LYMPHATIC FLOW IN HUMAN SUBJECTS AS INDICATED BY THE DISAPPEARANCE OF I^{131}-LABELED ALBUMIN FROM THE SUBCUTANEOUS TISSUE * †

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Prior studies in animals have indicated that the lymphatic system returns extravascular protein to the blood stream (1–8). Lewis injected horse serum subcutaneously into dogs and found that it appeared in the thoracic duct lymph before it was detected in the blood (4). Similar observations were made by Field and Drinker (5). Conklin, after injecting foreign proteins into the circulating blood of the frog, observed that they passed through the skin capillaries into the lymphatics (6). More recently Courtice, Simmonds and Steinbeck have shown that homologous serum protein labeled with Evans blue is absorbed by the lymphatics when introduced into the pleural or peritoneal cavities (7, 8).

In man, lymphatic function has been studied mainly by outlining the lymphatics visually with vital dyes injected into the tissues (9–11). The interpretation of these studies has been based primarily on the local distribution of the dyes in the tissues. The removal of particles and colloids by the lymphatics also has been studied (12–14). However, the results of these experiments have been influenced by the local reaction of the tissue to the injected foreign substance.

The present study was undertaken to determine whether the rate of disappearance of human serum albumin injected into the subcutaneous tissues might give some estimate of lymphatic function in man (15).

MATERIAL AND METHODS

Hospitalized patients with and without edema were studied. All patients had been at bed rest for 3 to 5 days without showing a variation in weight of more than 2 pounds or a measurable change in the size of the extremities. Throughout the study the patients remained at bed rest without significant change in body weight or in the circumference of the injected limb.

Fifteen patients in the control group had no history or physical findings suggesting cardiovascular or peripheral vascular disease. Their limbs were nonedematous and appeared normal.

Five patients had had lymphedema of an entire upper extremity for 3 to 15 weeks following radical mastectomy for carcinoma of the breast. One patient had had lymphedema of the lower extremity of 9 weeks' duration, resulting from a radical groin resection for malignant melanoma. In all 6 patients the edematous extremity pitted to local pressure. However, the venous pressure in the involved as well as in the opposite normal limb was in the normal range.

Seven patients had had edema, caused by peripheral venous obstruction, for 1 to 5 weeks. The edema of the involved extremities pitted to a marked degree. Venous obstruction was due to femoral or popliteal vein thrombosis in 4 patients, to compression of the inferior vena cava by an abdominal neoplasm in 2 other patients, and to narrowing of the superior vena cava by an inflammatory mediastinal lesion in another patient. In those cases of vena caval block, the venous pressure distally was over 350 mm of water.

Fifteen patients in severe congestive heart failure had enlargement of the heart, pulmonary congestion by X-ray, venous distention, marked pitting edema of the legs, elevated venous pressure and prolonged circulation time. The heart failure with peripheral edema had been present for 1 to 5 weeks and was being treated with salt restriction and maintenence of digitalis but without diuretics. Three patients with rheumatic heart disease, 2 with hypertensive heart disease and 1 with pulmonic stenosis were restudied after their edema had disappeared following further medical therapy with additional digitalis and frequent injections of mercurials. The hypertensive patients were also given antihypertensive drugs with return of their blood pressures close to normal

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levels. One other patient with constrictive pericarditis was restudied after pericardectomy at which time he was fully compensated, as revealed by the findings of right heart catheterization.

Six patients with Laennec's cirrhosis and 4 patients with nephrotic syndrome had peripheral edema associated with hypoproteinemia. The edema of the legs was of 2 to 7 weeks' duration and pitted markedly. The nephrotic syndrome was caused by amyloidosis in 2 patients, intercapillary glomerulosclerosis in 1 other patient, and glomerulonephritis in the remaining patient. All the patients had a normal venous pressure and were receiving a diet restricted in salt.

Twenty to 30 μc of a 1 per cent solution of I^{131}-labeled human serum albumin [1] was administered subcutaneously into an extremity in a volume of 0.03 to 0.05 ml using a 25 gage needle and a tuberculin syringe. In all studies on paired extremities and in repeat studies in cardiac patients the dose and volume of labeled albumin injected into the limbs was identical. The sites of injection were similarly controlled. To prevent leakage of the radioactive protein from the puncture wound, the labeled albumin was injected 30 to 35 mm from the site of puncture by advancing the needle subcutaneously. After the needle was withdrawn the puncture site was sealed with collodion. The site of injection was then externally monitored at intervals of 10 to 15 minutes for the first hour and every 2 to 3 hours for the next 12 hours. Thereafter, the site was monitored 4 times a day until the injected radioactivity had disappeared from the limb. The activity at the site of injection was corrected for "background" by subtracting the radioactivity of the uninjected limb from the activity of the injected limb. In certain cases, a scintogram scan of the entire limb was made and compared with that of the opposite control limb. The thyroid gland was externally monitored at intervals of 6 to 12 hours. Daily total urine collections were made and assayed for radioactivity. Plasma samples were drawn at times at which the limb was monitored. Total radioactivity, trichloroacetic acid (10 per cent) precipitable radioactivity, and the radioactivity in the supernatant were determined in the plasma samples. More than 96 per cent of the radioactivity was precipitable with the trichloroacetic acid, and all of the radioactivity was contained in the albumin fraction separated by electrophoresis. Radioactivity of plasma and urine samples was assayed in a well-type sodium iodide crystal. The measurements of radioactivity were related to appropriate standards.

In a number of subjects after the labeled albumin had completely disappeared from the tissue, 0.1 ml of a solution containing 10 to 15 μc of $Na^{24}Cl$ or of NaI^{131} was injected subcutaneously into an area which had previously been injected with I^{131}-labeled albumin. The rate of disappearance of these radioactive ions was determined by monitoring the injection site at intervals of 1 to 3 minutes. The activity at the site of injection was corrected for background (final plateau reached after clear-

[1] Obtained from Abbott Laboratories, Oak Ridge, Tenn.

TABLE I

Disappearance of I^{131}-albumin and NaI^{131} from a normal leg *

Subject	Age	Sex	BSA	I^{131}-alb. disapp. $T_{\frac{1}{2}}$	I^{131}-alb. appearance after			I^{131}-alb. diffusion after			Urinary excretion†	Thyroid uptake‡	Serum ppt. act.	NaI^{131} disapp. $T_{\frac{1}{2}}$
					6	24	48	72 hrs	0	6	24	48 hrs				
	yrs		*m²*	*hrs*	*% dose/L plasma*				*cm²*			*cm²*			*%*	*min*
R. H.	42	M	1.75	42	1.6	4.9	7.7	7.5	3.8	4.5	6.2	8	3.5	1.7	>97	9
N. B.	42	M	1.72	35	2.1	5.4	7.2	7.4	2.5	5.3	14	17	4.3	2.6	>98	18
B. M.	36	F	1.61	39									3.2	1.7		11§
A. R.	28	M	1.65	24	2.3	8.0	8.7	9.1	2.5	4.5	10	13	3.6	2.8	>97	
A. M.	55	F	1.79	48	2.0	4.0	5.8	6.2					5.7	1.5	>97	12
A. L.	61	M	1.82	42	1.8	5.8	7.2	7.4	3.1	6.2	13	14	4.6	1.4	>96	
E. S.	58	F	1.60	30	2.9	7.6	8.6	8.6	5.3		7.1	8.0	3.8	1.9		7§
R. B.	45	M	1.74	26	2.4	6.8	7.2	7.1					5.2	2.1	>97	10
L. P.	43	M	1.70	36	2.5	5.8	7.0	6.9	5.3	6.2	10	13	3.5	1.0	>97	
C. F.	46	F	1.58	20	2.7	8.2	9.0	8.6	3.1		9.1	11	4.8	2.2	>96	13
M. S.	53	F	1.75	32					3.1	5.3	11		4.1	1.8		15§
L. W.	52	M	1.67	38	2.8	6.5	8.4	7.9					3.9	1.4	>98	
R. S.	28	F	1.69	18	2.5	7.2	7.7	7.4	4.5	7.1	13	15	5.2	2.1	>97	
B. K.	52	M	1.74	45	2.1	6.0	7.5	7.4					4.6	1.6	<97	10
F. S.	41	F	1.54	24	3.2	9.4	10.5	10.2	4.5	8	20	23	4.2	3.1	>96	14
Mean	45.5		1.69	33.4	2.4	6.6	7.9	7.8	3.8	5.9	11.3	13.6	4.3	1.9	> 96.9	11.9
SD	±9.9		±0.08	±9.3	±0.5	±1.5	±1.2	±1.1	±1.1	±1.2	±4.0	±1.5	±0.7	±0.6	> ±0.6	±3.2

* I^{131}-alb. disapp. =disappearance of I^{131}-albumin from the limb; I^{131}-alb. diffusion =area of diffusion of I^{131}-albumin about the injection site; Serum ppt. act. =% of total plasma radioactivity precipitated by 10% trichloroacetic acid.
† Per cent of the I^{131}-albumin dose excreted in the urine per day.
‡ Per cent of the I^{131}-albumin dose taken up by the thyroid per day.
§ $T_{\frac{1}{2}}$ of $Na^{24}Cl$.

TABLE II

Disappearance of I^{131}-albumin and NaI^{131} from a lymphedematous and opposite normal limb

Subject	Age	Sex	BSA	Cause of lymph-edema	Limb	I^{131}-alb. disapp. $T_{\frac{1}{2}}$	I^{131}-alb. appearance after				I^{131}-alb. diffusion after				Urinary excre-tion*	Thyroid uptake†	Serum ppt. act.	NaI^{131} disapp $T_{\frac{1}{2}}$
							6	24	48	72 hrs	0	6	24	48 hrs				
	yrs		m^2			*hrs*	*% dose/L plasma*				*cm^2*		*cm^2*				*%*	*min*
E. A.	57	F	1.85	Radical mastectomy	Control	27	1.0	2.9	3.8	4.1	3.1	5.3	13	15	2.1	1.1	>96	7‡
					Edema§	140					2.5	4.5	10	15				8
M. G.	65	F	1.78	Radical mastectomy	Control	26	1.4	3.4	4.0	4.3					3.0	1.2	>97	6
					Edema	92												7
A. A.	54	F	1.82	Radical mastectomy	Control	21	1.2	3.2	3.6	3.9	3.8		7.1	8.0	2.4	0.8	>96	10‡
					Edema	90					3.8		9.1	11				10
D. B.	59	F	1.74	Radical mastectomy	Control	22									2.8	0.7		15
					Edema	101												14
J. D.	62	F	1.83	Radical mastectomy	Control	27	1.3	3.2	4.0	4.3	4.5	5.3	9.1	11	2.2	0.9	>97	11
					Edema	118					3.8	4.5	8.0	9.1				9
E. P.	32	F	1.65	Radical groin resection	Control	33	1.7	3.4	4.4	4.9	3.1	6.2	10	13	2.7	1.0	>96	12
					Edema	65					2.5	6.2	14	15				13
Mean	54.8		1.78			26.0					3.6	5.6	9.8	11.8				10.2
SD	±11.8		±0.08		Control	4.3	1.3	3.2	3.9	4.3	±0.7	±0.5	±2.4	±3.0	2.5	1.0	>96.4	±3.3
Mean					Edema	101.0	±0.3	±0.2	±0.3	±0.4	3.2	5.1	10.3	13.8	±0.4	±0.2	>±0.5	10.2
S.D.						±25.7					±0.8	±1.0	±2.6	±3.2				±2.8

* Per cent of I^{131}-albumin dose excreted in the urine per day.
† Per cent of I^{131}-albumin dose taken up by the thyroid per day.
‡ $T_{\frac{1}{2}}$ of $Na^{24}Cl$.
§ Lymphedematous.

ance) and for decay of the isotopes. In repeat studies in cardiac patients and in all studies on paired extremities, the administered dose and volume of the radioactive ion was the same. The sites of injection were also controlled.

Two types of collimation were employed to limit the detected radiation to the monitored areas. For the lower extremities, a shield of lead bricks, 2 inches thick, was constructed to provide a square opening (approximately 100 cm^2) over which the injection site could conveniently be placed in front of the radiation detector, which was recessed 15 cm below the opening of the shield. For monitoring the thyroid gland and the injection site in the upper extremities, a conical lead shield, 2 inches thick, with a round opening (approximately 175 cm^2) into which the radiation detector was recessed 40 cm, was used.

Duplicate determinations of the monitored radioactivity checked within 2 per cent. Tissue background radiation arising from circulating I^{131}-albumin at the injection site was assumed to be equal to that of a similar area in the opposite extremity.

RESULTS

The results are summarized in Tables I through VII.

Removal of I^{131}-albumin from the normal limb. The disappearance rate of injected labeled albumin from the subcutaneous tissue of a normal leg is illustrated in Figure 1. For the first 4 to 8 hours following the injection of I^{131}-albumin there was a relatively rapid decrease in radioactivity over the injected area in 10 of the 15 cases. During this period 10 to 28 per cent of the injected dose disappeared. Thereafter the radioactivity decreased more slowly. The half-time of disappearance ($T_{\frac{1}{2}}$), calculated from the slower exponential component of the disappearance curve, averaged 33.4 hours and ranged from 18 to 48 hours. In contrast to these values, the $T_{\frac{1}{2}}$ of NaI^{131} from the subcutaneous tissue of the same subjects averaged 11.9 minutes and ranged from 7 to 18 minutes. There was no consistent relationship between the $T_{\frac{1}{2}}$ of I^{131}-labeled albumin and that of NaI^{131}.

Repeated scanning over the site of injection revealed that I^{131}-albumin remained within the monitored area, although some diffusion occurred. Immediately after injection, the area occupied by the injected activity averaged about 3.8 cm^2, whereas 24 and 48 hours after the injection it averaged 11.3 and 13.6 cm^2, respectively. The relationship between the $T_{\frac{1}{2}}$ and the area of diffusion of the

TABLE III

Disappearance of I^{131}-albumin and NaI^{131} from paired normal limbs

Subject	Age	Sex	BSA	Limb*	I^{131}-alb. disapp. $T_{\frac{1}{2}}$	NaI^{131} disapp. $T_{\frac{1}{2}}$
	yrs		*m^2*		*hrs*	*min*
L. M.	52	F	1.77	R. F.	19	6
				L. F.	20	5
A. C.	57	F	1.84	R. F.	25	18†
				L. F.	27	20
C. F.	49	F	1.81	R. F.	25	10
				L. F.	25	11
M. C.	54	F	1.75	R. F.	22	15
				L. F.	22	13
E. B.	34	F	1.68	R. L.	31	
				L. L.	29	
E. K.	42	F	1.72	R. L.	34	14
				L. L.	32	14
N. B.	37	F	1.64	R. L.	28	9†
				L. L.	38	8
Mean	46.4		1.74	R. Ext.	26.3	12.0
SD	±8.9		0.08		±5.2	±4.4
Mean				L. Ext.	27.6	11.8
SD					±6.1	±5.2

* R.F. = right forearm; L.F. = left forearm; R.L. = right leg; L.L. = left leg; R. Ext. = right extremity; L. Ext. = left extremity.

† $T_{\frac{1}{2}}$ of $Na^{24}Cl$.

TABLE IV

Disappearance of I^{131}-albumin in edema due to venous obstruction

Subject	Age	Sex	BSA	Cause of edema	Leg	I^{131}-alb. disapp. $T_{\frac{1}{2}}$	I^{131}-alb. appearance after				I^{131}-alb. diffusion after				Urinary excretion*	Thyroid uptake†	Serum ppt. act.
							6	24	48	72 hrs	0	6	24	48 hrs			
	yrs		*m²*			*hrs*		*% dose/L plasma*			*cm²*			*cm²*			%
S. T.	59	F	1.62	Femoral vein thrombosis	Control	32	2.5	7.6	8.2	9.0					3.2	2.1	>97
					Edema	20											
E. C.	42	F	1.58	Femoral vein thrombosis	Control	41	2.3	7.5	8.7	9.2	4.5	7.1	13	15	4.5	1.8	>96
					Edema	18					3.1	8	17	23			
H. S.	48	M	1.92	Femoral vein thrombosis	Control	27	1.9	5.2	5.7	5.9					4.8	1.9	>97
					Edema	12											
A. S.	55	F	1.88	Popliteal vein thrombosis	Control	34					2.5	6.2	13	17	4.1	1.5	
					Edema	12					2.5	8	20	30			
D. M.	64	M	1.75	Inf. vena caval block	Edema	16	3.1	6.0	6.6	6.2					1.8	3.5	>98
R. M.	61	M	1.78	Inf. vena caval block	Edema	18	2.6	5.6	6.0	5.9	3.1	4.5	13		2.0	2.7	>96
A. R.	42	M	1.72	Sup. vena caval block	Control‡	36	2.0	6.4	7.0	7.7					3.3	2.9	>96
					Edema§	17											
Mean	53.0		1.75		Control	34.0	2.4	6.4	7.0	7.3					3.4	2.3	>96.7
SD	±9.1		±0.1			±5.1											
Mean						16.1	±0.4	±1.0	±1.2	±1.5					±1.2	±0.7	> ±0.8
SD					Edema	±3.1											

* Per cent of I^{131}-albumin dose excreted in the urine per day.
† Per cent of I^{131}-albumin dose taken up by the thyroid per day.
‡ Nonedematous legs.
§ Edematous forearm.

TABLE V

Disappearance of I^{131}-albumin in cardiac edema

Subject	Age	Sex	BSA	Diagnosis*	Edema grade	Venous pressure	I^{131}-alb. disapp. $T_{\frac{1}{2}}$	I^{131}-alb. appearance after				I^{131}-alb. diffusion after			Urinary excretion†	Thyroid uptake‡	Serum ppt. act.
								6	24	48	72 hrs	0	6	24 hrs			
	yrs		*m²*			*mm H_2O*	*hrs*		*% dose/L plasma*				*cm²*				*%*
F. R.	62	M	1.75	ASHD	4+	205	18	2.5	6.6	7.0	6.8	3.1	8	25	0.8	3.4	>97
M. M.	47	M	1.71	ASHD	4+	305	12	4.4	7.8	8.0	8.0	2.5	7.1	20	1.7	2.5	>96
M. L.	60	F	1.72	ASHD	3+	235	24	2.0	5.3	5.0	5.8	3.4		17	2.1	3.0	>97
E. J.	44	M	1.67	Hyp., ASHD	3+	205	10	5.2	9.6	9.4	9.0				1.9	2.3	>98
J. B.	64	M	1.70	Hyp., ASHD	4+	315	30	2.1	5.4	6.9	6.6				0.9	3.5	>96
W. B.	52	M	1.68	HCVD	3+	325	13	3.4	5.6	6.0	6.0				1.2	2.8	>96
A. S.	59	M	1.62	HCVD	4+	255	28					3.8	8	20			
M. A.	39	M	1.69	HCVD	4+	180	15	3.2	5.9	6.2	5.9				1.6	2.2	>97
M. F.	56	M	1.84	HCVD	3+	285	14					4.5	13	37	1.0	3.3	
G. L.	45	M	1.64	RHD MI, MS	4+	235	12	3.5	5.4	5.1	5.0	4.5	9.1	26	1.1	2.9	>96
C. J.	41	F	1.51	RHD, MI, MS	3+	275	12	4.4	9.5	9.0	8.8				2.2	3.1	>98
W. G.	43	M	1.58	RHD, AI, MI	4+	205	11	4.8	7.5	7.3	7.2	2.5	4.5	11	1.8	2.5	>97
W. M.	45	F	1.73	RHD., MI	4+	295	16	3.0	5.6	5.8	5.7	3.1	4.5	13	2.0	2.7	>97
R. L.	23	M	1.82	Pericard.	3+	340	15	3.1	5.7	6.1	6.0	3.1	10.1	30	0.6	2.8	>96
F. B.	41	M	1.60	Pul. sten.	3+	240	20	2.2	6.5	7.2	7.0	3.1	10	28	1.2	3.0	>97
Mean	48.1		1.68			260	16.7	3.4	6.6	6.9	6.7	3.4	8.3	22.9	1.4	2.8	>96.8
SD	±10.9		0.09			±49.9	±6.2	±1.1	±1.5	±1.3	±1.2	±0.7	±2.7	±8.4	±0.5	±0.4	>±0.8

* ASHD =arteriosclerotic heart disease; Hyp. =hypertensive; HCVD =hypertensive cardiovascular disease; RHD =rheumatic heart disease; Pericard. =pericarditis, chronic constrictive; MI = mitral insufficiency; AI =aortic insufficiency; MS =mitral stenosis; Pul. sten. =pulmonic stenosis.

† Per cent of I^{131}-albumin dose excreted in the urine per day.

‡ Per cent of I^{131}-albumin dose taken up by the thyroid per day.

TABLE VI

Disappearance of I^{131}-albumin and $Na^{24}Cl$ in cardiac edema following therapy

Subject	Treatment	Edema grade	Venous pressure	Decholin circ. time	I^{131}-alb. disapp. $T_{\frac{1}{2}}$	Na^{24} Cl disapp.
			mm H_2O	*sec*	*hrs*	
M. A.	None	4+	180	35	15	54
	After 8 days	0	80	14	31	15
M. F.	None	3+	285	38	12	62*
	After 7 days	0	75	15	25	14
G. L.	None	4+	235	33	12	
	After 7 days	0	100	24	40	
C. J.	None	3+	275	45	12	67
	After 8 days	0	185	40	24	60
W. G.	None	4+	205	31	11	27
	After 8 days	0	95	23	44	19
R. L.	None	3+	340	46	15	56
	After 15 days	0	90	13	24	16
F. B.	None	3+	240	34	20	52*
	After 7 days	0	145	27	34	41
Mean	None		251	37.4	13.9	53.0
SD			±54	±5.9	±3.1	±13.9
Mean	After		110	22.1	31.7	27.5
SD			±40	±9.5	±8.1	±18.9

* $T_{\frac{1}{2}}$ of NaI^{131}.

injected activity was inconsistent. Scans of the limbs also showed that the radioactivity throughout the injected limb, except for the injection site, was comparable to that in the opposite control limb and was not accompanied by a detectable increase of radioactivity over the regional lymph nodes.

As I^{131}-albumin disappeared from the site of injection, the radioactivity in the blood increased progressively in the first 18 to 36 hours and

TABLE VII

Disappearance of I^{131}-albumin in hypoproteinemic edema

Subject	Age	Sex	BSA	Diagnosis	Edema grade	Serum total protein	Serum albumin	I^{131}-alb. disapp. $T_{\frac{1}{2}}$
	yrs		*m^2*			*g %*	*g %*	*hrs*
C. S.	48	M	1.60	Cirrhosis	3+	6.9	2.4	10
C. C.	55	M	1.72	Cirrhosis	3+	4.2	1.9	13
M. C.	51	F	1.52	Cirrhosis	2+	5.4	1.8	20
R. C.	62	M	1.79	Cirrhosis	3+	5.0	1.8	16
M. R.	49	M	1.68	Cirrhosis	3+	5.2	2.2	14
A. P.	45	F	1.57	Cirrhosis	4+	4.8	1.8	17
C. M.	54	F	1.58	Nephrotic syndrome	4+	5.5	0.9	17
E. N.	38	M	1.74	Nephrotic syndrome	4+	5.1	1.3	18
T. R.	23	M	1.65	Nephrotic syndrome	4+	5.2	1.9	15
C. A.	65	F	1.82	Nephrotic syndrome	4+	5.7	1.2	12
Mean	49.0		1.67			5.3	1.7	15.2
SD	±12.0		±0.1			±0.7	±0.5	±3.0

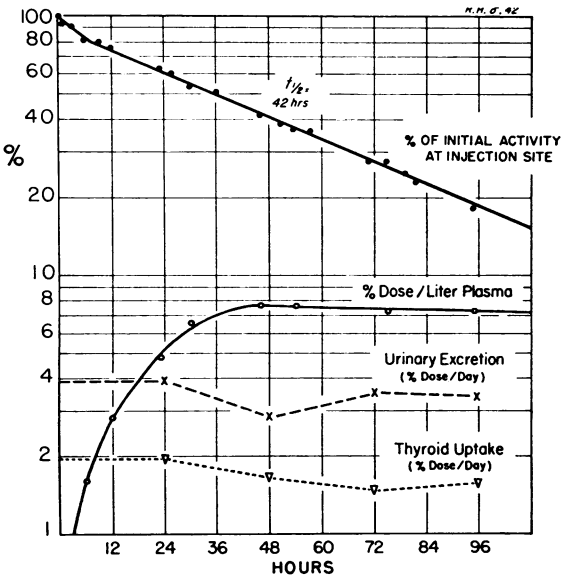

FIG. 1. THE DISAPPEARANCE OF I^{131}-ALBUMIN FROM THE SUBCUTANEOUS TISSUE OF A NORMAL LEG.

reached a plateau in 48 to 72 hours. The buildup of radioactivity in the plasma was more rapid in those subjects with a short than in those with a long $T_{\frac{1}{2}}$ of I^{131}-labeled albumin. Of the total activity in each plasma sample, more than 96 per cent was contained on the protein fraction precipitated by 10 per cent trichloroacetic acid.

Thyroid and urine radioactivities averaged, respectively, 1.9 and 4.3 per cent of the injected dose per day.

Removal of I^{131}-albumin from the lymphedematous limb. The clearance of I^{131}-albumin and NaI^{131} from the subcutaneous tissue of a lymphedematous limb due to a radical mastectomy and that of the opposite normal limb are compared in Figures 2 and 3.

In six patients I^{131}-albumin disappeared at a significantly slower rate from the lymphedematous limb than from the control limb ($p < 0.01$). The $T_{\frac{1}{2}}$ from the involved limb averaged 101 hours and ranged from 65 to 140 hours as compared to an average of 26 hours and a range of 21 to 33 hours from the opposite normal limb. Some diffusion of the injected albumin occurred in the tissue but was not appreciably different in the two limbs. In contrast to these results, the disappearance of NaI^{131} and $Na^{24}Cl$ did not differ significantly in the lymphedematous and opposite normal limb ($p > 0.9$) and was approximately 150 to 600 times more rapid than the disappearance of I^{131}-albumin. In control studies of paired normal limbs the disappearance of labeled albumin as well as of NaI^{131} and $Na^{24}Cl$ was approximately the same ($p = 0.4$ and $p = 0.8$, respectively).

Following the subcutaneous injection of I^{131}-albumin, radioactivity, more than 96 per cent of which was precipitable with 10 per cent trichloroacetic acid, appeared in the plasma. The average urinary excretion of radioactivity was 2.5 per cent per day, whereas the radioactivity taken up by the thyroid gland averaged 1 per cent per day.

Removal of I^{131}-albumin in edematous limbs caused by venous occlusion. The disappearance of I^{131}-albumin from an edematous limb caused by phlebothrombosis is compared with that of the opposite normal limb in Figure 4.

The disappearance of I^{131}-albumin in edema caused by venous obstruction in contrast to that caused by lymphatic obstruction was significantly increased as compared to the disappearance of I^{131}-albumin in control limbs ($p < 0.01$). In four subjects with venous obstruction, the mean disappearance time of I^{131}-albumin in the edematous limb was 16 hours, whereas in the opposite normal limb it was 34 hours. The diffusion of radioactivity from the subcutaneous injection site also appeared to be greater in the edematous than in the control limb. It is noteworthy that the re-

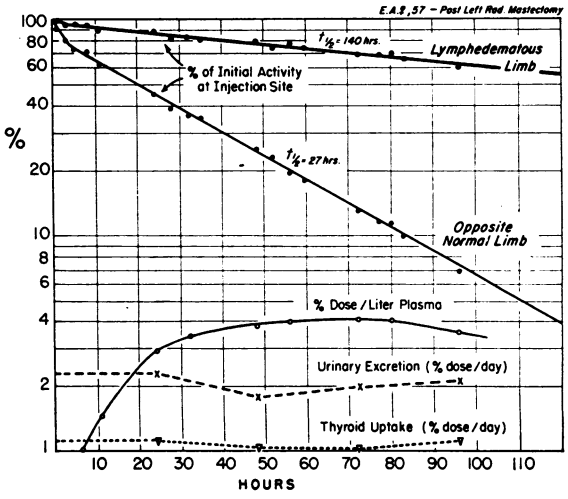

FIG. 2. THE DISAPPEARANCE OF I^{131}-ALBUMIN FROM THE SUBCUTANEOUS TISSUE OF A LYMPHEDEMATOUS FOREARM DUE TO A RADICAL MASTECTOMY FOR CARCINOMA OF THE BREAST AS COMPARED TO THAT OF THE OPPOSITE NORMAL FOREARM.

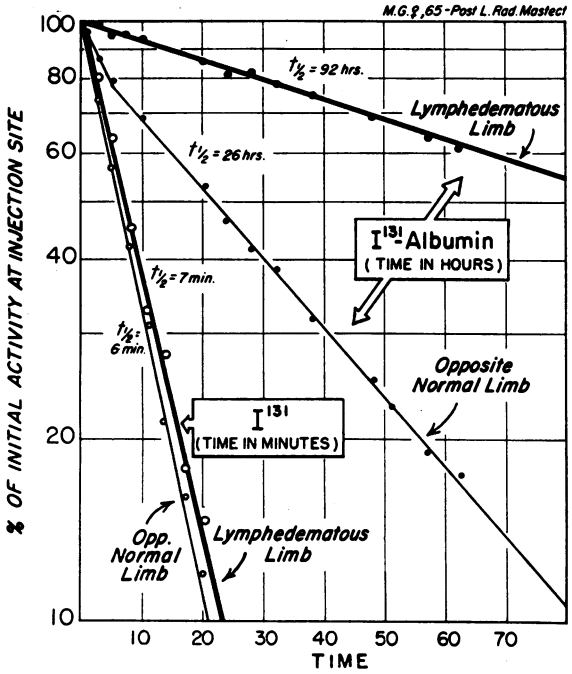

FIG. 3. THE DISAPPEARANCE OF I^{131}-ALBUMIN AND NaI^{131} FROM THE SUBCUTANEOUS TISSUE OF A LYMPHEDEMATOUS FOREARM DUE TO A RADICAL MASTECTOMY AND FROM THE OPPOSITE NORMAL FOREARM.

moval of I^{131}-albumin from the upper extremities in Patient A.R., with superior vena caval block, was unimpaired even though the antecubital venous pressure was 465 mm H_2O. The appearance

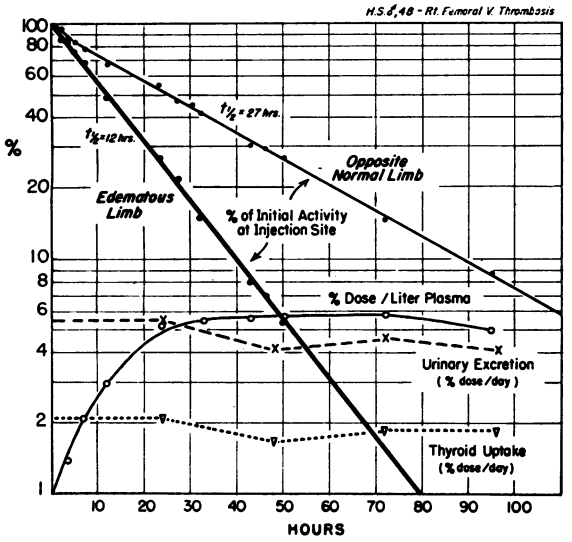

FIG. 4. THE DISAPPEARANCE OF I^{131}-ALBUMIN FROM THE SUBCUTANEOUS TISSUE OF AN EDEMATOUS LEG CAUSED BY FEMORAL VEIN THROMBOSIS AS COMPARED TO THAT OF THE OPPOSITE NORMAL LEG.

of radioactivity in the plasma, urine and thyroid gland was similar to that observed in the control group.

Removal of I^{131}-albumin in cardiac edema. The clearance of I^{131}-albumin and $Na^{24}Cl$ from the subcutaneous tissue of the leg during and following recovery from heart failure in a hypertensive patient is illustrated in Figure 5.

The disappearance and appearance curves of I^{131}-albumin in patients with cardiac edema were similar to those in normal subjects. However, the $T_{\frac{1}{2}}$, which averaged 16.7 hours and varied

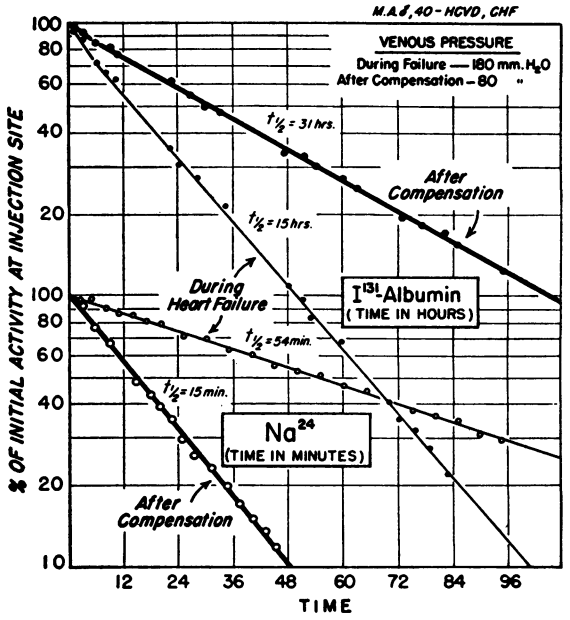

FIG. 5. THE DISAPPEARANCE OF I^{131}-ALBUMIN AND $Na^{24}Cl$ FROM THE SUBCUTANEOUS TISSUE OF A LEG OF A CARDIAC PATIENT DURING AND FOLLOWING RECOVERY FROM CONGESTIVE HEART FAILURE.

from 11 to 30 hours, was significantly shorter in the cardiac edematous than in the normal leg ($p < 0.01$). The buildup of activity in the blood also appeared to be more rapid in patients with cardiac edema than in normal subjects, although there was an inconsistent relationship between the actual level of radioactivity measurable in the plasma and the amount of I^{131}-albumin which had disappeared from the site of injection. The radioactivity excreted in the urine was significantly less ($p < 0.01$), whereas the radioactivity appearing in the thyroid gland was significantly greater ($p < 0.01$) than the values obtained in the control group. The area of diffusion of the injected

activity in the subcutaneous tissue was significantly greater in the cardiac edematous than in the normal limb ($p < 0.01$). Twenty-four hours following injection of the I^{131}-albumin, radioactivity of the injection site covered an average area of 22.9 cm^2 in edematous cardiac patients as compared to 11.3 cm^2 in normal subjects. Although the average rate of removal of $Na^{24}Cl$ in cardiac edema was about 50 times more rapid than that of I^{131}-albumin, it was significantly slowed, being about one-fourth as rapid as that in normal subjects ($p < 0.01$).

In seven subjects who were restudied after cardiac compensation, the removal of I^{131}-albumin from the subcutaneous tissue of the legs decreased noticeably toward the normal range. The $T_{\frac{1}{2}}$ averaged 31.7 hours as compared to 13.9 hours before treatment. The clearance of Na^{24} also returned toward normal after treatment, but in contrast to that of I^{131}-albumin, it was considerably more rapid after compensation than it had been during congestive failure. The $T_{\frac{1}{2}}$ of Na^{24} before treatment averaged 53 minutes, whereas after treatment it averaged 28 minutes. The return of Na^{24} clearance to the normal range after treatment was associated with "complete" compensation as indicated by the disappearance of peripheral edema and a decrease in venous pressure and circulation time to normal levels. However, normal clearance values for I^{131}-albumin occurred without "complete" compensation, but it was consistently associated with the disappearance of edema.

Removal of I^{131}-albumin in hypoproteinemic edema. The disappearance of I^{131}-albumin from the subcutaneous tissue of the legs of patients with hypoproteinemic edema caused by cirrhosis or nephrotic syndrome was significantly more rapid than that in the control group ($p < 0.01$). The $T_{\frac{1}{2}}$ averaged 15.2 hours and ranged from 10 to 20 hours.

DISCUSSION

I^{131}-albumin appeared to have a high degree of stability following injection into the subcutaneous tissue. The existence of a firm bond between the radioiodine and albumin molecules in the tissue is supported by the observation that the disappearance of I^{131}-albumin from the tissues was considerably slower than that of inorganic I^{131}. The amount of radioiodine excreted in the urine and taken up by the thyroid gland following the subcutaneous injection of the labeled albumin was small and consistent with the metabolic breakdown of plasma proteins, and over 96 per cent of the plasma radioactivity was contained on the protein fraction precipitated by trichloroacetic acid and was accounted for in the albumin fraction separated by electrophoresis.

The removal of labeled albumin from the resting limb was slow but occurred at an exponential rate. The explanation for the increased clearance of the labeled protein in the first 4 to 8 hours after injection is not clear but might be due to the injection of some radioactivity directly into the subcutaneous lymphatic capillaries or to a temporary injury to the lymphatic capillaries by the injection. Some dissociation of I^{131} from the albumin molecule has been reported to occur in certain batches of radioiodinated serum albumin (16). However, it is unlikely that the clearance of unbound I^{131} would account for the relatively rapid fall-off of activity at the injection site during the first 4 or more hours, since the removal of inorganic I^{131} was virtually complete in 1 hour.

The appearance of radioactivity in the blood did not parallel the disappearance of radioactivity from the site of injection even when appropriate corrections were made for the radioactivity which had been excreted in the urine or taken up by the thyroid gland. The dissimilarities in the disappearance and appearance curves are not surprising, since the level of radioactivity in the blood at a given time presumably is influenced not only by the amount of radioactivity appearing in the blood from the injection site but also by the volume of distribution of the radioactivity, which increases as the labeled albumin equilibrates with extravascular protein.

The marked reduction in the clearance of I^{131}-albumin from the subcutaneous tissue in lymphedema suggests that the principal route of removal of extravascular protein in man is through the lymphatic vessels and that the direct passage of interstitial protein across blood capillaries into the blood stream is negligible in such a limb. Studies with I^{131}-albumin in animals also support this view (17). The finding also suggests that the rate of disappearance of labeled albumin might be employed as an index of lymphatic "function," including flow, since it is likely that protein is ab-

sorbed by the lymphatic vessels along with a relatively fixed amount of interstitial fluid. In animal studies, Drinker and Field have concluded that the absorption of protein by the lymphatics is accompanied by salt and water in the same concentrations that exist in the tissue fluid (1).

The present observations that the disappearance of Na^{24} or I^{131} from subcutaneous tissue is much more rapid that that of I^{131}-albumin, that it is comparable in lymphedematous and normal limbs, and that it is influenced by the degree of cardiac compensation, are in line with the evidence that inorganic ions are principally cleared from the interstitial fluid by direct diffusion through the blood capillaries into the blood stream (18–21). The findings are also consistent with reports that the rate of disappearance of inorganic ions from the tissues may be a useful index of blood flow, especially through the capillaries (18–20).

With the exception of lymphatic edema, the disappearance of I^{131}-albumin in edema caused by other conditions such as venous obstruction, congestive heart failure, and hypoproteinemia was significantly more rapid than the removal of I^{131}-albumin from normal limbs. It is unlikely that the differences in albumin removal were due to differences of diffusion of the injected protein in the subcutaneous tissue, since the I^{131}-albumin disappeared at an exponential rate. It is also improbable that differences shown by the different groups in thyroidal and renal clearances of radioiodide liberated from degraded I^{131}-albumin influenced the disappearance of the injected albumin.

An increase in permeability to protein in the blood capillaries might also promote the removal of I^{131}-albumin by the blood stream. However, evidence for such a change is lacking in cardiac and nephritic edema, since the interstitial protein concentration in these conditions is only a small fraction of the plasma protein concentration and no greater than the protein content of interstitial fluid in normal limbs (22, 23). Furthermore, Ross and Walker have recently presented findings which indicate a decrease rather than an increase in capillary permeability to protein in congestive heart failure (24). Other present observations which argue against the blood stream as the major route of removal of subcutaneously injected albumin in cardiac edema are those showing that the clearance of I^{131}-albumin changed in an opposite direction to that of NaI^{131} or $Na^{24}Cl$ following recovery from heart failure.

These results are consistent with the presence of an increased lymphatic flow in cardiac edema. They also appear to indicate that edema fluid in heart failure is not "stagnant," but actually exchanges with the fluid and salt in the blood and circulates through the lymphatics at a fairly constant rate and more rapidly than does normal interstitial fluid.

As opposed to the present findings, McMaster has concluded from the local spread of vital dyes injected intradermally that lymphatic flow, although increased in nephritic edema, was negligible or absent in cardiac edema (10). Threefoot, using similar methods of study, has indicated a reduction in lymphatic flow within the skin of all edematous extremities (11). Since leakage of the vital dye from the cutaneous lymphatics was reported to occur in both these studies, it might have influenced the results of the experiments. Furthermore, the interpretation of these studies was made from the distribution and not from the actual rate of removal of the dye from the tissues.

In addition to the methods of measurement, other factors which might account for differences in results between those and the present studies include the age of the patients studied, duration of the edema, and differences in the cutaneous as compared with the subcutaneous lymphatic flow. In the current study the patients were younger and had a shorter history of heart failure than the patients studied by McMaster.

In edema, especially of long duration, a proliferation of interstitial tissue may occur and interfere with lymphatic drainage. This factor and differences in interstitial pressure may account for the variations in the clearance of labeled albumin from edematous tissues. It likewise appears from the observations in edematous states, especially in heart failure and superior vena caval block syndrome, that the interstitial pressure is an extremely important determinant of lymphatic flow, even when marked hypertension exists in the veins into which the main lymphatics drain.

SUMMARY

The disappearance of human serum albumin labeled with radioiodine from the subcutaneous

tissue of the forearm or leg described an exponential slope and was followed by the appearance of radioactive albumin in the blood. The rate of removal of I^{131}-albumin from the tissues was significantly reduced in edema caused by lymphatic obstruction, but significantly increased in edema caused by venous obstruction, congestive heart failure or hypoproteinemia. The disappearance of $Na^{24}Cl$ or of NaI^{131} from the tissues was considerably more rapid than that of I^{131}-albumin and was not significantly altered in lymphatic edema, although it was significantly reduced in cardiac edema. Following treatment of the heart failure, the clearance of labeled protein and inorganic ions changed in opposite directions and returned to or toward normal values.

These findings indicate that I^{131}-albumin injected into the subcutaneous tissue is removed mainly by lymphatic vessels and suggest that lymphatic flow is reduced in lymphedema but is increased in cardiac and other types of edema.

REFERENCES

1. Drinker, C. K., and Field, M. E. The protein content of mammalian lymph and the relation of lymph to tissue fluid. Amer. J. Physiol. 1931, **97**, 32.
2. Drinker, C. K., Field, M. E., and Homans, J. The experimental production of edema and elephantiasis as a result of lymphatic obstruction. Amer. J. Physiol. 1934, **108**, 509.
3. White, J. C., Field, M. E., and Drinker, C. K. On the protein content and normal flow of lymph from the foot of the dog. Amer. J. Physiol. 1933, **103**, 34.
4. Lewis, J. H. The route and rate of absorption of subcutaneously injected serum in relation to the occurrence of sudden death after injection of antitoxic horse serum. J. Amer. med. Ass. 1921, **76**, 1342.
5. Field, M. E., and Drinker, C. K. The permeability of the capillaries of the dog to protein. Amer. J. Physiol. 1931, **97**, 40.
6. Conklin, R. E. The formation and circulation of lymph in the frog. III. The permeability of the capillaries to protein. Amer. J. Physiol. 1930, **95**, 98.
7. Courtice, F. C., and Simmonds, W. J. Absorption of fluids from the pleural cavities of rabbits and cats. J. Physiol. (Lond.) 1949, **109**, 117.
8. Courtice, F. C., and Steinbeck, A. W. The lymphatic drainage of plasma from the peritoneal cavity of the cat. Aust. J. exp. Biol. med. Sci. 1950, **28**, 161.
9. McMaster, P. D. Changes in the cutaneous lymphatics of human beings and in the lymph flow under normal and pathological conditions. J. exp. Med. 1937, **65**, 347.
10. McMaster, P. D. The lymphatics and lymph flow in the edematous skin of human beings in the cardiac and renal diseases. J. exp. Med. 1937, **65**, 373.
11. Threefoot, S. A. The local spread of intradermally injected dye in edematous and nonedematous extremities. Clin. Res. 1958, **6**, 234.
12. Lemon, W. S., and Higgins, G. M. Pulmonary fibrosis: Experiments of short duration. Amer. J. med. Sci. 1932, **183**, 153.
13. Meneely, G. R., Kory, R. C., Auerbach, S. H., and Hahn, P. F. Distribution of radioactive colloidal gold following intrapulmonary administration. Fed. Proc. 1951, **10**, 365.
14. Hahn, P. F., and Carothers, E. L. Lymphatic drainage following intrabronchial installation of silver-coated radioactive gold colloids in therapeutic quantities. J. thorac. Surg. 1953, **125**, 265.
15. Hollander, W., Reilly, P., and Burrows, B. A. Lymphatic flow in human subjects as indicated by the disappearance of I^{131} labeled albumin from the subcutaneous tissues (abstract). J. clin. Invest. 1956, **35**, 713.
16. Berson, S. A., Yalow, R. S., Schreiber, S. S., and Post, J. Tracer experiments with I^{131} labeled human serum albumin: Distribution and degradation studies. J. clin. Invest. 1953, **32**, 746.
17. Patterson, R. M., Ballard, C. L., Wasserman, K., and Mayerson, H. S. Lymphatic permeability to albumin. Amer. J. Physiol. 1958, **194**, 120.
18. Kety, S. S. Measurements of regional circulation by local clearance of radioactive sodium. Amer. Heart J. 1949, **38**, 321.
19. Walder, D. N. The relationship between blood flow, capillary surface area and sodium clearance in muscle. Clin. Sci. 1955, **14**, 303.
20. McGirr, E. M. Tissue clearance of inorganic ions. Brit. med. Bull. 1952, **8**, 192.
21. Stone, P. W., and Miller, W. B., Jr. Mobilization of radioactive sodium from gastrocnemious muscle of dog. Proc. Soc. exp. Biol. (N. Y.) 1949, **71**, 529.
22. Epstein, A. A. Studies on the chemistry of serous effusions. J. exp. Med. 1914, **20**, 334.
23. Stead, E. A., Jr., and Warren, J. V. The protein content of the extracellular fluid in normal subjects after venous congestion and in patients with cardiac failure, anoxemia and fever. J. clin. Invest. 1944, **23**, 283.
24. Ross, R. S., and Walker, W. G. Decreased permeability of capillaries to protein in chronic congestive heart failure (abstract). J. clin. Invest. 1956, **35**, 732.