THE USE OF RADIOACTIVE LYSINE IN STUDIES OF PROTEIN METABOLISM

SYNTHESIS AND UTILIZATION OF PLASMA PROTEINS*,‡,§

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We have long been interested in protein metabolism, blood protein production and exchange, body protein needs as sustained by plasma protein or hemoglobin given parenterally, and the labile exchange between reserve protein stores and circulating plasma proteins. This adds up to a dynamic equilibrium in body protein production, storage, and utilization or exchange (9, 21, 22).

The availability of synthetic *dl*-lysine-ϵ-C^{14} with C^{14} in unequivocally known position (13) has enabled us to study not only the incorporation of this essential amino acid in the various body proteins, but also to follow the time course of the isotopically labeled blood proteins. In addition, the apparent rate of disappearance of the labeled plasma proteins injected into normal recipient animals has been studied in an attempt to define the comparative behavior of the plasma albumin and globulin fractions. It will be seen that in these experiments the globulin fraction is turned over more rapidly than the albumin fraction on a gram for gram basis.

EXPERIMENTAL METHODS

A description of dogs used in these experiments and their dietary histories, including the amounts of *dl*-lysine-ϵ-C^{14} fed, is included in individual experimental histories below. Whenever the term *specific activity* is used, it refers to the per cent administered C^{14} per gram carbon of the material under consideration.

The *dl*-lysine-ϵ-C^{14} was synthesized under the direction of Dr. R. W. Helmkamp and his collaborators in the Department of Organic Chemistry, The University of Rochester, according to their published method (13). The method of synthesis and the homogeneity of the labeled lysine on paper partition chromatography and subsequent autoradiography make the presence of significant amino acid or radioactive impurities very unlikely.

For the quantitative collection of expired carbon dioxide, the dogs were placed in a sealed

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metal chamber of about 8 cubic feet in volume. Fresh air was drawn from the outside through a flow meter at a rate of 130 to 140 cubic feet per hour into the chamber by a continuously operated suction pump. This pump was so connected to the chamber outlet by a Y-tube that the chamber air could be discharged to the outside directly, or shunted through a carbon dioxide absorption apparatus containing 40 per cent aqueous potassium hydroxide. The absorption of carbon dioxide was essentially quantitative as indicated by an aqueous barium hydroxide trap at the outlet of the absorption apparatus. In Experiments D-3-L and D-7-L expired carbon dioxide was collected for only the last 30 minutes of regular 2 hour intervals; the total carbon dioxide activity for rhe 2 hour interval was then approximated by multiplying the activity of the 30 minute period by four.

The dogs were removed from the gas collection chamber at the close of carbon dioxide collection periods to obtain blood samples when necessary. Blood was drawn from the external jugular veins and mixed with sodium oxalate or citrate. After proper centrifugalization the red blood cell hematocrits were recorded and the plasma removed. Where red blood cells were to be examined for radioactivity, they were twice suspended in isotonic saline and centrifugalized. The cells were then dried *in vacuo* before C^{14} assay was carried out.

Aliquots of plasma were taken for (*a*) total plasma protein estimation by semimicro Kjeldahl; (*b*) the separation of plasma albumin from the globulins by the method of Majoor (10), and chemical albumin to globulin ratios were based on semimicro Kjeldahl analysis of aliquots of the filtrate; (*c*) the determination of C^{14} activity by the method of Bale (1) which utilizes the quantitative conversion of organic material to CO_2 by the reagent of Van Slyke and Folch (19) and then transfers the carbon dioxide to an ionization chamber for radioactivity measurement.

In Experiments D-1-L, D-3-L, and D-7-L the dogs were sacrificed by viviperfusion with warm (38°C.) potassium-free Ringer's solution with 5 per cent glucose under light ether anesthesia.

The viscera were removed, care being taken to avoid contamination of tissues with gastrointestinal or urinary bladder contents. The gastrointestinal tract was opened and washed free of its contents by a stream of cold water. Tissue samples were taken and preserved by being kept frozen. Tissues for analysis were dried either by prolonged desiccation over sulfuric acid *in vacuo* or by the lyophile process.

The heparinized plasma obtained from Experiments D-3-L and D-7-L was used within a few hours in Experiments D-4-L and D-8-L respectively.

For the purpose of preparing protein-free filtrates, aliquots of plasma or albumin solution from the Majoor procedure were treated with sufficient 20 per cent aqueous trichloracetic acid solution to yield a final concentration of 6 per cent trichloracetic acid. The precipitated proteins, when needed for assay of C^{14} activity, were first washed with absolute alcohol, separated by centrifugalization, taken up with a small volume of dilute aqueous sodium hydroxide, and transferred to the flask used in C^{14} assay. Before assay the excess water was then removed by warming to about 35°C. and passing a slow stream of air through the flask until dry.

EXPERIMENTAL HISTORIES

Experiment D-1-L—Normal Blood Levels.—Dog 47-113, a normal, adult, female beagle mongrel weighing 10.0 kilos which had not previously been used for experimental purposes was fasted for 72 hours before feeding 1.022 gm. of *dl*-lysine incorporating 26.3 microcuries of C^{14} in the epsilon carbon position. The lysine was folded into 250 gm. of lean hamburg which was rapidly consumed in a few minutes. On the following day the diet consisted of 250 gm. of hamburg and subsequently of the usual kennel ration of table scraps. Urine samples were obtained by catheterization at 4, 8, 12, and 24 hours after lysine feeding and daily

urine collections were made from a metabolism cage for the following 4 days. Total blood volume 783 ml., plasma 471 ml. The first fecal specimen was obtained at 72 hours and subsequent specimens were collected at irregular intervals for a further period of 6 days. Blood samples were obtained by jugular puncture at intervals corresponding to urine collections during the first 3 days at which time plasmapheresis was instituted with the removal of 100 to 145 ml. of blood and return of 50 to 75 ml. of red cells on five occasions during the next 9 days. The dog was viviperfused under ether anesthesia on the 17th day of the experiment. No abnormalities were found at postmortem examination and various viviperfused tissues free of red cells were taken for C^{14} analysis. During the course of the experiment the hematocrit readings ranged from 37 to 44 per cent and the plasma protein concentration remained between 5.0 and 5.7 gm. per 100 ml. Blood removed totalled 695 ml.

Experiment D-3-L—2 Day Fast.—Dog 47-236, a normal, adult, female spaniel weighing 10.0 kilos. Food was withheld for a period of 48 hours before 2.0 gm. *dl*-lysine incorporating 51.24 microcuries of C^{14} in the epsilon carbon position was fed with 180 gm. of hamburg. The meal was eaten slowly over a period of one-half hour after which time the last portion was given by forced feeding. Four hours later several mucus-coated boli of hamburg were *vomited* and completely refed in 10 minutes. There was no subsequent vomiting. At the start of the experiment the animal was placed in a *gas collection chamber* from which CO_2 was collected during a half-hour period every 2 hours for a total of 24 hours. During this time blood samples were withdrawn at intervals of 2 hours for a total of 100 ml. and all urine excreted was pooled. Viviperfusion under ether anesthesia was carried out satisfactorily 25 hours after lysine feeding and blood-free tissues were removed for C^{14} analysis. No postmortem tissue or organ abnormalities were noted. Prior to perfusion 330 ml. of blood was withdrawn to provide labeled plasma for injection into another dog (Experiment D-4-L). Blood volume calculated 600 ml., hematocrit 50 per cent.

Experiment D-4-L—2 Day Fast.—Dog 44-98, a normal female, adult, short-haired, mongrel terrier was fasted for 48 hours prior to the intravenous injection of 133 ml. of plasma removed from dog 47-236 24 hours after a feeding of labeled *dl*-lysine (*cf.* Experiment D-3-L). The total transfused plasma had a C^{14} activity of 0.264 microcurie. Initially dog 44-98 weighed 8.4 kilos. The hematocrit was 56.8 per cent, the plasma protein concentration 7.4 gm. per 100 ml., and the plasma volume 346 ml. Five blood samples were withdrawn during the first 24 hours and daily samples were obtained until 96 hours had elapsed, in all 155 ml.

Experiment D-7-L—Normal.—Dog 44-98. Five months after an intravenous injection of labeled plasma (Experiment D-4-L) this normal, adult, female dog, having received a standard kennel diet in the interval, weighed 9.0 kilos. At this time the hematocrit was 52 per cent and the plasma protein concentration was 7.0 gm. per 100 ml. Without a preliminary period of fasting, 2.002 gm. of *dl*-lysine incorporating 57 microcuries was fed with about 150 gm. hamburg. This was consumed completely in a few minutes.

During the next 24 hours the animal was subjected to procedures identical with those described for Experiment D-3-L. About 6 hours later the dog vomited and was refed at once. With the dog in a *gas chamber* expired CO_2 was collected for one-half hour every 2 hours. Blood samples were withdrawn at 2 and 4 hourly intervals (Fig. 2) and totalled 89.4 ml. The total urinary excretion was pooled. At 27 hours the animal was viviperfused under ether anesthesia and tissues were taken for C^{14} analysis. No abnormalities were noted postmortem. Prior to perfusion 400 ml. of blood was withdrawn from which the labeled plasma was used for injection into another dog, Experiment D-8-L.

Experiment D-8-L.—Dog 46-S-A, a normal, adult, female spitz, weighing 6.7 kilos was fed a kennel diet up to the day before the experiment. Two hundred ml. of plasma which had been removed from dog 44-98 (24 hours after a feeding of labeled *dl*-lysine) was injected intravenously in a period of about 10 minutes. The total C^{14} activity in the transfused plasma was 0.478 microcurie. Following the transfusion blood samples were collected for C^{14}

analysis in total plasma, albumin, and globulin at intervals from 4 minutes to 144 hours. Blood removed in sampling totalled 183.3 ml. Total blood volume 478 ml., plasma volume 355 ml., hematocrit 30 per cent.

Experiment D-9-L—Repeat Anemia Plus Hypoproteinemia.—Dog 44-10. Approximately 10 months after the first feeding of labeled lysine the animal, in good physical condition, was again subjected to a period of plasma protein and red cell depletion. For 2 weeks a diet consisting of 300 to 400 gm. of low protein biscuit, 100 gm. of carrots, and 3 gm. of yeast was substituted for the kennel ration and bleeding was carried out at 48 hour intervals. Consumption of the diet averaged about 60 per cent and the amount of blood removed totalled 1460 ml. During this time the hematocrit was lowered from 59.0 to 32.7 per cent, the plasma protein concentration dropped from 6.5 to 5.29 gm. per 100 ml., and the weight fell from 15.7 to 13.5 kilos. At the end of this depletion period 1.576 gm. of *dl*-lysine incorporating 44.5 microcuries was divided into three approximately equal portions, each of which mixed with 200 gm. of cooked, ground liver and 200 gm. of basal biscuit was fed on each of 3 consecutive days. On the 4th day after the first lysine feeding the hematocrit had risen to 40 per cent and the plasma protein concentration to 7.6 gm. per 100 ml. On this day 200 ml. and on the following day 160 ml. of blood was withdrawn to provide plasma for a different type of experiment. For 3 days following the last lysine feeding, the food consisted of kennel diet plus liver and subsequently of kennel diet alone. Samples were obtained for C^{14} analysis at variable intervals up to 6 weeks totalling 156 ml. The hematocrit returned to normal in 4 weeks. Total blood volume before depletion 1133 ml., after depletion 921 ml. total blood volume, 4 days later total blood volume 1161 ml.

EXPERIMENTAL OBSERVATIONS

Experiment D-1-L.—Dog 47-113. *dl*-Lysine-ϵ-C^{14} 1.02 gm. (26.3 microcuries) was fed with diet protein. The dog excreted 34 per cent of the total dose of C^{14} in the urine collected over a period of 24 hours with an additional 2 per cent of the total dose in the urine of the subsequent 4 days. The feces contained only about 0.9 per cent of the total dose which indicates the completeness of absorption of the fed amino acid.

The relative quantitative distribution of the activity in the tissues of this dog sacrificed under ether anesthesia 17 days after the C^{14} lysine feeding can be indicated by dividing them into three groups:

Group 1: Tissues contain 0.030 to 0.015 per cent of the administered C^{14} per gm. carbon and include liver, kidney, spleen, and pancreas in order of decreasing activity.

Group 2: Tissues contain 0.015 to 0.005 per cent of the fed C^{14} per gm. carbon and include lung, cardiac muscle, stomach, skeletal muscle, colon, duodenum, jejunum, ileum, bone marrow, adrenal, and thyroid in order of decreasing activity.

Group 3: Tissues contain less than 0.005 per cent of the fed C^{14} per gm. carbon and include vertebral bodies, ribs, aorta, lymph node, and eye in order of decreasing activity. These tissues were blood-free as the result of viviperfusion. See Experimental Histories.

Fig. 1 presents the C^{14} activity of the blood cells and the plasma as estimated from samples taken over the first 12 days after the C^{14} lysine feeding. It is at

once apparent that the per cent fed C^{14} per gm. carbon 24 hours after the labeled lysine feeding is more than twenty times greater in plasma than blood cells. At this time 85 to 90 per cent of the plasma activity is found in the plasma proteins (as measured in later experiments), hence it may be said that in the non-anemic normal dog the red cell proteins acquire a considerably lower specific activity than the plasma proteins. Remembering that the blood of a normal dog

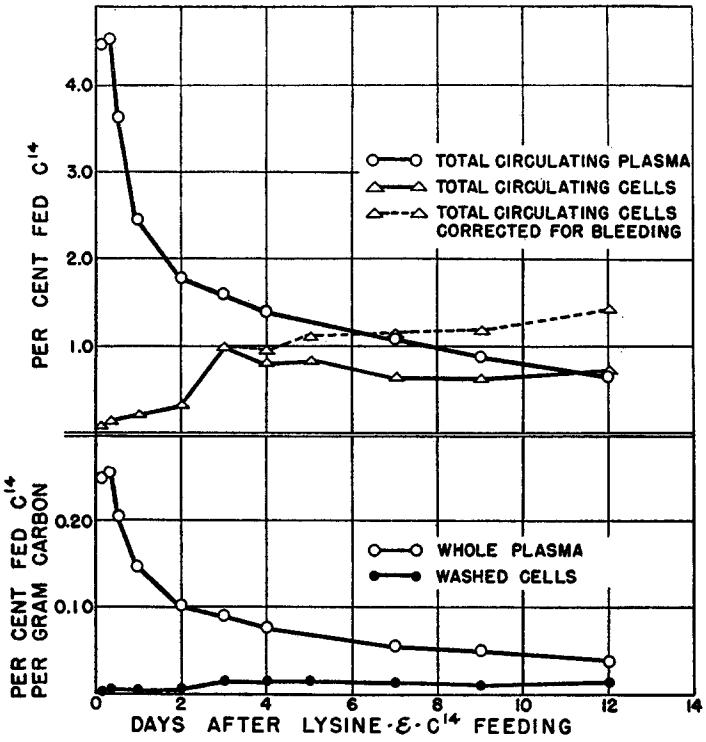

FIG. 1. C^{14} distribution in plasma and cells after lysine-ε-C^{14} feeding. Experiment D-1-L. Substantial amounts of blood were withdrawn 3, 4, 5, and 9 days after the feeding of labeled lysine with hamburg.

contains about five times as much red cell protein as plasma protein, we may conclude that the total C^{14} activity in the plasma proteins at 24 hours is about four to five times greater than that in the blood cells.

The specific activity of the plasma protein takes about 4 days to fall to a level equal to one-half that seen at 24 hours. This is in good agreement with estimates obtained from a study of the disappearance of transfused C^{14}-labeled plasma (see Experiments 3 and 4). Extrapolation of the plasma activity curve to the day of sacrifice places the final plasma activity at a level slightly below that of but one organ—the liver which has long been suspected as the organ

most actively involved in plasma protein production if not in plasma protein turnover and reconversion to other protein metabolites.

The blood cell C^{14} content rises to a maximum over a period of 3 days and is there maintained at a comparatively constant level during the course of the experiment.

Experiments D-3-L and D-7-L.—Dogs 47–236 and 44–98 represent a more detailed short term study of the varying distribution of C^{14} activity in the tissues and plasma protein fractions. Experiment D-3-L differed from D-7-L only in the fact that in D-3-L the dog was given only water for 48 hours be-

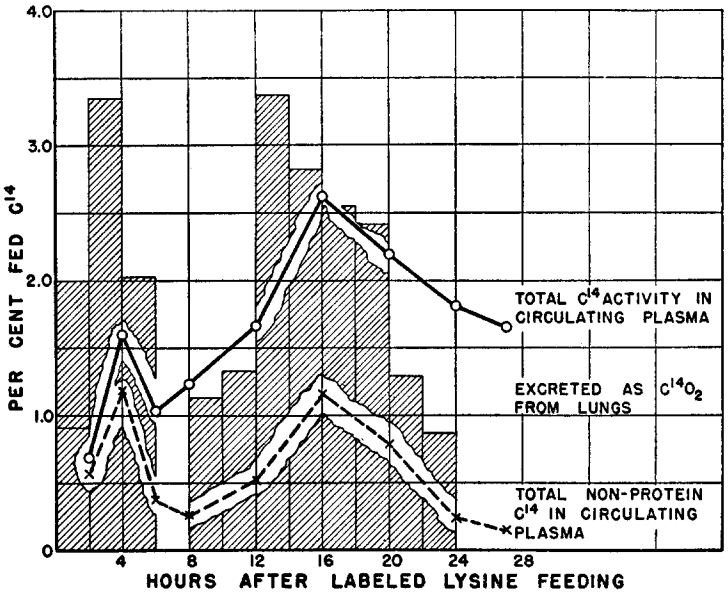

FIG. 2. Experiment D-7-L. Blood plasma C^{14} content compared with C^{14} in expired air (shaded columns). Drop in C^{14} values after 4 hours related to vomiting.

fore the feeding containing the C^{14}-lysine while in D-7-L the dog had received its regular daily ration of kennel diet in the days prior to the experiment.

After being fed 2.00 gm. of *dl*-lysine-ϵ-C^{14} (51.2 microcuries) with 180 gm. of lean hamburg, the dog in D-3-L excreted 28 per cent of the fed dose of C^{14} in the expired air and 33 per cent in the urine collected over the 24 hours of the experiment. The dog in D-7-L received a similar dose of *dl*-lysine-ϵ-C^{14} in about 150 gm. of lean hamburg and excreted 28.5 per cent and 25.1 per cent of the fed dose of C^{14} in the urine and expired air respectively.

Fig. 2 shows the $C^{14}O_2$ excretion and the non-protein C^{14} activity of the plasma in D-7-L. It is clear that the radioactivity of the exhaled $C^{14}O_2$ closely parallels the level of the non-protein C^{14} activity of the plasma. The minimum in both

occurs at a time closely corresponding to the point at which the dog regurgitated and was refed a large compact bolus of the food ingested at the start of the experiment. Fig. 2 is representative of D-3-L as well as of D-7-L.

The tissue distribution in these dogs in order of decreasing activity is qualitatively and quantitatively comparable as shown in Table I. Dogs killed 25 and 27 hours after feeding of *dl*-lysine-ϵ-C^{14}.

TABLE I

Blood-Free Tissues Arranged in Order of Decreasing Activity

	Tissue activity in per cent fed C^{14} activity per gm. carbon	Tissues of D-3-L	Tissue activity in per cent fed C^{14} activity per gm. carbon	Tissues of D-7-L
Group 1	0.052 to 0.020	Duodenum, jejunum, kidney, liver, gastric mucosa, colon, ileum, pancreas, parotid gland, esophagus, vertebra, spleen, cervical lymph node	0.087 to 0.020	Duodenum, jejunum, ileum, liver, kidney, lung, spleen, pancreas, colon, stomach, esophagus, cardiac muscle
Group 2	0.020 to 0.010	Lung, ovaries, thyroid, adrenals, GI content, cardiac muscle, sciatic nerve, thymus, temporal muscle, tongue, femur, eye (lens), rib, urinary bladder	0.020 to 0.010	Urinary bladder, temporal muscle, diaphragm
Group 3	Below 0.010	Diaphragm, trachea, uterus and bones, parietal bone, thigh muscle, bile, gall bladder, stomach muscle, spinal cord, mesentery (0.0)	Below 0.010	Brain, thigh muscle, spinal cord

The tissue distributions of these short experiments differ from the longer D-1-L experiment in the activity of the gastrointestinal tract tissues which are among the most active tissues of the short experiments and only of intermediate activity in the longer experiments. The liver, kidney, pancreas, and spleen are seen to maintain their positions of highest activity.

Fig. 3 shows the specific C^{14} activity of the plasma protein fractions (expressed in terms of the per cent fed C^{14} activity per 100 ml. of plasma) as a function of time; in the case of both dogs more total activity is found in the globulin than in the albumin of 100 ml. of plasma. The chemical A/G ratios showed no significant changes while the C^{14} A/G ratios for D-7-L showed progressive decrease. This indicates that the C^{14} ratio is decreasing while the

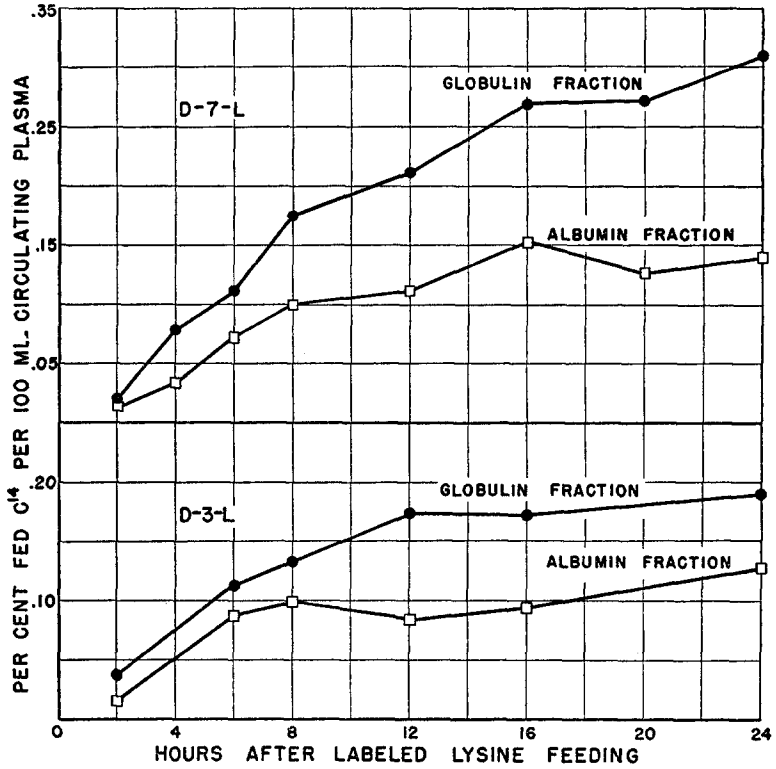

FIG. 3. Experiments D-3-L and D-7-L. Comparison of C^{14} incorporation in plasma albumin and globulin fractions following feeding of lysine-ϵ-C^{14} with hamburg.

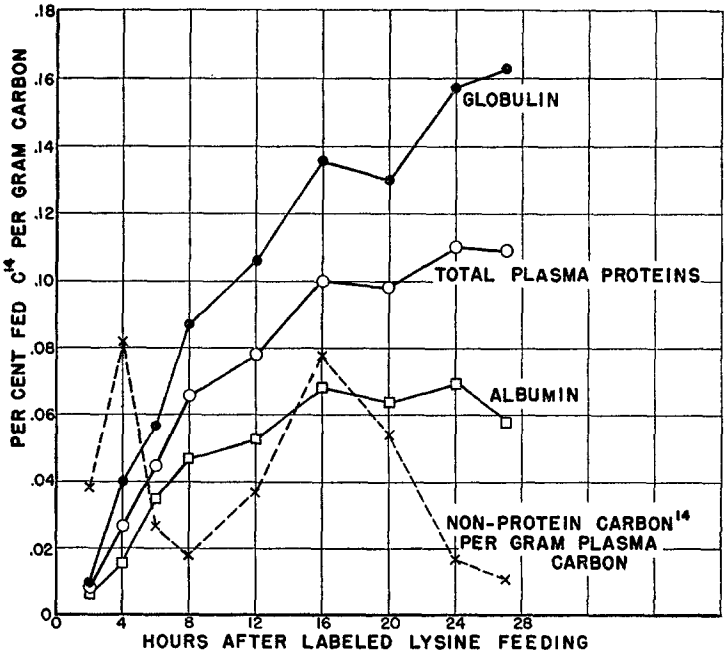

FIG. 4. Experiment D-7-L. Incorporation of C^{14} in plasma carbon fractions following feeding of lysine-ϵ-C^{14} with hamburg.

extent of C^{14} incorporation in both protein fractions is still increasing. This may be a result of a higher lysine content of the globulin fraction and a reflection of a greater rate of formation in grams of the globulin fraction. The results of Experiments D-4-L, D-8-L, and D-9-L point to the latter interpretation.

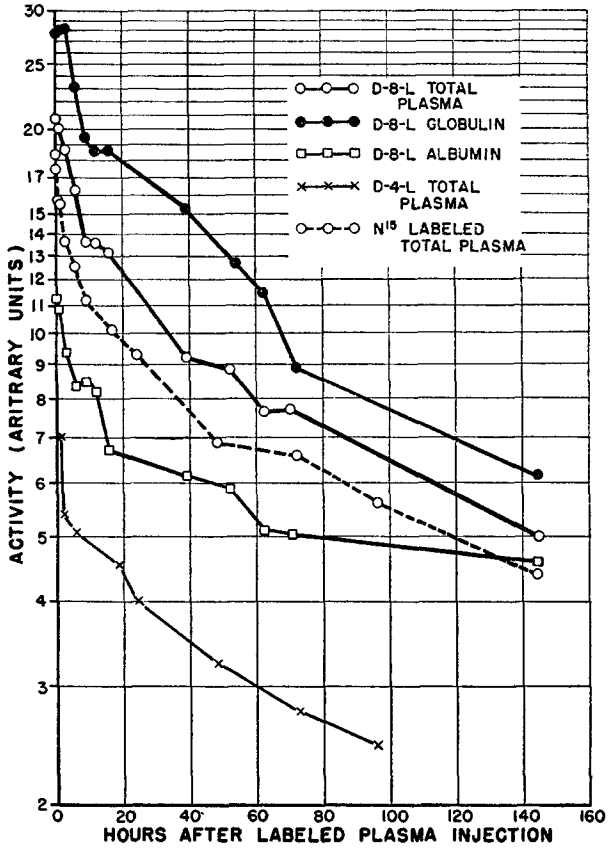

FIG. 5. Rate of decline in specific activity of plasma protein and protein fractions following transfusion of labeled plasma into normal dogs.

Although not shown in Figs. 3 and 4, the extent of incorporation of C^{14} into the fibrinogen fraction at 24 hours was found to be 0.12 per cent of fed C^{14} per gm. carbon.

Experiments D-4-L and D-8-L.—Fig. 5 presents the observations made on two normal dogs, 44-98 and 46-S-A, after they were given single, large (133 to 200 ml.) intravenous injections of heparinized plasma obtained immediately prior to sacrifice from dogs in Experiments D-3-L and D-7-L. Fig. 5 shows the disappearance curves of C^{14} specific activity for total plasma protein in the

recipient dogs. A similar disappearance curve established by the use of plasma protein labeled with N^{15} (4) is included for comparison. Disappearance curves of C^{14} specific activity in albumin and globulin fractions are shown for Experiment D-8-L. It is obvious that the C^{14} activity of the globulin fraction decreases at a greater rate than that of the albumin fraction. The data from protein fractionation from Experiment D-4-L, not included in the figure, were qualitatively similar.

Experiment D-9-L.—Dog 44-10. This experiment presents a long term (74 days) study of the plasma protein albumin and globulin fractions. The dog

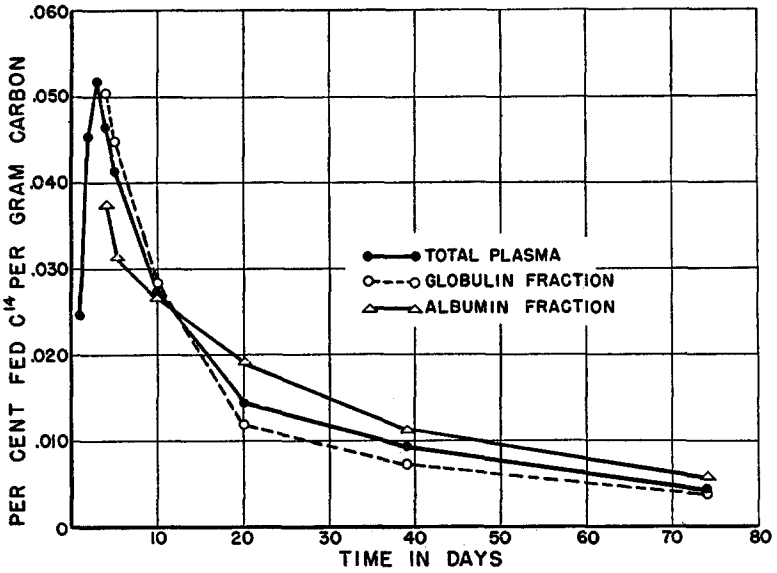

FIG. 6. Experiment D-9-L. Curve showing more rapid decline in specific activity of globulin fraction of plasma than of albumin fraction following the feeding of lysine-ε-C^{14} to a dog originally anemic and hypoproteinemic.

44-10 was depleted of plasma protein and hemoglobin (16) and then fed a total of 1.576 gm. *dl*-lysine-ε-C^{14} (44.5 microcuries) in three equal doses on successive days.

Fig. 6 shows the per cent of the fed C^{14} per gm. carbon of the plasma proteins as a function of time. It is at once clear that not only is a larger amount of the fed C^{14} incorporated into the globulin fraction, but also that the activity in the globulin fraction drops off more rapidly than the activity of the albumin fraction.

DISCUSSION

Little is known of the *in vivo* metabolism of the essential amino acid lysine. Schoenheimer and his colleagues (20), using *l*-lysine labeled with N^{15} in the

alpha amino group and deuterium along the carbon chain, found it unique among the amino acids they studied in that transamination did not occur. In their experimental animals, rats, no reamination or synthesis of the carbon chain took place. Also the unnatural *d*-lysine was not utilized as a source of protein lysine (14). These workers did not make a detailed study of the separate organ proteins in their experimental animals. Lysine was chosen for the investigative program here because this apparent metabolic individuality makes it distinctive as a protein label. Labeled lysine isolated from a protein will be the same entity that was fed or otherwise administered.

The data presented here bear on the metabolic relationship of tissue and blood proteins, the comparative rates of turnover of the plasma protein albumin and globulin fractions, and the time necessary for hemoglobin formation in the intramedullary maturation of the red cell.

Data from Experiments D-3-L and D-7-L show that the general metabolic fate of the C^{14} of the lysine-ϵ-C^{14} was similar in these two normal dogs sacrificed 25 and 27 hours respectively after the amino acid was fed. About one-third of the fed C^{14} was incorporated in blood and tissues, predominantly as protein, about one-third appeared in the expired CO_2, and the remainder appeared in the urine. The high urine content is undoubtedly related to the *d*-lysine component of the racemic mixture fed. Data from Experiment D-7-L presented in Fig. 2 show the parallel relationship between non-protein C^{14} circulating in the plasma and rate of $C^{14}O_2$ excretion in expired air. This finding was closely duplicated in D-3-L.

The pattern of C^{14} distribution in tissues is basically similar to the pattern reported by other investigators following the feeding of tagged amino acids (5, 15, 18). It confirms the expectation that the finding that an organ has a high early uptake of one amino acid will in general be repeated when studies on other amino acids are carried out.

The high C^{14} content noted in the tissues of the gastrointestinal tract, particularly the duodenum, in the animals sacrificed 1 day after labeled lysine feeding is similar to findings reported after feeding other labeled amino acids including glycine (5), phenylalanine (15), and methionine (18). Some have concluded that a high concentration in the duodenum of the labeled material fed was referable to its functional activity in secreting enzyme proteins. The generally high intestinal tract activity suggests that the very rapid normal rate of regeneration of the mucosa may be of even greater significance.

Data presented by us elsewhere (11, 12) in a preliminary form indicate that this C^{14} found in tissue is predominantly in protein and concentrated largely but not exclusively in the lysine residues of the protein. Significant amounts of C^{14} are found in other amino acids including the carbon chains of glutamic acid, aspartic acid, and for some tissues, arginine. More extensive data on this subject will be presented elsewhere.

Synthesis and Utilization of Plasma Protein.—The data presented here are

perhaps of primary interest because of the information that can be derived concerning the rate of production and utilization of the blood proteins, particularly the protein components of the plasma.

First let us consider the plasma proteins as a whole. In the two experiments, D-4-L and D-8-L, (Fig. 5) in which the C^{14} content of the plasma proteins was followed after the transfusion of C^{14}-labeled plasma to recipient animals, one notes that the decline in specific activity (the amount of C^{14} per gm. carbon) is rather rapid at first but that the rate of decline decreases with time. In the first 24 hours the rate of decline is such that if continued at the same average rate the level would have reached 50 per cent of its original value in 28 hours in Experiment D-4-L and 29 hours in Experiment D-8-L. The decline during the next 72 hours in Experiment D-4-L is such as to correspond to a rate of reduction of specific activity of 50 per cent in 100 hours. For Experiment D-8-L a rate of reduction of 50 per cent in 120 hours can be computed from the decline in specific activity between the 39th and 144th hours.

It is to be noted that results such as these can only be produced by a preferential loss of the injected protein or by a dilution of the circulating plasma proteins by newly formed unlabeled proteins in the recipient animal. A nonspecific mass movement of proteins out of the circulation would have no effect on C^{14} abundance per gm. carbon; *i.e.*, specific activity. This is pertinent since in these animals, otherwise essentially normal, 133 ml. and 200 ml. plasma respectively was injected in excess of these animals' normal plasma volume. Assuming that these animals treated the injected plasma proteins in a manner indistinguishable from their own proteins, one is led to the conclusion that they did dilute or replace the plasma proteins circulating at the time of injection by about 50 per cent new plasma proteins in 30 hours and about 75 per cent in 150 hours.

These results are not inconsistent with results previously reported following the injection of labeled plasma proteins into recipient animals. Fink and coworkers (4) have reported that in similar experiments in which plasma proteins labeled by N^{15} contained in lysine residue were injected into normal dogs, the N^{15} concentration per gm. plasma protein had decreased to about 50 per cent of the initial value in 24 hours and to about 25 per cent of the initial value in 6 days. Very similar results are reported by Fine and Seligman following the transfusion into dogs of proteins labeled with radioactive sulfur (3). Also Heidelberger and coworkers (7) found that the rate of loss of injected antibody protein in rabbits was such that of the amount circulating 20 minutes following the injection, 62 per cent remained in circulation after 48 hours and 17 per cent after 168 hours despite a large bleeding and replacement transfusion between the two determinations.

The fact that, in the experiments reported here as well as those mentioned above, the rate of decline in specific activity is not linear when plotted on a

semilog paper suggests either that different components of the plasma proteins are utilized and regenerated at substantially different rates, a question discussed in more detail later, or that the circulating plasma proteins as a whole first come into equilibrium with a labile pool of protein of about equal amount at about 24 hours after injection. The importance of the latter mechanism is suggested by the observation, shown in Fig. 5, that the two principal fractions of the plasma proteins, albumin and globulin, each show this more rapid early fall in specific activity.

There exists a considerable amount of extravascular, extracellular protein in solution that may be collectively designated as "lymph protein." Studies on this protein (2) indicate that it is in constant interchange with plasma proteins and that if the plasma proteins were to come into equilibrium with it, the dilution would roughly account for the more rapid early phase of the decline in plasma protein specific activity. The decline in specific activity of plasma proteins produced by such a simple mixing with soluble "lymph" proteins ought not in any fundamental sense to be considered a true metabolism or "turnover" of plasma proteins. Thus these data suggest that the true time during which half the amino acid residue of the plasma proteins are replaced, presumably by the synthesis of new plasma protein molecules, is of the order of 100 to 120 hours. This mobilization of new plasma proteins occurred in spite of a preceding 48 hour fast in the recipient animal of Experiment D-4-L and the injection of substantial excesses of plasma in both animals, factors that should suppress rather than stimulate the mobilization of new plasma proteins.

Estimates by other authors, usually made from measurements of the abundance of labeled plasma proteins as a function of time following the feeding or injection of a tagged protein precursor, have tended to suggest that the rate at which plasma proteins are utilized and replaced in the circulation is considerably slower than is consistent with the maximum 100 to 120 hour period for utilization and replacement of 50 per cent of the plasma proteins reported above and derived from labeled plasma injection studies. For example, Schoenheimer, Ratner, Rittenberg, and Heidelberger (17) suggest about 2 weeks as the time necessary for such replacement in the rabbit, according to their terminology, the half-life of the plasma proteins. Data of this type obtained following the feeding of C^{14}-labeled lysine are found in Experiments D-1-L (Fig. 1), D-9-L (Fig. 6), and in Figs. 1 and 2 of the accompanying paper.

Attempts to deduce a turnover rate from such data obtained following feeding of a labeled amino acid are likely to give the erroneous impression that the turnover rate is slower than is in fact the case. This is because the labeled plasma proteins as they are metabolized and removed from circulation are replaced not by entirely unlabeled proteins, but rather by proteins again incorporating some of the labeling material which was initially built into other tissue proteins.

This point is illustrated by Experiment D-1-L. Between the 3rd and the 9th days some 300 ml. of plasma was removed containing approximately 53 per cent of the C^{14} circulating at the beginning of this period. This total protein deficiency was made up by the animal so rapidly by the synthesis of new plasma protein that there was no appreciable drop in total plasma protein concentration. Since the plasma removed accounts for the whole of the decline in C^{14} concentration per gm. plasma protein carbon over this period, any plasma proteins leaving the blood stream for metabolic purposes must have been replaced by new plasma protein of virtually the same C^{14} content.

The figure of 120 hours for utilization and replacement of one-half of the plasma proteins corresponds to an upper average time of existence of a protein entity in the plasma of 174 hours. The period during which an amount of plasma protein is synthesized corresponding to the total circulating plasma protein probably is not greater than 240 hours.

In normal dogs the circulating plasma is equivalent to about 4 per cent of the animal's weight. This plasma contains 7 per cent protein. Thus for a 10 kilo dog there would be synthesized every 10 days at least 28 gm. circulating plasma proteins or 2.8 gm. per day. By considering the extracellular proteins in solution, the "lymph" proteins, as roughly equal in magnitude to the plasma proteins and as essentially rapidly equilibrating components of the same system the total daily synthesis of plasma proteins for a 10 kilo dog is probably not less than 5 to 6 gm. protein. This is a substantial amount; the equivalent, for example, of 12 to 15 per cent of the 43 gm. of protein estimated in the liver of a 10 kilo dog.

The data from Experiment D-8-L plotted in Fig. 5 and the essentially similar results of Experiment D-4-L not presented in detail here on the specific activities of albumin and globulin fractions after injection of labeled plasma show that there is a more rapid decrease of C^{14} concentration per gm. globulin than per gm. albumin. The two curves are alike in that neither is linear when plotted on semilog paper and that both show a more rapid initial decline in specific activity corresponding in magnitude to a replacement in the plasma during the first 20 hours of about 40 per cent of both circulating albumin and globulin. It was earlier suggested that this rapid initial decline represented a mixing with extracellular lymph protein. Over a period of 144 hours, however, the C^{14} abundance per gm. globulin carbon had declined to 22 per cent of its initial value, that of the albumin fraction to only 40 per cent of its initial value.

Data presented in Figs. 3 and 4 from Experiments D-3-L and D-7-L show that in presumably normal dogs 24 hours after labeled lysine feeding, the amount of C^{14} incorporated into the globulin fraction of plasma protein was greater than that incorporated into the albumin fraction—about 50 per cent greater in the first experiment, and 100 per cent greater in the second. Also in Experiment D-9-L (Fig. 6) a dog depleted of plasma and reserve proteins

showed a similar response. In this instance the amount of C^{14} per gm. carbon was about 40 per cent higher in the globulin than in the albumin fraction 24 hours after the last of three daily feedings of C^{14}-labeled lysine. Subsequently on a stock diet the specific activity of the globulin fell more rapidly than that of the albumin so that 6 days later they were equal and in another 9 days the specific activity of the globulin was only 63 per cent of the albumin activity.

Such relative changes in specific activity, namely the greater incorporation of C^{14} into the plasma globulin than albumin during the period when this material was available for synthesis and more significantly the considerably shorter period required for the disappearance of labeled globulin than albumin, indicate a more rapid metabolic turnover of the components comprising the globulin fraction than of the albumin fraction. This suggests that an animal forced to manufacture excessive amounts of plasma proteins by extensive plasmapheresis would be more able to maintain plasma globulin than plasma albumin at something approaching normal levels. That this is in fact true has been verified by studies of plasma albumin and globulin levels following plasmapheresis in dogs (8).

Synthesis of Blood Cell Proteins.—In Experiment D-1-L with a dog essentially normal except for a preceding 72 hour fast as shown in Fig. 1, about 70 per cent of the total incorporation of C^{14} that occurred in hemoglobin in a 12 day period took place in the first 3 days. In Experiment D-2-L described in an accompanying paper, following the feeding of C^{14}-labeled lysine to a dog both anemic and hypoproteinemic, of the maximum incorporation of some 6.8 per cent of the fed C^{14} in blood cells over a 22 day period, some 80 per cent took place in the first 5 days.

Hemoglobin is the predominant substance in the red cell into which the C^{14} administered as lysine would be incorporated. These data, therefore, suggest that the period of bone marrow maturation of the red cell during which hemoglobin is synthesized does not exceed 3 to 5 days.

This is consistent with the observation that following the feeding or injection of small amounts of radioactive iron into dogs made anemic by bleeding, the incorporation of most of this iron into circulating hemoglobin occurs over a period of 3 to 5 days (6). Also this period corresponds to the time interval before a maximum reticulocyte response after a single massive bleeding.

SUMMARY

Racemic lysine labeled with C^{14} in the epsilon carbon position was fed to dogs. The distribution of C^{14} in blood and tissue fractions is recorded. In normal dogs sacrificed at 24 hours, approximately one-third of the C^{14} was found in the urine, one-third in expired air, and one-third in the body, mostly in protein, predominantly as lysine residues. The rate of C^{14} excretion as CO_2, hour by hour, paralleled closely the amount of non-protein C^{14} in the blood plasma.

The liver, kidney, pancreas, and spleen all have high values for C^{14} in 24 hour and 17 day experiments. The gastrointestinal tract is significantly high in the 24 hour experiments.

Plasma protein from animals previously fed C^{14} containing lysine and thus in turn labeled, was transfused into other dogs and the rate of disappearance of albumin and globulin fractions from the circulation of the recipient dog followed. The results lead to the conclusion that as a whole, plasma proteins are utilized and replaced at a rate of at least 10 per cent per 24 hours.

This minimum rate is substantially faster than turnover rates commonly accepted and emphasizes the rôle played by the plasma proteins in the protein economy of the body. The exact rate determination is made uncertain by the lack of knowledge of the magnitude of the amount of protein in solution in extracellular and lymph spaces and its rate of equilibrium with circulating plasma proteins.

Evidence from these transfusion studies indicates that plasma globulin is metabolized at a significantly faster rate than plasma albumin. This is confirmed by the observation that following the feeding of labeled lysine to dogs, C^{14} is first incorporated in globulin in high concentration but that later it also disappears more rapidly from the globulin fraction.

These data suggest that the period of bone marrow maturation of the red cell during which time its related hemoglobin is synthesized does not exceed 3 to 5 days.

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