CONVERSION OF PLASMA PROTEIN TO TISSUE PROTEIN WITHOUT EVIDENCE OF PROTEIN BREAKDOWN

RESULTS OF GIVING PLASMA PROTEIN LABELED WITH CARBON[14] PARENTERALLY TO DOGS*, ‡, §

BY C. L. YUILE, M.D., B. G. LAMSON, M.D., L. L. MILLER, M.D., AND G. H. WHIPPLE, M.D.

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

(Received for publication, February 16, 1951)

Downloaded from http://rupress.org/jem/article-pdf/93/6/539/1184626/539.pdf by guest on 25 June 2021

The role of plasma proteins in the general protein metabolism of the body is one of fundamental importance and is particularly suited to investigation by the newer radioisotope techniques. By feeding labeled amino acids to donor dogs it is possible to obtain labeled plasma proteins which in turn can be injected into recipient animals. Earlier reports (5, 8), using *dl*-lysine labeled with N^{15} and C^{14} were concerned only with the apparent rates of disappearance of plasma proteins, the experiments being limited by low activity which did not permit analysis of tissues nor of excretion products derived from the injected plasma. The preparation of ϵ-C^{14}-labeled *dl*-lysine with a specific activity some 20 times greater than that of the material originally available has made possible a detailed study in dogs of the distribution of C^{14} activity in the blood protein fractions and tissues as well as the excretion of C^{14} in both expired air and urine for periods up to 7 days following intravenous injection of single doses of labeled plasma protein. Zeldis and Madden (15) have studied the short term fate of transfused plasma protein labeled with radioactive sulfur.

Previous studies in this laboratory have clearly demonstrated that plasma proteins introduced parenterally into dogs can supply all the body protein needs over long periods of time (2, 6, 10, 11). That this participation in body metabolism is accomplished without evidence of profound protein breakdown is attested by the relative conservation of nitrogen which occurs during and after periods of parenteral plasma administration and also by the marked difference in the behavior of plasma protein given orally or intravenously to phlorhizinized dogs (7).

* The authors wish to thank L. Bruce Anderson, Jr., Ruth Kurtz, Nancy Lodge, and Virginia Webber for valuable assistance in this work.

‡ 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.

Recent studies on the fate of intravenously administered plasma proteins, given usually in the form of serum albumin, to human subjects both normal and with various illnesses have been reported by Albright *et al.* (1), Eckhardt *et al.* (4), and Waterhouse, Basset, and Holler (13). Measurements of conversion of plasma protein to that of protoplasm have been made on the basis of decreased phosphorus or potassium excretion (1, 13), the assumption being that plasma proteins, lacking potassium, phosphorus, and other ingredients of tissue protein, must pick these up during the process of conversion. Increased nitrogen excretion, if present, is used as a measure of that amount of protein which is deaminized after breakdown into amino acids, and finally the difference between these two fractions and the total amount of protein administered is taken as that portion which remains unchanged. These procedures undoubtedly indicate over-all changes in the metabolic picture but give little if any clue to a state of dynamic equilibrium or fluid exchange between cell and plasma protein. In all the above mentioned reports the question is raised concerning the manner in which plasma protein is converted into tissue protein. Albright's group conclude that their data offer no direct evidence for or against the hypothesis of conversion without prior breakdown to amino acids although some indirect evidence in its favor is provided by their studies on calcium metabolism. Workers in this laboratory have felt for many years that a partial catabolism of injected plasma protein with reassembly of large aggregates to form other specific types of cell and tissue protein serves better to explain the observed facts. On the other hand Eckhardt *et al.* (4) claim that a slow degradation of plasma protein with gradual release and more or less complete conservation of amino acids precedes its utilization by organ and tissue cells.

In the present series of experiments the quantitative determinations of C^{14} activity transfer from plasma to tissue proteins indicate that this transfer occurs at a rate compatible with the disappearance of the labeled protein from the circulation and extravascular fluid. Such transfer is accompanied by the excretion of only very small quantities of C^{14} from the lysine fraction. This C^{14} conservation coupled with the repeatedly observed retention of nitrogen following parenteral plasma protein injection would seem to favor the theory of partial as opposed to complete catabolism in so far as the metabolism of plasma protein is concerned.

Methods

Descriptions of the dogs and the procedures carried out in each experiment are included in the individual experimental histories below.

Preparation of the Labeled Plasma.—(See experimental histories of donor dogs.) The *dl*-lysine-ε-C^{14} fed to the donor dogs was synthesized in the Department of Organic Chemistry, The University of Rochester, under the direction of Dr. R. W. Helmkamp (9).

Diet.—The low protein diet, a sucrose, lard, bone ash mixture with added vitamins, containing 0.12 gm. N per cent was fed in amounts calculated to supply approximately 100 calories per day per kilo of body weight.

Downloaded from http://rupress.org/jem/article-pdf/93/6/539/1184626/539.pdf by guest on 25 June 2021

Methods pertaining to the collection of expired carbon dioxide; the estimation of total plasma protein, albumin and globulin; the preparation of red blood cells and tissues for C^{14} analysis and the determination of C^{14} activity have been reported in a previous publication (8).

The protein content of each tissue was based on determinations of nitrogen by the Kjeldahl method after drying by the lyophile process and grinding. As a check, nitrogen and C^{14} determinations were made on 5 or 6 selected tissues from each dog after trichloracetic acid and acetone extraction. Essentially similar results were obtained by both procedures.

Urine samples were preserved under toluene at 4°C. Total nitrogen was determined by the macro-Kjeldahl method and urea and ammonia nitrogen by the aeration method of Van Slyke and Cullen (12). Aliquots of urine, plasma, and other fluids were prepared for C^{14} assay by passing a slow stream of air through the digestion flasks, warmed to about 35°C. until the material was dry.

The amino acids, lysine, glutamic acid, aspartic acid, and arginine were isolated from acid hydrolysates of plasma and liver protein by appropriate methods and their identity and purity were checked by paper chromatography.

Histories of the Experimental Animals

Donor dog 44-10 was a mongrel, adult, male collie. Over a period of 8 weeks a moderate degree of protein and hemoglobin depletion was induced by a diet almost devoid of protein and intermittent bleedings to a total of 1620 ml. during the first 4 weeks and 80 ml. during the second 4 weeks. The plasma protein concentrations fell from 6.69 to 4.92 gm. per 100 ml., hemoglobin,—which initially was 16.8 gm. per 100 ml.,—fell to 9.0 gm., and rose during the last few weeks to 12.0 gm. per 100 ml., while the weight fell from 16.8 to 13.8 kilos. At the end of this depletion period a total of 0.668 gm. of *dl*-lysine-ϵ-C^{14} incorporating 467 microcuries was fed in two approximately equal doses on consecutive days. The labeled lysine was mixed with 50 gm. of ground, cooked liver and several hours later an additional 40 gm. of liver and 100 gm. of the low protein diet were fed. Twenty-four hours after the second lysine feeding the animal received 200 gm. of liver and 100 gm. of low protein diet. At 48 hours 225 ml. of blood was withdrawn into heparin to supply 135 ml. of plasma for injection into dog 48-106 (Experiment 1).

Donor dog 49-117 was an adult, male, spaniel mongrel, weighing 14.0 kilos. Protein and hemoglobin depletion was induced by a low protein diet supplemented daily with 50 gm. of horsemeat and bleedings, at 3 to 4 day intervals, totalling 1050 ml., over a period of 4 weeks. Plasma protein concentration dropped from 5.54 to 4.33 gm. per 100 ml. and the hemoglobin from 16.5 to 9.2 gm. per 100 ml. The dog's weight remained constant. At the end of the depletion period the diet was changed to 100 gm. of low protein mixture plus 150 gm. of cooked, ground liver. On the 2nd day of this regime 229.1 mg. of *dl*-lysine-ϵ-C^{14} was added and on the 3rd day 312.5 mg. of *dl*-lysine-ϵ-C^{14} was added to the diet. The total activity fed as lysine amounted to 450 microcuries. Forty hours after the second feeding of labeled lysine, 250 ml. of blood was withdrawn to supply 133 ml. of plasma for injection into dog 50-37 (Experiment 2) and 48 hours later a second bleeding of 250 ml. supplied 137 ml. of plasma for injection into dog 49-131 (Experiment 3).

Experiment 1, dog 48-106, an adult, female, mongrel hound weighing 9.0 kilos, was placed on a diet of low protein content equal to 100 calories per kilo for 7 days. This was supplemented on the first 2 days with 16 gm. of horsemeat. After 5 days on a constant nitrogen intake of approximately 0.19 gm. per day during which time the urinary nitrogen output was relatively constant, averaging 1.13 gm. per day, 135 ml. of C^{14}-labeled plasma from donor dog 44-10 was injected intravenously after the removal of 100 ml. of blood. The protein content of the injected plasma was 7.13 gm. in which protein, 94.75 per cent of the total C^{14} activity of 3.15 microcuries was incorporated. During the next 2 days, low protein diet was

Downloaded from http://rupress.org/jem/article-pdf/93/6/539/1184626/539.pdf by guest on 25 June 2021

fed as before, and six blood samples were collected at times indicated in Table 1 for total protein nitrogen and C^{14} analysis.

Albumin and globulin determinations were made on the second, fourth, and sixth samples. Carbon dioxide was collected from the expired air during four periods of 1 hour on the 1st day and two periods on the 2nd day. Two 24 hour samples of urine were obtained. Urinary nitrogen output was not significantly increased over the baseline and the total C^{14} excretion was measured. After 49 hours the dog was viviperfused under light ether anesthesia with removal of 78 per cent of the circulating plasma protein and 77 per cent of circulating red cells in the perfusate. Aliquots of the relatively blood-free tissues listed in Table 5 were taken for analysis. Immediately before bleeding and labeled plasma injection the hematocrit reading, was 54 per cent, the estimated plasma volume 298 ml., and the plasma protein concentration 6.07 gm. per 100 ml. At the time of perfusion the hematocrit reading had fallen to 38 per cent, the plasma protein concentration to 5.45 gm. per 100 ml., and the estimated plasma volume was 372 ml.

Experiment 2, dog 50-37 was an adult, female, spaniel mongrel weighing 8.9 kilos. Prior to the injection of labeled plasma, this dog was fed low protein diet, approximately 100 calories per kilo for 6 days with 16 gm. of horsemeat added for 4 days. The animal was in negative nitrogen balance throughout this period with an average urinary nitrogen output of 1.31 gm. on each of the last 2 days. In the 7 days following labeled plasma injection the only food intake was the low protein diet as before, with complete consumption assured by spoon feeding when necessary. Urinary nitrogen excretion remained fairly constant at a daily average of 1.14 gm. The body weight remained unchanged.

After removal of 100 ml. of blood, 133 ml. of labeled plasma from donor dog 49-117 was injected intravenously. This contained 6.55 gm. labeled plasma protein incorporating 95.8 per cent of the 3.91 microcuries of total C^{14} activity. During the first 48 hours seven blood samples were drawn at intervals indicated in Table 2, for nitrogen and C^{14} determinations of total plasma protein, albumin and globulin. Daily samples were collected thereafter. The C^{14} activity of measured volumes of washed, packed red blood cells was determined daily. Carbon dioxide from the expired air was collected for periods of 1 hour, four times during the first 24 hours, twice during the second 24 hours, and daily from the 3rd to 6th days. All urine was collected, the bladder being catheterized and rinsed at the end of each period. The output of the 1st day was collected in 4 periods, of the 2nd day in 2 periods, and of the remaining 5 days for 24 hour periods. Total nitrogen, urea and ammonia nitrogen, and C^{14} activity were determined on each urine sample.

Seven days after the injection of labeled plasma the dog was viviperfused under light ether anesthesia with modified Ringer's solution plus glucose for 45 minutes. Estimated recovery of circulating plasma protein was 89 per cent and of red blood cells 85 per cent of the amount present at the time of perfusion. A complete autopsy was performed and aliquots of the relatively blood-free tissues were taken for nitrogen and C^{14} analysis.

The hematocrit reading which was 45.5 per cent before bleeding and plasma injection fell to 36.9 per cent on the 7th day. Plasma protein concentration, 5.70 gm. per 100 ml. initially, remained fairly constant (see Table 2). Plasma volumes at the beginning and end of the experiment were 395 ml. and 380 ml. respectively.

Experiment 3, dog 49-131 was a smooth-haired, female mongrel weighing 9.75 kilos. An attempt was made to produce a moderate degree of protein depletion by a combination of low protein diet, providing 100 calories per kilo, and 4 intermittent bleedings totalling 435 ml. over a period of 3 weeks. During this time the body weight fell to 8.20 kilos, the hematocrit reading decreased from 60 per cent to 35.5 per cent, and the plasma protein concentration from 6.15 to 5.90 gm. per 100 ml. However, there was no significant drop in the total amount of circulating protein. During the 3 weeks before injection of labeled plasma the dog was in negative

Downloaded from http://rupress.org/jem/article-pdf/93/6/539/1184626/539.pdf by guest on 25 June 2021

nitrogen balance. For the final 7 days the daily nitrogen output averaged 1.58 gm. per day with a daily intake of less than 0.2 gm. of nitrogen. Without prior bleeding except for sampling, 137 ml. of plasma from donor dog 49-117 was injected in 12 minutes. This contained 7.20 gm. protein incorporating 96.5 per cent of the C^{14} activity of 2.8 microcuries. The schedule of blood sampling, collection of urine and of CO_2 from expired air was essentially similar to that outlined for Experiment 2, dog 50-37.

Following the injection of labeled plasma, daily injections of normal dog plasma were given for 6 days. These injections ranging between 100 and 125 ml. contained from 6.45 to 7.70 gm. of plasma protein for a total of 41.1 gm. This was the sole source of nitrogen intake except for the small amount in 180 gm. of low protein diet consumed daily throughout the experimental period. During this week the dog showed a slight positive nitrogen balance with an average daily net intake of 1.21 gm. and urinary output of 0.97 gm. nitrogen. The latter figure is lower than during the control period indicating some retention of nitrogen. Plasma

TABLE 1

Disappearance of Labeled Plasma Proteins from Circulation

Experiment 1, dog 48-106. 135 ml. C^{14}-labeled plasma intravenously.

Time after injection	Plasma prot.	Per cent dose C^{14} per gm.			Alb./Glob. ratio-chem.	Alb./Glob. ratio-C^{14}	Total circulating activity
		Total plas. prot.	Plasma alb.	Plasma glob.			
	gm./100 ml.						*per cent of dose*
1 hr.	4.92	4.13	—	—	—	—	80
5 hrs.	5.38	3.57	3.00	3.95	0.74	0.56	75
11 "	5.12	3.22	—	—	—	—	62
23 "	5.18	2.42	1.84	3.02	0.88	0.54	50
30 "	4.94	2.37	—	—	—	—	—
49 "	5.45	2.10	1.76	2.65	0.90	0.47	36

protein concentrations in the 7 days after labeled plasma injection are listed in Table 3. Plasma volume before injection was 445 ml. and terminally 470 ml. The final hematocrit reading was 34.0 per cent. At the end of the experimental period (7 days) the dog was subjected to viviperfusion and autopsy as in Experiments 1 and 2. Estimated recovery of plasma protein was 89.5 per cent and of red blood cells 88.0 per cent of the amount estimated to be in circulation at the time of perfusion. Tissue samples for nitrogen and C^{14} analysis were taken as indicated below (Table 5).

EXPERIMENTAL OBSERVATIONS

Tables 1, 2, and 3 list the pertinent data from all three experiments relative to the decline in C^{14} activity in total plasma proteins and in albumin and globulin fractions. The decline in C^{14} activity per gram of total plasma protein in each instance resulted in a decrease to approximately 50 per cent of the original activity during the first 24 hours after injection of labeled plasma. Following this initial period the C^{14} concentration of the plasma protein decreased more slowly. In Experiment 1 (dog 48-106) which was terminated at the end of 2 days the C^{14} per gram plasma protein decreased 13 per cent during the final

Downloaded from http://rupress.org/jem/article-pdf/93/6/539/1184626/539.pdf by guest on 25 June 2021

TABLE 2

Disappearance of Labeled Plasma Proteins from Circulation

Experiment 2, dog 50-37. 133 ml. C^{14}-labeled plasma intravenously.

Time after injection	Plasma prot.	Per cent dose C^{14} per gm.			Alb./Glob. ratio–chem.	Alb./Glob. ratio–C^{14}	Total circulating activity
		Total plas. prot.	Plasma alb.	Plasma glob.			
	gm./100 ml.						*per cent of dose*
25 min.	4.57	3.30	2.36	4.10	0.84	0.48	80
3 hrs.	5.05	2.75	2.14	3.25	0.82	0.54	72
6 "	5.30	2.46	2.04	2.72	0.67	0.50	66
12 "	5.38	2.06	1.62	2.38	0.70	0.48	51
22 "	5.50	1.78	1.60	1.91	0.71	0.58	45
28 "	5.50	1.49	1.41	1.50	0.81	0.76	38
47 "	5.90	1.39	1.54	1.28	0.59	0.71	33
3 days	5.65	1.20	1.49	1.02	0.63	0.93	27
4 "	5.20	1.18	1.26	1.07	0.83	0.94	23
5 "	5.03	1.10	1.36	0.92	0.72	1.08	21
6 "	5.50	0.94	1.07	0.84	0.82	1.05	20
7 "	5.50	0.89	1.02	0.81	0.83	1.06	19

TABLE 3

Disappearance of Labeled Plasma Proteins from Circulation

Experiment 3, dog 49-131. 137 ml. C^{14}-labeled plasma intravenously.

Time after injection	Plasma prot.	Per cent dose C^{14} per gm.			Alb./Glob. ratio–chem.	Alb./Glob. ratio–C^{14}	Total circulating activity
		Total plas. prot.	Plasma alb.	Plasma. glob.			
	gm./100 ml.						*per cent of dose*
10 min.	5.79	3.03	2.90	3.02	0.54	0.51	99
1 hr.	6.22	2.46	2.08	2.62	0.56	0.45	83
5 hrs.	5.95	2.23	1.98	2.32	0.55	0.46	65
10 "	6.47	2.02	1.77	2.12	0.57	0.48	62
24 "*	6.15	1.75	1.59	1.80	0.49	0.43	48
28 "	5.93	1.64	—	—	—	0.48	45
2 days*	5.80	1.49	1.25	1.60	0.63	0.49	40
3 "*	6.75	1.04	1.08	1.00	0.64	0.69	33
4 "*	7.00	0.88	0.89	0.78	0.77	0.87	28
5 "*	7.66	0.70	0.81	0.61	0.66	0.88	25
6 "*	7.40	0.60	0.70	0.48	0.67	1.68	21
7 "	8.08	0.48	0.72	0.31	0.72	1.55	18

* Non-labeled plasma intravenously.

26 hours. In Experiment 2 (dog 50-37) the specific C^{14} activity of the plasma protein was reduced by 48 per cent during the last 6 days of the experiment.

Downloaded from http://rupress.org/jem/article-pdf/93/6/539/1184626/539.pdf by guest on 25 June 2021

In Experiment 3 (dog 49-131) in the equivalent time interval the specific C^{14} activity of the plasma protein showed a decrease of 73 per cent. This greater reduction of C^{14} concentration in the plasma protein in Experiment 3 was related to 6 daily injections of unlabeled plasma protein totalling 40 gm. associated with a rise in plasma protein concentration from 6.15 to 8.08 gm. per 100 ml.

The C^{14} concentration of both the albumin and globulin fractions showed a rapid decline, comparable to that of total plasma protein during the first 24 hours. Subsequently the specific activity of both fractions fell more slowly but the decrease in globulin activity was more rapid than that of albumin. In Experiment 2 the C^{14} concentration of albumin fell 37 per cent and the globulin activity 55 per cent during the last 6 days while in the same period of Experiment 3 the decreases in albumin and globulin activity were 57 and 83 per cent respectively. The incomplete data for Experiment 1 indicate a drop of 4 per cent for albumin and 12 per cent for globulin during the 2nd day. Albumin/globulin ratios show relatively little change while in the two longer experiments the ratio of albumin C^{14} per 100 ml. to globulin C^{14} per 100 ml. began to increase after remaining constant for 1 day in dog 50-37 and 2 days in dog 49-131. The disappearance curves of C^{14} activity of albumin and globulin (not shown) indicate that the initially higher concentration of C^{14} in globulin becomes equal to that of albumin at 36 hours in Experiment 2 and at 60 hours in Experiment 3. Variation in the initial albumin and globulin activities in the different animals is related to differences in the C^{14} albumin/globulin ratios in the donor plasma which were 0.47 for Experiment 1, 0.54 for Experiment 2, and 0.64 for Experiment 3.

The final column of Tables 1 to 3 lists the total circulating C^{14} activity as per cent of dose at the various time intervals. Values for Experiment 1 are based on calculated estimates of plasma volume while those in Experiments 2 and 3 were derived from direct measurements of plasma volume by the dye method, before the injection of labeled plasma and on the 7th day. There is a close correlation between the decreases in total circulating activity and activity per gram plasma protein.

Fig. 1 illustrates the rate of excretion of C^{14} in the carbon dioxide of expired air following the injection of labeled plasma in the 3 experiments. The curves are derived by plotting the amount of C^{14}, expressed as a percentage of the total dose, contained in the carbon dioxide expired during each 1 hour collection period, against time. The relatively high rate of $C^{14}O_2$ excretion during the first 6 hours by dogs 48-106 (Experiment 1) and 50-37 (Experiment 2) is attributed to the slightly higher percentage of non-protein C^{14} in the plasma received by these two animals, 5.25 and 4.20 per cent respectively, as compared to the plasma given to dog 49-131 which contained 3.5 per cent non-protein C^{14}. Plasma used for Experiments 1 and 2 was the first to be withdrawn from

Downloaded from http://rupress.org/jem/article-pdf/93/6/539/1184626/539.pdf by guest on 25 June 2021

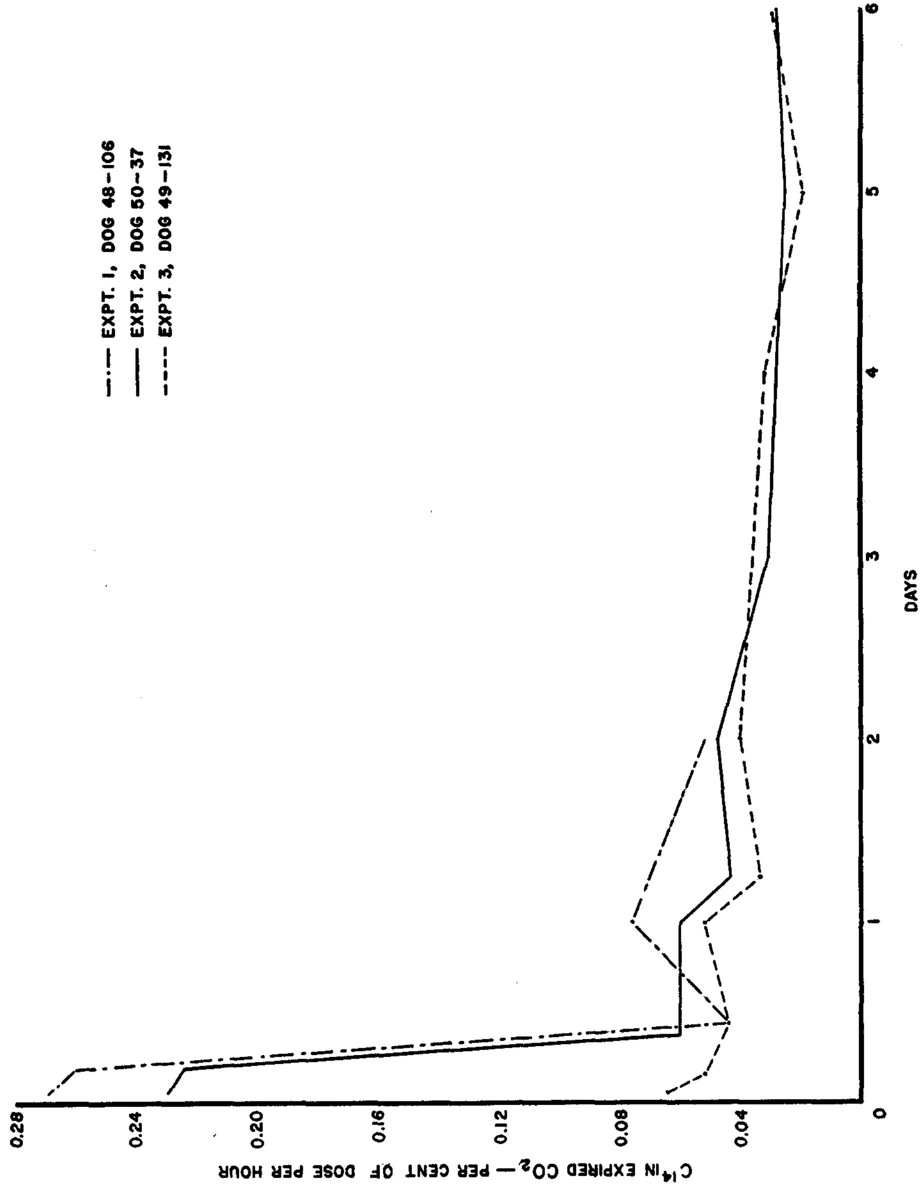

FIG. 1. Rate of C^{14} excretion in expired air. Experiments 1, 2, and 3.

Downloaded from http://rupress.org/jem/article-pdf/93/6/539/1184626/539.pdf by guest on 25 June 2021

donor dogs 44-10 and 49-117, 40 and 48 hours after they had been fed C^{14}-labeled *dl*-lysine, whereas the plasma given to dog 49-131 was obtained from donor 49-117 after a further lapse of 48 hours when a smaller amount of non-protein C^{14} would be expected. After the 2nd day the rate of C^{14} excretion in the expired CO_2 reached a relatively constant low level in Experiments 2 and 3.

The urinary excretion of C^{14} in Experiment 1, dog 48-106, amounted to 0.40 per cent of the total activity in the injected plasma during the first 24 hour period and 0.13 per cent from 24 to 48 hours, while data relative to C^{14} excretion in the urine for Experiments 2 and 3 are shown in Fig. 2. Fractional col-

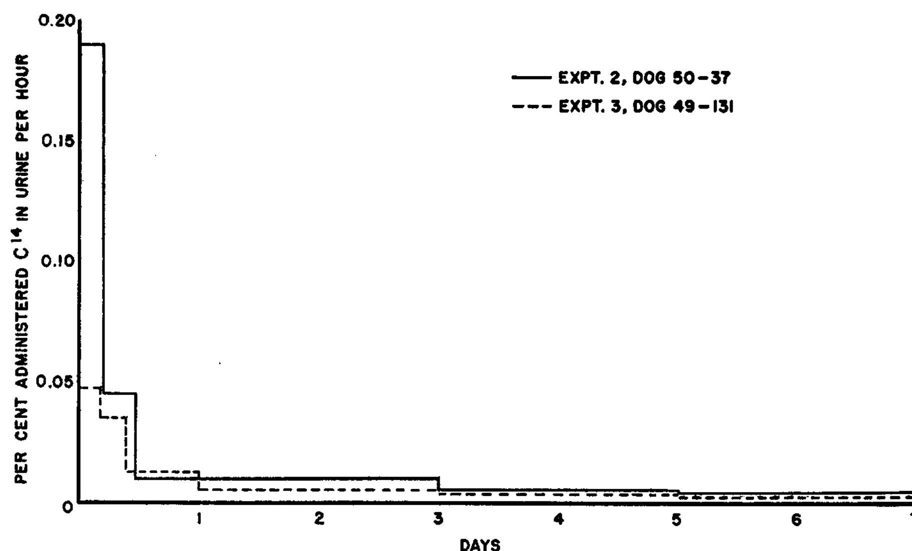

FIG. 2. Excretion rate of C^{14} in urine. Experiments 2 and 3.

lection of urine samples during the first 48 hours indicated a rapid decrease in the hourly excretion rate of C^{14} within the first 12 hours and a subsequent very slow decline. As with $C^{14}O_2$ excretion this is probably related to residual non-protein activity and possibly to the presence of traces of *d*-lysine in the injected plasma. In Experiment 2 the total 7 day urinary excretion of C^{14} was 2.5 per cent of the dose, 60 per cent being accounted for by the output in the first 24 hours and in Experiment 3 the total was 1.3 per cent of which 41 per cent appeared during the 1st day.

The data on urinary nitrogen excretion are summarized in Table 4. It will be noted that in no instance does the urinary nitrogen increase significantly and that there is definite conservation of nitrogen by the animal receiving daily plasma injections (Experiment 3). The proportional excretion of urea and ammonia nitrogen (not listed) showed no significant alteration in Experiment

Downloaded from http://rupress.org/jem/article-pdf/93/6/539/1184626/539.pdf by guest on 25 June 2021

2 but fell from 75 to 60 per cent in Experiment 3. In the second to last column of Table 4 are listed the protein equivalents of the total nitrogen excreted during each experiment. Presumably most if not all of this nitrogen was derived from blood or tissue protein in view of the almost negligible dietary intake. The figures in the final column, Table 4, raise an interesting question concerning the source of this urinary nitrogen, since the total amount of C^{14} excreted, including the relatively large fraction lost in the first few hours after injection, corresponds to the activity of considerably less than 1 gm. of

TABLE 4

Summary of Urinary Nitrogen Excretion Data

Dog No.	Duration of experiment	Plas. prot. injected	Control urine N-average	Experimental period-urine N-average	Total urine N output	Prot. equivalent	Plas. prot. equivalent of total urinary C^{14}
	days	*gm.*	*gm./day*	*gm./day*	*gm.*	*gm.*	*gm.**
48-106 (Expt. 1)	2	7.1 labeled—1 injection	1.13	1.35	2.70	16.9	0.12
50-37 (Expt. 2)	7	6.55 labeled—1 injection	1.31	1.14	7.97	50.5	0.70
49-131 (Expt. 3)	7	7.20 labeled—day 1					
		41.1 unlabeled—6 daily injections	1.58	0.97	6.80	42.5	0.50

* Corresponds to highest level of plasma protein activity on 1st day of experiment.

plasma protein in the circulation of each animal at the beginning of the experiment. This point will be discussed in more detail below.

The *relative tissue activities* found in each experiment are illustrated in Figs. 3 to 5. They are expressed as per cent administered C^{14} per gram of tissue protein and correspond in a general way to the distribution following labeled lysine feeding, reported previously (8). A notable exception is the adrenal which is consistently one of the most active tissues. However, in the earlier experiments C^{14} activity was related to total carbon content, while the present figures are based on nitrogen content of fat-free tissue.

Table 5 lists the total percentage of injected C^{14} found in the various organs and tissues. It must be borne in mind that values include the activity of all plasma proteins in the extravascular, extracellular fluid, and in any blood not

Downloaded from http://rupress.org/jem/article-pdf/93/6/539/1184626/539.pdf by guest on 25 June 2021

removed by viviperfusion, as well as the activity actually incorporated into the tissue proteins. This fact must be taken into consideration in any attempt to estimate the actual transfer of activity to tissue proteins and it indicates that in the short term experiment on dog 48-106, the tissue protein activities shown in Fig. 3 and Table 5 are relatively much too high, since the plasma

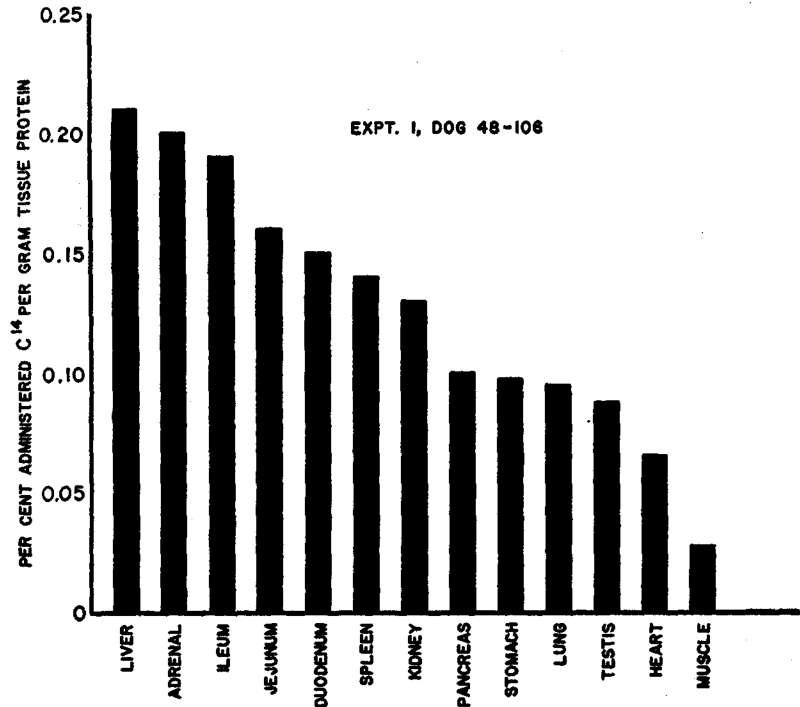

FIG. 3. Dog 48-106, Experiment 1. Relative C^{14} activity of blood-free tissues 49 hours after intravenous injection of C^{14}-labeled plasma. The values shown include all extravascular fluid protein activity.

protein activity at the time of perfusion of this animal was 4 to 5 times greater than that at the termination of Experiments 2 and 3.

This point is further illustrated in Table 6 in which a final balance is struck between the C^{14} administered intravenously as labeled plasma and the total activity recovered. While the bony skeleton, bone marrow, and lymphoid tissues were not included in any of the total tissue figures, determinations of skin and areolar tissues were omitted in Experiment 1. The sum of these two items, calculated from estimates of total organ weight as a percentage of body weight, accounted for about 16 per cent of the activity in Experiments 2 and 3. The figures for plasma and perfusate represent 78, 89, and 89.5 per cent of the es-

Downloaded from http://rupress.org/jem/article-pdf/93/6/539/1184626/539.pdf by guest on 25 June 2021

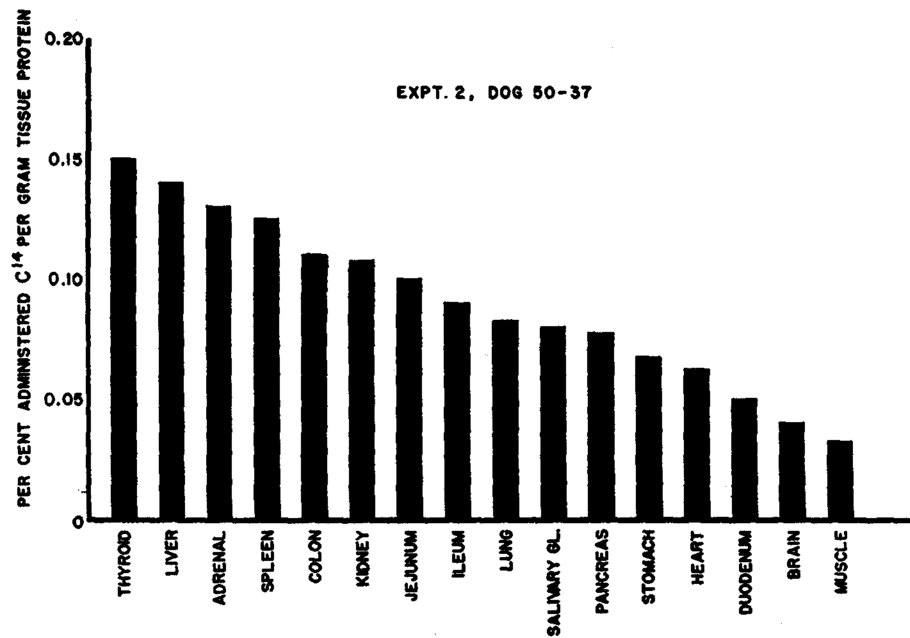

FIG. 4. Dog 50-37, Experiment 2. Relative C^{14} activity of blood-free tissues 7 days after intravenous injection of C^{14}-labeled plasma. Extravascular fluid protein activity is included.

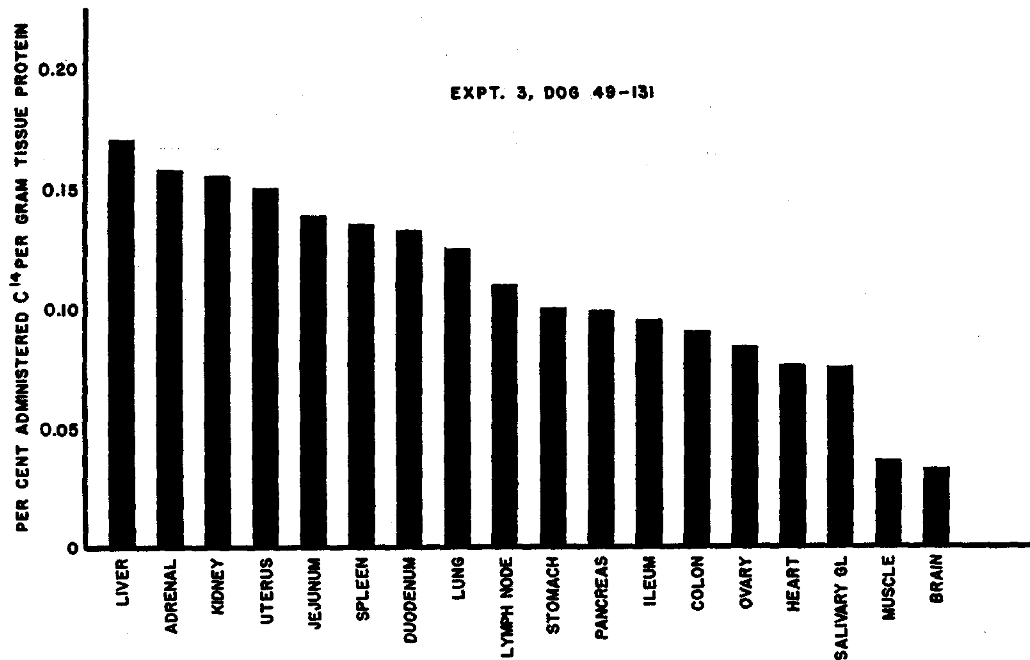

FIG. 5. Dog 49-131, Experiment 3. Relative C^{14} activity of blood-free tissues 7 days after intravenous injection of C^{14}-labeled plasma. Extravascular fluid protein activity is included.

Downloaded from http://rupress.org/jem/article-pdf/93/6/539/1184626/539.pdf by guest on 25 June 2021

timated circulating plasma protein activity in Experiments 1, 2, and 3 respectively.

An attempt to measure the activity in a small sample of red blood cells at the end of Experiment 1 was unsatisfactory. However, in the two longer experiments larger samples were used and a definite pattern emerged. Very little activity was detected at 24 hours and at 48 hours it was less than 0.01 per cent

TABLE 5

Total C^{14} Tissue Recovery after Intravenous Injection of Labeled Plasma

Source of C^{14}	Per cent administered C^{14}		
	Dog 48-106 (49 hrs.)	Dog 50-37 (7 days)	Dog 49-131 (7 days)
Heart	0.64	0.73	0.69
Lungs	0.88	0.68	1.12
Spleen	0.25	0.32	0.78
Stomach	1.82	0.85	1.14
Duodenum	0.60	0.31	0.36
Jejunum	1.57	0.66	1.11
Ileum	2.36	1.47	1.80
Colon		0.50	0.61
Pancreas	0.12	0.19	0.22
Liver	7.62	7.20	7.05
Adrenals	0.06	0.03	0.02
Kidneys	0.85	0.56	0.79
Salivary glands		0.07	0.12
Thyroid		0.04	
Skeletal muscle	21.90	22.60	28.75
Skin		13.80	12.60
Brain		0.28	0.21
Uterus			0.18
Testes	0.10		
Fat		2.45	4.10
Total	38.77	52.74	61.65

of the dose per gm. hemoglobin. During the next 5 days there was a progressive apparently linear increase to a level of about 0.08 per cent administered C^{14} per gm. hemoglobin. This was approximately equal to that of the lower intermediate tissue activities and on the basis of total circulating hemoglobin accounted for about 4 per cent of the dose, as seen in Table 6.

The four amino acids, lysine, glutamic acid, aspartic acid, and arginine, were separated from hydrolysates of plasma and liver protein from each of the three dogs, the plasma proteins being precipitated from the fluid removed at the time of viviperfusion. In each instance the specific activities of glutamic and aspartic acid derived from plasma protein were less than 1 per cent of the

Downloaded from http://rupress.org/jem/article-pdf/93/6/539/1184626/539.pdf by guest on 25 June 2021

specific activity of lysine from the same source while no significant activity was detected in any of the plasma arginine samples. Of the four amino acids isolated from liver protein only lysine contained measurable C^{14} activity. If the specific activities of the other amino acids in the liver protein in relation to lysine activity were proportional to those found in plasma protein, detectable activity would not be expected in samples small enough to use in the C^{14} measuring apparatus, and no significant activity was found on measurement. Ratios of C^{14} specific activity of lysine to total protein were approximately equal for both plasma and liver in each experiment.

TABLE 6

Total C^{14} Recovery after Intravenous Injection of Labeled Plasma Protein

Source of C^{14}	Experiment 1	Experiment 2	Experiment 3
	Dog 48-106 49 hrs.	Dog 50-37 7 days	Dog 49-131 7 days
	Per cent administered C^{14}		
Tissues	38.77*	52.74	61.65
Plasma and perfusate	34.50	16.00	16.70
Plasma removed in sampling	5.80	5.60	7.90
Expired CO_2	5.20	6.73	4.92
Urine	0.50	2.50	1.31
Red blood cells	‡	4.00	4.33
Total recovery	84.77	87.57	96.81

* Brain, bone marrow, skeleton, integument, colon, lymphoid, and areolar tissues not sampled.

‡ C^{14} activity in 1 ml. packed red blood cells insignificant.

DISCUSSION

The data presented relative to the disappearance rates of C^{14} activity from the total protein, albumin and globulin, of the plasma following labeled plasma injection confirm the previously reported observations of a rapid initial decline followed by a slower phase during which the specific activity of globulin falls more rapidly than that of albumin (8). It has been suggested, on the basis of the 50 per cent drop in specific activity of the total protein as well as of the albumin and globulin fractions during the first 24 hours, that equilibrium is reached during this time largely by simple mixing with a quantity of protein in the extravascular, extracellular fluids qualitatively similar to and approximately equal to the circulating plasma proteins.

Added confirmation that such an estimate of the mass of non-circulating plasma protein at any given time is reasonably correct is obtained from Experiment 3 (dog 49-131) in which injections of unlabeled plasma were given on each

Downloaded from http://rupress.org/jem/article-pdf/93/6/539/1184626/539.pdf by guest on 25 June 2021

of the 6 days immediately following the administration of labeled plasma. The combined plasma protein in these 6 injections amounted to 41.1 gm. On the final day of the experiment the specific activity of this dog's plasma proteins had fallen to 28 per cent of that found on the 2nd day, as contrasted with a decline of 50 per cent during the same time interval of Experiment 2 (dog 50-37) in which only a single injection of labeled plasma was given. A summation of the estimated metabolic utilization of the plasma protein added in each injection, based on the observed rate of disappearance of labeled protein and the assumption that equilibrium is reached within 24 hours with a mass of plasma protein roughly twice the size of that in circulation, indicates that approximately 15 gm. would remain in the circulation on the 7th day. The estimated total circulating plasma protein at this time was 38 gm. and the specific C^{14} activity was slightly more than half that observed on the same day in dog 50-37 which had received no additional plasma by vein. This would represent a dilution by unlabeled plasma of about 17 gm. The fact that this figure corresponds closely to that derived by the above calculation lends strength to the assumptions on which the calculation was based.

It has been shown in human subjects that repeated injections of serum albumin may increase the volume of extravascular, extracellular fluid to a degree proportionally greater than that occurring in the circulating plasma (14). While such a shift due to repeated plasma injections in Experiment 3 might have increased the total protein content of the extravascular fluid to some extent it would not have affected the dilution of C^{14} in the plasma proteins remaining in equilibrium in the two compartments.

In the two longer experiments the magnitude of C^{14} activity transfer from plasma proteins to tissue proteins can be stated with reasonable accuracy. The total tissue activity values shown in Table 6, of 52.7 per cent for Experiment 2 and 61.7 per cent for Experiment 3 include in addition to C^{14} activity actually in protoplasm all the activity of proteins in solution in the extravascular, extracellular fluid as well as small amounts of residual circulating plasma protein activity not removed by perfusion. As previously discussed, it would seem justifiable to assume that the extravascular protein portion of the total body activity is at least equal to the activity in the circulating plasma. By applying the necessary corrections the total maximum net tissue activities are therefore 33.2 and 41.0 per cent of the administered C^{14} in Experiments 2 and 3 respectively.

Approximately similar figures for tissue activity can be arrived at by a different type of calculation. In Experiment 2 (dog 50-37), roughly 23 per cent of the dose disappeared from the circulation during the last 6 days of the experiment and presumably a similar amount was removed from the extravascular fluid compartment making a total of 46 per cent. During this time about 12 per cent of the administered C^{14} could be accounted for by incorporation

Downloaded from http://rupress.org/jem/article-pdf/93/6/539/1184626/539.pdf by guest on 25 June 2021

into red blood cells, excretion, and sampling, leaving 34 per cent for conversion into protoplasm. In Experiment 3 (dog 49-131), with a loss of approximately 30 per cent of the injected activity from the circulation during the last 6 days and 13 per cent accounted for by red cell incorporation, excretion, and sampling, a figure of 47 per cent for tissue conversion is obtained. These values are in the same range as those arrived at by subtracting the activity found in circulation at the time of perfusion from the activity of all the tissues measured. One is still left in doubt about the cause of the difference in net tissue activities in the two 7 day experiments. The somewhat higher over-all tissue activity in the animal which received daily plasma protein injections could be due either to increased conversion of plasma to tissue protein or to an increase in total "lymph" protein activity associated with a shift of fluid to the extravascular compartment. In view of the incomplete tissue data for Experiment 1 as well as the much greater plasma to tissue activity ratio after only 2 days it is impossible to make any accurate estimate of actual tissue incorporation although an absolute maximum would appear to be approximately 9 per cent.

While the above figures indicate the total conversion of plasma to tissue protein in a given period of time they provide little information regarding the rate at which such transfer occurs. By computing the percentage decline in circulating activity for each 24 hour period after the 1st day it is found that smaller and smaller proportions of the total circulating activity leave the plasma as time goes on. In Experiment 2, 20 per cent of the activity in circulation at 24 hours had disappeared by 48 hours while the rate of removal during the 7th day was only 5 per cent. Corresponding figures for Experiment 3 associated with daily plasma injections were 20 and 13 per cent. Differences in the rate of conversion of plasma protein to protoplasm are probably related to a variety of factors such as the state of protein repletion, the presence or absence of other substrates, and possibly the influence of certain hormones.

The relative tissue activities shown in Figs. 4 and 5 indicate that in general those organs which would be expected to have the greatest metabolic activity are found to have the highest C^{14} activity per gram of protein. The rough parallelism between the degree of activity found in any given organ or tissue and the reported values for protein concentration of lymph from the same organ or tissue (3) suggests a relationship between these two factors. It is difficult to arrive at more than a relative idea of the specific activities of tissue proteins in the short term experiment, Fig. 3, in view of the relatively high activity of plasma and "lymph" protein, which undoubtedly accounts for a considerable proportion of the total observed values. In this experiment the specific activity of plasma protein was 10 times greater than that of liver and 84 times that of muscle whereas the ratios of plasma to liver protein activity were 1–5.4 in Experiment 2 and 1–3.5 in Experiment 3. Corresponding plasma to muscle ratios were 1–42 and 1–15. It is interesting to note that, despite the low specific

Downloaded from http://rupress.org/jem/article-pdf/93/6/539/1184626/539.pdf by guest on 25 June 2021

activity of muscle protein, the total muscle mass accounts for the highest individual percentage of administered C^{14} after 1 week.

A final point of discussion concerns the mechanism of transfer of C^{14} activity from plasma protein to tissue protein. There has never been any serious controversy about the quantitative difference between the utilization of oral or parenteral protein. However, opinions differ sharply regarding the degree to which plasma proteins must be degraded within the body before they become available to cells and tissues. While the present experiments do not provide a final answer to the question, several points are worthy of note. Within a period of 7 days, 30 to 40 per cent of the C^{14} activity of intravenously injected plasma protein, tagged with radioactive lysine, is incorporated into the tissue and organ proteins of dogs. This conversion is accompanied by the loss in either urine or expired air of only a small fraction of administered C^{14}, even when the excretion of activity in the first 12 hours, related to non-protein C^{14} in the injected plasma is taken into consideration. The activity remains in the lysine residue of the liver proteins as demonstrated, and very probably of other proteins, and the ratio of lysine activity to total protein activity in liver and plasma is unaltered. The very considerable amount of urinary nitrogen, in terms of protein catabolized, excreted by these dogs in 7 days compared with the insignificant quantity of C^{14} in the urine suggests either that this nitrogen is derived from as yet unlabeled protein, from tissue or red cell breakdown, or that the lysine released from plasma and tissue proteins is almost completely conserved as such. In view of the relatively high content of C^{14} in the expired air following oral feeding of labeled plasma or tracer doses of *l*-lysine added to a casein digest (data to be published) it is difficult to believe that complete breakdown to the amino acid level at a rate sufficient to account for the observed transfer of C^{14} from plasma to tissues could occur without a greater degree of C^{14} loss.

It may be worth while to summarize our belief relative to the dynamic equilibrium which obtains between the plasma protein and cell protein. When a dog is maintained (11) in active health and normal weight for 3 months by being given carbohydrate, fat, and accessories by mouth and whole dog plasma by vein, it seems that the plasma proteins must supply all the protein requirements of the body. There is no excess of urinary nitrogen to indicate any unusual protein breakdown. Liver cells can manufacture and release albumin, fibrinogen, and probably many other related proteins. If these large molecules pass *out* through the cell membrane there is no reason to suppose that large protein molecules cannot pass *into* the cell through the cell membrane when plasma proteins supply body needs under the conditions noted above. Labeled proteins containing C^{14} are found in all tissue cells.

It appears that the problem is pushed back *into the cell*—for example the liver cell—in this study of protein equilibrium. There are no known forces

Downloaded from http://rupress.org/jem/article-pdf/93/6/539/1184626/539.pdf by guest on 25 June 2021

outside the cell capable of *significant* protein degradation and, or protein construction in the dog. This shift of plasma proteins to cell proteins of various types in various organs—liver, adrenal, muscle, and so on—means production of a great variety of substances of protein nature. At present some workers think that the plasma proteins are degraded on the cell surface or within the cell to amino acids but one might suggest the possibility of more nitrogen escape in the urine under such assumed conditions. We prefer to think of the cell as effecting this shift of one protein to another by a less drastic cleavage and reassembly; polypeptide size being concerned or cleavage of that order.

One need not apologize for differences of opinions regarding processes that go on in relation to the cell. In the maelstrom which is the functioning liver cell so very little is known about the complex processes which bring into being so very much new protein material.

SUMMARY

Labeled plasma proteins obtained from donor dogs, previously fed ϵ-C^{14}-*dl*-lysine, have been given intravenously to recipient dogs.

The disappearance of labeled globulin from the plasma at a rate considerably faster than albumin has been confirmed.

Evidence suggesting that the mass of protein in solution in the extravascular, extracellular fluid is approximately equal to the plasma proteins in circulation has been derived from a study of the dilution of labeled plasma protein by repeated injections of non-labeled plasma protein.

In a period of 7 days the transfer of C^{14} from plasma to tissue proteins amounted to between 30 and 40 per cent of the activity in the labeled plasma protein injected intravenously. The conversion was accompanied by a very small loss of activity in the urine and expired air and the activity remained in the lysine residue of the liver and probably of other tissues.

The data presented favor the view that plasma proteins are utilized in the body economy after partial catabolism within the cell area and provide no evidence of complete breakdown to the amino acid level.

BIBLIOGRAPHY

1. Albright, F., Forbes, A. P., Bartter, F. C., Reifstein, E. C., Jr., Bryant, D., Cox, L. D., and Dempsey, E. F., Symposia on Nutrition of the Robert Gould Foundation, Vol. **2**, Plasma Proteins, (J. B. Youmans, editor), Springfield, Illinois, Charles C. Thomas, 1950.
2. Daft, F. S., Robscheit-Robbins, F. S., and Whipple, G. H., *J. Biol. Chem.*, 1938, **123**, 87.
3. Drinker, C. K., and Yoffey, J. M., Lymphatics, Lymph, and Lymphoid Tissue, Cambridge, Harvard University Press, 1941.
4. Eckhardt, R. D., Lewis, J. H., Murphy, T. L., Batchelor, W. H., and Davidson, C. S., *J. Clin. Inv.*, 1948, **27**, 119.

Downloaded from http://rupress.org/jem/article-pdf/93/6/539/1184626/539.pdf by guest on 25 June 2021

5. Fink, R. M., Enns, T., Kimball, C. P., Silberstein, H. E., Bale, W. F., Madden, S. C., and Whipple. G. H., *J. Exp. Med.*, 1944, **80,** 455.
6. Holman, R. L., Mahoney, E. B., and Whipple, G. H., *J. Exp. Med.*, 1934, **59,** 269.
7. Howland, J. W., and Hawkins, W. B., *J. Biol. Chem.*, 1938, **123,** 99.
8. 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.
9. Olnyk, P., Camp, D. B., Griffith, A. M., Woislawski, S., and Helmkamp, R. W., *J. Org. Chem.*, 1948, **13,** 465.
10. Pommerenke, W. T., Slavin, H. B., Kariher, D. H., and Whipple, G. H., *J. Exp. Med.*, 1935, **61,** 283.
11. Terry, R., Sandrock, W. E., Nye, R. E., Jr., and Whipple, G. H., *J. Exp. Med.*, 1948, **87,** 547.
12. Van Slyke, D. D., and Cullen, G. E., *J. Biol. Chem.*, 1916, **24,** 117.
13. Waterhouse, C., Basset, S. H., and Holler, J. W., *J. Clin. Inv.*, 1949, **28,** 245.
14. Waterhouse, C., personal communication.
15. Zeldis, L. J., and Madden, S. C., *Am. J. Path.*, 1950, **26,** 726.

Downloaded from http://rupress.org/jem/article-pdf/93/6/539/1184626/539.pdf by guest on 25 June 2021