

MYOCARDIAL TRANSFER OF SODIUM AND POTASSIUM: EFFECT OF ACETYL STROPHANTHIDIN IN NORMAL DOGS^{1, 2}

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Although the hemodynamic events attending low-output congestive heart failure have been well defined, the biochemical changes in the myocardium responsible for these events and the response to cardiac glycosides remain largely unknown. Evidence to date suggests that in the failing heart there is normal substrate oxidation but an impairment of energy utilization (1). Recent data would indicate an altered state of the myocardial protein-complex, actomyosin (2), but whether this is the primary event in the evolution of congestive failure remains to be demonstrated.

Post-mortem tissue analyses for cation content of human heart muscle in failure have shown a lowered content of potassium (3, 4). Moreover, after digitalis, in therapeutic (5) and toxic doses (5, 6), the potassium content diminishes, although some disagreement over the former exists. That these changes may have functional significance is suggested by the work of Hajdu (7), who, from data obtained with a frog heart-muscle preparation, has inferred that the positive inotropism of digitalis is effected by the reduction in internal ionic content produced by the transfer of potassium from the cell.

The pronounced effect of digitalis on the mechanical efficiency of the failing myocardium (8, 9) suggests that this drug might provide data re-

lating the movement of myocardial cations to cardiac hemodynamic function *in vivo*. Consequently, a study of these parameters was undertaken in the experimental animal, employing the rapid-acting digitalis analogue, acetyl strophanthidin.

METHODS

The experimental design of this study involves assumptions which appear sufficiently valid to justify their use. The coronary arterio-venous (A-V) differences of sodium and potassium were employed as a measure of their net uptake by, or release from, the myocardium. It is assumed that the shifting ions move quickly enough across the capillary to result in almost instant equilibration between interstitial fluid and effluent plasma (10). Whereas increased blood flow will dilute down or diminish A-V difference, decreased flow will augment it. Although no measurements of coronary blood flow have been made after strophanthidin, a separate study has revealed no change in myocardial oxygen A-V difference after this drug, suggesting that substantial changes in coronary flow do not occur. In other unpublished work, we have increased coronary blood flow by other experimental means and noted no change in electrolyte A-V difference. Thus we feel that the coronary flow changes accompanying strophanthidin dosage are insufficient in magnitude to bring about the electrolyte A-V differences to be reported.

Eight mongrel male dogs, weighing 14 to 21 kilograms, were anesthetized with sodium pentobarbital, 25 mg. per kg., supplemented as needed to maintain light anesthesia. A Cournand needle was placed in the femoral artery and single lumen catheters, filled with heparin, were placed via the external jugular vein in the coronary sinus to obtain blood samples and in the right auricle for injection of the drug. Heparin, 5 mg. per kg., was administered to prevent clotting, and pure oxygen delivered into an endotracheal tube to exclude the effects of hypoxia on plasma electrolytes (11).

Acetyl strophanthidin,⁵ dissolved in 60 per cent ethyl alcohol (0.5 mg. per cc.) was infused into the right auricle in dosage of 0.05 to 0.1 mg. per kg. in volumes of

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TABLE I
Electrolyte, hemodynamic and EKG findings

Dog No.	Dosage mg./kg.	Potassium mEq./L.			Sodium mEq./L.			Cardiac output cc./min./kg.	Pulse	Mean B.P.	Left vent. stroke work gm./M.	EKG	
		Fem-oral artery	Coro-nary sinus	Arterio-venous difference	Fem-oral artery	Coro-nary sinus	Arterio-venous difference						
104	0.1	Control	3.62	+0.07	Control	156	155	+1.0	172	100		Normal	
		Maximum change 7 min.	5.15	-1.60	Maximum change 3 min.	157	150	+7.0	174	180		Ventricular tachycardia	
		Recovery 30 min.	4.00	+0.10	Recovery 30 min.	151	149	+2.0	180	140		Normal	
106	0.05	Control	3.94	+0.18	Control	144	145	-1.0	162	130	32.0	Normal	
		Maximum change 6 min.	4.81	-0.49	Maximum change 6 min.	156	148	+8.0	93	152	18.3	P wave diminished	
		Recovery 27 min.	4.52	-0.27	Recovery 27 min.	151	149	+2.0	160	80		Normal	
108	0.05	Control	4.68	+0.02	Control	147	154	-7.0	82	115	89	15.3	Normal
		Maximum change 15 min.	4.19	-0.44	Maximum change 7 min.	151	146	+5.0	113	117	83	19.5	P wave diminished and slight bradycardia
		Recovery 30 min.	4.04	+0.16	Recovery 17 min.	145	145	0.0	123	79		Normal	
109	0.05	Control	4.80	+0.13	Control	159	164	-5.0	130	132	134	38.8	Normal
		Maximum change 6 min.	5.57	-0.49	Maximum change 18 min.	162	158	+4.0	97	96	120	33.0	P wave diminished
		Recovery 40 min.	4.37	+0.25	Recovery 40 min.	150	157	-7.0	101	111		Normal	
113	0.05	Control	4.00	+0.08	Control	159	162	-3.0	132	186	124	18.7	Normal
		Maximum change 1 min.	4.49	-0.97	Maximum change 2 min.	154	146	+8.0	78	170	122	11.9	Nodal rhythm
		Recovery 6 min.	5.12	+0.16	Recovery 6 min.	153	153	0.0	205	110		Normal	

TABLE I—Continued

Dog No.	Dosage mg./kg.	Potassium mEq./L.			Sodium mEq./L.			Cardiac output cc./min./kg.	Pulse	Mean B.P.	Left vent. stroke work gm./M.	EKG		
		Time	Fem-oral artery	Coro-nary sinus	Arterio-venous difference	Time	Fem-oral artery						Coro-nary sinus	Arterio-venous difference
114	0.05	Control	3.31	2.92	+0.39	Control	146	152	-6.0	136	102	33.6	Normal	
		Maximum change 6 min.	4.18	4.76	-0.58	Maximum change 6 min.	147	137	+10.0	115	174	118	18.3	Nodal and ventricular rhythm
		Recovery 20 min.	3.77	3.78	-0.01	Recovery 20 min.	149	151	-3.0	120	121	121	Normal	
		Control	4.63	4.70	-0.07	Control	152	162	-10.0	113	198	162	17.2	Normal
115	0.05	Maximum change 1 min.	5.28	6.68	-1.40	Maximum change 6 min.	156	159	-3.0	58	173	138	8.6	Nodal and ventricular rhythm
		Recovery 25 min.	5.26	5.28	-0.02	Recovery 25 min.	149	151	-2.0	148	88	88	Normal	
		Control	4.16	4.18	-0.02	Control	150	148	+4.0	109	160	98	11.7	Normal
		Maximum change 5 min.	5.27	6.18	-0.91	Maximum change 8 min.	146	140	+6.0	59	102	107	10.9	P wave diminished
Averages	Standard deviations	Recovery 15 min.	4.86	4.71	+0.15	Recovery 15 min.	143	142	+1.0	125	140	140	Normal	
		Control	4.15	4.05	+0.10	Control	151	154	-3.0	131	154	119	14.8	
		(±0.14)			(±4.5)									
Averages	Standard deviations	Maximum change 6 min.	4.87	5.73	-0.86	Maximum change 6 min.	153	147	+6.0	88	133	117	11.2	
		Control	(±0.44)			(±4.0)								

0.6 to 1.0 cc. over a period of 15 seconds. This rapid acting aglycone was selected so that its abrupt and short duration of action (12) might facilitate detection of electrolyte changes in coronary sinus blood, which might otherwise not be found with slower acting preparations. The hemodynamic properties of this drug appear to be qualitatively the same as the glycoside, Ouabain (13, 14), and it is presumed that its physical chemical properties are not essentially different.

Determination of cardiac output by a modification (15) of the Stewart-Hamilton dye method was made 10 to 20 minutes prior to drug injection and 10 minutes after, at which time, the hemodynamic effects of the drug are near maximal (12). Phasic and mean arterial pressures were obtained at 5-minute intervals with a Sanborn electromanometer and recorded on the Sanborn polyviso. Stroke work against pressure was calculated as the product of the mean femoral artery pressure and stroke output, and expressed in gram-meters (16). The electrocardiogram (Lead AV_F) was recorded on the polyviso just prior to giving the drug and at one-minute intervals for 15 minutes; thereafter, at 5-minute intervals until the control state was re-established. The respiratory rate was noted simultaneously.

Eight milliliters of whole blood were drawn in chemically clean, dry syringes simultaneously from the coronary sinus and femoral artery for control electrolyte samples, 2 to 10 minutes before drug administration. In four dogs, two sets of controls were drawn 10 to 15 minutes apart and were not significantly different. After strophanthidin injection through the right auricular catheter, 8 milliliter samples were taken from artery and coronary sinus at 2, 5, 8, 15, 30, 45, 60, and occasionally at 90, and 120 minutes. Timing was not precise in some instances, but remained simultaneous. After centrifuging at 1,500 r.p.m. for 20 minutes, plasma determinations of sodium and potassium were done on a Beckman flame photometer, with an error of less than 2 per cent on duplicate readings. The occasional hemolyzed samples were discarded. In four experiments, simultaneous blood samples from the artery and coronary sinus were secured in oiled syringes containing small amounts of heparin for immediate delivery to a Beckman glass-electrode pH meter maintained at constant temperature of 38° in the water bath, and in which duplicate readings agreed to 0.02 pH unit. Samples were taken during control periods and at 2, 8, and 15 minutes. Hematocrits were run on the same samples.

In determining the statistical significance of the electrolyte changes, utilizing standard methods (17), the maximum change in A-V difference of sodium and potassium was selected and compared with the control A-V difference. Intervening A-V differences generally represented increasing and decreasing gradations of these maximum values, as shown in Figure 1. The variability in time of occurrence of this maximum change is probably consistent with the variability of the hemodynamic time-response to cardiac glycosides. The figures for recovery, given in Table I, signify the first A-V dif-

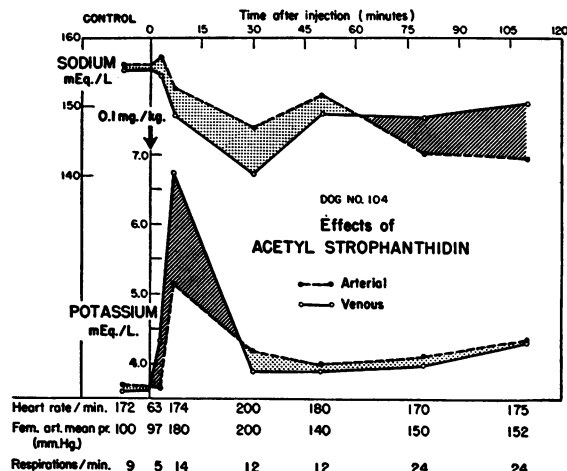


FIG. 1. MYOCARDIAL CATION EXCHANGE IN AN INDIVIDUAL EXPERIMENT TYPICAL FOR GROUP ILLUSTRATING SEQUENTIAL CHANGES AFTER ACETYL STROPHANTHIDIN

Venous refers to coronary sinus blood. Arterial refers to systemic artery. Stippling indicates positive A-V difference or myocardial uptake and diagonal shading indicates negative A-V difference or release of cation.

ference values after the maximum response to approximate the control values.

RESULTS

A typical individual experiment of this study is depicted in Figure 1, demonstrating the acuteness of the cation responses and their divergent movement as illustrated by the simultaneous positive sodium and negative potassium A-V differences. The reversal of these initial changes after 30 minutes, as seen in this experiment, was not a consistent finding in the group as a whole. The mean control potassium values for the group were $4.15 \pm .52$ mEq. per L. in the artery, and $4.05 \pm .62$ mEq. per L. in the coronary sinus, so that the mean A-V difference of $0.10 \pm .14$ was not significant in view of the error of determination. After the infusion of acetyl strophanthidin, there was, as shown in Figure 2, an abrupt loss of potassium in seven animals, a somewhat slower loss in the eighth, with the maximum change occurring in an average of six minutes for the group. The mean arterial potassium at this time was 4.87 ± 0.53 mEq. per L., and the coronary sinus value 5.73 ± 0.82 mEq. per L., so that a mean negative A-V difference of $0.86 \pm .44$ mEq. per L. was present, which, when subject with the control values to the t test, was found significant ($P = <$

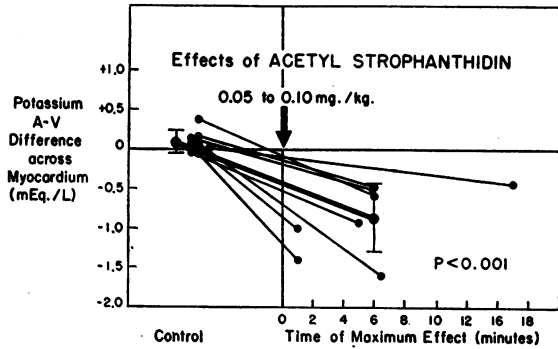


FIG. 2. THE EFFECT OF ACETYL STROPHANTHIDIN ON POTASSIUM A-V DIFFERENCE

Control and maximum A-V differences in individual animals represented by closed circles. Mean values for group represented by targets. Brackets indicate standard deviation.

.001). There was in general a less rapid reversion to control values of the potassium A-V difference (Figure 4), but variation was evident with a range of 6 to 50 minutes (Table I). The rise in arterial potassium was greater than could be accounted for by myocardial loss alone and presumably was derived from skeletal muscle in accord with previous tissue studies (3).

The mean value for the control arterial sodium was 151 ± 6 mEq. per L., and for the coronary sinus 154 ± 8 mEq. per L., so that a slight negative A-V difference of -3 ± 4 mEq. per L. was present. As seen in Figure 3, approximately concurrent with the potassium loss from the myocardium and despite the varied control values, there occurred increasing positivity of the A-V difference in each case, suggesting uptake of sodium. The mean A-V difference at the point of maximal change was $+6 \pm 4$ mEq. per L., which represents an increase of about 9 mEq. per L. over the control state. The arterial value was 153 ± 5 mEq. per L., the coronary sinus, 147 ± 8 mEq. per L. A comparison with the control figures gave a P value of < 0.01 . Reversion to negligible A-V difference required, as with potassium, a variable time ranging from 6 to 50 minutes. Although more precise electrolyte measurement would be preferable, it would appear from the graphing of the composite values seen in Figure 4 that there was excess sodium ingress over potassium loss, suggesting that the ionic content of the myocardium increased, or that other cation was lost

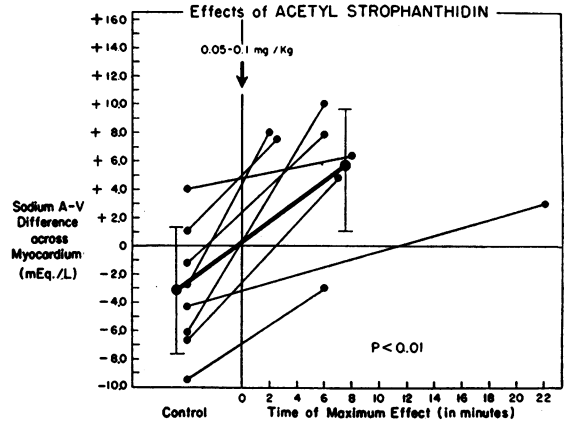


FIG. 3. THE EFFECT OF ACETYL STROPHANTHIDIN ON SODIUM A-V DIFFERENCE

Symbols same as in Figure 2.

if this represents a complete exchange phenomenon.

The transient respiratory depression induced by the drug reduced ventilation by about 80 per cent for thirty seconds with rapid recovery and was accompanied by a decline in mean arterial pH from 7.46 to 7.36 in the 4 animals in which this was measured. In two dogs in whom there was no significant change in pH, electrolyte changes were of the same order as those with a substantial fall. Moreover, in another study (18), the same ionic changes were seen in animals in which respiratory alteration was prevented by vagotomy.

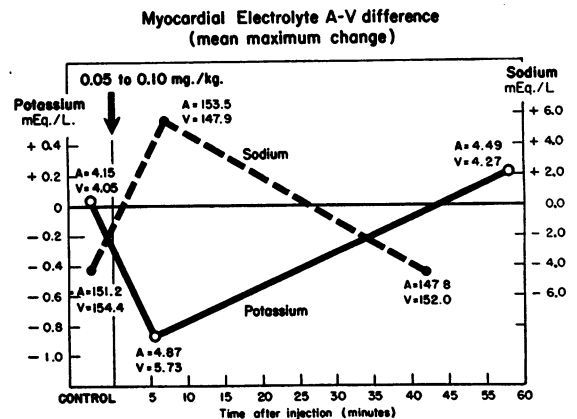


FIG. 4. COMPOSITE OF CONTROL, MAXIMUM AND RECOVERY MEAN A-V DIFFERENCE VALUES OF CATIONS FOR GROUP

Interrupted line, sodium; solid line, potassium. A refers to arterial and V refers to coronary sinus. Mean concentration of electrolytes in mEq. per L. at times indicated.

The difference between the coronary sinus pH and that of the arterial blood remained essentially the same during the experiment, except in the group of dogs with the more severe EKG changes, where the mean control pH A-V difference was +.05 and the maximum change was +.11 pH units, suggesting that the myocardium may release H⁺ during the toxic response to strophanthidin.

The mean cardiac output fell from a control of 131 cc. per min. per kg. to 88 cc. per min. per kg. (Figure 5); the rise in one animal was apparently associated with an unsteady state, as the systemic arterial pressure was fluctuating throughout the experiment. This decrease was due to a diminished stroke volume of 14.8 cc. to 11.2 cc., and a decline in pulse rate from 154 to 133 beats per minute. Since the mean arterial blood pressure for the group remained the same, the stroke work change paralleled that of the stroke volume, decreasing from 23.8 gm. meters in the control to 17.7 gm. meters after acetyl strophanthidin.

Electrocardiographic changes in this group of animals appeared related to the magnitude of potassium transfer. Four dogs with minimal changes of bradycardia with or without P wave alteration had negative potassium A-V differences ranging from -0.44 to -0.91 mEq. per L. (average -.58). As the coronary sinus blood represents predominantly drainage from the left ven-

tricle (19) and these animals gave no evidence of any electrocardiographic change referable to the ventricle, the potassium increment in the coronary sinus blood may be said to be derived from tissue exhibiting no electrical sign of toxicity. The four dogs with toxic electrocardiographic alterations manifested by ectopic beats, nodal and/or ventricular tachycardia had negative potassium A-V differences of -0.58 to -1.60 mEq. per L. (average -1.14). There was no correlation between the electrocardiographic changes and myocardial uptake of sodium.

No significant change in hematocrit values across the heart was observed during the course of these experiments, suggesting that there was no substantial transfer of water.

Digitalis preparations and ethyl alcohol may destroy the red cell membrane and release hemoglobin and potassium into the plasma (20). To exclude the alcohol vehicle as a cause of the observed electrolyte changes, 1 cc. of 60 per cent ethyl alcohol was injected into 3 dogs and produced no significant change in myocardial potassium A-V difference. Plasma hemoglobin determinations (21) were performed on the same plasma used for electrolyte analysis. Although there was a slight increase in plasma hemoglobin, the calculated increase in plasma potassium of .05 mEq. per L. as a result of the hemolysis would not significantly modify the plasma electrolyte concentration, as would be expected from the relatively low potassium content of 10 mEq. per L. in the canine red cell (19). Moreover, if there was any prozone effect on the red cells, it is unlikely that this would distort the arterio-venous difference values of potassium in such a manner as to contribute to the changes observed.

DISCUSSION

A direct action of cardiac glycosides on heart muscle has been demonstrated in a variety of ways, ranging from the improvement in contractile force of the fatigued papillary muscle of the cat (22), to the hemodynamic studies in the human subject with congestive failure in which increase in stroke output is the primary effect of the drug (8, 9). Since this improvement in the mechanical efficiency of the failing heart in low output failure occurs without significantly altering the supply

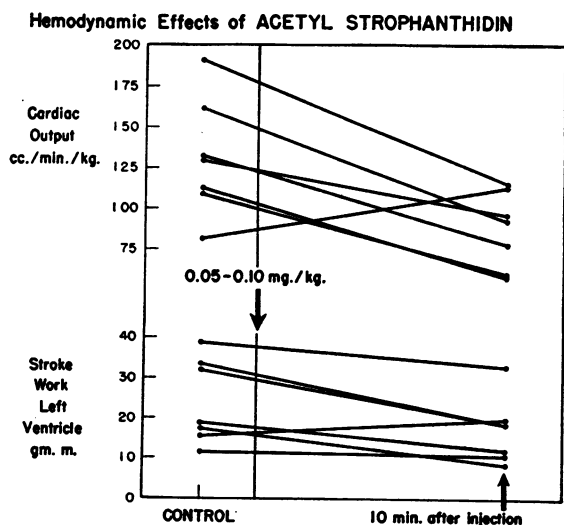


FIG. 5. THE EFFECTS OF ACETYL STROPHANTHIDIN ON CARDIAC OUTPUT AND STROKE WORK

of chemical energy (23, 24), attention has been drawn to the direct action of digitalis upon the contractile protein.

That the contractile elements in heart muscle are the agents of this action has been suggested by Horváth, Király, and Szerb (25), who concluded that the basic action of the glycosides may be on the polymerization of actin prior to its union with myosin. In support of this general view, Robb and Mallov (26) have found that the actomyosin thread shortened more rapidly and to a greater degree under the influence of Ouabain. These reactions, however, might be secondary to changes in the ionic media since such changes have considerable influence upon the actomyosin system as has been suggested by Szent-Györgyi (27).

From the isolated heart muscle studies of Hajdu (7), it has been inferred that the positive inotropic effect of digitalis depends upon the diminished internal ionic content effected by the movement of potassium from the cell. Assuming that the only alterations occurring were in those ions measured, as suggested by tissue analyses (4), the normal dog heart in this study apparently underwent an increase in internal ionic content due to the greater net uptake of sodium over egress of potassium. It would appear possible that this alteration in the intracellular ionic atmosphere may represent the physico-chemical process responsible for the decline in stroke work in the normal animal (28, 29). That an increment in intracellular sodium associated with potassium loss may be deleterious to the heart is borne out by studies of Cannon, Frazier, and Hughes (30), in which this electrolyte situation was produced in the rat by dietary control, resulting in areas of focal necrosis mainly in the subendocardium.

While ionic changes may be but one of the determinants of myocardial contraction, and may exist to the extent observed here only under pharmacologic influence, it is quite possible that they participate in the physiologic control of cardiac output that attends altered ventricular filling pressure and cardiac muscle stretch. Hercus, McDowall, and Mendel (31) have found that stretch of the rat left ventricle promotes sodium loss and that increase in sodium extrusion may contribute to the efficiency of stretched muscle. The diminished efficiency found following impairment of the sodium extrusion mechanism would appear to

parallel the findings in this study of strophanthidin in the normal animal, in which intracellular sodium accumulation was associated with diminished stroke work. While this ionic change in both instances may be but a by-product of the altered mechanical ability of the myocardium, the observation by Ranney (32) of a direct inhibitory effect of sodium at almost all concentrations in glycerol-extracted myocardial muscle would tend to support the view of an intimate relation between sodium accumulation and impaired mechanical ability.

The myocardial potassium loss seen in these animals, quantitatively greater in the four with toxic electrocardiographic changes, compares closely to that found by Conn (33) in normal dogs after digitoxin administration and amounted to 15 per cent of the total myocardial intracellular potassium by approximate calculation. Substantially the same myocardial potassium egress has occurred in patients reacting therapeutically to strophanthidin (34) so that the reduced potassium content of the untreated failing heart reported by other workers (3, 4) would appear to be enhanced by glycoside therapy.

The evidence provided by Wilde, O'Brien, and Bay (35) that there is potassium transfer across the heart with each cardiac cycle, egress of this ion occurring during repolarization, raises the question whether the potassium loss induced by glycosides is but an enhancement of this normal phenomenon, since the characteristic electrocardiographic manifestations of digitalis agents also takes place during repolarization. There is, however, suggestive experimental evidence from the isotopic study of Conn (33) that the return of potassium to the cell might be impeded since a reduced influx rate was found in dogs treated with digitoxin.

As it is possible that the electrolyte changes associated with strophanthidin have no influence upon myocardial performance, it would seem important to devise a method for blocking this ionic transport, preferably in the failing heart preparation where the effects on performance are more readily discerned. Moreover, if the same ionic pattern is found in the positive inotropic response of the failing heart as is reported here in the normal heart, then the lack of specificity of these ionic changes would militate against their having

a major role in the determination of myocardial performance.

SUMMARY

By simultaneous collection of blood samples from a systemic artery and coronary sinus, the myocardial transfer of sodium and potassium has been measured in eight anesthetized dogs before and after the administration of the digitalis analogue acetyl strophanthidin in doses of 0.05 to 0.1 mg. per kg. To relate cation transfer to cardiac performance, cardiac stroke work was assessed during the control period and at the time of maximal drug response.

There was no significant A-V difference of potassium during the control period. After strophanthidin, there was an abrupt increase in coronary sinus potassium with less rise in arterial potassium concentration, giving a mean maximum negative A-V difference of 0.86 ± 0.44 mEq. per L., indicating loss of potassium from the myocardium. Concurrently, there appeared to be a greater uptake of sodium with a positive A-V difference of 6.0 ± 4.0 mEq. per L. Both cation A-V differences resumed approximate control values at about 30 minutes. Animals with minor EKG changes after strophanthidin had less potassium loss than those with toxic EKG changes. No such correlation with sodium was found.

The stroke work of the left ventricle in this group of animals declined from a control value of 23.8 gm. meters to 17.7 gm. meters after strophanthidin. It is suggested that the negative inotropic effect of the digitalis analogues in the normal animal may be affected by the increase in internal ionic content of the myocardium due to the greater influx of sodium over egress of potassium.

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