TRAUMATIC SHOCK. I. THE PRODUCTION OF RADIOACTIVE PLASMA PROTEIN FROM AMINO ACIDS CONTAINING RADIOACTIVE SULFUR

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In order to study the fate of the plasma proteins in shock, it was considered desirable to tag these proteins with radioactive elements. Sulfur was selected because of (*1*) its favorable half life (88 days), (2) its position of relative stability in the protein molecule, and (*3*) the significant role which sulfur-containing amino acids play in the generation of plasma proteins, as shown by Whipple *et al.* (1, 2). The preparation of radioactive sulfur-containing amino acids (cystine, methionine, and homocystine) from radioactive sulfur is reported by one of us in the following article in this same journal (3). This communication reports our experience in the production of radioactive plasma proteins by dogs fed radioactive sulfur-containing amino acids.

It has long been known that l-cystine is an amino acid essential for maintenance and growth of animals. Du Vigneaud *et al.* (4, 5), found that the l-cystine component of the meso form can be utilized by growing rats, but that d-cystine cannot be utilized. l-Cystine, necessary for the growth of rats, can be replaced by both d- and l-methionine (6) and by d- and l-homocystine (*7*) if choline or betaine are added to the diet (8). Tarver and Schmidt (9) isolated radioactive cystine from the fur of rats 3 weeks after feeding d,l-methionine containing radioactive sulfur.

Whipple *et al.* (1, 2) showed that hypoproteinemic dogs, which had been kept on a basal diet and depleted of "reserve stores" of plasma protein by long continued plasmapheresis, regenerated plasma protein at a markedly increased rate when l-cystine was fed, to a lesser extent when d,l-methionine was fed, and to a still lesser extent when l-tyrosine or l-tryptophane was fed. Homocystine was not studied. Whipple demonstrated that a hypoproteinemia of 4 grams per cent was the optimum level for maximum regeneration of plasma protein. A lower level of plasma protein did not permit maintenance of a satisfactory state of health.

With these facts as a basis, we studied the utilization of small amounts of radioactive d,l-cystine,[1] d,l-methionine, and d,l-homocystine by hypoproteinemic dogs, for the production of radioactive plasma protein.

METHOD

Dogs were rendered hypoproteinemic after the manner described by Whipple (1), *i.e.* by the use of protein deficient diets and plasmapheresis. In most cases, however, the plasmapheresis was not carried on for the length of time required, according to Whipple, to deplete the "reserve stores" of plasma protein.

Diet. Dogs were fed a low protein diet for 2 weeks, followed by a non-protein diet for 1 week unless otherwise specified in the individual protocol. The diet was essentially that recommended by Cowgill (10). Each dog was given 70 to 80 calories per kilogram of body weight per day.

Plasmapheresis was performed aseptically 3 or 4 times weekly by removal of 25 to 30 per cent of the total blood volume (6 cc. of 5 per cent sodium citrate per 100 cc. blood as anticoagulant) with immediate return of the unwashed red cells in 2.5 per cent glucose in physiological saline solution.

Protein levels were determined by the specific gravity method. When a level of or close to 4 grams per cent was reached, an amount of plasma was removed during the following week which was just sufficient to maintain this level. The early death of some of the dogs is explained by the fact that much more plasma was removed with each plasmapheresis after the radioactive amino acids were given than before.

Amino acids containing radioactive sulfur were converted to the hydrochloride and then fed or, as in 2 cases, injected intravenously. Recorded weights are of free amino acids. At the same time, the diet was usually supplemented with 50 grams of casein daily (forced feeding when necessary). In most cases, l-tyrosine (1 gram)

[1] Although no utilization of d-cystine was expected, no attempt was made to resolve the radioactive d,l-cystine because of the small amounts available and the likelihood of some loss of the laevo form. A method for resolution of d,l-cystine is reported by du Vigneaud (4).

and in some cases l-tryptophane (0.25 gram) was also fed daily. Dogs receiving homocystine were given betaine (4 grams) daily (8).

Radioactivity determinations. Because radioactive sulfur has a soft radiation, material containing it absorbs the radiation in proportion to the mass of the sample being measured. Because of this self-absorption, it is necessary to utilize the thinnest possible layer for measurement of the radioactivity. The most convenient form in which to obtain the sulfur from biological material for measurement is by oxidation of the material and precipitation of the sulfur as either barium or benzidine sulfate. As recommended by Tarver and Schmidt (9), the barium sulfate layer should not exceed 3 mgm. per sq. cm.

Plasma or tissue was prepared for analysis of radioactive potency by oxidation of 1 cc. of plasma or 1 gram of tissue with concentrated nitric acid and superoxol in a 100 cc. Kjeldahl flask. This required about 1.5 to 2.0 hours. Care was taken to avoid ignition when the mixture approached dryness. The non-protein fraction of plasma was similarly prepared for analysis after removal of the protein fraction by precipitation with an equal volume of 20 per cent trichloroacetic acid. The resulting ash in both cases was dissolved in several cc. of water containing 1 or 2 drops of concentrated hydrochloric acid.

When isolation of the sulfur as $BaSO_4$ was desired, the solution was transferred to a large test tube, diluted to 10 cc. and heated to boiling. An equal volume of 0.1 per cent $BaCl_2$ solution brought to the boiling point was added to the hot sulfate solution and the mixture was digested for 1 hour and allowed to stand overnight. It was again digested for 1 hour at 100° C., and the precipitate was collected while hot.

When isolation of the sulfur as benzidine sulfate was desired, the solution was transferred to a 50 cc. beaker, evaporated to 3 cc., and diluted with an equal volume of alcohol. To this was added 1 cc. of a saturated solution of benzidine dihydrochloride in 50 per cent alcohol, containing a few drops of concentrated hydrochloric acid. The mixture was allowed to sit in the ice box half an hour and the precipitate was collected.

Either precipitate was collected in a filtration apparatus, similar to the one described by Tarver and Schmidt (9) except that the precipitate covered a 1.54 sq. cm. area. It was washed with 50 per cent alcohol, water, and finally with acetone.

Advantages of the benzidine method are (*1*) the precipitates (7 mgm.) are easier to collect than the barium precipitates (4.5 mgm.), (*2*) the particle size is more uniform, and (*3*) the procedure can be carried out more quickly and efficiently.

The weight of the precipitate was determined in every case. Since analyses of urine and feces were made on aliquots treated in the same way as plasma, care was taken to select the size of the aliquot that would yield approximately the same weight of sulfate precipitate.[2]

Standards were prepared by oxidizing a known weight of the radioactive amino acid fed, and dissolving the ash in a given volume of Na_2SO_4 solution which was equivalent to 4.5 mgm. $BaSO_4$ per cc. Several dilutions of the radioactive solution were made with this non-radioactive Na_2SO_4 solution. In this way, standard precipitates of the same weight, containing known dilutions of the original radioactive amino acid, were obtained (Table I).

TABLE I

Radioactivity measurements of the standards in one experiment

Dilutions of radioactive standard	Cystine equivalent	Weight of benzidine sulfate	Radioactivity	Ratio of radioactivity to background	Deviation from linearity
	mgm.	*mgm.*	*divisions per second*		*per cent*
1	0.80	10.5	0.033	250	−13
25	0.032	9.7	0.0015	10	−1.3
100	0.008	9.5	0.00038	3	0
1000	0.0008	9.3	0.00028	0.2	−26

All measurements were made with a modified Lauritzen electroscope, described in another publication (11). In subsequent protocols, radioactivity is expressed in divisions per second (each division equals 20 small divisions on the scale of the electroscope). The background of our instrument varied from 0.00006 to 0.00009 division per second.

In the experiments which follow, the total amount of radioactive plasma protein removed from the dog is given as percentage of the amino acid fed. By so doing, a basis is provided for estimating the radioactive potency of the amino acids necessary to obtain any desired level of radioactivity in the plasma protein.

[2] We found a rough practical method of doing this was to use $\frac{1}{400}$ to $\frac{1}{500}$ of a 24-hour output.

EXPERIMENTS WITH RADIOACTIVE CYSTINE, HOMOCYSTINE, AND METHIONINE

TABLE II

Radioactive cystine

Dog No. S-11; weight 6.8 kgm.; hematocrit 40 per cent; non-protein diet 6 days, low protein diet 6 days; plasmapheresis 5 times; lowest plasma protein level 4.5 grams per cent; weight loss 1 kgm.; radioactive d,l-cystine (200 mgm.) fed; low protein diet supplemented with casein (50 grams) and l-tyrosine (1 gram) daily; circulatory collapse after third blood withdrawal; restored with saline and plasma; dog decorticate until termination of experiment 2 days later; weight 5 kgm.; hematocrit 25 per cent.

Specimen	Duration of experiment	Total protein	Radioactive plasma removed	Radioactivity per cc. plasma			Radioactive cystine in plasma protein		Ingested radioactive l-cystine (100 mgm.) utilized
				Plasma	Non-protein fraction	Protein fraction	Per cc. plasma	Total plasma removed	
	days	*per cent*	*cc.*		*divisions per second*		*mgm.*	*mgm.*	*per cent*
Plasma	0	4.5							
Plasma	1	5.1	20	0.00153	0.00043	0.00110	0.024	0.48	
Plasma	2	5.5	15	0.00109	0.00018	0.00091	0.020	0.30	
Plasma	3	5.5	150	0.00089	0.00008	0.00081	0.018	2.70	
Plasma	5	6.8	175	0.00045		0.00045	0.010	1.75	
						Total		5.23	5.2

Specimen	Duration of experiment	Total protein	Volume	Radioactivity per cc.	Radioactive cystine		Ingested radioactive l-cystine (100 mgm.) utilized
					Per cc.	Total specimen	
	days	*per cent*	*cc.*	*divisions per second*	*mgm.*	*mgm.*	*per cent*
Urine	2		400	0.00807	0.18	72†	36*
Feces	2		250	0.00044	0.0099	2.5	1.2*

Specimen	Duration of experiment	Total protein	Weight organ	Radioactivity per gram	Radioactive cystine		Ingested radioactive l-cystine (100 mgm.) utilized
					Per gram	Total organ	
	days	*per cent*	*grams*	*divisions per second*	*mgm.*	*mgm.*	*per cent*
Liver	5		250	0.00090	0.020	5.0	5.0
Lung	5		63	0.00097	0.021	1.3	1.2
Kidney	5		50	0.00096	0.021	1.0	1.0
Bowel	5		200	0.00090	0.020	4.0	4.0
Heart	5		42	0.00068	0.015	0.6	0.6
Brain	5		50	0.00012	0.003	0.2	0.2
Leg muscle	5		2000	0.00030	0.007	14.0	14.0

Standard from 0.0080 mgm. cystine had activity of 0.00036 divisions per second.

* Calculated on basis of d,l-cystine ingested.

† Excreted as sulfate.

TABLE III

Radioactive cystine

Dog No. S-19; weight 9.1 kgm.; low protein diet 22 days, non-protein diet 9 days; plasmapheresis 16 times; lowest plasma protein level 5.8 grams per cent; weight loss 1.3 kgm.; radioactive d,1-cystine (400 mgm.) fed in 2 doses at 24-hour interval; non-protein diet supplemented with casein (50 grams), 1-tyrosine (1 gram), and 1-tryptophane (0.25 gram) daily; dog died 1 week after last sample withdrawn.

Specimen	Duration of experiment	Total protein	Radioactive plasma removed	Radioactivity per cc. plasma			Radioactive cystine in plasma protein		Ingested radioactive 1-cystine (100 mgm.) utilized
				Plasma	Non-protein fraction	Protein fraction	Per cc. plasma	Total plasma removed	
	days	*per cent*	*cc.*	*divisions per second*			*mgm.*	*mgm.*	*per cent*
Plasma	0	5.8							
Plasma	1	5.1	164	0.00220	0.00124	0.00096	0.020	3.30	
Plasma	2	5.5	152	0.00301	0.00105	0.00196	0.040	6.10	
Plasma	3	5.5	134	0.00278	0.00052	0.00226	0.046	6.20	
Plasma	4	5.8	150	0.00260	0.00072	0.00188	0.038	5.70	
Plasma	5	5.1	191	0.00178	0.00041	0.00137	0.028	5.30	
Plasma	8	6.2	172	0.00118	0.00027	0.00091	0.019	3.25	
						Total		29.85	14.8

			Volume	Radioactivity per cc.	Radioactive cystine		d, 1-Cystine
					Per cc.	Total specimen	
			cc.	*divisions per second*	*mgm.*	*mgm.*	*per cent*
Urine	3		500	0.00728	0.15	75*	19

Standard from 0.0080 mgm. cystine had activity of 0.00039 divisions per second.

* Excreted as sulfate.

TABLE IV

Radioactive cystine

Dog No. S-18; weight 14.5 kgm.; non-protein diet 5 days; plasmapheresis once; radioactive d,1-cystine (174 mgm.) fed; non-protein diet supplemented with casein (50 grams), 1-tyrosine (1 gram), and 1-tryptophane (0.25 gram) daily; dog died 3 days later with bronchopneumonia.

Specimen	Duration of experiment	Total protein	Radioactive plasma removed	Radioactivity per cc. plasma			Radioactive cystine in plasma protein		Ingested radioactive 1-cystine (87 mgm.) utilized
				Plasma	Non-protein fraction	Protein fraction	Per cc. plasma	Total plasma removed	
	days	*per cent*	*cc.*	*divisions per second*			*mgm.*	*mgm.*	*per cent*
Plasma	0	7.2							
Plasma	1	6.8	118	0.000529	0.000272	0.000257	0.0068	0.80	
Plasma	2	5.2	130	0.000339	0.000107	0.000232	0.0062	0.81	
Blood	3		5	0.000122					
						Total		1.61	1.84

			Volume	Radioactivity per cc.	Radioactive cystine		d,1-Cystine
					Per cc.	Total specimen	
			cc.	*divisions per second*	*mgm.*	*mgm.*	*per cent*
Urine	3		1000	0.00219	0.058	58*	34

Standard from 0.0080 mgm. cystine had activity of 0.00030 divisions per second.

* Excreted as sulfate.

TABLE V

Radioactive homocystine

Dog No. S-24; weight 8.4 kgm.; low protein diet 10 days, non-protein diet 19 days; plasmapheresis 10 times; lowest plasma protein level 4.1 grams per cent; radioactive d,l-homocystine (500 mgm.) fed; non-protein diet supplemented with gelatin (30 grams) and betaine hydrochloride (4 grams) daily; dog died suddenly 5 days later; weight 7.1 kgm.

Specimen	Duration of experiment	Total protein	Radioactive plasma removed	Radioactivity per cc. plasma			Radioactive cystine in plasma protein		Ingested radioactive d,l-homocystine (500 mgm.) utilized
				Plasma	Non-protein fraction	Protein fraction	Per cc. plasma	Total plasma removed	
	days	*per cent*	*cc.*		*divisions per second*		*mgm.*	*mgm.*	*per cent*
Plasma	0	4.1							
Plasma	1	3.9	15	0.00030					
Plasma	2	4.2	150	0.00033	0.00028	0.00005	0.0016	0.24	
Plasma	3	3.8	15	0.00014					
Plasma	4	4.1	15	0.00011	0.00001	0.00010	0.0032	0.05	
Plasma	5	4.3	150	0.00011	0.00001	0.00010	0.0032	0.49	
						Total		0.78	0.15

			Volume	Radioactivity per cc.	Radioactive homocystine		
					Per cc.	Total specimen	
			cc.	*divisions per second*	*mgm.*	*mgm.*	
Urine	2		200	0.0250	1.55	310*	62.0
Urine	3		200	0.0013	0.08	16*	3.2
Urine	4		200	0.0005	0.017	3.3*	0.7
Feces	2		50	0.0032	0.19	9.8	2.0
					Total		68

			Weight of organ	Radioactivity per 1.9 grams	Radioactive homocystine		
					Per 1.9 grams	Total organ	
			grams	*divisions per second*	*mgm.*	*mgm.*	
Liver	5		336	0.00034	0.0110	1.9	0.4
Lung	5		59	0.00026	0.0084	0.26	0.05
Skin	5		920	0.00018	0.0059	2.8	0.6
Muscle	5		2400	0.00012	0.0039	4.9	1.0

Standard from 0.102 mgm. homocystine had activity of 0.00166 divisions per second.
Standard from 0.026 mgm. homocystine had activity of 0.00080 divisions per second.

* Excreted as sulfate.

TABLE VI

Radioactive homocystine

Dog No. S-25; weight 8.4 kgm.; hematocrit 45 per cent; low protein diet 17 days; plasmapheresis 10 times; lowest plasma protein level 3.8 grams per cent; hematocrit 20 per cent; radioactive d,l-homocystine (50 mgm.) intravenously; low protein diet supplemented with casein hydrolysate (50 grams) daily; dog died 6 days later with bronchopneumonia, pulmonary edema, liver necrosis, and edema.

Specimen	Duration of experiment	Total protein	Radioactive plasma removed	Radioactivity per cc. plasma			Radioactive homocystine in plasma protein		Injected radioactive d,l-homocystine (50 mgm.) utilized
				Plasma	Non-protein fraction	Protein fraction	Per cc. plasma	Total plasma removed	
	days	*per cent*	*cc.*	*divisions per second*			*mgm.*	*mgm.*	*per cent*
Plasma	0	3.8							
Plasma	1		10	0.00007					
Plasma	2	4.1	150	0.00003	0.00001	0.00002	.0031	0.47	0.9
Plasma	3	3.9	150	0	0	0	0	0	0

			Volume	Radioactivity per cc.	Radioactive homocystine		
					Per cc.	Total specimen	
			cc.	*divisions per second*	*mgm.*	*mgm.*	
Urine	2		200	0.00011	0.017	3.4*	6.8
Urine	5		200	0	0	0*	0

Standard from 0.025 mgm. homocystine had activity of 0.00016 divisions per second.

* Excreted as sulfate.

TABLE VII

Radioactive methionine

Dog No. S-26; weight 9 kgm.; non-protein diet 15 days; plasmapheresis twice; lowest plasma protein level 4.1 grams per cent; radioactive d,l-methionine (50 mgm.) fed; non-protein diet supplemented with casein hydrolysate (50 grams) daily, and non-radioactive l-cystine (50 mgm.) twice; experiment terminated after 11 days.

Specimen	Duration of experiment	Total protein	Radioactive plasma removed	Radioactivity per cc. plasma		Radioactive methionine in plasma protein		Ingested radioactive d,l-methionine (50 mgm.) utilized
				Plasma	Protein* fraction	Per cc. plasma	Total plasma removed	
	days	*per cent*	*cc.*	*divisions per second*		*mgm.*	*mgm.*	*per cent*
Plasma	0	4.1						
Plasma	1	3.8	15	0.000057				
Plasma	2	3.6	150	0.000036	0.000005	0.00017	0.025	
Plasma	6	3.7	15	0.000029				
Plasma	11	4.6	15	0.000010	0.000010	0.00034	0.005	
						Total	0.03	0.06

			Volume	Radioactivity per cc.	Radioactive methionine		
					Per cc.	Total specimen	
			cc.	*divisions per second*	*mgm.*	*mgm.*	
Urine	2		400	0.00058	0.02	8†	16
Urine	4		200	0.00014	0.005	1†	2
Feces	4		100	0.00069	0.024	2.4	5

Standard from 0.0028 mgm. methionine had activity of 0.000082 divisions per second.
Standard from 0.028 mgm. methionine had activity of 0.00081 divisions per second.

* Determination of radioactivity made on dialysed specimen.

† Excreted as sulfate.

TABLE VIII

Radioactive methionine

Dog No. S-27; weight 8.0 kgm; hematocrit 45 per cent; non-protein diet 16 days; low protein diet 10 days; non-protein diet 26 days; plasmapheresis 10 times; lowest plasma protein level 4.0 grams per cent; weight loss 1.2 kgm; radioactive d,1-methionine (150 mgm.) intravenously; non-protein diet supplemented with gelatin (50 grams) and 1-tyrosine (1 gram) daily; experiment terminated after 7 days; hematocrit 24 per cent.

Specimen	Duration of experiment	Total protein	Radioactive plasma removed	Radioactivity per cc. plasma		Injected radioactive d,1-methionine (150 mgm.) utilized
				Plasma	Protein fraction	
	days	*per cent*	*cc.*	*divisions per second*		*per cent*
Plasma	0	4.0				
Plasma	1		6	0.000035	0	
Plasma	2	4.1	15	0.000018	0	
Plasma	3	4.6	30	0.0000035	0	
Plasma	7	4.6	15	0	0	0
		Volume	Radioactivity per cc.	Radioactive methionine		
				Per cc.	Total specimen	
		cc.	*divisions per second*	*mgm.*	*mgm.*	
Urine	2	400	0.00070	0.040	16*	11
Urine	4	300	0.000065	0.008	2.4*	1.6
Urine	7	600	0.000021	0.003	1.8*	1.2
Feces	7	200	0.000012	0.001	0.2	0.1

Standard from 0.0029 mgm. methionine had activity of 0.000024 divisions per second.
Standard from 0.029 mgm. methionine had activity of 0.00052 divisions per second.

* Excreted in sulfate.

TABLE IX

Summary of the results of the individual experiments

Dog number	Table number	Amino acid	Weight fed	Total plasma removed	Highest concen. of amino acid in protein fraction of plasma	Ingested amino acid		Diet supplements and remarks
						Incorporated in plasma protein	Excreted in urine as sulfate	
			mgm.	*cc.*	*mgm. per cc.*	*per cent*	*per cent*	
11	II	l-Cystine	100	360	0.024	5.2	36†	Casein, tyrosine
19	III	l-Cystine	200	963	0.046	14.8	19†	Casein, tyrosine, tryptophane
24	V	d,l-Homocystine	500	345	0.0032	0.15	66	Gelatin, betaine
25	VI	d,l-Homocystine	50*	310	0.0031	0.9	7	Casein hydrolysate, infection
26	VII	d,l-Methionine	50	195	0.00034	0.06	18	Casein hydrolysate, l-cystine
27	VIII	d,l-Methionine	150*	66	0.0000	0.00	14	Gelatin, l-tyrosine

* Injected intravenously.

† Calculated on basis of d,l-cystine.

RESULTS

A summary of the results of the individual experiments is given in Table IX. The most efficient utilization of sulfur-containing radioactive amino acids in the production of plasma protein was obtained from cystine. Calculations were made on the basis of the l-cystine content of the ingested d,l-cystine, since d-cystine is not utilized. A comparison of dog 11 and dog 19 (Tables II and III) shows that the ingestion of 2 doses of cystine at a 24-hour interval resulted in a concentration of cystine in plasma protein twice that obtained with one dose. Maximum levels were obtained in 24 hours. However, the total percentage utilization, which is calculated on the basis of the amount of plasma which can be removed without injury, was 3 times as great. This result may have been due to the fact that dog 19 was an animal in better condition than dog 11. That dog 19 was a superior plasma producer was evident from the fact that repeated plasmapheresis reduced the plasma protein to 5.8 grams per cent slowly.

The importance of hypoproteinemia as a stimulus to plasma protein production and to the incorporation of cystine in the plasma proteins is suggested by results from dog 18 (Table IV). The plasma total protein was 7.2 grams per cent (plasmapheresis for induction of hypoproteinemia was not done) at the time radioactive cystine was fed. Only 1.8 per cent of the l-cystine fed was incorporated in the plasma protein withdrawn, in contrast to 5 per cent and 15 per cent in dogs 11 and 19. But since dog 18 developed a fatal infection, the latter may also have been an important factor in producing poor utilization of cystine.

Much poorer utilization was obtained with homocystine and methionine than with cystine. This confirms Whipple's observations on the relative importance of methionine and cystine in plasma protein production by hypoproteinemic dogs kept on a low protein basal diet (2). Supplementary betaine did not improve the utilization of small amounts of homocystine (*cf.* dogs 24 and 25 (Tables V and VI)). In comparing dogs 26 and 27 (Tables VII and VIII), inactive cystine may have exerted a slightly beneficial effect, but tyrosine exerted none. No evidence was obtained in the experiments with homocystine and methionine that intravenous administration of the amino acids increased their utilization in the production of plasma protein.

The concentration of radioactive cystine in liver, lung, kidney, and bowel were found to be about the same in one experiment. Less was found in muscle, and much less in brain. Similar data are given for homocystine in one experiment.

SUMMARY

Dogs made hypoproteinemic, according to Whipple's method (1) of low protein intake and plasmapheresis, were fed cystine, homocystine, or methionine containing radioactive sulfur. Incorporation of these amino acids in the plasma protein was obtained in each case. The highest concentration of amino acid in the plasma protein was obtained with cystine. The largest percentage utilization of amino acid in the production of radioactive plasma protein was obtained with cystine.

Data are provided in Table IX for estimating the radioactive potency of the amino acids necessary to obtain any desired level of radioactivity in the plasma protein.

CONCLUSION

A technique for the preparation of plasma protein tagged with radioactive sulfur, by the utilization of dogs made hypoproteinemic according to Whipple's technique and fed radioactive amino acids, has been developed. From dogs so prepared, the plasma, withdrawn and dialysed, provides a source of radioactive sulfur-containing plasma proteins in sufficient quantity to make possible their use in any study involving the movement of plasma protein into or out of the circulation.

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