolic portions of the plasma disappearance curves back to zero time, as illustrated in the composite graphs of Fig. A.

Using the group on the essentially protein-free diet (LPD) as the basis of comparison it is seen that prolonged protein depletion shortens the half-life

TABLE III

Plasma Protein Half-Life and Total Pool

Dog No.	Protein intake	Half-life — t½ days			Total plasma protein pool
		Plasma protein	Albumin	Globulin	
	gm./kg./day				*gm./kg.*
51-171	LPD and depletion	5.2			3.00
51-36	" " "	5.4			3.66
52-132	" " "	6.4	(8.0	4.8)*	3.10
53-154	" " "	6.4			2.64
50-37	LPD				6.10
P. B.	"	(7.8	12.5	6.0)‡	7.35
53-243	"				7.60
54-313	"				6.45
54-233	0.6	6.5	6.6	3.1	5.85
54-354	1.0	5.9	—	—	5.38
54-224	1.5	5.2	7.5	4.0	4.40
51-135	2.0	5.0	6.7	4.2	5.85
55-1	3.0	4.6	6.7	3.2	7.26
54-9	3.9	4.2	6.2	3.3	6.12
53-244	5.7	5.1	5.4	4.4	7.30
53-250	6.1	5.3	7.0	3.4	6.45

* Average for the group of four depleted dogs, low protein diet.

‡ Average for the group of four normal dogs, low protein diet.

and markedly lowers the level of total protein in the pool. The presence of protein in the diet also shortens the half-life to a fairly level plateau averaging 4.8 days, with supplements above 2 gm. per kilogram of body weight per day but does not significantly alter the size of the protein pool.

Plasma protein turnover rates may be obtained either graphically from the slow portion of the isotope disappearance curves or computed mathematically (7). Values expressed as a percentage of the total pool and as grams metabolized per kilogram of body weight per day are shown in Table IV. The smallest amounts of protein metabolized per day, in the depleted group, are a reflection of reduced pool size whereas the increased metabolism seen with high protein feeding is due largely to a shorter half-life.

If the sum of the plasma protein equivalents of C^{14} lost daily in urine and expired air (Table III) is subtracted from the total grams of plasma protein metabolized per day the difference must equal the net plasma protein equivalent of C^{14} transferred to tissues. Table IV lists the equivalent amounts of plasma protein involved in such transfer, both as per cent of total pool and as grams per kilogram per day.

TABLE IV

Plasma Protein Turnover and Tissue Exchange

Dog No.	Protein intake	Turnover rate		C^{14} transferred to tissue		
		Per cent plasma protein pool per day	Plasma protein metabolized	Per cent plasma protein pool per day	Plasma protein equivalent	Per cent total dose—7 days
	gm./kg./day		*gm./kg./day*		*gm./kg./day*	
51-171	LPD and depletion	13.6	0.49	13.3	0.40	48.9
54-36	" " "	12.8	0.57	12.3	0.45	47.6
52-132*	" " "	11.0	0.35	9.7	0.30	49.2
51-154	" " "	11.2	0.41	11.4	0.30	41.1
50-37	LPD 0.03 ±	8.8	0.62	8.2	0.50	38.8
P. B. (4 days)	" "	8.8	0.63	6.8	0.50	23.2
53-243	" "	8.9	0.73	5.9	0.45	29.3
54-313	" "	9.6	0.68	7.9	0.51	38.6
54-233	0.6	10.7	0.71	8.5	0.50	33.2
54-354	1.0	11.5	0.65	9.2	0.50	46.1
54-224	1.5	13.3	0.59	9.3	0.41	41.6
51-135	2.0	13.8	0.92	12.0	0.70	46.2
55-1	3.0	15.1	1.10	10.7	0.78	46.8
54-9	3.9	16.5	1.00	11.5	0.70	48.2
53-244	5.7	13.7	1.10	8.4	0.61	36.2
53-250	6.1	13.2	1.04	9.4	0.61	38.2

* Male.

Also shown are values for the total per cent of injected C^{14} converted from plasma to tissue protein. The figures in this column were derived by correctng measured C^{14} in all organs and tissues for estimated C^{14} in residual plasma and lymph protein and correspond closely to values obtained by calculation from data pertaining to excretion and loss from the plasma protein pool.

Relative individual tissue specific activities were found to be essentially similar to those previously observed after intravenous injection or feeding of labeled plasma or lysine (10, 13). Specific activities of all tissues were highest in the depleted animals and lowest in those receiving high protein diets.

Tissue protein-specific activities based on nitrogen and C^{14} determination

before and after extraction with trichloracetic acid and acetone were essentially similar. This indicated that, at most, insignificant quantities of C^{14}, from the lysine-labeled plasma proteins originally injected, were transferred to fat or carbohydrate within the experimental period of 7 days. Hemoglobin-specific C^{14} activity at the end of each experiment was determined and found to be approximately equal to the average activity of all tissue proteins. The values

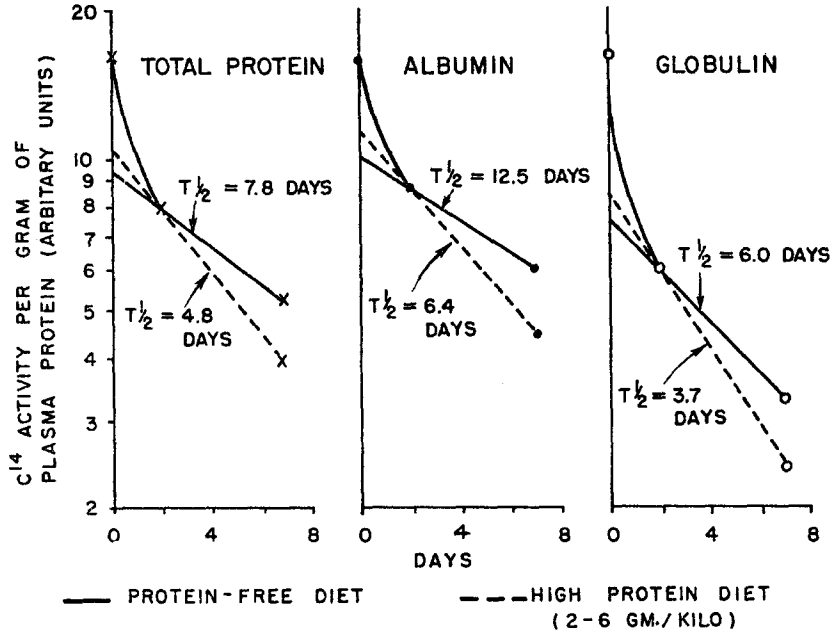

FIG. A. Composite curves illustrating the disappearance rates of C^{14} activity from plasma total protein, albumin, and globulin in dogs fed 2 to 6 gm. of dietary protein per kilogram daily and dogs on a very low protein diet. Initial rapid fall is similar in both groups but the longer half-life of all proteins due to the essentially non-protein diet is apparent.

were roughly one-tenth those of the corresponding plasma proteins at the same time.

There was no indication in these experiments of reincorporation into plasma protein of isotope previously transferred to tissues. Because of the high dilution factor involved, direct observations for periods greater than 7 days after intravenous injection of C^{14}-labeled plasma proteins were generally not feasible. However, in one animal (dog 53-243), receiving the non-protein diet, the experiment was continued for 2 weeks and during the 2nd week the turnover rate of total plasma protein remained unchanged. Excretion of C^{14} in urine and expired air continued to fall until the 14th day and total tissue activity reached a level higher than the 7 day average. These findings suggest that in the animal de-

prived of protein by mouth and given labeled protein intravenously, the amount of tissue C^{14}, if any, returned to plasma protein in a 14 day period is too small to be detected. After this time interval most of the C^{14} is found in low activity areas such as muscle and skin while the specific activity of even the highest tissue is many times lower than that of the plasma proteins (10). From the data presented by Goldworthy and Volwiler (2) it would appear that re-utilization of isotope following intravenous injection of labeled plasma into dogs receiving an adequate diet does not become significant until about the 4th week.

In animals receiving the isotope by mouth in the form of C^{14}-labeled lysine on the other hand, some tissue proteins such as liver have initial specific activities equal to or greater than the plasma proteins, and total tissue activity may be 5 to 10 times greater than the amount in blood and interstitial fluid proteins (13).

Four dogs in this category, previously fed relatively large amounts of DL-lysine-6-C^{14} and used as donors of labeled plasma protein, provided an opportunity to compare the half-lives of plasma protein in animals having highly labeled tissues with those already described in which the tissues were initially unlabeled. The ex-donor dogs had received C^{14}-labeled lysine in various amounts, according to various time schedules, and had been subjected to multiple bleedings. Each was permitted to stabilize on an adequate diet for periods up to several months when hemoglobin and plasma protein concentrations were normal. All 4 animals were then studied with respect to changes in C^{14} content of total plasma protein, plasma albumin, and plasma globulin, expired CO_2, and urine, during periods of controlled dietary intake of protein and calories. These periods ranged from 7 to 14 days, following a preliminary 4 to 5 days for equilibration. Each dog was observed on the standard very low protein diet and again when 3 gm. per kilogram of body weight of liver protein was substituted for equivalent calories each day. Fig. B. shows plasma protein C^{14} disappearance curves obtained from scatter graphs under each of the 2 dietary regimes. Curves based on average disappearance rates after intravenous labeled protein injection are included for comparison. With no appreciable oral protein intake, the plasma protein C^{14} level in these dogs tended to remain relatively constant. The shortest half-life observed was 45 days while an actual increase in C^{14}-specific activity was obtained in several instances. All dogs receiving adequate protein by mouth showed a remarkably constant rate of decline of plasma protein C^{14} with a half-life averaging 14 days.

These values contrast sharply with those, shown in Table II, following intravenous injection of labeled plasma, when the half-life on a very low protein diet was 7 to 8 days and 5 days on a diet containing 3 gm. of protein per kilogram of body weight. Albumin and globulin curves tended to parallel those for

total protein but no consistent pattern emerged. Results of measuring expired $C^{14}O_2$ and urinary C^{14} were inconclusive although when the protein-containing diet was fed there was a definite increase in C^{14} activity excreted relative to plasma protein C^{14} activity.

The only information suggesting particular tissue sources from which C^{14} might recycle into plasma proteins and cause such marked slowing of the isotope

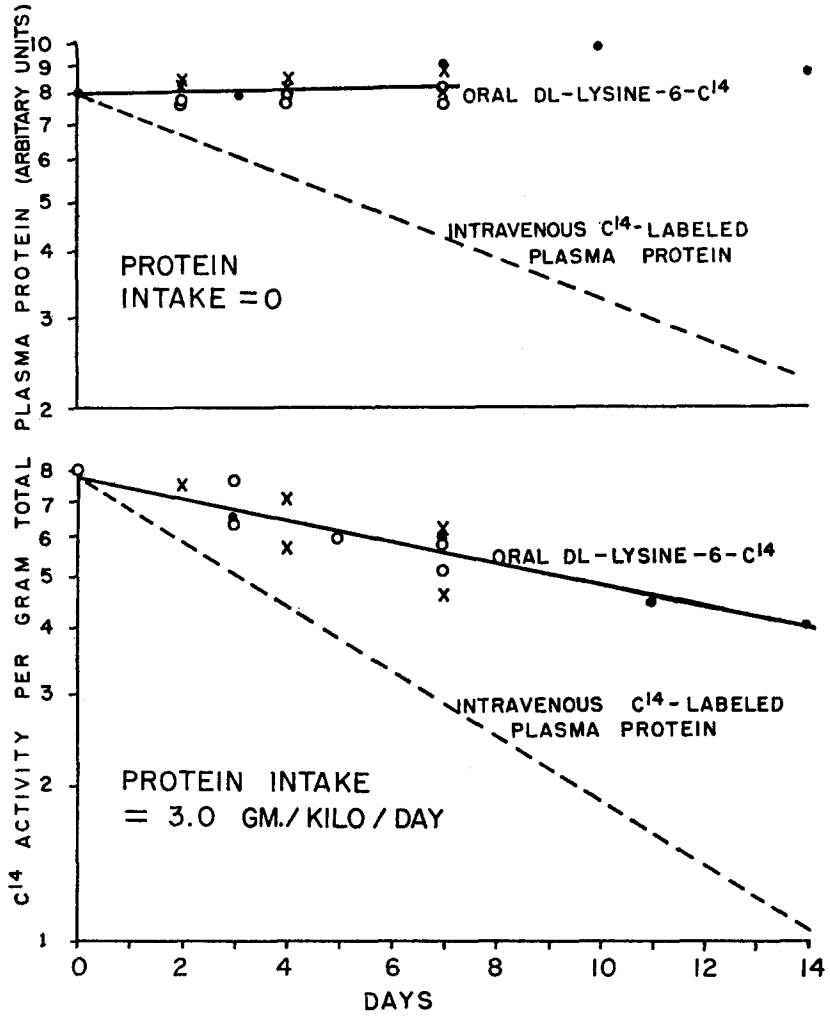

FIG. B. Graphs illustrating and comparing the effects of dietary protein (0 and 3 gm. per kilogram per day) and method of C^{14} labeling (feeding labeled lysine or injecting labeled plasma proteins intravenously) on the rate of disappearance of C^{14} from total plasma proteins. The slower rates observed after lysine feeding are attributed to recycling of C^{14} from tissues initially labeled by this procedure.

disappearance curves was supplied by one of the above donor dogs, killed 8 months after a single feeding of approximately 300 microcuries of DL-lysine-6-C^{14}. Approximately 17 per cent of the labeled L-lysine available for metabolism (140 microcuries after correcting for C^{14} removed by bleeding) was recovered from the animal at the time of sacrifice. Over 80 per cent of this residual activity was found in striated muscle protein whereas less than 0.02 per cent was found in the liver and only 0.01 per cent in the circulating plasma proteins. It is noteworthy that the ratio of liver protein to plasma protein activity was roughly 1 in this animal as well as in 6 animals killed a short time after labeled lysine feeding (11). The ratio of muscle protein to plasma protein activity, however, had shifted from 0.26 to 1.97 during the 8 month period. These findings indicate that significant endogenous contributions to the formation of plasma protein probably come ultimately from large tissue masses such as striated muscle.

DISCUSSION

The basic assumption on which interpretation of the foregoing and other comparable data rests is that the half-life of a labeled circulating protein, obtained from the slower exponential portion of the isotope disappearance curve is a true indication of its metabolic turnover rate. The validity of estimates of the size of the total plasma protein pool also depends on this assumption.

When the possibility of isotope recycling has been eliminated or minimized, the above concept is generally accepted for the normal, steady state. McFarlane (4), however, has suggested that significant variations might occur if plasma protein synthesis and/or breakdown occurred in only one compartment of the total pool. That this would create a great error seems unlikely in view of the relatively small amount of plasma protein metabolized (Table IV) compared to the daily exchange of protein between circulating plasma and extravascular extracellular fluids necessary to account for the equilibrium which uniformly occurs in these dogs within 24 to 48 hours. The great variations in the length of this initial rapid phase of isotope disappearance, of from 1 to 7 days, reported in the literature are difficult to reconcile. While the rate of this exchange has not been quantitated accurately it is probably at least several times the protein content of the total pool (Table III) per day as reflected by an approximate half-life of 10 to 12 hours. It is with full awareness of the possibility that abnormal conditions such as protein depletion and excess protein feeding may modify the slopes of specific activity curves and lead to erroneous estimates of pool size that the preceding calculations have been made. Nevertheless, it is felt that the quantitative aspects of turnover rate and exchange between plasma and tissue proteins are approximately correct even if not exact.

The effects of increasing dietary protein on plasma protein metabolism in these dogs differ in detail somewhat from those reported by Steinbock and Tarver (6) and Jeffay and Winzler (3). Major differences are possibly related to

differences in animal species and isotope used, as well as to variations in experimental design.

The data outlined in this paper show a much more rapid rate of turnover of plasma proteins with 2 gm. of dietary protein per kilogram per day than with an isocaloric diet devoid of protein, but fail to demonstrate any further increase in turnover rate with increments of from 2 to 6 gm. of protein per kilogram (approximately 10 to 30 per cent protein).

Steinbock and Tarver (6) reported a decrease in half-life not only when the protein of the diet was increased from 0 to 25 per cent but also a significant further increase when the protein content was changed to 65 per cent. Secondly, accelerated turnover of both albumin and combined globulins was noted in the present experiments with dogs while only serum albumin turnover was found to be dependent on the level of dietary protein in rats by Jeffay and Winzler (3).

Of particular interest are the computations of interchange between plasma and tissue proteins under the varied experimental conditions (Table IV). All animals irrespective of protein intake were in a relatively steady state with insignificant changes in plasma protein concentration or albumin/globulin ratio during the experimental period. This implies that plasma protein breakdown and synthesis must have been balanced and that in animals, both depleted and normal, receiving practically no dietary protein, all materials necessary for plasma protein synthesis must have come from body tissue sources. However after accounting for the C^{14} excreted in urine and expired air it was found that only 75 per cent of the total activity lost daily from plasma proteins was transferred back to tissues. Thus, it follows that tissue proteins are subjected to a constant though small net loss at the expense of maintaining plasma protein levels when protein is withheld from the diet. This and the associated findings of reduced urinary nitrogen and expired $C^{14}O_2$ are consistent with conservation of protein by a general slowing of protein metabolism under conditions of protein deprivation.

Under conditions of adequate or excessive protein intake and injection of labeled proteins, it is apparent that the C^{14} transferred from plasma to tissues is equivalent to an average of 70 per cent of the plasma protein metabolized daily (Table IV). Since reutilization of isotope has little effect on the plasma disappearance curves for several weeks in this type of experiment no indication of the tissue contribution if any to plasma protein synthesis is obtainable. On the other hand when all body proteins are labeled, the tissues do make a significant contribution to plasma protein synthesis even in the face of adequate dietary protein. This contribution is sufficient to cause a threefold increase in the apparent plasma protein half-life in dogs with both plasma and tissue proteins labeled by feeding the isotope incorporated in an amino acid,

compared to the half-life when only the plasma proteins are tagged by injection of labeled proteins, (Fig. B).

The rate of isotope recycling necessary to account for this apparent decrease in plasma protein turnover can be derived mathematically[1] and is found to be approximately 9 per cent of the total plasma protein pool per day. This corresponds closely to the amount transferred in the opposite direction from plasma protein to tissues (Table IV) and suggests a relationship to the continuing 1/1 ratio of plasma to liver protein-specific activities observed after feeding a labeled amino acid.

In these dogs, subjected to limited activity, nitrogen balance was attained with an intake of 1 gm. of protein per kilogram of body weight per day. However, the finding that the plasma protein turnover rate did not reach a maximum until the diet contained between 2 and 3 times this much protein, raises the question of which is the better measure of an optimal diet.

SUMMARY

The rate of plasma protein turnover is more rapid in dogs receiving adequate dietary protein than when a diet devoid of protein is fed. Both albumin and combined globulins are involved in this change.

The difference in turnover is reflected in a total protein half-life of 4.8 days with protein feeding *versus* 7.8 days without protein in the diet and in the metabolism of 1.0 and 0.65 gm. per kilogram of body weight per day on the respective diets.

Additions of dietary protein from 10 to 30 per cent caused no further increase in the rate of plasma protein turnover.

With protein depletion due to plasmapheresis and a very low protein diet there is evidence of reduced protein metabolism as indicated by nitrogen retention as well as a reduction in total plasma protein breakdown and interchange of isotope between plasma and tissue proteins.

Following introduction of labeled plasma protein into the circulation the net amount of isotope transferred to tissues has been computed from the

[1] Fig. B, lower graph. It is assumed that the difference between the apparently slower plasma protein turnover rate after labeled lysine feeding, ($t'_{\frac{1}{2}}$ = 14 days) and the actual turnover rate obtained after intravenous labeled protein injection ($t''_{\frac{1}{2}}$ = 5 days) is due to recycling of C^{14} from the tissues. Since both curves are exponential it follows that the rate of transfer of C^{14} from tissues to plasma protein is also exponential and is proportional to the level of isotope in the total pool. Therefore, the rate of turnover of plasma protein after feeding labeled lysine (K_1) minus the rate of turnover after injection of labeled protein (K_2) equals the rate of appearance in the blood of C^{14} recycled from the tissues (K_3).

$$K_1 - K_2 = K_3$$

$$\frac{\ln 2}{t'_{\frac{1}{2}}} - \frac{\ln 2}{t''_{\frac{1}{2}}} = K_3$$

$$0.139 - 0.049 = 0.09 = 9 \text{ per cent per day.}$$

difference between total plasma protein breakdown and combined C^{14} excretion in urine and expired air. In animals receiving adequate dietary protein, tissue transfer amounts to 70 per cent of the total lost from the plasma proteins each day while the percentage rises to 85 in depleted dogs deprived of protein.

In dogs with both plasma and tissue proteins labeled it can be estimated that, under conditions of protein feeding, an amount of C^{14} approximately equal to that lost from the plasma must recycle to account for the observed decrease in apparent plasma protein turnover rate, ($t\frac{1}{2}$ of 15 *versus* 5 days). Without protein in the diet the isotope contribution of the tissues to the maintenance of plasma protein levels must be as great as or greater than that transferred in the opposite direction.

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