ent in the nodes at any time. This would reconcile the apparent discrepancy between the short circulating life calculated from thoracic duct outputs and the long life obtained from DNA labeling. To clarify this further the present experiments are being repeated using tritiated thymidine as DNA precursor. To date excellent agreement has been obtained between adenine and thymidine data. Thoracic duct lymphocytes are labeled to a degree consistent with the above concept (Schooley, Bryant, Kelly, to be published).

Summary. 1. Twenty-four hours are required for maturation of the neutrophil from the last division stage in the marrow. 2. The data are best explained by a finite life of ⅔ day for mature neutrophils in marrow, followed by a peripheral life span of 1 to 1⅓ days. 3. Lymphocytes fall into two classes—medium and large cells, which are rapidly renewed, and small cells, which live at least a week.

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Effect of Endotoxin on Vascular Reactivity to Epinephrine in the Perfused Dog Forelimb and Lung.* (24463)

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Recent publications have indicated that endotoxin may have a direct effect on blood vessels, altering their reactivity to epinephrine (1-4). Changes in reactivity to epinephrine were reported to occur in blood vessels of the isolated perfused rabbit ear and in the mesoappendix of the rat following administration of small doses of endotoxin. Injections of larger doses resulted in vascular hyperreactivity to epinephrine followed by hyporeactivity. Arterioles and venules became completely refractory to epinephrine, while increased reactivity persisted in larger arteries and veins. The work of Zweifach, Nagler and Thomas(1) suggested very large changes in vascular reactivity after endotoxin in the rat mesentery, ranging from 100 times less, to 700 times greater than the pre-endotoxin response. It was postulated that changes in reactivity to epinephrine might be responsible for damaging effects of endotoxin on the vascular systems of intact animals and could account for certain resemblances between endotoxin reactions and "intoxication" by epinephrine(2). It was suggested that the end result of altered reactivity to epinephrine was pooling of blood in distended capillaries and venules(1). A number of vascular responses to endotoxin have been reported from this laboratory(5-8). Constrictions of blood vessels in a variety of organs have been observed

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in vivo and in the isolated perfused state(9-12). Changes in vascular tone after endotoxin have been suggested by decrease in peripheral resistance in eviscerated dogs(13). In view of findings of Zweifach and others it was believed that the lethal effect of endotoxin on the dog might be partially explained on the basis of altered vascular reactivity to epinephrine. A series of experiments was therefore carried out on isolated, perfused lung and forelimb of the dog. Results from these studies fail to indicate significant degree of altered response to intra-arterially injected epinephrine after administration of endotoxin to the isolated perfused dog forelimb and lung.

Methods. Adult mongrel dogs, anesthetized with sodium pentobarbital, 30 mg/kg body weight, were used. Freshly drawn heparinized homologous blood (5 mg heparin/100 cc blood) was obtained from donor animals. Perfusion studies included the following preparations: (a) Pump-perfused lungs, and pump-lung perfusions with an intact dog providing blood for the lung inflow circuit; (b) pump-lung perfused forelimbs, and pump-perfused forelimbs with intact dog providing arterial blood for the limb. In the first experiments, lungs were removed from heparinized dogs and perfused at constant flow with a sigmamotor pump as previously described (12). Lungs were perfused at highest flows compatible with isogravimetric state and were continuously weighed by a strain gauge weighing device(12). Perfusion pressure was measured in the pulmonary artery and a polyethylene catheter was advanced in retrograde manner into a small pulmonary vein without wedging. Pressures in the pulmonary artery and small pulmonary vein and changes in lung weight were continuously registered on a Sanborn Polyviso 4-channel direct writing recorder. The perfusion apparatus contained approximately one liter of blood maintained at temperatures 35-38°C by means of controlled water bath. Lungs were ventilated with a gas mixture of 95% oxygen and 5% CO_2 which maintained the blood pH at approximately 7.4. Injections of epinephrine and endotoxin were made intra-arterially in each study. In the second group of lung studies an intact heparinized dog was connected in series with the lung perfusion circuit. Arterial blood from the aorta was perfused at constant flow through the lung by a sigmamotor pump after appropriate cannulations in dog and lung were made. Blood from the lung was pumped to an elevated reservoir and returned *via* gravity flow to the jugular vein of the dog. Flow to the dog was maintained constant by means of a preset screw clamp. The mean carotid artery pressure of the dog was continuously monitored on the Sanborn recorder. An injection of endotoxin, 1.25 mg/kg body weight, was intravenously administered to the dog in each study, and the arterial blood pressure fell to hypotensive levels due to pooling effect of endotoxin(5). Experiments were terminated at approximately one hour after endotoxin. Further aspects of these experiments were identical to the pump perfused lung studies described above. Two control experiments of approximately 90 minutes each were also included to determine stability of the preparation. In the last experiments, amputated forelimbs of dogs were separately perfused with blood oxygenated by a perfused lung or pumped from a donor dog to which the venous blood was subsequently returned. Limbs were perfused at constant flow in each instance. The experimental preparation was carried out by a procedure recently described (14). Pressures in the brachial artery and small branch of the cephalic vein and changes in limb weight were continuously recorded. Limbs were maintained in the isogravimetric state prior to injection of endotoxin. Five mg of endotoxin were intra-arterially injected in the pump-lung perfused limb experiments and 1.25 mg/kg body weight were intravenously injected in the intact dog-pump-limb experiments. Freshly diluted, refrigerated synthetic epinephrine (Winthrop-Stearns, N. Y.) was used in all studies described above. Epinephrine was given in dosages sufficient to obtain accurately measurable responses. Purified *E. coli* endotoxin, prepared as previously described(5) was used in all experiments.

Results. Table I shows the results in 5 pump-perfused isolated lungs. Injections of

TABLE I. Vascular Responses of Pump-Perfused Dog Lung to Epinephrine before and after Injections of Endotoxin.

Exp.	Drug	Dose (μg)	Time (min.)	Pulm. art. press. (mm Hg) Control	Pulm. art. press. (mm Hg) Max △	Pulm. vein press. (mm Hg) Control	Pulm. vein press. (mm Hg) Max △
1	epi	5	-14	12.5	+4.5	6.5	+ .5
Lung wt, 312 g	Endotoxin		0				
Flow, 220 cc/min.	(5 mg)						
	epi	5	+14	17.0	+4.0	7.5	+ .3
	"	5	+27	16.0	+2.5	8.0	+ .5
2	epi	5	-43	17.5	+3.5	8.5	+3.5
Flow, 190 cc/min.	"	5	-27	16.5	+3.0	8.5	+2.0
	Endotoxin (5 mg)		0				
	epi	5	+13	19.0	+3.5	10.0	+3.5
	"	5	+47	18.5	+3.0	8.5	+4.0
3	epi	4	- 2	12.0	+5.5	5.0	+1.5
Lung wt, 194 g	Endotoxin		0				
Flow, 180 cc/min.	(5 mg)						
	epi	4	+13	14.0	+1.0	9.0	+2.0
4	epi	2	-37	15.5	+1.5	none	
Lung wt, 39 g	"	5	-30	15.5	+2.5		
Flow, 94 cc/min.	"	3	- 9	17.0	+2.0		
	Endotoxin (1 mg)		0				
	epi	2.5	+ 2	18.0	+1.5		
	"	"	+ 8	16.5	+1.5		
	"	"	+19	20.0	+ .5		
	"	"	+35	18.0	+1.0		
5	epi	4	-10	16.0	+1.5	none	
	"	2	- 6	16.0	+1.5		
	Endotoxin (1 mg)		0				
	epi	2	+ 8	17.5	+1.5		
	"	4	+13	17.5	+1.0		

epinephrine were made before, and 2 to 47 minutes after administration of 1-5 mg of endotoxin. No significant alterations in vascular reactivity to epinephrine after endotoxin were observed, as evidenced by changes in arterial and venous pressures.

The results from 3 pump-lung experiments provided with an intact dog in the perfusion circuit are shown in Table II. Injections of epinephrine into the pulmonary artery inflow were made before and after endotoxin, administered to the dog. No marked changes in pulmonary artery response to epinephrine were observed from 5 to 65 minutes after endotoxin, although in 2 instances (Exp. 2 and 3) somewhat smaller responses were recorded than were obtained in control injections. Pulmonary venous responses were somewhat decreased in Exp. 1, and increased to a small degree in Exp. 3. However, although pre-endotoxin vascular responses to epinephrine were fairly constant in the perfused lung, spontaneous alterations in response did occur, as is readily seen in Table III. It is noted that the maximal difference in response after endotoxin (Table II) is a 50% change and that a similar change is also observed in control experiments shown in Table III, when endotoxin was not administered.

Tables IV and V illustrate results in 6 experiments on the perfused dog forelimb. In Table IV are shown 3 experiments in which injections of epinephrine were made into the perfusion tubing of a perfused lung-forelimb preparation before, and from 9 to 51 minutes after endotoxin. No changes in vascular reactivity to epinephrine were seen as evidenced by arterial and venous pressure responses.

TABLE II. Vascular Responses of the Dog-Pump-Perfused Lung to Epinephrine before and after Injections of Endotoxin.

Exp.	Drug	Dose (μg)	Time (min.)	Mean art. B. P. of dog (mm Hg)	Pulm. art. press. (mm Hg) Control	Pulm. art. press. (mm Hg) Max Δ	Pulm. vein press. (mm Hg) Control	Pulm. vein press. (mm Hg) Max Δ
1	epi	15	−25	100	18.5	+1.5	7.5	+5.0
Lung wt, 70 g	"	"	−15	90	17.5	+1.0	8.0	+4.0
Flow, 110 cc/min.	"	"	− 4	90	"	+1.5	9.0	"
Dog wt, 24 kg	Endotoxin (30 mg)		0					
	epi	"	+ 8	85	18.5	+2.0	8.0	+2.5
	"	"	+20	80	"	+1.5	6.0	+2.0
	"	"	+28	70	19.0	+1.0	6.5	+1.5
	"	"	+36	70	"	+1.5	7.5	"
	"	"	+54	67	19.5	"	7.0	"
2	epi	40	−24	90	20.5	+3.0	8.5	+3.0
Lung wt, 42 g	"	"	− 8	125	22.5	+2.0	10.0	"
Flow, 90 cc/min.	Endotoxin		0					
Dog wt, 16 kg	(20 mg)							
	epi	"	+ 9	50	22.5	+3.5	12.0	+2.0
	"	"	+22	65	21.5	+1.0	11.0	"
	"	"	+35	90	22.5	+1.5	8.5	+2.5
	"	"	+64	60	20.5	+3.0	9.5	+3.0
3	epi	20	−27	165	11.0	+4.0	5.0	+1.5
Lung wt, 125 g	"	"	−13	170	12.0	+4.5	6.0	"
Flow, 120 cc/min.	"	"	− 3	160	12.5	+4.0	6.0	+2.0
Dog wt, 24 kg	Endotoxin (30 mg)		0					
	epi	"	+ 5	35	15.0	+2.5		
	"	"	+12	95	16.5	+3.0	12.5	+1.0
	"	"	+28	120	13.5	"	8.0	+3.0
	"	"	+45	90	12.0	+4.0	7.0	+4.0
	"	"	+58	70	11.5	+3.5	6.0	"
	"	"	+65	45	12.5	+2.5	7.0	+3.0

Table V shows 3 experiments on the isolated perfused forelimb with an intact dog providing arterial blood for the limb. No significant differences in vascular response to epinephrine were observed from 16 to 60 minutes after endotoxin, except that in one experiment (#1) the limb artery response to epinephrine decreased by about 50%. Dogs

TABLE III. Vascular Responses of Dog-Pump-Perfused Lung to Epinephrine in Absence of Endotoxin (Controls).

Exp.	Drug	Dose (μg)	Time (min.)	Mean art. B. P. of dog (mm Hg)	Pulm. art. press. (mm Hg) Control	Pulm. art. press. (mm Hg) Max Δ	Pulm. vein press. (mm Hg) Control	Pulm. vein press. (mm Hg) Max Δ
1	epi	20	0	115	16.5	+ 3.5	6.0	+7.5
Lung wt, 41 g	"	"	+ 6	120	17.0	+ 3.0	5.5	"
Flow, 85 cc/min.	"	"	+15	117	"	"	6.5	+9.0
Dog wt, 26 kg	"	"	+30	140	16.0	"	4.0	+7.0
	"	"	+45	145	15.0	+ 3.5	3.5	"
	"	"	+58	128	"	"	4.0	"
	"	"	+71	130	"	+ 4.5	"	"
	"	"	+83	135	"	"	"	"
2	epi	20	0	130	18.0	+ 6.5	10.0	+3.0
Lung wt, 75 g	"	"	+11	133	16.0	+ 9.5	"	"
Flow, 175 cc/min.	"	"	+25	125	15.0	+10.0	8.0	+4.0
Dog wt, 17 kg	"	"	+44	130	14.0	+ 9.5	9.0	+2.0
	"	"	+55	127	13.5	+ 9.0	8.5	+3.5
	"	"	+71	110	13.0	"	7.0	+4.0
	"	"	+80	115	14.0	+ 7.5	8.5	"
	"	"	+92	133	13.5	+ 8.5	6.0	+5.0

TABLE IV. Vascular Responses of Pump-Lung-Perfused Dog Forelimb to Epinephrine before and after Injections of Endotoxin.

Exp.	Drug	Dose (μg)	Time (min.)	Brach. art. press. (mm Hg) Control	Brach. art. press. (mm Hg) Max △	Cephalic vein press. (mm Hg) Control	Cephalic vein press. (mm Hg) Max △
1	epi	1	-35	55	+18	14.0	-3.5; +2.0
Limb wt, 397 g	Endotoxin		0				
Flow, 30 cc/min.	(5 mg)						
	epi	1	+32	53	+19	15.0	-2.0; +5.5
2	epi	1	-17	55	+25	9.0	-1.0; +6.0
Flow, 53 cc/min.	Endotoxin		0				
	(5 mg)						
	epi	1	+ 9	"	+31	"	-2.0; +2.0
	"	1	+36	"	+33	10.0	" ; "
	"	1	+51	57	+32	9.5	-2.5; +5.5
3	epi	.1	-37	90	+23	5.0	+.5
Flow, 19 cc/min.	"	.1	-14	113	+46	3.0	"
	Endotoxin		0				
	(5 mg)						
	epi	.1	+12	107	+30	4.0	"

were markedly hypotensive following injection of endotoxin.

Discussion. These experiments fail to show a significant degree of altered vascular reactivity to epinephrine following injection of endotoxin in the isolated perfused dog forelimb and lung. The largest differences in response were of a 2:1 magnitude, however,

TABLE V. Vascular Responses of the Dog-Pump-Perfused Forelimb to Epinephrine before and after Injections of Endotoxin.

Exp.	Drug	Dose (μg)	Time (min.)	Mean art. B. P. of dog (mm Hg)	Brach. art. press. (mm Hg) Control	Brach. art. press. (mm Hg) Max △	Cephalic vein press. (mm Hg) Control	Cephalic vein press. (mm Hg) Max △
1	epi	.25	-32	137	36	+ 47	11.5	+ 9.0
Limb wt, 595 g	"	"	-24	125	39	+ 34		
Flow, 44 cc/min.	"	.50	-15	132	50	+ 75	18.0	+13.0
Dog wt, 30 kg	"	"	- 8	127	44	+ 60	20.0	+ 5.5
	Endotoxin		0					
	(40 mg)							
	epi	"	+17	65	100	+ 30	29.0	+11.0
	"	"	+39	58	84	+ 26	30.5	+ 8.5
	"	"	+55	107	48	+ 32	33.0	+ 9.0
	"	"	+60	110	45	+ 35	31.5	+11.6
	"	1.0	+65	100	48	+ 56	"	+16.5
2	epi	.5	-16	142	72	+118	10.0	+ 9.5
Limb wt, 392 g	"	"	- 9	150	"	+128	10.5	+11.5
Flow, 39 cc/min.	Endotoxin		0					
Dog wt, 19.2 kg	(24 mg)							
	epi	"	+18	65	155	+ 85	14.5	+ 8.5
	"	"	+33	103	127	+ 76	"	+ 8.0
	"	"	+44	102	80	+106	11.0	+ 9.0
	"	"	+60	142	75	+108	14.0	+11.0
3	epi	1.0	-33	115	110	+ 52	23.0	+12.5
Flow, 20 cc/min.	"	"	-27	100	84	+ 44		
Dog wt, 28 kg	"	"	-21	90	104	+ 66	16.5	+17.0
	"	"	-10	98	114	+ 51	19.0	+10.5
	Endotoxin		0					
	(35 mg)							
	epi	"	+16	50	108	+ 50	20.5	+13.5
	"	"	+22	10	82	+ 40	20.0	+11.0

these do not appear significant in view of similar spontaneous changes. Experiments were not extended beyond one hour after endotoxin and do not therefore rule out possible later changes in vascular response to epinephrine. It is of interest to note however, that since a significant fall in peripheral resistance occurs within 30 minutes after endotoxin in the eviscerated dog(13), data of this study fail to suggest a relationship between progressive decline in total peripheral resistance and an altered reactivity of blood vessels to circulating epinephrine.

It should be pointed out that rather large amounts of endotoxin were used. However, large changes in vascular reactivity have been reported in other studies in which the endotoxin dosage was large enough to produce death(1). Zweifach, Nagler, and Thomas reported 100-fold changes in vascular reactivity with 2 mg injections of endotoxin in the rat mesentery(1), and even larger masses of tissue and greater volumes of blood were involved in the dog limb and lung studies than in the rat experiments.

Additional experiments were performed in which combinations of epinephrine, nor-epinephine and 5-hydroxytryptamine were injected before and after endotoxin, but no potentiation or depression of response to these simultaneously injected drugs occurred in the doses and ratios of doses employed. Single intra-arterial injections of nor-epinephrine and 5-hydroxytryptamine were also separately made in isolated perfused lungs, limbs and kidneys of dogs before and after injections of endotoxin. No changes in vascular response were observed up to one hour after endotoxin.

A complicating factor in the present study is that endotoxin may increase vessel tone (Exp. 3, Table II) so that pressure responses following subsequent injection of epinephrine, may not be directly comparable to those obtained before administration of endotoxin. Nonetheless, since organs were perfused at constant flow, changes in perfusion pressure after epinephrine represent changes in resistance. These changes can be compared with each other although they do not necessarily represent comparable changes in the tone of the vascular smooth muscle.

It may be that the differences between findings of the present study and those of Zweifach and others are due either to the kind of experimental preparation used or to the species employed. In any event it appears that alterations in vascular reactivity to epinephrine may not be observed in all species and are probably unimportant in the dog.

Summary. Experiments were carried out on isolated perfused lungs and forelimbs of adult dogs. There was no significant change in vascular response to epinephrine following injection of endotoxin.

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