tisone had no effect on the papillary concentration of sodium. However, injection of Pitressin into adrenalectomized animals which did not receive adrenal hormones, resulted in a large decrease in the papillary concentration of sodium. This reduction in concentration of sodium was statistically significant when expressed on a dry weight or wet weight basis.

The paradoxical effect of Pitressin in the adrenalectomized animal is somewhat difficult to explain. However, a possibility exists which we believe is likely to be responsible for the changes noted. Adrenalectomized animals generally show a reduction in renal blood flow if not given adrenal hormones(8). Such a reduction in blood flow is not enough to alter the efficiency of the countercurrent multiplier system significantly. ADH also reduces medullary blood flow(9). It seems to us that this reduction imposed on an animal already having reduced blood flow could easily embarrass the countercurrent multiplier system. Severe restrictions in renal blood flow have been shown to reduce the ability of the kidney to form a concentrated urine(10).

The increase in tissue hydration may also be related to reduced medullary blood flow. If the flow were reduced enough the medullary tissue might suffer a reduction in metabolic rate sufficient to cause swelling of the cells. Naturally it is not possible from the present data to determine if the increase in tissue water is due to intracellular swelling or to extracellular changes. It is important to note that Pitressin in the adrenalectomized animal did not increase cortical hydration, but reduced it. This is consistent with the finding that ADH alters medullary blood flow alone(9).

Summary. It appears that the conflicting reports in the literature regarding the effects of adrenalectomy on the concentration of water and electrolytes in the kidney may be due to different levels of circulating ADH in the animal at the time of experiment, or to differences in the protocols of the several sets of experiments.

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Tissue Catecholamine Levels in Prolonged Oligemic Hypotension.* (30327)

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Stress situations have been shown to result in high circulating levels of catecholamines (1), presumably accompanied by depletion of tissue stores(2). *A priori* one might expect that such depletion would affect all organs indiscriminately. On the other hand, one could formulate a working hypothesis to the effect that not all organs suffer equally as a consequence of the imposed stress situation

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and that only those which find themselves forced to perform under adverse conditions exhibit alterations in catecholamine content. Our data would lend support to such an hypothesis.

Methods. The tissues used in this study were derived mostly from the donor and experimental dogs which had been used in the dye-dilution curve experiments described elsewhere(3). Additional experimental animals were maintained until death or sacrifice at an oligemic hypotension of about 30 mm Hg similar to the above group, either with or without catheters in place in the right heart, but uniformly without receiving the periodic "I-infusions."† Some additional controls were unbled dogs which were observed for about 6 hours either with catheters in place or receiving "I-infusions" at hourly intervals.

At time of death or sacrifice the chest was opened quickly, the great vessels clamped off and the heart and other organs collected as follows: the whole heart, the right lobe of the lung, a section of the right lobe of the liver, the whole spleen, one kidney and a section of the jejunum about 3 feet below the pylorus. The organs were perfused with ice cold saline as rapidly as possible to remove excess blood. Suitable portions were then cut off, blotted and stored frozen. The skull was opened, the brain removed, trimmed of meninges, rinsed in saline and stored frozen. All tissues were thus put away within 60 minutes after the death of the dog.

Catecholamines were determined by the method of Anton and Sayre(4) using an Aminco-spectrophosphorimeter-fluorometer. A few early estimations were made by the Shore and Olin(5) procedure. The normal and shock tissues were paired and worked up within a week of the experiment. They were minced with scissors and blotted between filter papers as they thawed out. Appropriate amounts were quickly weighed on a Roller-Smith balance and transferred to a conical glass homogenizer containing 6 ml 0.4 N perchloric acid. Homogenization was carried out in an icebath, the contents transferred quantitatively to a polypropylene centrifuge tube and spun in the highspeed attachment of a refrigerated International centrifuge. The supernate was decanted into a graduated cylinder and suitably diluted depending upon tissue and sample size. Ten ml aliquots were removed for duplicate analyses as well as recoveries of known amounts of adrenaline and noradrenaline. The frozen brain was separated into hemisphere and midbrain sections before mincing. The latter comprised the corpora quadrigemina, the pons and immediately adjacent areas. Adsorption and elution and subsequent oxidations at pH 2 and pH 7 were carried out in groups of about 20 samples with standard curves and recoveries included in each run. Concomitant blood analyses were performed by the same method using 5 or 10 ml aliquots of plasma.

Catecholamines were found to survive storage in the deepfreeze in the original tissue for at least 6 months and in the perchloric acid extract and the eluate 2 weeks.

Results. Table I summarizes the results. Lung and liver were evidently not depleted during the prolonged oligemic hypotension which resulted in death of the dog. The cerebral hemisphere, spleen, and right atrium and, to a lesser extent, the left ventricle were profoundly affected. The other organs took an intermediary position.

Since late shock as produced in our laboratory was associated with respiratory difficulties(3) and hypoxia as such is known to set up stress situations(6) we analyzed our data as indicated in Table II. Column 3 refers to dogs which died in irreversible hemorrhagic shock. Column 2 comprises individuals which survived 6 to 11 hours of hypotension. They were sacrificed by excision of the heart, usually after having experienced the first episode of acute respiratory arrest which ushers in the subterminal phase of shock. The terminal blood catecholamine levels observable at time of death or sacrifice were compared with the initial values obtained prior to induction of the hypotensive state in these 2 experimental groups. The control levels in Column 1 were estimated at the beginning and the end

† The term "I-infusion" referred to the rapid injection of 400 ml blood into a hypovolemic dog in order to render him near-normovolemic for a period of 5 minutes. This procedure permitted measurement of cardiac functional parameters as described in(3).

of the observation period in unbled dogs.

The similarity of the blood pictures in Groups 2 and 3 would indicate that all experimental animals reacted in an analogous manner regardless of survival time whereas the controls appeared to remain in a non-alarmed state throughout with uniformly low circulating catecholamine concentrations. The tissue data thus gain added significance and suggest that the depletion of the hemisphere is a terminal phenomenon while that of the spleen appears to proceed in a progressive

TABLE I. Catecholamine Levels in the Tissues of Normal and Shock Dogs in μg/g Wet Weight.

	No.	Normal, mean ± S.D.	No.	Shock, mean ± S.D.	P-value*
		Total			
Left ventricle†	16	1.08 ± .40	11	.70 ± .34	.018
Right atrium†	4	3.63 ± .60	5	1.91 ± .55	.002
Midbrain	11	.28 ± .09	9	.20 ± .09	.067
Hemisphere	10	.09 ± .03	8	.04 ± .02	.008
Spleen	10	4.75 ±2.13	6	1.31 ± .85	.001
Jejunum	10	.33 ± .26	6	.16 ± .06	.150
Kidney	10	.38 ± .19	7	.19 ± .09	.073
Lung	10	.06 ± .03	7	.08 ± .07	
Liver	8	.27 ± .19	7	.24 ± .18	.557
		Norepinephrine			
Left ventricle	12	.89 ± .28	7	.57 ± .38	.053
Midbrain	11	.26 ± .07	7	.16 ± .08	.106
Hemisphere	10	.08 ± .03	7	.03 ± .02	.003
Spleen	10	4.40 ±1.72	6	1.24 ± .81	.003
Jejunum	10	.32 ± .22	6	.15 ± .02	.115
Kidney	10	.35 ± .18	6	.17 ± .07	.093
Lung	10	.05 ± .03	6	.06 ± .07	
Liver	10	.26 ± .17	6	.20 ± .19	.441
		Epinephrine			
Left ventricle	12	.08 ± .13	7	.07 ± .08	
Midbrain	11	.01 ± .01	7	.02 ± .01	
Hemisphere	10	.01 ± .01	7	.01 ± .004	
Spleen	10	.35 ± .35	6	.06 ± .06	.063
Jejunum	10	.01 ± .01	6	.01 ± .01	
Kidney	10	.03 ± .03	6	.01 ± .02	
Lung	10	.01 ± .02	7	.01 ± .01	
Liver	9	.02 ± .01	6	.03 ± .02	

* Obtained by t-test.

† Some ventricular and all of the atrial data were obtained in early experiments by the Shore and Olin method(5) and have been published in part(22).

TABLE II. Blood and Tissue Catecholamine Levels in Dogs Sacrificed or Dead After Prolonged Oligemic Hypotension.

		1 Controls	2 Sacrificed	3 Died	P-value* died *vs* sacr.
			μg/liter plasma		
Blood epinephrine	Initial	2.80	2.55	2.16	
	Terminal	.76	13.62	14.98	
			μg/liter plasma		
Blood norepinephrine	Initial	1.94	3.37	1.90	
	Terminal	1.74	11.79	10.38	
			μg/g wet weight		
Left ventricle		1.08	.97	.78	.212
Spleen		4.40	2.99	1.24	.055
Hemisphere		.077	.070	.030	.004
Midbrain		.26	.38	.18	.028

* Obtained by t-test.

manner throughout the hypotensive period.

Discussion. The hypothesis that organs may suffer differential alterations in catecholamine content as an expression of localized stress has been accepted implicitly in a number of studies, notably those of Meerson on cardiac hypertrophy(7). Experiments with guinea pigs exposed to simulated high altitude conditions have furnished further evidence along these lines(8). Coleman and Glaviano analyzing tissues of rabbits subjected to oligemic hypotension found heart, brain, spleen and liver depleted but the gluteal muscle unaffected(9). The data presented here lend themselves to interpretation in the light of observations made on organ metabolism and function.

Metabolic studies conducted at the level of the mitochondria, the tissue slice and the whole perfused organ have indicated that the liver suffers no permanent injury during prolonged oligemic hypotension(10). Other investigators also(11,12) have observed that the organ taken from an animal dying in shock and tested *in vitro* performed normally. *In situ* in the body of the hypotensive animal, however, the liver does not function properly: its mitochondria swell, its glycogen stores remain depleted while lactate accumulates in the blood. Hepatic circulation is poor(13) and the organ engorged with blood when examined post mortem(14). At the same time, the liver is known to be able to survive long periods of severe hypoxia without suffering permanent functional impairment(15,16). It is our impression, therefore, that the organ finds itself in a cul-de-sac situation during prolonged oligemic hypotension, cut off from the central circulation to a greater or lesser extent. In these circumstances it shifts to a more anaerobic type of metabolism without apparently experiencing any particular functional stress. Its norepinephrine levels remain normal but its mitochondria swell in response to the prevailing hypoxia. Selkurt(17) and Engel(16) have come to similar conclusions regarding the role of the liver in shock.

The lung has never been seriously implicated in shock. Wiggers(18) reported continued adequate functional adjustments and Rothe and Selkurt(19) observed no significant changes in intrathoracic pressures during oligemic hypotension. Histologically the lungs of our experimental dogs were not found to have sustained marked alterations (3). Goldring *et al*(6) have been unable to demonstrate a relationship between artificially induced hypoxia and the concomitant vascular changes on the one hand and the pulmonary levels of norepinephrine or the pulmonary response to circulating norepinephrine on the other. This may be relevant to our negative lung data. At the same time, however, the possibility exists that humoral factors other than catecholamines are operative in the lung.

In contradistinction to lung and liver the heart of dogs in oligemic hypotension is forced to perform in a stressful situation: the right atrium is working in a tachycardia and the ventricles have to cope with conditions of low circulating volume and elevated peripheral and pulmonary resistances(3). This may well be the reason for the observed depletion of cardiac catecholamines. Low cardiac noradrenaline levels in shock have been reported by Glaviano and Coleman(20), Coleman and Glaviano(9), Zetterstrom *et al*(21) and Hift and Campos(22). Interestingly enough elevations in catecholamine content were observed in cases of early hypertrophy by Meerson(7) and Richtarik(8), *i.e.*, under conditions which may be expected to favor anabolic over catabolic reactions. In spite of adverse working conditions prevailing in oligemic hypotension the heart was found to be able to adjust its performance fairly adequately until late in shock(3,19) and correspondingly the atrial norepinephrine levels were not found to approach the critical value of 0.2 μg/g which has been described as the minimum consistent with adequate cardiac function in dogs(23).

The depletion in the cerebral hemisphere appears to be terminal and may, therefore, be associated with the episodes of respiratory arrest experienced by the dogs in late shock. Cerebral metabolic difficulties in oligemic hypotension have, however, been described by Kovach(24) and Moulton *et al*(25).

The spleen data indicate not only an unusually high content of norepinephrine but

also a progressive depletion. Considering the amounts of the hormone stored in the organs examined here and the depletions observed, it seems possible that the spleen furnished the major portion of the circulating noradrenaline. The situation, if true, may, however, be peculiar to the dog. The spleen has been implicated in shock by Hardaway(26).

Summary. Catecholamine levels were determined in tissues obtained from dogs subjected to prolonged oligemic hypotension. The lung and liver were found to have retained their normal complement of catecholamines while the cerebral hemisphere, spleen and heart were depleted. In the brain the depletion appeared to be a terminal phenomenon, possibly associated with the respiratory difficulties experienced by the animal at that time. In the spleen the depletion seemed to take place progressively throughout the experimental period. An attempt is made to reconcile these data with known metabolic and functional parameters of the organs studied.

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Renal Responses to Acetylcholine.* (30328)

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We reported recently that malachite green, a cationic triphenylmethane dye, acts directly on the kidney to increase excretion of calcium, phosphate, sodium, potassium, chloride and water(1). Evidence that triphenylmethanes inhibit cholinesterase(2) suggested

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