and an increase in its excretion(5), we have interpreted their data differently and have ourselves found a renal mercury content slightly greater in albumin-injected rats than in saline-injected controls when both sets were sacrificed 24 hours after mercuric chloride injection(11).

Summary. Rats given 8 or 12 daily injections of 6-dimethylamino purine, 3-amino-d-ribose, develop increase in proteinuria, hypoproteinemia, edema and ascites. Such rats survive an intravenous dose of mercuric chloride lethal to normal rats. The mercury contents of kidneys, collected urine and collected feces, 24 hours after intravenous injection of $Hg^{203}Cl_2$, are not significantly different in rats with the experimental nephrotic syndrome from those in normal rats given the same dose.

1. Frenk, S., Antonowicz, I., Craig, J. M., and Metcoff, J., PROC. SOC. EXP. BIOL. AND MED., 1955, v89, 424.

2. Havill, W. H., Lichty, J. A., Jr., and Whipple, G. H., *J. Exp. Med.*, 1932, v55, 627.

3. Holman, R. L., and Donnelly, G. L., *ibid.*, 1942, v76, 511.

4. Lippman, R. W., PROC. SOC. EXP. BIOL. AND MED., 1949, v72, 682.

5. Lippman, R. W., Finkle, R. D., and Gillette, D., *ibid.*, 1951, v77, 68.

6. Lowry, O. H., and Hunter, T. H., *J. Biol. Chem.*, 1945, v159, 465.

7. Martin, H. F., and Reid, N. H., PROC. SOC. EXP. BIOL. AND MED., 1951, v78, 863.

8. Peters, J. P., and Van Slyke, D. D., *Quantitative Clinical Chemistry,* Vol. II. Methods. Williams & Wilkins Co., Baltimore, 1932, p683.

9. Ray, C. T., Burch, G. E., Threefoot, S. A., and Kelley, F. J., *Am. J. Med. Sci.*, 1950, v220, 160.

10. Surtshin, A., *Am. J. Physiol.*,

11. Surtshin, A., and Parelman, A. G.,

Received February 18, 1957. P.S.E.B.M., 1957, v95.

Effect of Hypothermia on Ventricular Fibrillary Threshold. (23311)

BENJAMIN G. COVINO* AND WILLIAM R. BEAVERS (Introduced by K. Rodahl)
Arctic Aeromedical Laboratory APO 731, Seattle, Wash.

Cardiac arrhythmias occur with alarming frequency under conditions of experimental hypothermia(1,2). A hyperirritable ventricle brought about by the hypothermic process is believed responsible for these cardiac irregularities, of which ventricular fibrillation is the most common. Direct measurements of ventricular threshold thus far have failed to reveal any difference between excitability of the normo- and hypothermic heart (3,4). However, no information is available as yet on the actual threshold for ventricular fibrillation at various body temperatures. Thus quantitative data on ventricular fibrillary threshold during progressive hypothermia appear necessary to confirm or refute the hypothesis of a hyperirritable hypothermic heart. During our study, an attempt was made to measure ventricular fibrillary thresholds by determining the minimum electrical stimulus needed to induce a fibrillary state in dogs subjected to immersion hypothermia. In addition, the effect of pH and prolonged cold exposure on ventricular fibrillary threshold was evaluated. Previous studies have revealed that cold acclimatization(5) and maintenance of normal pH during general body cooling(6) significantly reduce the incidence of hypothermic ventricular fibrillation. Thus, ventricular fibrillary thresholds were measured in cold acclimatized normocapneic dogs during hypothermia to determine if these procedures exert their beneficial effect by direct action on the myocardium.

Method. Eight apparently healthy dogs, ranging 13 kg to 25 kg, were anesthetized with intravenous pentobarbital sodium (30 mg/kg). The left common carotid artery was exposed and cannulated for measurement of mean arterial blood pressure via a mercury manometer. Samples of venous blood were obtained from the left jugular vein at rectal

* Present address: Department of Pharmacology, Tufts Medical School, Boston, Mass.

temperatures of 38°, 30°, 22°C for determination of serum pH in a Cambridge pH meter equipped with a water jacket. This made possible the measurement of pH at the actual temperature of the blood. A maximum time interval of 1 minute elapsed between blood sampling and pH measurement to insure a minimum change in blood temperature. Standard limb leads were attached and electrocardiographic tracings (lead 2) were obtained on a Sanborn poly-viso cardiette. All animals were intubated with endotracheal tube and artificial respiration (push-pull) with room air was instituted as rectal temperature fell below 30°C. Rectal temperatures in °C were recorded continuously on a Leeds-Northrup iron-constantin potentiometer. Three dogs were rendered hypothermic following 4 weeks of continuous exposure to an environmental temperature of 0 to −40°C. This was done to evaluate the influence of cold acclimatization on the ventricular fibrillary threshold. Direct quantitative measurements of the ventricular threshold were made as described previously(7,8). During our study, however, both the driving and testing electrode were attached to the left ventricle. Two Grass square wave stimulators connected in tandem were used to drive and test the threshold of the ventricle. Anodal stimuli, varying from 0-15 mamp. and duration from 3-10 msec., were delivered at 10 msec. intervals throughout the cardiac cycle to determine the excitability recovery curve of the ventricle. The minimum stimulus required to produce a single extrasystolic beat was taken as a measure of the basic ventricular threshold. The ventricular fibrillary threshold was determined in a similar fashion by delivering at 10 msec. intervals throughout the cardiac cycle anodal stimuli of 0-15 mamps. and 3-10 msec. duration. It was found, however, that ventricular fibrillation could only be produced when impulses were applied to the ventricle immediately prior to diastole. Thus, the fibrillary threshold represents the minimum stimulus, in terms of strength of current and duration, which can produce ventricular fibrillation when applied during the prediastolic period of the cardiac cycle. Stimulation studies were carried out initially at normal body temperature, following which the dogs were immersed in a recirculating cold water bath of 3.5°C. The studies were then repeated at rectal temperatures of 30°C and 22°C.

Results. The basic threshold of the normothermic ventricle was determined in all cases prior to measuring the fibrillary threshold. The excitability recovery curve was essentially similar to that reported previously by Hoffman *et al.*(9). A period of absolute refractoriness, during which a maximum stimulus of 15 mamp. was ineffective, occurred immediately following ventricular depolarization. This interval was followed by a relative refractory period during which the ventricular theshold fell sharply within 20-70 msec. from a level of >15 mamp. to 1.2-2.6 mamp. A period of relative hyperexcitability immediately prior to diastole was observed and corresponded to the major dip of Hoffman *et al.*(9) and the vulnerable period of Wiggers and Wegria(10). No minor dip early in the cardiac cycle was observed. This may be due to the fact that our maximum strength of stimulus was 15 mamp. and anodal in nature.

Attempts to fibrillate a normothermic ventricle by gradually increasing the strength of stimulus to a maximum of 15 mamp., and the duration of stimulus from 3 to 10 msec. were uniformly unsuccessful. In 2 instances, a stimulus of 10 mamp. applied immediately prior to diastole did produce multiple ectopic beats but in no case was ventricular fibrillation initiated. Impulses delivered during the relative refractory period or diastole never induced more than a single extrasystolic beat.

At a rectal temperature of 30°C, no change in ventricular excitability recovery curve was observed. The 3 phases of the cardiac cycle, absolute refractory period, relative refractory period, and diastole, were slightly prolonged due to the decrease in heart rate at this temperature. However, the threshold levels during these various periods were similar to those observed in the normothermic heart. Again ventricular fibrillation could not be produced by application of stimuli of maximum strength to the ventricle at any point in the cardiac cycle. As rectal temperature fell to 22°C

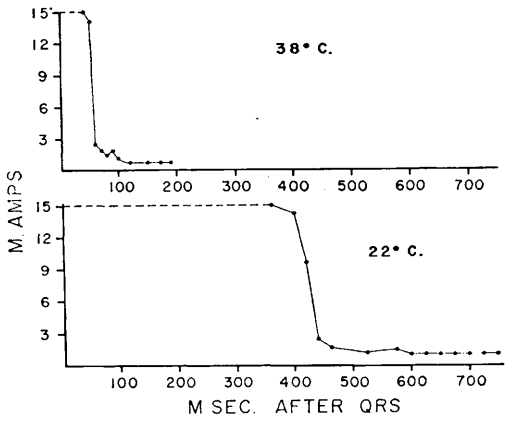

FIG. 1. Comparison of excitability recovery curve at rectal temperatures of 38°C and 22°C.

and the mean serum pH to 7.18, the average heart rate decreased to 42 beats per minute. The results of this bradycardia was a prolongation of the cardiac cycle. Yet no significant difference obtained between the basic ventricular threshold at 38°C and 22°C (Fig. 1). However, the ventricular fibrillary threshold was greatly diminished. At this temperature a stimulus of 2.5-5.2 mamp. was sufficient to initiate ventricular fibrillation in all dogs. Table I reveals the actual changes in diastolic threshold and ventricular fibrillary threshold which occurred during cooling in each of the 5 experimental animals. The average diastolic threshold remained quite constant, 1.32 mamp. at 38°C and 1.42 mamp. at 22°C, yet, the average fibrillary threshold decreased from >15.0 mamp. at 38°C to 3.44 mamp. at 22°C. Thus, the basic ventricular threshold is not affected by the hypothermic episode, while at least a 5 fold decrease in mean fibrillary threshold occurred

TABLE I. Comparison of Diastolic and Ventricular Fibrillary Thresholds at Rectal Temperatures of 38°C and 22°C, in 5 Dogs.

	38°C		22°C	
	Diastolic threshold (mamp.)	V.F. threshold (mamp.)	Diastolic threshold	V.F. threshold
	.7	>15	1.1	3.5
	.8	"	1.4	2.5
	1.9	"	1.9	5.2
	2.05	"	1.8	3.4
	1.2	"	1.9	2.6
Avg	1.32	"	1.42	3.44

TABLE II. Comparison of Diastolic and Ventricular Fibrillary Thresholds at Rectal Temperatures of 38°C and 22°C in 3 Cold Acclimatized, Normocapneic Dogs.

	38°C		22°C	
	Diastolic threshold (mamp.)	V.F. threshold (mamp.)	Diastolic threshold	V.F. threshold
	2.0	>15	2.0	3.5
	.9	"	.7	4.0
	.8	"	1.3	2.5
Avg	1.23	"	1.33	3.33

when rectal temperature was lowered to 22°C.

Effect of pH and cold acclimatization. Three dogs were exposed to an environmental temperature of 0 to −40°C for 4 weeks and then rendered hypothermic. The serum pH was maintained between 7.30 and 7.50 in all dogs via controlled artificial respiration. Table II shows that the diastolic threshold again was unaffected by the reduction in body temperature. The ventricular fibrillary threshold, however, fell from >15 mamp. at 38°C to a mean of 3.33 mamp. at 22°C. No difference in fibrillary threshold existed at 22°C between acclimatized, normocapneic dogs and non-acclimatized hypercapneic dogs. Thus, neither cold acclimatization nor maintenance of a normal pH during hypothermia was capable of preventing the fall in ventricular fibrillary threshold produced by the general reduction of body temperature.

Discussion. Our data demonstrate quite conclusively that the nembutalized hypothermic dog is highly susceptible to cardiac arrhythmias and particularly ventricular fibrillation, and affords the first direct evidence that a decrease in ventricular fibrillary threshold actually does occur during hypothermia. Although the fibrillary threshold was significantly diminished during general body cooling, no difference could be demonstrated between the basic ventricular threshold of the normo- and hypothermic heart. The latter also has been reported by Brooks *et al.*(3) and Hegnauer *et al.*(4). These findings indicate the fallacy of attempting to evaluate the efficacy of various antifibrillary procedures by direct determination of the diastolic ventricular threshold alone. It is apparent from our results that the latter may

not serve as an accurate index of the hypothermic heart's susceptibility to ventricular fibrillation. This situation, however, may be peculiar to hypothermia since Hoffman *et al.* (9) have reported that both the diastolic threshold and the fibrillary threshold of the normothermic ventricle are altered in a similar fashion following injection of epinephrine.

The failure to observe a change in diastolic threshold suggests that cellular processes responsible for the resting excitability of ventricular musculature are effected minimally by the degree of hypothermia utilized in this study. The ventricular fibrillary threshold, however, can be altered by factors other than changes in tissue excitability. Disturbances in cardiac conduction, refractoriness, and pacemaker activity are known to increase the vulnerability of the heart to fibrillation(3). At body temperatures below 25°C ventricular conduction is depressed in a heterogenous fashion(12) and pacemaker activity is 20% of normal(2). Thus, conditions existing within the hypothermic ventricle are ideal for initiation of disorganized activity. In such circumstances an extrinsic stimulus which is not effective at normal temperature could induce ventricular fibrillation at low temperatures due to the presence of favorable intrinsic conditions. The normal diastolic threshold and reduced fibrillary threshold observed in hypothermia tend to support this thesis. These altered intrinsic factors may also be responsible for spontaneous hypothermic ventricular fibrillation. Potential ectopic foci may discharge more readily in the presence of a depressed pacemaker. In addition, the heterogenous ventricular conduction pattern would allow the reentry of ectopic impulses and so produce repetitive firing and fibrillation.

It is interesting to note that 2 procedures, which are capable of decreasing the incidence of spontaneous fibrillation during hypothermia were ineffective in checking the sharp reduction of ventricular fibrillary threshold. Maintenance of a normal serum pH(5) and prolonged cold exposure(6) both have been shown to influence in a beneficial manner the terminal cardiac event in hypothermic dogs. Thus, the results obtained during our study indicate that the protective effect of these procedures is not due to a direct action on the ventricular myocardium.

Summary. Direct quantitative measurements of both basic ventricular threshold and ventricular fibrillary threshold were made in dogs prior to and during progressive immersion hypothermia. No change in basic ventricular threshold was observed as rectal temperature fell from 38°C to 22°C. At the same time, ventricular fibrillary threshold showed a 5-fold decrease. Neither maintenance of a normal serum pH during cooling nor prolonged cold exposure prior to the hypothermic episode altered the decrease in ventricular fibrillary threshold.

1. Bigelow, W. G., Lindsay, W. K., and Greenwood, W. F., *Ann. Surg.*, 1950, v132, 849.

2. Covino, B. G., Charleson, D. A., and D'Amato, H. E., *Am. J. Physiol.*, 1954, v178, 148.

3. Brooks, C. McC., Hoffman, B. F., Suckling, E. E., and Orais, O., *Excitability of the Heart.* Grune and Stratton, N. Y., 1955.

4. Hegnauer, A. H., and Covino, B. G., *Am. J. Physiol.*, 1956, v186, 511.

5. Covino, B. G., and Hegnauer, A. H., *Cir. Res.*, 1955, v3, 373.

6. Covino, B. G., and Beavers, W. R., *Am. J. Physiol.*, in press.

7. Covino, B. G., and Charleson, D. A., *ibid.*, 1955, v181, 377.

8. Covino, B. G., and Hegnauer, A. H., PROC. SOC. EXP. BIOL. AND MED., 1955, v89, 659.

9. Hoffman, B. F., Gorin, E. F., and Wax, F. S., *Am. J. Physiol.*, 1951, v167, 88.

10. Wiggers, C. J., and Wegria, R., *ibid.*, 1939-40, v138, 500.

11. Hoffman, B. F., Siebens, A. A., Cranefield, P. F., and Brooks, C. McC., *Circ. Res.*, 1955, v3, 140.

12. Garcia, Ramos G., *J. Thoracic Surg.*, 1956, v31, 635.

Received March 22, 1957. P.S.E.B.M., 1957, v95.