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Effect of Anoxemia on Myocardium of Isolated Heart of the Dog.

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Although the ultimate effects of anoxemia on tissue are clearly disorganization and death, the steps by which this final disorientation is produced have not been established. The heart-lung preparation seemed to be well suited to the study of the effects of anoxemia on the electrolytes of the heart. It has previously been shown that the production of temporary ischemia in localized portions of the heart of an intact animal results in a subsequent increase in the extracellular ions, sodium and chloride, and in water in the affected regions.[1] To test the effects of anoxemia rather than of ischemia, it would be desirable to reduce the oxygen supply to the heart without interfering with the circulation of blood. Although this could be accomplished with the intact animal, serious complications might result from the effects of anoxemia on the rest of the body. The use of the heart-lung preparation avoids these difficulties.

Observations were made on the electrolytes of the myocardium of the dog taken from heart-lung preparations in (a) 2 control experiments lasting about 1 hour, (b) 2 experiments in which the oxygen supplied to the lung of the heart-lung preparation was partially or completely replaced by nitrogen, and (c) 2 experiments in which the myocardium was poisoned, in one instance with sodium amytal and in the other with potassium.

The original electrolyte data have been used to calculate the relative amounts of the extra- and intracellular portions of the myocardium, and the concentrations of water, potassium, magnesium, and bicarbonate, as well as the pH in the cardiac fibers themselves. This type of histochemical calculation has become a well established procedure.

The electrolyte changes observed following an extended period of partial anoxemia were comparable to those produced following temporary ischemia in the intact animal. There is no evidence of significant change in the electrolytes of the fibers themselves.

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1 Hastings, A. B., Blumgart, H. L., Lowry, O. H., and Gilligan, D. R., *Trans. Assn. Am. Physicians*, 1939, **54**, 237.

Experimental. The heart-lung preparation was made according to Patterson and Starling's technic[2] with certain modifications which have been previously described.[3] Chloralose, 0.09 g per kg body weight, was used as anesthetic for the operation. Defibrinated blood served as perfusion fluid.

Gas Mixture. The gas supplied to the lungs of the preparations consisted of (a) 5% CO_2/95% air in one control experiment, (b) air alone in the second control experiment and in the experiments with amytal and potassium poisoning, (c) 5% CO_2/95% N_2 in the first anoxemia experiment, and (d) 2% O_2/98% N_2 in the second.

At the end of each experiment, a sample of blood was centrifuged under paraffin oil and the serum saved for the determination of pH, chloride, sodium, total CO_2, and water.

Separate samples of myocardium were taken rapidly from the right and left ventricles at the termination of the experiment, while the heart was still beating. The tissue was analyzed for blood, fat, total CO_2, chloride, sodium, potassium, calcium, and magnesium. The determinations of blood, fat, chloride, sodium, and potassium were performed essentially as previously described.[4] The tissue CO_2 was measured by the method of Danielson.[5] The calcium was determined acidimetrically after precipitation as the oxalate and conversion to the carbonate. Magnesium was determined colorimetrically from the phosphate in the magnesium ammonium phosphate precipitate.

The procedures used for the calculation of data of histochemical interest have been described previously.[4]

Results. In Table I are shown the electrolyte and water concentrations observed in the myocardium in 5 experiments. All of the results are reported on a blood-free, fat-free basis. For comparison, average values obtained from the myocardium of 14 adult dogs are included in the table. These data have been reported previously.[1] The average values for bicarbonate and pH are from unpublished data of Dr. W. M. Wallace on the hearts of 3 young dogs.

Control Experiments. In the tissue obtained from the control experiments, the electrolyte pattern probably falls within the normal range. However, the sodium, chloride, and water concentrations approach the upper limit of normal, indicating an amount of extra-

[2] Patterson, S. W., and Starling, E. H., *J. Physiol.*, 1913, **48**, 357.

[3] Krayer, O., *Arch. f. exp. Path. u. Pharmakol.*, 1931, **162**, 1.

[4] Lowry, O. H., and Hastings, A. B., *J. Biol. Chem.*, 1942, **143**, 351.

[5] Danielson, I. S., and Hastings, A. B., *J. Biol. Chem.*, 1939, **129**, 547.

TABLE I.

E_{Cl}, E_{Na}, and observed data (except pCO_2), reported per kg of blood-free, fat-free tissue, $(H_2O)_c$ reported per kg of fibers, and $[K]_c$, $[Mg]_c$ and $[HCO_3]_c$ reported per kg of fiber H_2O.

		Observed data									Derived data						
Experiment		H_2O g	Cl mE	HCO_3^- mE	Na mE	K mE	Ca mE	Mg mE	Total base mE	pCO_2 mm	E_{Cl} g	E_{Na} g	$(H_2O)_c$ g	$[K]_c$	$[Mg]_c$	$[HCO_3^-]_c$	pH_c
Control	Serum	924.2	115.2	12.33	145.2					41.3						13.4*	7.10†
5% CO_2/	L.V.	795.6	30.8		38.2	92.8	1.62	22.4	155	(41.3)	265	266	753	166	40		
95% air	R.V.	797.5	32.5	10.54	42.3	95.3				(41.3)	284	298	755	176		13.2	7.14
Control	Serum	924.2	121.8	8.45	146.5					12.2						9.1*	7.47†
air	L.V.	786.1	30.2	10.07	41.4	95.8	1.47	20.5	159	(12.2)	247	285	741	174	37	14.5	7.71
	R.V.	793.5	32.6	8.98	39.8	92.0				(12.2)	270	278	754	166		12.2	7.64
5% CO_2/	Serum	917.3	116.3	14.28	148.3					38.5						15.5*	7.20†
95% N_2	L.V.	800.0	30.0	9.66	38.8	83.4	1.62	21.3	145	(38.5)	255	262	761	146	39	10.5	7.10
	R.V.	795.5	31.8	11.27	40.0	89.0				(38.5)	273	274	757	156		13.4	7.17
2% O_2/	Serum	914.9	115.6	6.68	145.7					9.3						7.3*	7.49†
98% N_2	L.V.	802.9	36.6	6.44	45.6	83.6	1.82	21.3	152	(9.3)	309	312	752	159	40	8.3	7.58
	R.V.	801.1	37.7	6.86	43.8	85.7				(9.3)	322	304	754	164		8.2	7.58
Potassium	Serum	915.6	114.7	5.78	141.4					8.7						6.3*	7.46†
poisoning	L.V.	780.0	29.6	7.36	28.3	100.2	1.41	23.4	153	(8.7)	254	203	743	171	40	11.4	7.75
air	R.V.	779.5	29.7	7.53	30.7	101.0				(8.7)	259	223	744	175		10.9	7.73
Normal dog	L.V.	785.9	25.4	13.3§	31.1	89.0					220	223	750	152		13.5§	7.09§
	σ	6.3	3.0		4.3	2.4					17	19	7	4			
myocardium‡	R.V.	789.1	26.6		32.8	94.8					232	242	754	165			
	σ	7.5	2.5		4.1	6.0					18	26	8	10			

*Concentration per kg serum H_2O.
†pH of serum.
‡Data previously published.[1]
§Unpublished data of Dr. W. M. Wallace on 3 young dog hearts.

cellular tissue, E_{Cl} or E_{Na}, a little above the average for the heart of the intact dogs. The total base concentrations, estimated as the sum of the individual cations, averaged 157 mE per kg of tissue, or 208 mE per kg of fiber water.

The bicarbonate concentrations in the tissue as a whole, and in the fibers themselves, differed but little in the 2 control experiments in spite of a marked difference in the CO_2 tensions of the respective sera.

Anoxemia. Complete replacement of oxygen by nitrogen in the gas supplied to the heart-lung preparation resulted in failure of the heart within 15 minutes. Actually, complete anoxemia probably obtained for less than 10 minutes since some time was required for the lungs to blow off all of the oxygen in the blood of the preparation. This heart showed little change except for a moderate decrease in potassium in the left ventricle (Table I). In the belief that this heart may have stopped too promptly to allow electrolyte rearrangements to take place, another preparation was given a gas mixture consisting of 2% O_2/98% N_2 which permitted the heart to beat for an hour, at which time fibrillation occurred. This heart showed distinct increases in water, sodium, and chloride, and a small decrease in potassium. Calcium and magnesium were not significantly altered. From a histochemical interpretation of these data, there appears to have been an increase in the extracellular portion of the myocardium amounting to about 20% when compared to the controls. The concentrations *in the fibers* of potassium, magnesium, and water, $(H_2O)_c$, do not appear to be significantly altered by this sublethal degree of anoxemia. Similarly, the bicarbonate concentrations in the fibers, and the fiber pH are comparable in the control and hypo-oxemia experiment when due allowance is made for differences in the composition of the respective sera. Apparently as long as the heart is able to continue beating, the degree of anoxemia has not been sufficiently severe or prolonged to destroy the original electrolyte configuration in the fibers.

Effects of Poisoning. The injection of 20 cc of isotonic KCl during the course of an hour into a heart-lung preparation brought the serum level of potassium to 9.5 mE per liter, and nearly stopped the heart. In the myocardium, an almost normal electrolyte configuration was observed (Table I). Sodium appeared to be somewhat decreased in comparison with the chloride, and a somewhat increased potassium concentration was found. The possibility exists that the small amount of intracellular sodium originally present had been replaced by potassium, although the concentration of potassium within the fibers did not appear to be greatly increased.

The administration of 160 mg of sodium amytal in divided doses during a period of an hour to another preparation nearly stopped the heart, and produced slight changes in the electrolyte concentrations in the heart that were very similar to those produced by the potassium.

It would appear, therefore, that with decreasing amounts of oxygen, or with increasing amounts of potassium or amytal, the general impermeability of the cardiac fibers to electrolytes is maintained at least as long as the heart continues to beat.

Summary. 1. The myocardium from a heart-lung preparation respiring air or a mixture of 5% CO_2 and air presents a nearly normal electrolyte pattern. 2. Heart failure from oxygen deficiency in such a preparation results in an increase in sodium, chloride, and water, and a decrease in potassium of the myocardium. 3. These changes are interpreted as indicating an extracellular edema without significant change in the fibers themselves. 4. Sufficient potassium or amytal to produce incipient heart failure produced only small changes in the cardiac electrolytes.

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Urinary Gonadotrophins in Normal Men.

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There is a dearth of information as to the gonadotrophins found in the urine of normal men. This is to some extent due to the difficulty of preparing non-toxic concentrates, for high doses are necessary to reveal the low titer of gonadotrophins characteristic of normal male urine. Recently investigators employing alcohol, tannic acid, or tungstic acid as precipitation agents, appear to be agreed that the level of excretion of gonadotrophins in normal male urine is approximately 5 to 25 mouse-uterus units[1] per liter.

Witschi[2] has pointed out that the ratio of follicle stimulating to luteinizing or interstitial cell stimulating activities in any given gonadotrophin may be characteristic of that gonadotrophin. Fraenk-

[1] Levin, L., and Tyndale, H. H., PROC. SOC. EXP. BIOL. AND MED., 1935, **34**, 516.

[2] Witschi, E., *Endocrinol.*, 1940, **27**, 437.