ALBUMIN AND GLOBULIN CIRCULATION IN EXPERIMENTAL ASCITES

Relative Rates of Interchange between Plasma and Ascitic Fluid Studied with C^{14}-Labeled Proteins*, ‡

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The interchange of C^{14}-labeled plasma protein between blood and ascitic fluid, designated ascitic fluid circulation, has been investigated and reported previously (8). These preliminary experiments and the present study of the mobility and rate of transfer of the major plasma protein components, albumin and globulin, in experimental ascites are part of the broad and perplexing problem of the passage of large molecules of protein across living membranes. While the physiological fact of such transfer is recognized, many controversial questions remain. Among these are the degree of degradation, if any, of the protein molecule required for transmembrane passage and the physiological likenesses and differences between the membrane of an individual cell and a partially permeable tissue barrier made up in greater or less part of cells, as for example a capillary wall, the peritoneum, the glomerulus, and the placenta.

Few quantitative data are available concerning this problem of protein diffusion across living tissue membranes. Such diffusion is influenced by many forces, including, no doubt, all those which limit the transport of other materials in the body as well as by more specific factors, such as molecular size. In regard to this latter factor, the kidney is known to excrete small protein molecules more rapidly and in greater amounts than larger protein molecules, muscle hemoglobin 25 times more rapidly than blood hemoglobin (13), and albumin more readily than globulins in dogs with experimentally induced proteinuria (12).

Previous experiments designed to measure the amount and rate of protein passage in the living animal have been handicapped by the difficulty of recognizing the test protein throughout its course in the body, an obstacle circum-

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vented satisfactorily by radioactive labeling. Also, the transfer between two large, but definitely separate, pools of protein-containing fluid—the circulating blood and the ascitic fluid—puts at the investigator's disposal sufficient, readily accessible material for frequent and satisfactory measurement.

In this series of experiments the circulation and interchange of protein in both directions between the circulating blood and ascitic fluid are reaffirmed, and, in addition, evidence is presented which indicates that albumin is constantly passing back and forth across the peritoneal membrane, at least 3 times more rapidly than is globulin. While equilibrium between labeled plasma introduced into either circulating plasma or ascitic fluid is not reached until from 1 to 3 days after injection, at no time is there any appreciable change in the over-all albumin/globulin ratio.

Methods

A description of the dogs used in these experiments together with a chronological report of the procedures involved in administering the C^{14}-labeled plasma either intraperitoneally or intravenously is contained in the experimental histories below. The experimental ascites was produced in each case by constricting the inferior vena cava above the diaphragm as described in detail in previous reports (4–6).

The radioactive plasma used in the experiments was produced by feeding lysine, labeled with C^{14} in the epsilon position, to 2 hypoproteinemic and anemic dogs. The animals incorporated the tagged material in their body proteins and served as donors of radioactive plasma for the test animals. Details of this procedure appear elsewhere (9, 10, 14). The plasma obtained from bleeding donor animals was separated by centrifugation under sterile conditions and used immediately when administered to the test animal intravenously. Plasma for intraperitoneal injection was obtained in a similar manner, rapidly frozen and kept at −4°C. for 2 to 3 months before being used. Liquaemin (Hoffman-La Roche Inc.) was used as an anticoagulant (1.2 cc. per 300 cc. whole blood).

As indicated in the individual experimental histories, the amounts of C^{14} activity administered as albumin and globulin vary somewhat from experiment to experiment. These variations in donor plasma reflect differences in time of collection after labeled lysine feeding, which ranged from 8 to 17 days, and the number of prior bleedings as related to the previously reported observation that the metabolic turnover of globulin is significantly faster than that of albumin (9).

In one experiment on each of the test animals, the plasma was given intraperitoneally, and in the second experiment on each dog, the plasma was given intravenously.

In the course of the experiments, samples of blood and ascitic fluid were taken at time intervals indicated on the charts. Aliquots of both plasma and ascitic fluid were assayed by the method of Bale (1) for radioactivity measurement.

Determinations of the concentration of total protein C^{14} activity in plasma or ascitic fluid were made directly. Determinations of the concentration of albumin C^{14} activity in plasma or ascitic fluid were made after isolating the albumin by salting-out with 26.8 per cent sodium sulfate according to the method of Majoor (3). In general the determinations of the concentration of globulin C^{14} activity in plasma or ascitic fluid were made by difference, but in some instances these determinations were checked by direct determination of both albumin and globulin, the globulin being redissolved and its radioactivity measured. Chemical determinations of total protein of both plasma and ascitic fluid were made by the macro Kjeldahl

method. Chemical determinations of albumin of both plasma and ascitic fluid were made by the micro-Kjedahl method, using the same filtrate from the 26.8 per cent sodium sulfate salting-out that was used for the radioactive determinations. Specific activity of C^{14} per gram of protein was derived by dividing the C^{14} activity per 100 cc. by the grams of protein per 100 cc.

EXPERIMENTAL HISTORIES

Dog 50-5. This mongrel female had the aluminum band placed about its inferior vena cava above the diaphragm 6 weeks prior to these experiments, and had been used only in standardization experiments to test ascitic fluid productability.

In the first experiment (Table 1), 100 cc. of C^{14}-labeled plasma was injected intravenously. This contained 5.6 gm. of protein with a C^{14} activity of approximately 1.5 μc. Of this activity 35 per cent was incorporated in 2.0 gm. of albumin and 65 per cent in 3.6 gm. of globulin. Samples of blood (20 cc. each) and ascitic fluid (15 cc. each) were taken at 0, 8, 21, 30, 45, 54, and 72 hours. Three days prior to the administration of this plasma, the animal received daily 3 to 4 gm. of sodium chloride (1 gm. enteric coated tablets) orally, and the dose of 3 gm. of sodium chloride was continued for the 3 days of the experiment. This dose insured the accumulation of ascitic fluid at the start of the experiment and the continued formation of ascites for the duration of the experiment. The animal received a high protein diet before and during the test period to provide an ascitic fluid of high protein content. This daily diet consisted of 250 gm. of cooked horsemeat plus 50 gm. of a standard mixture of sucrose, lard, bone ash, yeast, cod liver oil, and liver powder (11) plus 2 cc. of a choline–nicotinic acid solution. Plasma and blood volumes, determined earlier on the day of labeled plasma injection using Evans blue dye (T-1824) (2) were calculated to be 340 cc. and 618 cc. respectively.

At the beginning of the experiment, the animal weighed 9.1 kilos, and 1400 cc. of ascitic fluid was estimated to be present. Weight in kilos on successive days during the experiment was as follows: 8.3, 9.2, 9.1, and 7.4 (following paracentesis). At the end of the experiment 1680 cc. of ascitic fluid was removed and less than 50 cc. was estimated to remain. During the 72 hour experiment, the hematocrit reading varied between 44.6 and 42.5 per cent. The total plasma protein concentration varied between 6.4 and 5.5 gm. per cent, and the total ascitic fluid protein between 4.0 and 3.8 gm. per cent. The plasma albumin varied between 3.7 and 2.9 gm. per cent, and that of the ascitic fluid between 1.7 and 1.4 gm. per cent.

In the second experiment (Fig. B) the radioactive tagged plasma was given *intraperitoneally.* 80 cc. of plasma containing a total protein of 4.9 gm., of which 1.9 gm. was present as albumin and 3.0 gm. as globulin, was injected into the peritoneal cavity following removal of 95 cc. of ascitic fluid. The total C^{14} activity given amounted to 0.80 μc. of which 46 per cent was incorporated in albumin and 54 per cent in globulin. Samples of blood (20 cc.) and ascitic fluid (15 cc.) were taken at 0, 2, 4, 8, 12, 24, 48, and 72 hours for measurement for C^{14} activity.

At the beginning of the experiment, the animal weighed 9.2 kilos. About 1600 cc. of ascitic fluid (estimated) was present. The weight of the animal in kilos on successive days following the sodium chloride administration was as follows: 7.8, 8.1, 8.5, 9.2 (C^{14}-labeled plasma injected intraperitoneally), 9.3, 9.3, and 7.5 (following paracentesis). At the end of the experiment 1850 cc. of ascitic fluid was removed and approximately 50 cc. was estimated to remain. During the 72 hour experiment, the hematocrit reading dropped from 61.3 to 46.6 per cent. The total plasma protein concentration varied between 5.7 and 4.7 per cent, and that of the ascitic fluid between 3.1 and 2.7 per cent. Albumin of the circulating plasma varied between 3.9 and 3.1 gm. per cent, while the albumin of the ascitic fluid varied between 2.8 and 2.3 gm. per cent.

Dog 49-140. This mongrel female had been a member of the ascitic colony for 4 months prior to these experiments, and had participated in other studies (7). In the first experiment

(Fig. A), 95 cc. of C^{14}-labeled plasma containing a total protein of 5.1 gm. of which 1.7 gm. was present as albumin and 3.4 gm. as globulin, was given *intravenously*. The albumin accounted for 39 per cent and the globulin 61 per cent of the 1.16 μc. of C^{14} activity administered. Samples of blood (15 cc.) and ascitic fluid (15 cc.) were taken at 0, 8, 21, 30, 45, 53, and 72 hours for determinations of activity of total protein, albumin, and globulin. The plasma volume was calculated to be 432 cc. and the total blood volume 928 cc. Preparation with sodium chloride and the diet was similar to that in the previous experiments. The daily weight of the animal in kilos during the preparatory and experimental periods was 9.8, 9.8, 10.3, 10.7 (C^{14}-labeled plasma given intravenously), 10.7, 10.6, 10.5, and 8.3 (following paracentesis). At the end of the experiment 2420 cc. of ascitic fluid was removed with about 50 cc. estimated to remain. The hematocrit reading varied between 43.7 and 39.8 per cent during the experiment. Total plasma proteins varied between 5.2 and 4.4 gm. per cent, and the ascitic fluid proteins between 4.3 and 4.1 gm. per cent. Albumin of the circulating plasma varied between 3.0 and 2.5 gm. per cent, and albumin of the ascitic fluid between 2.5 and 2.2 gm. per cent.

At the termination of the experiment, this dog, 49-140, was placed on the usual high protein diet without sodium chloride, to discourage the accumulation of asctic fluid. After 2 weeks the animal unexpectedly was found to have reversed plasma and ascitic fluid albumin/globulin ratios of 0.5 and 0.4 whereas the previous averages had been 1.36 (plasma) and 1.39 (ascitic fluid). Occasionally the ratio rose to 1.0 during the next 4 weeks, after which it remained consistently below 0.5. Despite this spontaneous inversion of the chemical albumin/globulin, ratios, which was confirmed by electrophoretic studies and which persisted for over a year, the general condition of the animal and its ascitic fluid production remained essentially unchanged.

In the second experiment (Table 2) the animal received the C^{14}-labeled plasma *intraperitoneally*, 3 months after completion of the first experiment with intravenous labeled plasma and 10 weeks after the disturbed albumin/globulin ratio was first observed. After removal of 110 cc. of ascitic fluid, an equivalent amount of labeled plasma was injected into the peritoneal cavity. This plasma contained 6.0 gm. of total protein, of which 2.5 gm. was present as albumin, and 3.5 gm. as globulin, in which were incorporated approximately 0.86 μc. of C^{14} (40 per cent in albumin and 60 per cent in globulin). Samples of blood (15 cc.) were taken at 8, 18, 24, 31, 48, 54, and 72 hours, and determinations were made on ascitic fluid removed before injection and at the conclusion of the experiment at 72 hours. The preparation with sodium chloride and the diet were similar to that described above. On the day of the C^{14}-tagged plasma injection, the plasma volume was 515 cc. and the total blood volume 813 cc. The weight at the time of the test plasma injection was 9.5 kilos and about 1000 cc. (estimated) of ascitic fluid was present. The daily weights in kilos of the animal during the experiment were 8.8, 9.0, 9.0, 9.5 (C^{14}-labeled plasma given intraperitoneally) 10.1, 10.1, 10.1, and 8.7 (following paracentesis). At the end of the experiment 1500 cc. of ascitic fluid was removed and about 50 to 100 cc. was estimated to remain. During the 72 hour experiment the hematocrit reading varied between 36.2 and 29.4 per cent. The plasma protein concentration varied between 6.2 and 5.3 gm. per cent, and the plasma albumin between 1.7 and 0.9 gm. per cent. Determinations of ascitic fluid total proteins and albumin were made only at the beginning and the end of the experiment. Similar values, 4.5 gm. of total protein and 1.3 gm. of albumin per 100 cc. were obtained for each sample.

Urine excreted during the experiment was not examined for protein but no proteinuria was detected subsequently. The dog was sacrificed and a complete autopsy performed 13 months after completion of this experiment. At autopsy about 300 cc. of reddish serous ascitic fluid was present. The viscera were within normal limits with the exception of the liver which weighed 660 gm. (Weight of the animal at sacrifice was 8.6 kilos.) Several thin web-like adhesions were present between the liver and diaphragm, omentum, and bowel, and between the lobes of the liver, though the adhesions were not as tough or numerous as noted in other

experimental ascitic animals. On section the liver was greatly congested and a large amount of dark blood oozed from each cut surface. The lobulation was distinct, although in the subcapsular area, the tissue was so congested that no normal architecture was discerned. In the deeper areas nodules of pale yellowish liver tissues were noted, and still deeper the organ assumed a true nutmeg pattern. The gall bladder was thin walled and filled with thick dark bile. The extrahepatic ducts were patent. Adhesions were present in the right thoracic cavity between the pleurae. The vena cava, while narrowed and fibrotic with the aluminum band still in place and embedded in dense fibrous tissue, was nevertheless patent to the passage of a small probe.

TABLE 1

Disappearance from Plasma and Appearance in Ascitic Fluid of C^{14}-Labeled Plasma Proteins after Intravenous Injection

Ascitic dog 50-5, weight 9.0 kilos, 100 ml. C^{14}-labeled plasma intravenously.

Time after injection	PLASMA								
	Total protein			Albumin			Globulin		
	Gm. per 100 cc.	Per cent dose C^{14} per 100 cc.	Per cent dose C^{14} per gm. protein	Gm. per 100 cc.	Per cent dose C^{14} per 100 cc.	Per cent dose C^{14} per gm. albumin	Gm. per 100 cc.	Per cent dose C^{14} per 100 cc.	Per cent dose C^{14} per gm. globulin
hrs.									
0	5.91	22.80	3.95	3.66	8.01	2.18	2.25	14.75	6.45
8	5.75	12.80	2.23	3.14	5.39	1.72	2.61	7.40	2.84
21	6.45	6.50	1.01	4.09	3.28	0.80	2.36	3.26	1.38
30	5.95	4.86	0.81	3.96	2.85	0.72	1.99	2.00	1.00
45	5.50	3.58	0.65	3.00	1.75	0.59	2.50	1.83	0.73
54	6.20	3.72	0.60	2.92	1.52	0.52	3.28	2.20	0.67
72	5.72	3.16	0.55	—	—	—	—	—	—
	ASCITIC FLUID								
8	3.94	1.70	0.43	1.41	0.94	0.67	2.53	0.76	0.30
21	3.85	2.58	0.67	1.73	1.55	0.90	2.12	1.03	0.48
30	3.88	2.54	0.65	1.45	1.52	1.07	2.43	1.02	0.42
45	3.78	2.28	0.61	—	1.48	—	—	0.80	—
54	4.00	2.58	0.65	—	1.44	—	—	1.14	—
72	3.94	2.82	0.71	1.62	1.62	1.00	2.32	1.20	0.52

Microscopically, the liver showed the severe congestion and the almost hemangiomatous pattern described elsewhere (5). Beneath the capsule little liver tissue remained, while more centrally the tissue was less congested and atrophied and appeared normal. The other viscera were microscopically normal. No anatomical or histological basis was discovered for the unexpected, but persistent, reversal of the albumin/globulin ratio. The changes observed in the liver were in all respects similar to those observed in our entire series of experimental ascitic dogs sacrificed or biopsied to date.

EXPERIMENTAL OBSERVATIONS

Data pertaining to the two experiments in which the labeled plasma was administered intravenously are shown in Table 1 and Fig. A. Table 1 lists the

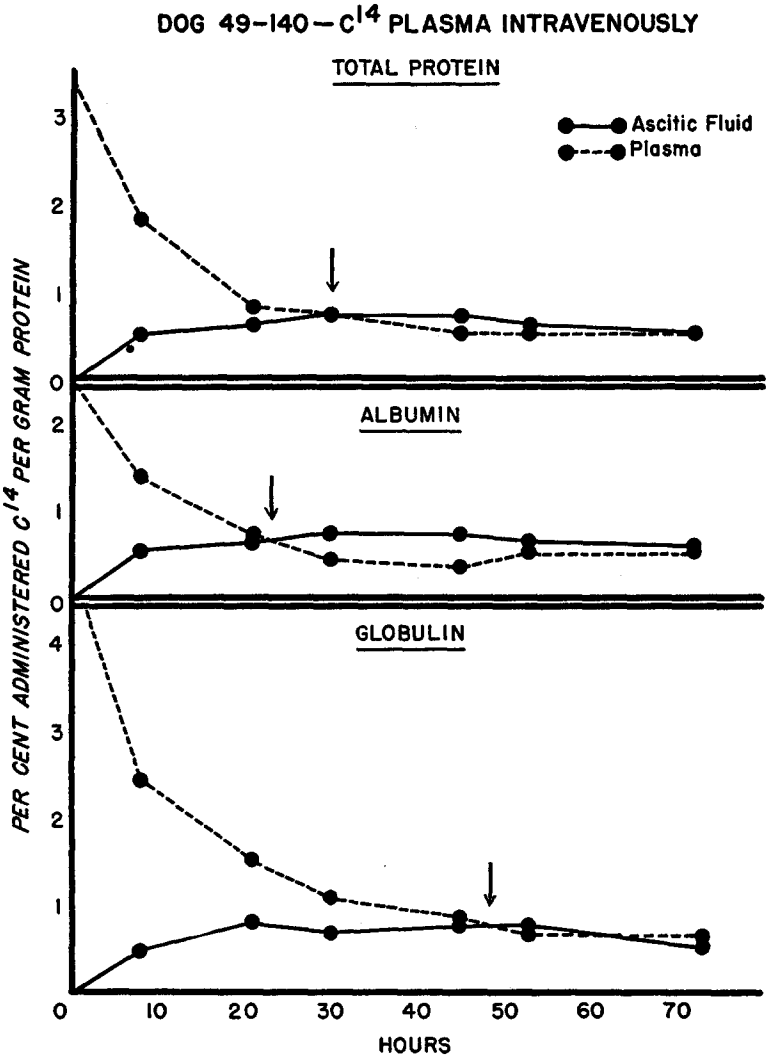

FIG. A. *Dog. 49-140.* C^{14} activity of plasma and ascitic fluid albumin, globulin, and total protein following *intravenous* injection of labeled plasma. Values plotted represent C^{14} per gram of albumin, globulin, or total protein expressed as per cent of administered C^{14} activity. Times at which equilibrium is reached between C^{14}-labeled proteins in plasma and ascitic fluid are indicated by arrows.

chemically determined concentration, the C^{14} content per 100 cc. and the C^{14} per gm., of total protein, albumin, and globulin in both plasma and ascitic fluid at various time intervals during the 72 hours of the experiment on dog 50–5, after *intravenous* injection of the C^{14}-labeled plasma. Fig. A illustrates

the changes in C^{14} content per gm. of total protein, albumin, and globulin in a similar experiment in which dog 49–140 was used. Despite some quantitative variations the results of the two experiments are essentially the same. A rapid drop in specific activity of the total *plasma* proteins as well as the albumin and globulin fractions is noted during the first 24 hours. While this is comparable to previously reported observations in normal dogs (9, 14), given labeled plasma intravenously, in that all three components decline at approximately the same rate, it is noteworthy that in these dogs with ascites there is a reduction in the specific activities of over 70 per cent in contrast to a reduction of 45 to 50 per cent in normal animals during the same period of time. After the first day the plasma protein activity in the dog with ascites falls as in the normal, at a much slower rate, which, however, is slightly greater for globulin than for albumin. Specific activities of total protein, albumin, and globulin in the ascitic fluid increase most rapidly during the first 8 hours after injection, more slowly for approximately 24 hours more, and then tend to remain constant or fall off slightly. The times at which the total proteins, albumin, and globulin in plasma and ascitic fluid reach equilibrium, as indicated in Fig. A (dog 49–140) are 30, 24, and 48 hours respectively. Comparable figures for dog 50–5 (Table 1) are total proteins 45 hours, albumin 20 hours, and globulin 60 hours.

Fig. B and Table 2 illustrate the findings in the same two dogs for the experiments involving the *intraperitoneal* injection of C^{14}-labeled plasma. From Fig. B (dog 50–5) it can be readily seen that there is a gradual decline in the C^{14} activity of the ascitic fluid protein throughout the experiment. This is in contrast to the sharp initial drop when the tagged plasma is injected intravenously, attributed to mixing of the fluid and solutes of the rapidly circulating plasma with extravascular fluid, so that it is not surprising to find a slower, more uniform, decrease in activity from the relatively stagnant pool of ascitic fluid. Associated with this decline in ascitic fluid activity, the C^{14} activity per gm. of plasma albumin, globulin, and total protein increases steadily until an approximately constant level is reached between 24 and 30 hours. In dog 50–5 (Fig. B) the curves representing C^{14} activity in the plasma and ascitic fluid do not meet within the experimental period, presumably because of the continued drain on the plasma protein, not only in interchange with and augmentation of the ever, however slowly, accumulating ascitic fluid, but for metabolic needs and maintenance of equilibrium with protein in lymph and extravascular fluid.

As indicated in the experimental history of dog 49–140, some metabolic disturbance, characterized by inversion of the albumin/globulin ratio, occurred in this animal about 6 weeks before the experiment involving intraperitoneal injection of labeled plasma. Sampling of ascitic fluid only before and 72 hours after injection provided a further complicating factor making comparison with intraperitoneal injection in dog 50–5 difficult. The incomplete

data from this experiment are shown in Table 2. The values for C^{14} activity in ascitic fluid total protein, albumin, and globulin at 0 hours are approximations based on the activity injected and the estimated volume of fluid in the peritoneal cavity. While passage of labeled albumin and globulin from ascitic

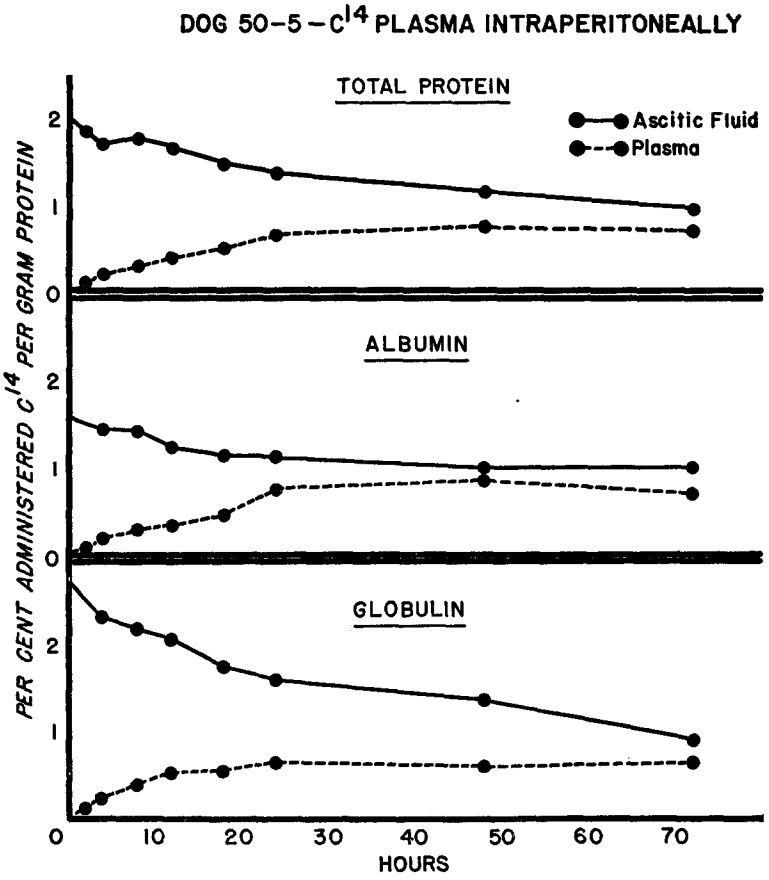

FIG. B. *Dog 50-5.* C^{14} activity of plasma and ascitic fluid albumin, globulin, and total protein following *intraperitoneal* injection of labeled plasma. Values plotted represent C^{14} per gram of albumin, globulin, or total protein expressed as per cent of administered C^{14} activity.

fluid to plasma was observed in both dogs after intraperitoneal injection of plasma, the specific activity per gm. of plasma albumin rose more rapidly and to a much higher level than did that of plasma globulin in dog 49–140 or that of either protein fraction in dog 50–5 (Fig. B). This discrepancy, related to the low plasma concentration of albumin in dog 49–140, occurred despite the low circulating C^{14} albumin activity, indicated in column 6, Table 2, listing the

per cent of dose as albumin per 100 cc. These values average about 50 per cent of those found in the comparable experiment on dog 50–5. It will also be noted in Table 2, that the C^{14} activity per gm. albumin in the ascitic fluid falls from 3.15 to 1.11 per cent of the dose between 0 and 72 hours, a drop of 65 per cent compared to a reduction in ascitic fluid globulin activity of 60 per cent. Following intraperitoneal injection of labeled plasma in dog 50–5, with a normal albumin/globulin ratio, the ascitic fluid albumin activity fell 36 per cent and the globulin activity 65 per cent, during a similar period of time. Taken together the above findings point to a rapid loss of albumin from the blood

TABLE 2

Appearance of C^{14}-Labeled Proteins in Plasma after Intraperitoneal Injection of Labeled Plasma

Ascitic dog 49-140, weight 10.0 kilos, 110 ml. C^{14}-labeled plasma intraperitoneally.

Time after injection	PLASMA								
	Total protein			Albumin			Globulin		
	Gm. per 100 cc.	Per cent dose C^{14} per 100 cc.	Per cent dose C^{14} per gm. protein	Gm. per 100 cc.	Per cent dose C^{14} per 100 cc.	Per cent dose C^{14} per gm. albumin	Gm. per 100 cc.	Per cent dose C^{14} per 100 cc.	Per cent dose C^{14} per gm. globulin
hrs.									
8	5.3	1.65	0.32	1.70	0.77	0.46	3.60	0.85	0.23
18	5.7	2.97	0.53	1.68	1.18	0.70	3.97	1.73	0.43
24	5.9	3.46	0.58	1.59	1.53	0.96	4.31	1.64	0.39
31	6.0	4.05	0.68	1.03	1.43	1.38	4.93	2.62	0.54
48	6.2	4.11	0.67	1.06	1.43	1.34	5.14	2.70	0.53
54	5.7	4.25	0.75	0.96	1.41	1.47	4.70	2.84	0.61
72	5.8	4.12	0.71	0.95	1.13	1.19	4.87	3.00	0.62
	ASCITIC FLUID								
0	4.5	10.00	2.22	1.30	4.10	3.15	3.20	5.90	1.84
72	4.5	3.80	0.84	1.31	1.45	1.11	3.19	2.34	0.74

stream of dog 49–140. Loss of albumin in the urine can be more or less excluded by the lack of proteinuria subsequent to the experiment and the finding of normal kidneys at autopsy. It is suggested that damage to the liver by constriction of the vena cava in this dog may have interfered with the production of albumin, to a greater extent than in other animals rendered ascitic by this procedure, resulting in a more rapid loss of injected albumin and leading to the differences observed in the two otherwise comparable experiments.

DISCUSSION

It is apparent from data previously published (8) and from the foregoing observations that proteins are constantly passing into and out of both plasma

and ascitic fluid under the experimental conditions obtaining, and that following the injection of labeled plasma into either the blood stream or the peritoneal cavity, the rates of protein transfer in opposite directions tend to become equal as the concentrations of tagged proteins in the two compartments approach an even distribution. However, in the early stages of each experiment, when labeled proteins are first appearing and increasing in plasma or ascitic fluid, depending on the site of injection, the flow of labeled proteins will be approximately unidirectional. It is thus possible to estimate minimum relative rates of transfer of the two major protein fractions, albumin and globulin, across the peritoneal membrane in the first few periods after labeled plasma injection.

From Table 1 and similar data for the two experiments on which Figs. A and B are based, the average C^{14} activity, which represents 1 gm. of albumin and 1 gm. of globulin in the compartment into which the injection was made, can be readily calculated for each time interval. Then from the increases in albumin and globulin C^{14} activity per 100 cc. in the other compartment the number of grams of each protein transferred during the same period can be determined.

The following is a sample calculation for the 0 to 8 hour period, dog 50-5, following intravenous labeled plasma:—

Average C^{14} activity equivalent to 1 gm. of plasma albumin (0–8 hrs.) ..... 1.95 (per cent dose)

Increase in ascitic fluid albumin C^{14} per 100 cc. ..... 0.94 (per cent dose)

Albumin transferred to ascitic fluid $= \frac{0.94}{1.95}$ ..... 0.48 gm. per 100 cc.

Albumin transferred to ascitic fluid per hr. $= \frac{0.48}{8}$ ..... 0.06 gm. per 100 cc.

.....

Average C^{14} activity equivalent to 1 gm. of plasma globulin (0–8 hrs.). 4.65 (per cent dose)

Increase in ascitic fluid globulin C^{14} per 100 cc. ..... 0.76 (per cent dose)

Globulin transferred to ascitic fluid $= \frac{0.76}{4.65}$ ..... 0.16 gm. per 100 cc.

Globulin transferred to ascitic fluid per hr. $= \frac{0.16}{8}$ ..... 0.02 gm. per 100 cc.

Table 3 lists the relative rates of transfer of the two protein fractions in all periods in which such calculations were possible. The zero values at the bottom of each column refer to the first periods during which no increase in activity was observed in the compartment which initially contained no labeled protein. While the relative minimum rates of transfer, three times more albumin than globulin per hour in all the 0 to 8 hour periods, are similar, it is apparent that these values have already been modified to some extent by the return of some labeled albumin and globulin, at different rates, in the opposite direction. In fact, a rate difference of 4 to 1 in favor of albumin was observed for 0 to 4

hours in one instance (dog 50–5, after intraperitoneal plasma) when samples were collected before the lapse of 8 hours. The passage of at least three times more albumin than globulin across the barrier during the same period of time in terms of grams indicates a much wider spread in terms of molecules. From this difference in the rates of transfer, apparently similar in both directions, it is clear why the labeled globulin requires a longer time to reach or approximate equilibrium than does labeled albumin in these experiments. It is interesting to contrast this slower rate of membrane passage of globulin with its metabolic turnover in plasma which is appreciably more rapid than that of albumin. Dog 49–140 could not be included in Table 3 owing to lack of activity measurements of ascitic fluid proteins throughout the course of the experiment.

TABLE 3

Relative Minimum Rates of Transfer of Albumin and Globulin between Plasma and Ascitic Fluid

Dog 50-5 C^{14} plasma intravenously			Dog 50-5 C^{14} plasma intraperitoneally			Dog 49-140 C^{14} plasma intravenously		
Time interval	Transfer to ascitic fluid		Time interval	Transfer to plasma		Time interval	Transfer to ascitic fluid	
	Gm. albumin per 100 cc. per hr.	Gm. globulin per 100 cc. per hr.		Gm. albumin per 100 cc. per hr.	Gm. globulin per 100 cc. per hr.		Gm. albumin per 100 cc. per hr.	Gm. globulin per 100 cc. per hr.
hrs.			*hrs.*			*hrs.*		
0–8	0.06	0.02	0–8	0.09	0.03	0–8	0.08	0.03
8–21	0.04	0.01	8–18	0.05	0.02	8–21	0.02	0.01
After 21	0	0	18–24	0.07	0.03	21–30	0.03	0.01
			After 24	0	0	After 30	0	0

Finally, the different conditions affecting the disappearance of labeled proteins from the plasma and ascitic fluid undoubtedly explain in large measure the observed variations in the curves seen after intravenous or intraperitoneal administration of labeled plasma. The rapid equilibria reached after intravenous injection—20 to 24 hours for albumin and 48 to 60 hours for globulin—as well as the failure to reach equilibrium within 72 hours after intraperitoneal injection are probably both related to constant circulatory and metabolic opportunities available for removal of proteins from the plasma, which are greater than those for ascitic fluid.

SUMMARY

Plasma containing carbon^{14}-labeled albumin and globulin, obtained by feeding ϵ-C^{14} D,L-lysine to a donor dog, has been injected intravenously and intraperitoneally into recipient dogs with experimental ascites.

The circulation and interchange of total plasma protein between circulating

blood and ascitic fluid have been confirmed and the participation of both albumin and globulin in this interchange has been demonstrated.

Labeled albumin tends to reach equilibrium in plasma and ascitic fluid in a shorter period of time (1 to 2 days) than does globulin (2 or more days), after administration of labeled plasma by either route.

Evidence is presented that the rate of transfer of albumin across the peritoneal membrane is at least three times faster than that of globulin in terms of weight.

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