In Vivo Assessment of Pulmonary Vascular Integrity in Experimental Pulmonary Edema

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ABSTRACT During single pass indicator studies across the lungs [^{14}C]urea remains in the vascular compartment, but its molecular size and solubility suggest it might escape abnormally permeable vessels. To test the hypothesis that [^{14}C]urea might be used to distinguish pulmonary edema due to acutely increased intravascular pressure from that due to vascular damage by alloxan, we studied [^{51}Cr]erythrocytes (r), [^{125}I]albumin (a), [^{14}C]urea (u), and tritiated water as dilution indicators in the pulmonary circulation of anesthetized dogs. In addition, the adequacy of albumin as an intravascular indicator was evaluated.

Indicator curves, blood gases, hematocrit, and vascular pressures were determined during a base-line period and repeated 30 and 60 min after treatment in five groups of dogs: (a) saline control, (b) alloxan edema, (c) epinephrine infusion, (d) volume overload, and (e) left atrial (LA) balloon obstruction.

Groups b, d, and e developed a similar degree of edema judging by wet/dry lung weights and histology. Groups a and c did not develop edema. In alloxan edema, differences between the mean transit time volume of u and r (V_{u-r}) increased over base line at 30 ($P < 0.01$) and 60 min ($P < 0.02$); the differences between the mean transit time volume of a and r (V_{a-r}) increased slightly at 30 ($P < 0.03$) and 60 min ($P < 0.02$); and V_{u-r} significantly exceeded V_{a-r} at 30 (mean difference = 9 ml, $P < 0.02$) and 60 min (mean difference = 11, $P < 0.04$). In none of the other groups did V_{u-r} significantly exceed V_{a-r}. Thus, comparison of V_{u-r} with V_{a-r} may permit distinction between "high pressure" and "increased permeability" pulmonary edema.

Albumin was not a consistently reliable indicator of intravascular volume as compared with composite red cell and albumin curve.

Received for publication 25 September 1972 and in revised form 5 April 1973.

INTRODUCTION

Most of the information available about movement of water across permeable vessels in the pulmonary circulation suggests that, as in other organs, the Starling hypothesis (1) is valid (2). Pulmonary edema develops when the net rate of fluid movement from the vessels to the lung interstitium exceeds the rate at which the lymphatics return fluid to the systemic circulation (2). This situation may occur as a result of an increase in the net transmural pressure gradient in the permeable vessels or as a result of an increase in permeability of the vascular bed to protein (and presumably to smaller hydrophilic molecules as well) (2–5).

The most frequent cause of altered pressure relationships favoring increased filtration of fluid in the lungs is heart failure. This "high pressure" edema has been produced experimentally by a variety of maneuvers (6–9) and much of the data describing pulmonary edema has been obtained in such preparations.

Lung edema develops in humans without apparent heart failure after administration of certain drugs (10, 11), during traumatic (12) or septic (13, 14) shock, after thoracentesis (15), or blood transfusion (16), and during the course of several acute illnesses (17). The study of pathogenesis in these clinical situations is hampered partially by a lack of in vivo methods for detecting altered vascular permeability either when present as an isolated process or when present together with increased intravascular pressures.

We have attempted to detect altered pulmonary vascular permeability using indicator dilution techniques because they can be used in vivo. Urea was chosen as the test indicator because of its physical characteristics and its behavior in the pulmonary vascular bed. Urea is a hydrophilic substance with a molecular radius ($r \simeq$ 2.6Å) and a free diffusion coefficient ($D_{37°C} = 1.95 \times 10^{-5} cm^2 s^{-1}$) only slightly different from water ($r \simeq$

1.5Å; $D_{37°C} = 3.4 \times 10^{-5} cm^2 s^{-1}$), and most investigators have assumed that it passively crosses vessel walls (18). However, unlike water, urea behaves essentially like an intravascular indicator in a single transit through the lungs of normal dogs (19, 20). When lung vascular permeability is increased, urea should escape more readily from the vascular space and the relationship between its single pass dilution and the curve of a less "diffusible" intravascular indicator might change. Since hydrostatic pressure only minimally affects diffusive flux, similar changes might not be expected to result from elevating intravascular pressure.

A number of experimental models have been described in which pulmonary edema has been produced by chemical agents in living animals (21–27) or in isolated perfused lungs (28, 29). For this study alloxan-induced pulmonary edema in dogs was chosen because it is the most thoroughly studied (6, 7, 30). Since alloxan alters vascular pressures very little (6, 7), does not increase capillary blood volume (7), and increases permeability to protein in isolated lungs (29), the edema is apparently a result of loss of vascular integrity.

Chinard, Enns, and Nolan measured "pulmonary extravascular water volume" in living dogs by comparing indicator dilution curves of tritiated water and an intravascular indicator across the lungs (31), and many investigators have used this method to measure pulmonary edema. The distribution volumes of lung water, determined by this technique, are not limited by the diffusion rate of tritiated water (32–34), but are uniformly smaller than lung water content determined by postmortem dessication (6, 35, 36). This discrepancy has been attributed to hypoperfusion and "inaccessible" water (6, 34), and since most investigators have used either radioactively labeled albumin or dyes that bind to plasma proteins as the intravascular indicator, two questions remain unanswered. First, does albumin leak from the vascular compartment during one transit through edematous lungs? If it does, extravascular lung water may be underestimated. Second, does neglecting the fraction of water in the faster moving red cells, when calculating intravascular transit time, result in a marked underestimate of water in edematous lungs? Several investigators have studied this problem in dogs without edema (37, 38) and Goresky, Cronin, Lawson, and Wangel reported preliminary studies in edematous lungs (39), but careful comparisons of red cell and albumin transits in edematous animals have not been reported. Because of the marked changes in flow and hematocrit in some of our experimental groups, we felt it necessary to evaluate the reliability of albumin as an intravascular indicator.

In this study we have compared dilution curves of four indicators during a single transit through the lungs of dogs with and without pulmonary edema to test the hypothesis that [^{14}C]urea used as a dilution indicator in the pulmonary circulation will permit distinction between "high pressure" and "increased permeability" pulmonary edema. In addition, we have determined the adequacy of albumin as an intravascular indicator in both kinds of edema.

METHODS

We studied mongrel dogs anesthetized with 30–35 mg/kg sodium pentobarbital given intravenously. The animals were restrained supine and ventilated by a Harvard respirator (Harvard Apparatus Co., Inc., Millis, Mass.) with room air through a cuffed endotracheal tube. The lungs were hyperinflated every 7 min to 30 cm H_2O pressure. Catheters were passed through the external jugular veins and femoral arteries into the right atrium, pulmonary artery, terminal aorta, and left ventricle under fluoroscopic observation. Aortic, left ventricular and mean pulmonary arterial pressures were measured continuously by model P23Gb Statham strain gauges (Statham Instruments, Inc., Oxnard, Calif.) and recorded on a Sanborn model 350 recorder (Sanborn Div., Hewlett-Packard Co., Waltham, Mass.) was also used to record an electrocardiogram. 15 ml of the dog's blood was mixed with 3 ml special formula acid citrate dextrose solution (Abbott Laboratories, North Chicago, Ill.) in a siliconized plastic tube and incubated at room temperature with 40 μCi of [^{51}Cr]sodium chromate for 45 min. The cells were then washed twice with normal saline and resuspended in saline to the original volume. 12 μCi [^{125}I]human serum albumin (^{125}I-ALB), 40 μCi tritiated water (THO), and 10 μCi [^{14}C]urea were added to these labeled cells. This injectate was prepared immediately before the first dilution curve was run and kept refrigerated for the duration of the study to prevent breakdown of urea. Immediately before each dilution curve, arterial blood samples were taken for determination of background activities of the isotopes and hematocrit, and an anaerobic sample collected for measurement of P_{CO_2}, P_{O_2}, and pH that were determined with Instrumentation Laboratory model S113 blood gas analyzer (Instrumentation Laboratory, Inc., Lexington, Mass.). For each dilution curve, 3.0 ml of the injectate was rapidly injected as a bolus into the right atrial catheter (catheter vol = 0.43 ml), afterwards 1.2-ml samples were drawn at 1-s intervals from the catheter in the terminal aorta using an automatic syringe sampler described by Ramsey, Puckett, Jose, and Lacy (33).

A 0.5 ml portion of each blood sample and of the injectate diluted 1 : 51 in the dog's base-line blood was counted for duplicate 2-min periods in a two channel Packard Tri-Carb model 3002 crystal scintillation spectrometer (Packard Instrument Co., Inc., Downers Grove, Ill.) for ^{125}I and ^{51}Cr activity. Corrections were made for the overlap of ^{51}Cr into the ^{125}I channel. Each sample was then mixed with 3.0 ml absolute ethanol, centrifuged, and a 2.0 ml portion of the supernate placed in Bray's solution (40) for determination of ^{14}C and ^{3}H activity by duplicate 2-min counts in a Packard Tri-Carb model 4312 liquid scintillation detector (Packard Instrument Co., Inc.). Quenching was monitored using an external standard and the overlap of ^{14}C into the ^{3}H channel was determined for samples with similar quenching. The mean of duplicate counts was used for all calculations.

Relative concentration, expressed as a ratio of counts for each indicator in each sample to the total counts of that indicator injected, was plotted on a logarithmic scale against a linear time scale; the downslopes were extrapolated linearly using a least-squares line and calculations of area, flow, and mean transit time were made as described by Ramsey et al. (33). The difference between mean transit time volumes of any two indicators was taken as the product of the difference in their mean transit times and flow determined from the red cell curves. The intravascular mean transit time for a "composite" curve of labeled red cells and albumin was calculated according to the formulas of Goresky, Cronin, and Wangel (37), using the hematocrit determined before each dilution curve and assuming the water content of red blood cells as 0.70 g H_2O/ml cells and the water content of plasma as 0.93 g H_2O/ml plasma.

In each study a base-line dilution curve was run and curves were repeated at 30 and 60 min after treatment. Immediately after the last dilution curve the dog's chest was opened, the left hilum cross-clamped, the animal rapidly exsanguinated, and the lungs removed. After blotting them free of excess blood, the total wet lung weight and the wet weight of the right lung were determined. The left lung was then prepared for histological examination and the right lung was dried to constant weight suspended in a well-ventilated hood at room temperature. Approximately 3 wk were required for drying.

The left lung was fixed under 30 cm H_2O intrabronchial pressure with 1.5% glutaraldehyde in isotonic saline overnight and the sections from the posterior portion of the lower lobe were transferred to 10% buffered formalin solution and subsequently sectioned and stained with hematoxylin and eosin for light microscopy.

Six dogs only received an injection of 10–20 cm^3 normal saline into the right atrium following the base-line curves (saline control group). Seven dogs received 125 mg/kg alloxan (Fisher Scientific Co., Pittsburgh, Pa.) dissolved in 10–20 cm^3 normal saline injected into the right atrium following the base-line curves (alloxan edema group). Five dogs received a slow infusion (about 200 ml/h) of a 0.003% solution of epinephrine in normal saline for the 60 min study period after the base-line curves (epinephrine group). Six dogs received a liter of 6% dextran (average molecular weight 70,000) in saline over a 15 min period after the base-line curve, followed by an infusion of 0.003% epinephrine in dextran (about 200 ml/h) regulated to maintain the left ventricular end diastolic pressure at 35–50 mm Hg (volume overload edema group). Two additional dogs receiving alloxan and one receiving dextran and epinephrine died before the studies could be completed and are not included.

In addition to these four primary study groups, pulmonary edema was induced in four animals by inflating a balloon in the left atrium. These preparations were identical with those described above except that no catheter was placed in the left ventricle, and before the base-line indicator dilution studies, a left thoracotomy was performed and two catheters were fixed in the left atrium through an incision in the atrial appendage. One catheter had a 40 ml capacity inflatable balloon on its distal end and the other, fixed cephalad to the balloon, was used for recording mean left atrial pressures throughout the studies. The chest was not closed. The base-line studies were carried out with the balloon deflated. After this, 100–150 ml of 6% dextran in normal saline was infused through the right atrial catheter and the left atrial balloon was slowly inflated with about 15–20 ml of air to maintain left atrial pressure at 25–35 mm Hg. Indicator dilution studies were repeated at 30 and 60 min after inflation of the balloon. After the last dilution study, the thoracotomy incision was extended, the animal was exsanguinated, and both lungs were removed, separated, blotted, weighed, and dried to constant weight in room air. Histologic examination of these lungs was not done.

The significance of differences between observations in the same animals was evaluated with a paired t test and the significance of differences between observations in different groups of animals was evaluated using a t test for independent groups (41).

RESULTS

Table I defines the abbreviations we have used in presenting and discussing our data.

Blood gas, pH, hematocrit, and pulmonary arterial pressure values for each of the four primary study groups are summarized in Table II. Systemic arterial pressure in the control and alloxan edema groups was stable throughout the study period at about 150/100 mm Hg. In the epinephrine groups, systemic pressure rose from a base-line mean of 157/110 to 200/133 mm Hg by 60 min. Systemic pressure in the volume overload group rose from a base-line mean of 170/119 to 268/167 mm Hg at 60 min.

TABLE I
Abbreviations Used

$\dot{Q}$	Flow (ml/s) determined from [^{51}Cr]erythrocyte dilution curve.
V_{a-r}	Difference between the mean transit time volumes of [^{125}I]human serum albumin and [^{51}Cr]erythrocytes (ml).
V_{u-r}	Difference between the mean transit time volumes of [^{14}C] urea and [^{51}Cr]erythrocytes (ml).
V_{w-r}	Difference between the mean transit time volumes of tritiated water and [^{51}Cr]erythrocytes (ml).
V_{w-a}	Difference between the mean transit time volumes of tritiated water and [^{125}I]human serum albumin (ml).
V_{w-comp}	Difference between the mean transit time volumes of tritiated water and a composite intravascular indicator (ml) (see Methods).
W/D	Ratio of wet lung weight to dry lung weight uncorrected for residual blood.
histo*	Predominant pattern on light microscopic examination.
0	No edema
1+	Perivascular and/or peribronchial edema without alveolar flooding.
2+	Intraalveolar edema.

Flow determined from the [^{51}Cr]erythrocyte dilution curve was used for the mean transit time volume calculations for all of the indicators.
* See reference 7.

TABLE II

Mean Arterial Blood Gas, pH, Hematocrit, and Pulmonary Arterial Pressure Values in the Four Primary Study Groups

Group	Study time	P_{O_2}	P_{CO_2}	pH	Hct	P_{PA}
		mm Hg	*mm Hg*		%	*mm Hg*
Saline control	Base line	104±6	32±3	7.50±0.04	42±2	15±1
	30 min	107±5	32±3	7.50±0.04	40±2	15±1
	60 min	106±6	32±3	7.51±0.04	39±2	14±1
Alloxan edema	Base line	104±4	33±3	7.46±0.04	42±3	12±1
	30 min	85±4	35±3	7.37±0.04	42±1	12±1
	60 min	83±6	34±3	7.39±0.03	41±1	12±1
Epinephrine	Base line	104±6	28±3	7.54±0.04	42±2	15±1
	30 min	96±9	38±4	7.33±0.02	54±3	24±2
	60 min	94±9	38±4	7.28±0.01	55±4	25±2
Volume overload edema	Base line	115±1	30±4	7.51±0.03	44±3	12±1
	30 min	101±6	40±5	7.34±0.01	32±2	53±3
	60 min	82±8	47±8	7.24±0.04	36±2	48±3

All values are mean ±SE.

Recovery of [^{125}I]albumin and tritiated water relative to [^{51}Cr]erythrocytes was similar in all study groups. For 84 sets of curves, mean recovery of [^{125}I]albumin was 101.6%±4.4 SD and tritiated water was 98.8% ±5.6 SD. In 40 control and base-line studies, mean recovery of [^{14}C]urea was 95.4%±3.2 SD. Urea recoveries in the other study groups were: alloxan (n = 14) 92.3%±4.7 SD, volume overload (n = 12) 97.3% ±4.4 SD, epinephrine (n = 10) 98.6%±4.7 SD, and left atrial balloon (n = 8) 94.0%±6.6 SD.

V_{w-r} remained stable in the control and epinephrine groups but increased in each of the edema groups. Although there was variability within groups, mean W/D was higher than control values in all of the

TABLE III

Summary of Data—Control Group

Dog no.	Weight	Study time	$\dot{Q}$*	V_{a-r}*	V_{u-r}*	V_{w-r}*	W/D*	histo*
	kg							
1	21.6	Base line	43	16	16	81		
		30 min	39	16	15	91		
		60 min	41	13	17	74	4.28	0
2	17.3	Base line	33	12	11	70		
		30 min	32	12	13	75		
		60 min	29	10	12	71	4.41	0
3	21.1	Base line	62	23	22	107		
		30 min	64	20	24	108		
		60 min	66	32	26	120	4.48	0
4	19.1	Base line	39	14	14	82		
		30 min	46	18	19	84		
		60 min	40	11	10	74	4.44	0
5	23.2	Base line	102	24	21	118		
		30 min	97	25	20	120		
		60 min	89	22	23	120	4.34	0
6	19.1	Base line	45	19	27	103		
		30 min	47	23	40	121		
		60 min	41	13	21	109	4.24	0
Mean ±SE	20.2±0.9	Base line	54±10	18±2	18±2	93±8		
		30 min	54±10	19±2	22±4	100±8		
		60 min	51±9	17±4	18±3	95±10	4.36±0.04	

* See Table I for explanation of abbreviations.

TABLE IV

Summary of Data—Alloxan Edema Group

Dog no.	Weight	Study time	$\dot{Q}$*	V_{a-r}*	V_{u-r}*	V_{w-r}*	W/D*	histo*
	kg							
1	14.1	Base line	19	11	17	83		
		30 min	18	13	17	89		
		60 min	17	12	19	83	4.69	1+
2	18.6	Base line	52	17	19	111		
		30 min	46	24	30	125		
		60 min	57	23	23	138	5.75	1+
3	15.9	Base line	38	7	12	82		
		30 min	35	11	31	122		
		60 min	31	14	41	158	6.34	2+
4	21.8	Base line	40	14	13	77		
		30 min	39	16	27	125		
		60 min	41	27	38	151	5.34	2+
5	22.0	Base line	39	20	22	85		
		30 min	32	21	28	107		
		60 min	26	21	43	172	6.54	2+
6	17.0	Base line	44	9	13	90		
		30 min	32	11	22	90		
		60 min	40	14	18	97	5.51	2+
7	22.3	Base line	98	26	19	96		
		30 min	95	26	24	116		
		60 min	93	31	33	140	4.93	1+
Mean ±SE	18.8±1.2	Base line	47±9	15±2	16±1	89±4		
		30 min	42±9	17±2	26±2	111±6		
		60 min	43±10	20±3	31±4	134±12	5.58±0.26	

* See Table I for explanation of abbreviations

TABLE V

Summary of Data—Epinephrine Group

Dog no.	Weight	Study time	$\dot{Q}$*	V_{a-r}*	V_{u-r}*	V_{w-r}*	W/D*	histo*
	kg							
1	25.7	Base line	56	26	15	132		
		30 min	99	44	34	126		
		60 min	93	32	25	126	4.36	
2	18.0	Base line	33	22	29	89		
		30 min	108	39	25	100		
		60 min	87	28	12	73	4.22	0
3	23.4	Base line	81	29	18	102		
		30 min	142	24	16	101		
		60 min	64	23	16	96	4.15	0
4	18.6	Base line	69	21	23	79		
		30 min	106	30	24	81		
		60 min	82	20	11	75	4.08	0
5	21.6	Base line	60	16	13	90		
		30 min	87	35	24	94		
		60 min	88	43	34	106	4.18	0
Mean ±SE	21.5±1.4	Base line	60±8	23±2	19±3	99±9		
		30 min	108±9	34±4	24±3	100±7		
		60 min	82±5	29±4	20±4	95±10	4.20±0.05	

* See Table I for explanation of abbreviations.

TABLE VI

Summary of Data—Volume Overload Edema Group

Dog. no.	Weight	Study time	$\dot{Q}$*	$V_{a\to r}$*	$V_{u\to r}$*	$V_{w\to r}$*	W/D*	histo*
	kg							
1	26.1	Base line	28	10	12	78		
		30 min	126	20	20	105		
		60 min	118	27	20	111	5.28	2+
2	13.9	Base line	35	15	16	75		
		30 min	87	25	23	94		
		60 min	93	27	19	106	5.16	2+
3	15.0	Base line	45	12	11	69		
		30 min	86	17	17	97		
		60 min	38	12	12	94	4.91	2+
4	16.4	Base line	52	13	14	86		
		30 min	157	37	39	166		
		60 min	104	25	23	320	5.83	2+
5	18.0	Base line	38	16	12	59		
		30 min	64	13	11	129		
		60 min	67	19	16	141	6.43	2+
6	18.2	Base line	54	28	21	98		
		30 min	136	30	23	118		
		60 min	136	49	42	135	4.44	1+
Mean ±SE	17.9±1.8	Base line	42±4	16±3	14±2	78±5		
		30 min	109±15	24±4	22±4	118±11		
		60 min	92±14	26±5	22±4	151±35	5.34±0.29	

* See Table I for explanation of abbreviations.

edema groups and the mean values in edema groups were similar. Histological data confirmed the other measures of edema.

Assessment of vascular integrity (Tables III–VII). Cardiac output was essentially stable in the control, alloxan edema, and left atrial balloon groups. As expected, $\dot{Q}$ tended to increase in animals receiving either epinephrine or epinephrine and dextran.

Figs. 1 and 2 are examples of dilution curves obtained in control and alloxan edema studies. There were

TABLE VII

Summary of Data—Left Atrial Balloon Group

Dog no.	Weight	Study time	$\dot{Q}$*	$V_{a\to r}$*	$V_{u\to r}$*	$V_{w\to r}$*	W/D*
	kg						
1	12.5	Base line	28	23	24	101	
		30 min	29	70	74	204	
		60 min	26	77	78	306	5.91
3	13.6	Base line	39	21	28	107	
		30 min	40	21	7	170	
		60 min	37	92	90	273	4.95
3	19.1	Base line	28	17	17	80	
		30 min	30	42	40	147	
		60 min	36	65	64	222	5.05
4	19.8	Base line	21	18	15	63	
		30 min	42	63	49	299	
		60 min	28	55	54	241	4.33
Mean ±SE	16.2±1.9	Base line	29±4	20±1	21±3	88±10	
		30 min	35±3	49±11	42±14	205±33	
		60 min	32±3	72±8	72±8	260±18	5.06±0.32

* See Table I for explanation of abbreviations.

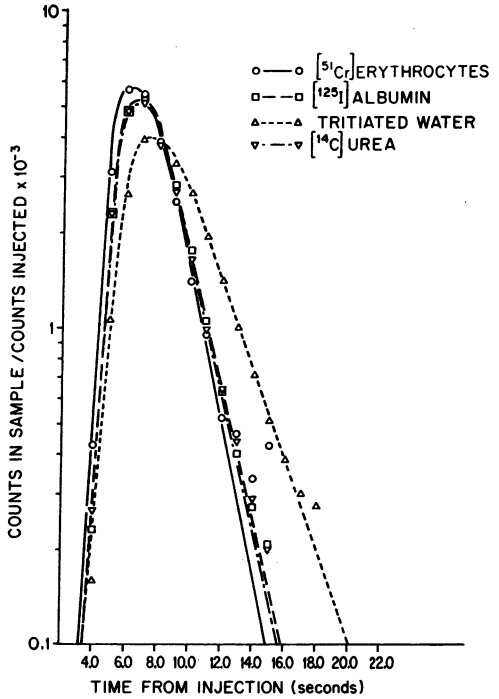

FIGURE 1 Indicator dilution curves for four indicators from a saline control experiment. The curves for [^{125}I]-albumin and [^{14}C]urea are essentially identical.

no significant changes from base-line V_{a-r} in the control group. The animals with alloxan edema showed small but significant increases at 30 ($P < 0.03$) and 60 min ($P < 0.02$). V_{a-r} tended to increase in both the epinephrine and volume overload groups, but the only significant changes from the base line were in the volume overload group at 60 min ($P < 0.03$). There were consistent large increases in V_{a-r} in the animals with inflated left atrial balloons.

The control group showed no significant changes in V_{w-r} during the study period, but in the alloxan group, V_{w-r} was increased from base line at 30 ($P < 0.01$) and 60 min ($P < 0.02$). There were consistent large increases in this value in the left atrial balloon group; the only other significant changes were in the volume overload group at 60 min ($P < 0.05$).

V_{w-r} was not significantly different from V_{a-r} at any study period in the control group of animals. This was also true for base-line studies in all of the experimental groups. However, in the alloxan edema group, V_{w-r} significantly exceeded V_{a-r} at 30 (difference = 9 ml, $P < 0.02$) and 60 min (difference = 11 ml, $P < 0.04$). In the epinephrine group, V_{w-r} was significantly less than V_{a-r} at 30 ($P < 0.01$) and 60 min ($P < 0.01$). In the volume overload group these values were not significantly different at 30 min, but at 60 min V_{w-r} was smaller ($P < 0.02$). V_{w-r} did not substantially exceed V_{a-r} in any of the studies in the left atrial balloon group.

Figs. 3 and 4 illustrate the changes in calculated volumes from base line in each of the primary study groups.

Evaluation of intravascular indicators (Table VIII). Table VIII compares the extravascular water volume calculations in each of the experimental groups using albumin alone as the intravascular indicator with that using a composite intravascular curve of red cells and albumin, and shows the means, ranges, and coefficients of variation of the ratios of these two values in each group. The greatest differences are apparent in the epinephrine group, where hematocrit was increased, and in the left atrial balloon group, where the separation of red cells and albumin was greatest. In these two groups, the mean difference in calculated extravascular water volume is 15–20% and some values greater than 25% different were seen.

DISCUSSION

Assessment of vascular integrity. Although small hydrophilic molecules cross capillaries relatively rapidly (42–44), indicator dilution studies suggest that many

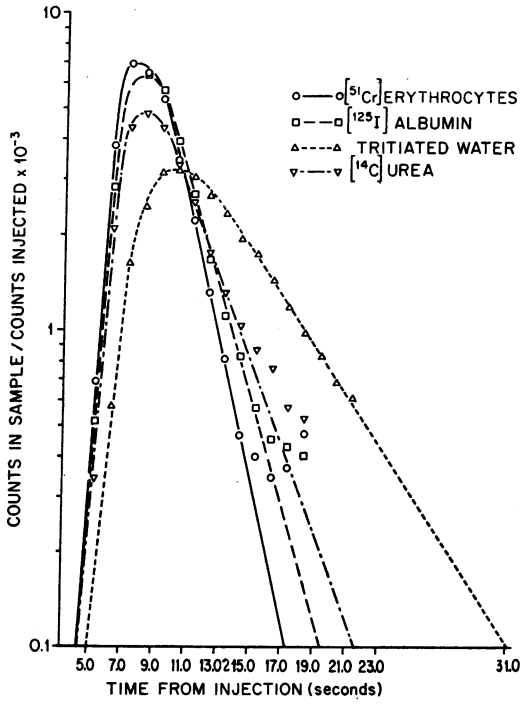

FIGURE 2 Indicator dilution curves for four indicators from an alloxan edema study. The peak of the [^{14}C]urea curve is lower than that of [^{125}I]albumin, and the downslope for [^{14}C]urea is less steep, suggesting a larger urea distribution volume.

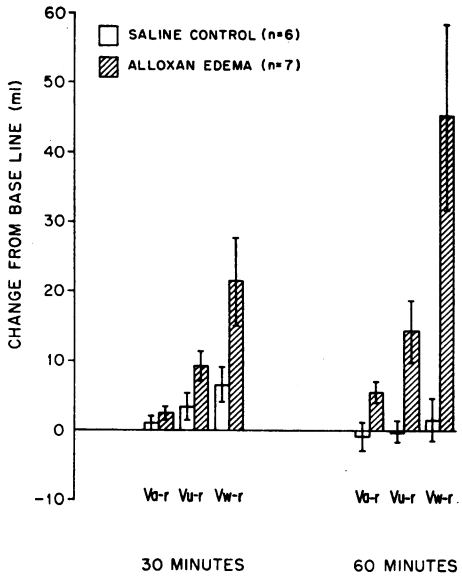

FIGURE 3 Changes in calculated distribution volumes for [^{125}I]albumin, [^{14}C]urea, and tritiated water from base line in saline control and alloxan edema groups. Abbreviations are explained in Table I, values are means ±SEM.

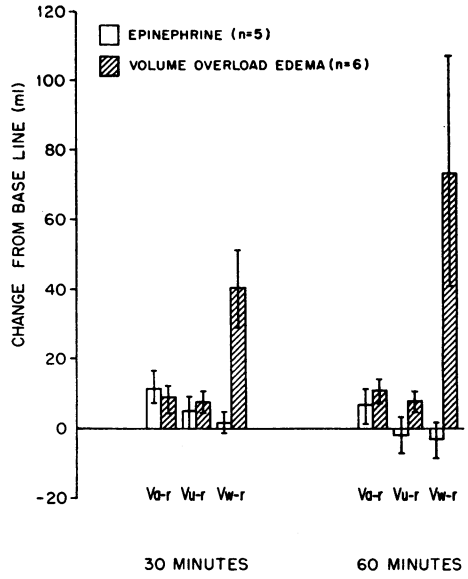

FIGURE 4 Changes in calculated distribution volumes for [^{125}I]albumin, [^{14}C]urea, and tritiated water in epinephrine and volume overload edema groups. Abbreviations are explained in Table I, values are means ±SEM.

such substances are almost completely confined to the vascular space in one transit through the lungs (9, 19, 20). In an effort to gain more information about the pulmonary vascular bed, several investigators have used small water-soluble substances as dilution indicators in humans and animals with pulmonary edema.

Lilienfield, Freis, Partenope, and Morowitz recovered less thiocyanate relative to an intravascular indicator in patients with congestive heart failure than in patients with normal lungs (45). Bauman, Rothschild, Yalow, and Berson obtained similar results with sodium as an indicator in humans with heart failure (46). Freinkel, Schreiner, and Athens recovered slightly less ^{24}Na in two patients with heart failure than in normals (47). Pearce studied sodium as an indicator in dogs with volume overload edema and alloxan edema and found decreased recovery in both situations with greater mean loss of sodium in his alloxan group (9). Gump, Mashima, Jorgensen, and Kinney compared sodium and labeled albumin dilution curves in surgical patients with respiratory failure and found decreased recovery of sodium compared with patients with normal lungs (48). The authors interpreted this as evidence for vascular damage, but pressures were not reported and no patients with "high pressure" edema were studied for comparison.

Since the shape of single transit dilution curves for small hydrophilic molecules reflects primarily diffusive movement, it would be expected that a change in permeability of these vessels might change the shape of the

TABLE VIII
Extravascular Water Volume Comparison

Study group	N	V_{w-e}* (Mean ±SE)	V_{w-comp}* (Mean ±SE)	V_{w-e}/V_{w-comp} Mean ±SD	Range	C‡
					%	
Controls	36	74±2	80±3	91.5±3.4	84–96	3.7
Alloxan edema	14	103±7	110±7	93.8±2.5	89–97	2.7
Epinephrine	10	66±5	81±5	81.1±5.8	74–90	7.2
Volume overload edema	12	110±18	116±18	94.0±4.0	85–98	4.3
Left atrial balloon	8	172±16	196±16	87.5±6.7	72–94	7.7

* See Table I for explanation of abbreviations.
‡ C = coefficient of variation.

dilution curves of such substances relative to those of intravascular indicators. Increases in hydrostatic pressure affect diffusion across permeable vessels very little (18), and the dilution curves might not be affected in situations where increased filtration of water is due to increased intravascular pressure. This does not seem to be the case for the substances discussed above, and Pearce has postulated that sodium is "bound" by abnormal vascular endothelium in his edematous dogs (9). We found the distribution volume of [^{14}C]urea in excess of that of [^{51}Cr]erythrocytes (V_{u-r}) to be greater than this "excess volume" for [^{125}I]albumin (V_{a-r}) during alloxan-induced edema, when permeability is increased but hemodynamic variables are constant. V_{u-r} did not exceed V_{a-r} in animals with pulmonary edema resulting from increased intravascular pressure whether flow was increased (volume overload edema) or not (left atrial balloon group). These findings are consistent with the concept that vessel walls impose the main diffusion barrier to the exit of urea from the vascular space, and that acutely increased hydrostatic pressure has less effect on transvascular flux of urea than increased vascular permeability. We found no evidence that urea behaves other than passively in the pulmonary circulation in these studies.

A comparison of the indicators we have used permits distinction between alloxan pulmonary edema and "high pressure" pulmonary edema. Therefore, the technique should detect an increased vascular leak in pathological states in humans in which there is loss of integrity of the pulmonary vessels whether or not pressures are increased. Similar studies with other indicators have been carried out in living humans with little morbidity (33, 34).

Chinard, Goresky, Enns, Nolan, and House (49) felt that distribution volumes for urea across the renal circulation in dogs were smaller than expected because capillary transit time was short relative to the time required for equilibration of urea between red cells and plasma, so that some of the urea indicator was "trapped" in red cells and not available for extravascular distribution during a single transit through the circulation. If this "trapping effect" were substantial in our control studies, and if alloxan either prolonged mean capillary transit time or markedly decreased the variability of small vessel transit times, V_{u-r} might exceed V_{a-r} only because the "trapping" effect was less. The average pulmonary capillary transit time of 0.8 s in normal dogs calculated by Staub and Schultz (50) is several times the $t_{\frac{1}{2}}$ for red cell-plasma urea equilibration (0.2–0.3 s) quoted by Chinard et al. (49). This suggests that the "trapping" effect should be minimal in the lung when hemodynamics are normal, but it is possible that individual capillary transit times are distributed widely about the mean with the overall effect of shortening urea mean transit time due to red cell trapping. Alloxan has little effect on vascular pressures and does not increase capillary blood volume (7), and since flow changed very little in our alloxan-treated animals, mean capillary transit time after alloxan should not be longer than at base line. It is also unlikely that the distribution of individual small vessel transit times was greatly altered. In addition, V_{u-r} did not exceed V_{a-r} in our left atrial balloon group where both low flow and increased pulmonary blood volume would prolong mean capillary transit time. We conclude that the best explanation for V_{u-r} in excess of V_{a-r} in alloxan edema is not decreased red cell trapping of urea, but rather increased vascular permeability. In our experimental groups with high flow (epinephrine and volume overload), V_{u-r} was less than V_{a-r}. This may have resulted from red cell "trapping" due to shortened capillary transit time or from increased separation of red cells and albumin at higher flows making differences between V_{u-r} and V_{a-r} more apparent than in control studies. The possibility of lack of equilibration of urea between red cells and plasma during transit through small pulmonary vessels poses some problems in interpreting urea indicator dilution curves. It is possible that a small hydrophilic substance, which does not enter red cells, would be a more useful indicator of increased permeability.

The mean recovery of the urea indicator was greater than 90% in all of our study groups although recovery was slightly lower than control values in animals receiving alloxan and slightly higher in both the epinephrine and volume overload edema groups where flow was increased. As Lassen and Sejrsen have pointed out (51), extrapolation of the downslope of the dilution curve assumes that all of the indicator returns to the vascular compartment as a single exponential function of time which is defined before recirculation occurs. If this is not the case, the magnitude of the error in the volume calculation as we have made it depends upon the time course of the return of the rest of the indicator. It is impossible in our studies to determine whether the unrecovered fraction of urea was "lost" or whether its return to the vascular space was obscured by recirculation. If the latter is the case, our volume calculations are underestimates of the true single pass distribution volume for urea. In addition, we calculated distribution volumes in excess of [^{51}Cr]-erythrocytes using whole blood flow. If the fraction of whole blood flow representing flow for the indicator was used, the volumes would be smaller. However, since recoveries were relatively high (52), and since the volume calculation as we have made it seemed to best

describe the changes in shape of the dilution curves, we have expressed the results this way.

Some investigators have calculated extracted fractions (E) and permeability-surface area products from the differences between the first portions of the dilution curves of intravascular- and diffusion-limited indicators (19, 53–55). We sampled at 1-s intervals resulting in relatively few points on the early part of the curves so that such calculations are less precise than when sampling is more frequent. We calculated mean urea-extracted fractions compared with the composite intravascular curve between appearance and intravascular indicator peak as suggested by Crone (56). E averaged 0.10–0.12 in all control and base-line studies, increasing to 0.20 at 60 min in the alloxan edema group. In both the epinephrine and volume overload edema groups (where flow was increased), E declined as expected (E at 60 min in the epinephrine group averaged 0.08 and E at 60 min in the volume overload edema group averaged 0.07). Since the extracted fraction decreased from appearance to peak in all groups, permeability-surface area products calculated from these data are difficult to interpret.

Evaluation of intravascular indicators. While it is clear that albumin leaks from the vascular space in all forms of pulmonary edema, it has been generally assumed that the rate of leak is too slow to affect the single pass indicator dilution curve, and albumin labels have been used almost exclusively as the intravascular indicator when extravascular water volumes are measured. If the mean transit time volume for albumin was larger than the intravascular volume in edematous animals, extravascular water would be underestimated.

In addition, the transit time of red blood cells through the pulmonary circulation is shorter than that of albumin (37). Since water distributes in both blood phases, the intravascular transit time used for extravascular water calculations should take this into account. Goresky et al. have shown that using albumin transit time alone will result in a smaller volume for extravascular water than when a "composite" intravascular transit time, calculated from the water content of red cells and plasma, is used (37). The same workers have demonstrated that alterations in hemodynamics affect the degree of red cell-albumin separation in dogs without edema (37).

In our group of dogs with alloxan edema, the separation of red cells and albumin increased as edema developed even though flow and pressure changes were minimal. This suggests that the albumin mean transit time volume may include part of the extravascular space after alloxan. However, the changes were small, and the difference between extravascular water volumes using albumin alone and using a composite intravascular indicator was similar to control values (Table VIII). Because most of these animals were severely edematous, it is unlikely that increased permeability in any situation compatible with life will produce a leak sufficient to markedly affect the single passage distribution volume of albumin in the lungs.

Our observations of a tendency toward increased separation of red cells and albumin when flow was increased, whether or not the animals were edematous, are consistent with those of Goresky et al. (37). However, we observed the greatest separation of red cells and albumin in animals with left atrial balloons when flow was normal or low. Although the changes in this group were large, we believe that the separation was intravascular since the differences were similar to those observed by Goresky et al. (37) in exercising normal dogs and since urea and albumin mean transit times were similar. If the difference between red cells and albumin is a real difference in intravascular volumes, it presumably results from the separation of the two blood phases in small vessels described by Fåhraeus and Lindqvist (57). In vitro studies of this phenomenon in glass capillaries suggest that the degree of separation depends more upon large vessel hematocrit and capillary size than upon flow (58). Our studies do not define a single hemodynamic variable that permits reliable prediction of the magnitude of the difference in red cell and albumin mean transit times across the lungs of living dogs.

The difference between extravascular water volume using albumin as the sole intravascular indicator and using a "composite" intravascular indicator was about 8% in control animals and in animals with either alloxan or volume overload edema, and the difference was fairly constant. However, the large discrepancies in the two values in the epinephrine and left atrial balloon groups and their wide variability suggest that when indicator dilution studies of lung water are compared between situations in which either pulmonary hemodynamics or blood hematocrit is different, both red cell and albumin labels should be used. The use of albumin alone could obscure large differences in extravascular water.

ACKNOWLEDGMENTS

This study was supported by U. S. Public Health Service Grant HL08195 and by Pulmonary Academic Award HL-11321.

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