PLASMA PROTEIN LABELED WITH LYSINE-ε-C^{14}

ITS ORAL FEEDING AND RELATED PROTEIN METABOLISM IN THE DOG*, ‡, §

BY C. L. YUILE, M.D., A. E. O'DEA, M.D., F. V. LUCAS, M.D., AND G. H. WHIPPLE, M.D.

(From the Department of Pathology, The University of Rochester School of Medicine and Dentistry, Rochester, New York)

(Received for publication, June 4, 1952)

Previous knowledge that differences exist between the metabolism of protein administered orally and that of protein administered parenterally has rested largely on studies of urinary nitrogen and sugar excretion (3, 8). The methods now available for labeling plasma proteins with C^{14} (radiocarbon) provide more direct ways and means of following the metabolic and excretory pathways of these proteins and their split products as well as comparing the results obtained after administration by oral and intravenous routes. Labeled plasma is prepared by feeding lysine-ε-C^{14} to a donor dog which some days later is bled to furnish the labeled plasma proteins in adequate amounts. The present paper deals with the oral feeding of dog plasma proteins *labeled* with *lysine*-ε-C^{14} and also an amino acid digest mixed with a tracer dose of labeled L-lysine-ε-C^{14} to dogs. A comparison of results obtained after *oral administration* with previously published data concerning *intravenous injection* of similarly labeled dog plasma proteins (9) indicates a marked difference in the metabolic picture following administration by these two routes. On the other hand, the almost identical behavior of C^{14} after ingestion of either homologous labeled plasma protein or an amino acid digest plus L-lysine-ε-C^{14} favors the concept of a more or less complete breakdown of the fed plasma protein in the gastrointestinal tract before absorption occurs.

Methods

The animals used in these experiments were healthy, female, mongrel dogs which had been under observation for periods ranging from months to years. Three of the dogs had been used previously for standard metabolic experiments.

The standard kennel ration was omitted for 1 day before and on the 1st day of each experiment, being resumed about 30 hours after each plasma feeding.

* Work supported jointly by the Office of Naval Research and the United States Atomic Energy Commission under contracts with the University of Rochester, N6ori-126, Task VIII and W-7401-eng-49.

‡ We are also indebted to Eli Lilly and Company for aid in conducting this work.

§ We are indebted to Merck and Co., Inc., for the amino acid mixture.

Labeled plasma was prepared by feeding D-L-lysine-ε-C^{14} to a donor dog (9). Blood from the donor was collected in heparin by jugular puncture at intervals of from 3 to 34 days after labeled lysine feeding and the separated plasma was rapidly frozen and stored at −4°C. for variable periods up to 5 months. Before administration to test animals, the plasma was thawed and any clotted fibrin was removed by centrifugation. Sterile precautions were used throughout and the final product was a clear, pale yellow fluid. Slight variations in protein concentration and C^{14} activity before and after freezing could be accounted for by the loss of fibrinogen. Labeled plasma was given by stomach tube, and 50 ml. of distilled water was used for rinsing. In one experiment, dog 43-141, an aqueous solution of 7.9 gm. of an amino acid digest (Vuj) (6) to which 7.3 mg. of L-lysine-ε-C^{14} had been added, was fed instead of plasma.

All methods pertaining to chemical determinations of plasma protein, albumin and globulin, collection of expired carbon dioxide, and preparation of samples for radioactivity have been described in detail elsewhere (5, 9).

Blood volume estimates were made on the basis of 80 ml. per kilo of body weight.

Fecal C^{14} was measured in aliquots of a KOH emulsion.

Determinations were made of C^{14} activity and nitrogen content of lyophilized, ground tissue samples from one dog, 51-137.

EXPERIMENTAL OBSERVATIONS

Details relating to body weight, hematocrit reading, plasma protein concentration, estimated plasma volume and C^{14} dose for the dogs used in each of the five experiments as well as the type and quantity of material fed are given in Table 1.

The per cent of administered C^{14} found in the circulating plasma at time intervals ranging from 45 minutes to 72 hours after feeding is listed in Table 2 in the first column under each dog number. *Maximum levels* were attained in from 7 to 10 hours after feeding labeled plasma protein or L-lysine-ε-C^{14} with an amino acid digest, and subsequently there was a slow decline.

The second column under each dog number (Table 2) indicates the per cent of circulating activity present as non-protein C^{14} at different time intervals. In the earliest samples, collected at 45 minutes, most of the plasma activity was found in the non-protein fraction, whereas after 7 hours all the activity was present as protein except in the two dogs (48-222 and 50-176) receiving larger amounts of plasma by mouth. In these two experiments a small per cent of the total plasma C^{14} was still in the non-protein fraction at 10 hours.

In all but the first experiment, dog 49-23, the C^{14} activity was measured in both albumin and globulin fractions. Values for total circulating albumin and globulin activity when plotted against time give curves which are in general parallel to those for total plasma protein activity, derived from the figures in Table 2. As with total plasma protein the maximal values for albumin and globulin activity are reached between 7 and 10 hours after labeled plasma feeding.

These maximal values for C^{14} activity in albumin and globulin are listed in Table 3. Several points are worthy of note. In the first place more activity

TABLE 1

Plasma Protein, Plasma Volume, and Labeled Plasma Fed

Dog No.	Weight	Initial hematocrit reading	Initial plasma protein	Plasma volume	Labeled plasma fed	Labeled plasma protein fed	C^{14} activity fed
	kilo	*per cent*	*gm./100 ml.*	*ml.*	*ml.*	*gm.*	*μc.*
51-137	9.6	50.0	6.14	435	79	4.0	0.77
49-23	13.9	44.4	7.60	656	74	4.3	0.94
48-222	13.7	52.5	7.10	535	130	8.2	0.98
50-176	10.5	52.6	7.3	440	200	11.6	0.68
43-141	13.1	51.0	7.0	565	100 H_2O	*	1.04

* Amino acid digest (Vuj) 7.2 gm. + 7.3 mg. L-lysine-ε-C^{14} in 100 ml. H_2O.

TABLE 2

Total and Non-Protein C^{14} Activity in Circulating Plasma after Feeding Labeled Plasma

Sample	Dog 51-137		Dog 49-23		Dog 48-222		Dog 50-176		Dog 43-141	
Time after feeding	Total circulating C^{14}, *per cent dose*	Non-protein C^{14}, as *per cent of total*	Total circulating C^{14}, *per cent dose*	Non-protein C^{14} as *per cent of total*	Total circulating C^{14} *per cent dose*	Non-protein C^{14}, as *per cent of total*	Total circulating C^{14}, *per cent dose*	Non-protein C^{14}, as *per cent of total*	Total circulating C^{14}, *per cent dose*	Non-protein C^{14}, as *per cent of total*
45 min.	—	—	2.86	71 (1 hr.)	1.39	93	—	—	3.35	84
2½ hrs.	5.3	26	—	—	4.15	59	3.60	65	5.70	35
4½ "	7.5	20	6.55	0	4.82	13	6.12	45	6.97	7
7 "	—	—	7.72	0	5.77	—	—	—	7.62	0
10 "	8.0	0	7.61	0	5.96	4	6.67	7	7.80	0
24 "	7.4	0	7.52	0	5.45	0	5.67	0	7.02	0
48 "	6.4	—	5.76	0	4.60	0	4.47	0	5.67	0
72 "	4.5 (96 hr.)	0	5.40	0	3.33	0	3.90	0	4.75	0

TABLE 3

Maximum Albumin and Globulin C^{14} Activity after Feeding Labeled Plasma

Dog No.	Material fed	Maximum total circulating C^{14} as albumin, *per cent dose*	Maximum total circulating C^{14} as globulin, *per cent dose*	Corresponding albumin/globulin ratios	
				C^{14}	Chemical
43-141	Amino acids 7.2 gm.	3.2	5.0	0.65	0.70
51-137	Plasma protein 4.0 "	3.3	5.0	0.66	0.89
48-222	" " 8.2 "	2.7	3.3	0.82	0.69
50-176	" " 11.6 "	2.7	3.4	0.79	1.75

was found in the globulin than in the albumin fractions in all four experiments. Also, in the two dogs fed larger quantities of plasma protein, of lower

specific activity, there was less total incorporation of C^{14} into both fractions and the specific activity of globulin was relatively lower than that of albumin as indicated by the ratios of albumin/globulin C^{14} shown in Table 3. The latter finding may well be due to a more prolonged elevation of the circulating amino acid level tending to favor albumin synthesis as suggested by Miller and Bale (4).

Table 4 summarizes the cumulative elimination of C^{14} as carbon dioxide in the expired air throughout the first 48 hours of each experimental period. In all experiments, carbon dioxide was collected during the 2nd, 4th, 7th, 10th,

TABLE 4

Cumulative Total C^{14} Excreted as Carbon Dioxide—Per Cent Dose Fed

Dog No.	0–2 hrs.	0–7 hrs.	0–12 hrs.	0–24 hrs.	0–48 hrs.
51-137	6.2	17.6	20.6	23.2	25.3
49-23	5.0	15.6	17.2	18.9	20.8
48-222	4.1	20.9	23.1	25.4	27.9
50-176	2.6	9.8	11.7	13.8	15.8
43-141	6.1	13.6	15.2	16.8	18.8

TABLE 5

Urinary and Fecal Excretion of C^{14}. Expressed as Per Cent of Dose Fed

Dog No.	Urinary excretion			Fecal excretion
	0–24 hrs.	24–48 hrs.	Total 48 hrs.	
42-23	0.56	0.39	0.95	0.15
48-222	1.84	0.42	2.26	0.61
51-137	1.25	0.80	2.32	—
50-176	0.21	0.99	1.20	—
43-141	1.39	0.20	1.59	0

24th, and 48th hours. The figures in Table 4 were derived from curves drawn through the points so obtained. In the two experiments involving small plasma protein feeding and in the control L-lysine feeding experiment, the maximal *hourly rate* of $C^{14}O_2$ excretion (4 per cent of dose) occurred between 1 and 2 hours. After ingestion of larger amounts of protein the peak was not reached until the 4th hour. At 7 hours, the rates of excretion had fallen to relatively low levels and subsequently all curves were practically identical.

Table 5 lists the amounts of C^{14} as per cent of activity fed which was excreted in the urine in each experiment and in the feces in three experiments. With one exception, dog 50-176, a major part of the relatively small total urinary excretion was present in the samples collected during the 1st day and insignificant C^{14} residues were detected in the feces.

Dog 51-137 was killed 96 hours after labeled plasma feeding and aliquots of most tissues were analyzed for C^{14} and protein content. The relative activities of the various tissues were found to be essentially the same as those previously described after feeding D-L-lysine-ε-C^{14} or after intravenous administration of C^{14}-labeled plasma protein (5, 9). Since no attempt was made to remove blood from this animal by perfusion, the total measured tissue activity which amounted to 68.4 per cent of the dose included all activity in protein from the plasma and extravascular fluids. At the time of sacrifice total circulating plasma protein activity amounted to 4.5 per cent of the dose. Since it would appear that under normal conditions, lymph and other extravascular, extracellular fluids contain an approximately equal amount of similar protein (7, 9), the residual net tissue activity in this dog was about 60 per cent of the administered dose. It is of interest that all the liver activity in this instance was present in the solid fraction after treatment with trichloroacetic acid and acetone and that the ratio of liver protein to plasma protein activity was 0.7 in contrast to a ratio of about 0.1 at a similar time after *intravenous injection* of labeled plasma.

On the basis of the findings in dog 51-137, it can be assumed that the tissue activity at various times after feeding labeled plasma or amino acids is equal to the difference between the activity fed and the activity accounted for by excretion and incorporation into proteins of the circulating plasma and extravascular, extracellular fluids. Estimates of the over-all tissue activity derived by application of such calculations to the 24 hour period of each experiment range from 60 to 74 per cent of the administered C^{14}. No significant changes in tissue activity were found at 48 hours in the four surviving dogs and in dog 51-137 the estimated tissue activity at 24 hours was essentially the same as that found after sacrifice at 96 hours. The remarkable constancy of these values in a given animal indicates the speed of tissue incorporation of the lysine fed but does not preclude the possibility of activity shifts within individual organs and tissues.

DISCUSSION

The present data establish a well defined pattern for C^{14} utilization and excretion by the dog after the radioactive isotope is given orally as homologous plasma protein labeled with L-lysine-ε-C^{14}. In addition, it is possible to make some interesting comparisons relative to the administration of various C^{14}-labeled materials by different routes. One such comparison, namely between intact plasma protein labeled *in vivo* with lysine-ε-C^{14} and a protein digest with L-lysine-ε-C^{14} added, is incorporated in the experimental observations outlined above. The almost identical behavior of C^{14}, in these experiments at least, after giving either of these two materials by mouth, would appear to rule out the possibility, suggested by Dent and Schilling (2), that

homologous plasma proteins can be absorbed from the intestine without appreciable change. The 2 hour delay in maximal $C^{14}O_2$ excretion and the lower plasma C^{14} levels attained after feeding larger amounts of protein also point to delayed absorption due to a longer period required for digestion (dogs 48-222 and 50-176).

A direct quantitative comparison of the results obtained in this study with the previously published findings after feeding D-L-lysine-ϵ-C^{14} to dogs (5) is not possible because of the presence of the unnatural isomer in the latter and the fact that the lysine in the earlier experiments was fed with a large excess of protein. However, the data from both series of experiments are qualitatively similar in all respects.

Abdou and Tarver (1 *a* and *b*) have described somewhat comparable experiments in the rat involving the oral administration of plasma protein labeled by feeding either serine-β-C^{14} or C^{14}-labeled *Rhodospirillum rubrum* organisms to donors. Despite the differences in animal species and labeled amino acids used, it is particularly interesting relative to the validity of this experimental approach that the results in dogs fed plasma proteins labeled only with lysine-ϵ-C^{14} are essentially similar to those in rats receiving plasma proteins in which many of the amino acids were labeled.

Finally, the greatest contrast in the distribution of C^{14} is seen when a comparison is made between oral and intravenous administration of lysine labeled plasma protein in the dog. After labeled plasma feeding, incorporation of the label into the plasma proteins of the recipient occurs rapidly but the maximum, attained between 7 and 10 hours, is only 6 to 8 per cent of the activity fed. After intravenous injection of labeled plasma, on the other hand, there is a rapid drop in plasma protein activity followed by a slow, gradual decline so that as much as 20 per cent of the dose may still be present in the circulation after 7 days (9).

Marked differences are also observed in the rate and magnitude of incorporation of the ϵ carbon atom of lysine into tissues after the administration of labeled plasma by either the oral or intravenous route. When plasma is fed, the maximum total tissue activity levels of 60 to 74 per cent appear to be reached within 24 hours and to remain unchanged at least until the 4th day. It should be stressed that this refers to the sum of all body tissues, since the observations of Abdou and Tarver (1 *a* and *b*) indicate an early rapid turnover in the more actively metabolic organs which, however, have a small mass in relation to the whole animal.

Following parenteral administration of labeled plasma protein there is a gradual increase in total tissue activity which amounts to approximately 40 per cent of the injected activity after 7 days in the normal dog (9, 10). The increase is associated with a decline in plasma protein activity and probably continues until the rate of transfer of C^{14} from plasma to tissue protein is balanced by the relatively small amount of C^{14} lost in the expired air.

The most important *excretory pathway* of C^{14} after giving labeled plasma protein by mouth or by vein is through the lungs. After oral administration 16 to 28 per cent of the label has been recovered as C^{14} O_2 in 48 hours with about 80 per cent of the total excreted during the first 12 hours (Table 4). Since variations in total C^{14} O_2 excretion, due entirely to differences occurring within the first 7 hours, are not related to the amount of C^{14} incorporated into plasma proteins, they probably reflect different degrees of uptake of C^{14} by the tissues during the early stages of the experiment. Intravenous injection of lysine-ϵ-C^{14}–labeled plasma proteins in dogs results in a much smaller loss of activity in the expired air. If the variable initial peaks, attributed to residual non-protein C^{14} in the donor plasma, are excluded, the rates of C^{14} O_2 excretion decline gradually from maxima of about 0.1 per cent of the dose per hour and in a 2 day period approximately 2.5 per cent of the injected activity is eliminated in this manner (9, 10).

Relatively small amounts of activity appear in the urine of these dogs irrespective of the route of administration of lysine-labeled plasma. An average 48 hour urinary C^{14} excretion of 1.66 per cent after plasma feeding (Table 5) and one of 1.0 per cent or less after intravenous injection (9, 10) have been found in this laboratory.

SUMMARY

The metabolism of homologous plasma proteins, labeled with lysine-ϵ-C^{14}, after oral administration to dogs has been investigated.

The speed of the various processes involved is indicated by the maximum rate of C^{14} O_2 excretion which is attained within 1 to 4 hours, the prompt appearance of protein activity in the plasma and disappearance of non-protein activity from it, both virtually complete in 7 to 10 hours, as well as the rapid incorporation of a large percentage of the fed-C^{14} into tissues.

There are no essential differences between the behavior of labeled plasma and that of an amino acid digest containing ϵ-C^{14} labeled lysine when these two materials are given orally.

At the end of 48 hours after labeled plasma feeding, a CO_2 elimination of 16 to 28 per cent of the fed C^{14} is noted. In contrast, after 48 hours following labeled plasma by vein, a CO_2 elimination of only 2.5 per cent is recorded—almost a 10 to 1 ratio. We believe this, together with the data concerning plasma and tissue protein activity, represents a significant difference in the metabolic process. The evidence favors a complete breakdown of plasma protein to the amino acid level when given orally but not when given by vein.

BIBLIOGRAPHY

1. Abdou, I. A., and Tarver, H., (*a*) *J. Biol. Chem.*, 1951, **190**, 769, 781; (*b*) *Proc. Soc. Exp. Biol. and Med.*, 1952, **79**, 102.
2. Dent, C. E., and Schilling, J. A., *Biochem. J.*, London, 1949, **44**, 318.

3. Howland, J. W., and Hawkins, W. B., *J. Biol. Chem.*, 1938, **123,** 99.
4. Miller, L. L., and Bale, W. F., *Fed. Proc.*, 1952, **11,** 260.
5. Miller, L. L., Bale, W. F., Yuile, C. L., Masters, R. E., Tishkoff, G. H., and Whipple, G. H., *J. Exp. Med.*, 1949, **90,** 297.
6. Robscheit-Robbins, F. S., Miller, L. L., and Whipple, G. H., *J. Exp. Med.*, 1947, **85,** 243.
7. Sterling, K., *J. Clin. Inv.*, 1951, **30,** 1228.
8. Terry, R., Sandrock, W. E., Nye, R. E., and Whipple, G. H., *J. Exp. Med.*, 1948, **87,** 547.
9. Yuile, C. L., Lamson, B. G., Miller, L. L., and Whipple, G. H., *J. Exp. Med.*, 1951, **93,** 539.
10. Unpublished data.