Sensitive Test for Detection of Early Viral Myocarditis in Mice[1] (34883)

JER-SHOUNG LIN,[2] RAMON RODRIGUEZ-TORRES, SUMNER BERKOVICH,[3] AND ALICE C. YAO
(Introduced by R. J. Watson)
Department of Pediatrics, State University of New York, Downstate Medical Center, Brooklyn, New York 11203

The electrocardiogram can be altered by changes in intra- and extracellular ionic concentration (1–3). It seems likely, therefore, that any insult to the myocardial fiber which modifies membrane permeability with resultant changes in ionic concentration can be detected by electrocardiographic analysis (4, 5). Although gross change can be easily recognized, minor alterations in membrane permeability might be overlooked. The administration of glucose and insulin, however, is known to produce significant change in the ionic concentration across the cell membrane (6, 7); these changes might be magnified if the cell membrane was already damaged.

To test this hypothesis, adult mice were infected with a cardiotropic agent, Coxsackie B virus, Type 1, and electrocardiographic recordings were made at frequent intervals. The tracings of the infected animals were not unlike those obtained from uninfected controls; however, following the infusion of glucose and insulin, the virus-infected animals exhibited significant changes in the P–R interval. The difference observed was probably due to cell membrane damage related to viral infection which was exaggerated by the glucose–insulin infusion. The data that support this conclusion are presented below.

Materials and Methods. Mice. Mature female "Swiss albino" mice (Albany strain) were used throughout the study. Weight ranged from 15 to 20 g.

Virus. The preparation and origin of the virus used has been described in detail (8). Each experimental animal received an intraperitoneal injection of 0.2 ml of the virus inoculum containing 10–100 tissue culture infectious doses $(TCID)_{50}$/0.1 ml.

Virologic Studies. Virus isolations and serum antibody determinations were performed in monkey kidney cell cultures by standard techniques. Blood for culture was obtained in heparinized microhematocrit tubes from the retro-orbital plexus.

Glucose–Insulin Infusion. Each animal received, through the tail vein, 0.3 ml of a 20% glucose solution in water containing 1 unit of regular insulin/g of glucose. The infusion was given over a period of 10 to 15 sec.

Electrocardiographic Studies. Recordings were made with a Sanborn Stethocardiette (recorder model No. 4560, preamplifier No. 350-2700 C) with a paper speed of 100/mm/sec standardized to 1.5-cm deflection for 1 mV input. Three standard leads, 3 unipolar limb leads, and 3 precordial leads (right anterior axillary line, midsternal and left anterior axillary line at the level of the xiphoid) were recorded. Needle electrodes were used. The test mice were anesthetized with a combination of sodium pentobarbital (0.15 ml of a 0.6% solution in glucose and water) and chlordiazepoxide hydrochloride (0.15 ml of a 0.5% solution in glucose and water) given intraperitoneally. Lead II of the electrocardiogram was used for measurement of heart rate and P–R interval; each value obtained was the mean result of at least 3 determinations on each tracing. The measured P–R interval was corrected for heart rate by the following formula $[(P\text{–}R)/(R\text{–}R)^{1/2}]$. The P–R index was calcu-

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[2] Fellow in pediatric cardiology, State University-Kings County Medical Center.

[3] Career Scientist of the Health Research Council of the City of New York under contract No. I-173.

lated by dividing the corrected P–R interval obtained at the time the animal was admitted to the study by the corrected P–R interval obtained before the infusion of glucose and insulin. Determinations were repeated 30 and 60 min after the infusion was begun. The values obtained were compared by the Student's *t* test.

Study Plan. During each experiment no more than 10 mice were caged together. Room temperature was maintained at approximately 24° with 12 hr of darkness and 12 hr of light.

Expt. 1. Two groups of mice were used, 19 control and 18 virus-infected animals. Each animal had an electrocardiogram at the start of the study. Seventy-two hr after injection of 0.2 ml of an inoculum containing 10–100 $TCID_{50}$/0.1 ml of the test virus, or an equal volume of control fluid from uninfected monkey cell cultures (mixture 199 with antibiotics but without serum), and again 30 and 60 min after the infusion of glucose–insulin, the electrocardiogram was repeated. Three days after inoculation with virus or control fluid, all of the animals were bled. Heparinized bloods were obtained for virus isolation studies.

Expt. 2. For this study 20 animals were included in each group. The mice were treated as outlined in Expt. 1, except that the virus inoculum contained only 10 $TCID_{50}$/0.1 ml.

Expt. 3. Nineteen animals were studied in each group. The glucose–insulin infusions were done 7 days after the inoculation of 0.2 ml of a preparation containing 10–100 $TCID_{50}$/0.1 ml of the test virus, or an equal volume of control fluid. Neutralizing antibodies to the Coxsackie B_1 test virus were determined on sera obtained at the same time.

Results. Virologic Observations. Expt. 1. Coxsackie B_1 virus was recovered from blood samples obtained from all of the virus-infected animals. No viral agent was detected in the uninfected controls.

Expt. 2. The test agent was not recovered from any of the samples tested.

Expt. 3. All of the virus inoculated animals in contrast to the uninoculated controls had Coxsackie B_1 antibodies (1:4 or greater) 7 days after infection.

Electrocardiographic findings. The mean P–R indices calculated for the 58 uninfected controls and the 37 virus-inoculated animals before the administration of glucose and insulin are reviewed in Table I. The average values obtained for the 2 experimental groups are not significantly different ($p > 0.05$). Only a small percentage, 3.4%, had a P–R index value greater than 1.30 (mean P–R index: 1.01 ± 0.128 SD). Therefore, in the normal animal a P–R index greater than 1.30 is more than 2 standard deviations from the mean and is abnormal.

Table II summarizes the results of data obtained from study of all 3 experimental groups. In all of the experiments, except one, Expt. 2, there was a significant difference at 30-min postglucose–insulin infusion between the average P–R index obtained for infected and uninfected mice ($p < 0.005$). The difference was even more significant at 60 min. The findings were similar to those obtained in Expt. 3, 1 week after virus infection. It is obvious that a significant prolongation of the P–R interval had occurred after infusion in the infected group. A representative electrocardiogram from one of the study animals is

TABLE I. P–R Indices in Uninfected Control and Virus-Infected Mice Before Infusion of Glucose and Insulin.

			P–R index > 1.30	
	No. of mice	P–R index [mean value ± SD (range)]	No. of mice	(%)
Uninfected control	58	1.01 ± 0.128 (0.77–1.49)	2	3.4
Virus animals				
Viremic phase (3rd day)	18	1.11 ± 0.183 (0.89–1.66)	2	11.1
Postviremic (7th day)	19	0.98 ± 0.081 (0.83–1.13)	0	0

TABLE II. P–R Indices After Glucose–Insulin Infusion.

Exp. no. (day of glucose–insulin infusion)[a]	Group	No. of mice	30 min after glucose–insulin: P–R index [mean value ± SD (range)]	30 min after glucose–insulin: P–R index > 1.30, No. of mice	30 min after glucose–insulin: P–R index > 1.30, (%)	60 min after glucose–insulin: P–R index [mean value ± SD (range)]	60 min after glucose–insulin: P–R index > 1.30, No. of mice	60 min after glucose–insulin: P–R index > 1.30, (%)
1[b] (3rd day)	Control	19	1.17 ± 0.171 (0.91–1.49)	6	31.5	1.03 ± 0.150 (0.76–1.40)	2	10.0
	Virus	18	1.35 ± 0.154 (1.15–1.68)	11	61.1	1.38 ± 0.186 (1.05–1.77)	11	61.1
			$p < 0.005$[e]			$p < 0.005$		
2[c] (3rd day)	Control	20	1.08 ± 0.174 (0.84–1.59)	2	10.0	1.06 ± 0.180 (0.76–1.43)	2	10.0
	Virus	20	1.19 ± 0.133 (1.02–1.50)	3	15.0	1.19 ± 0.151 (0.97–1.54)	5	25.0
			NS[f]			NS		
3[d] (7th day)	Control	19	1.13 ± 0.170 (0.91–1.51)	3	15.7	1.13 ± 0.177 (0.88–1.51)	3	15.7
	Virus	19	1.35 ± 0.151 (1.07–1.71)	12	63.1	1.42 ± 0.147 (1.21–1.81)	15	79.0
			$p < 0.005$			$p < 0.005$		

[a] Time elapsed after virus inoculation of test animals.

[b] Coxsackie B_1 virus recovered from blood of infected animals on day of glucose–insulin infusion.

[c] No viremia detected in infected animals on day of infusion.

[d] No detectable viremia on day of infusion; however, virus-infected animals had measurable levels (1:4 or greater) of Coxsackie B_1 neutralizing antibodies at this time.

[e] The p values represent the significance of the observed differences in mean values between the control and virus-infected group.

[f] Not significant.

shown in Fig. 1. The calculated mean P–R index, 60 min after infusion of glucose and insulin, was greater than 1.30 in more than 60% of the virus infected mice in Expts. 1 and 3 as compared to less than 15% in the uninfected controls (Table II). By contrast, in Expt. 2 there was no such difference.

Discussion. Electrocardiography should be one of the most sensitive tools for the diagnosis of myocardial disease, but the changes recorded are frequently not constant. A reason for this may be that minor changes at the level of the myocardial cell are not recognizable by presently available techniques. It would be desirable to intensify these minor abnormalities, which probably exist, so that they can be detected. Cell membrane permeability is probably damaged in myocarditis. If this is so, then the infusion of glucose and insulin which produces changes in the ionic concentration across a cell membrane should further intensify the damage that has already occurred from whatever etiologic agent is involved. This would fit the theory postulated by Sodi-Pallares *et al.* (7) that with myocardial damage there is an exodus of intracellular potassium. The glucose and insulin, however, might upset the equilibrium at the cell level, allowing potassium to migrate in the opposite direction. Following the infusion, therefore, previously normal or borderline electrocardiographic recordings would become abnormal and would reflect an imbalance in the transmembrane potassium gradient. This would be detected in the surface electrocardiogram as changes in repolariza-

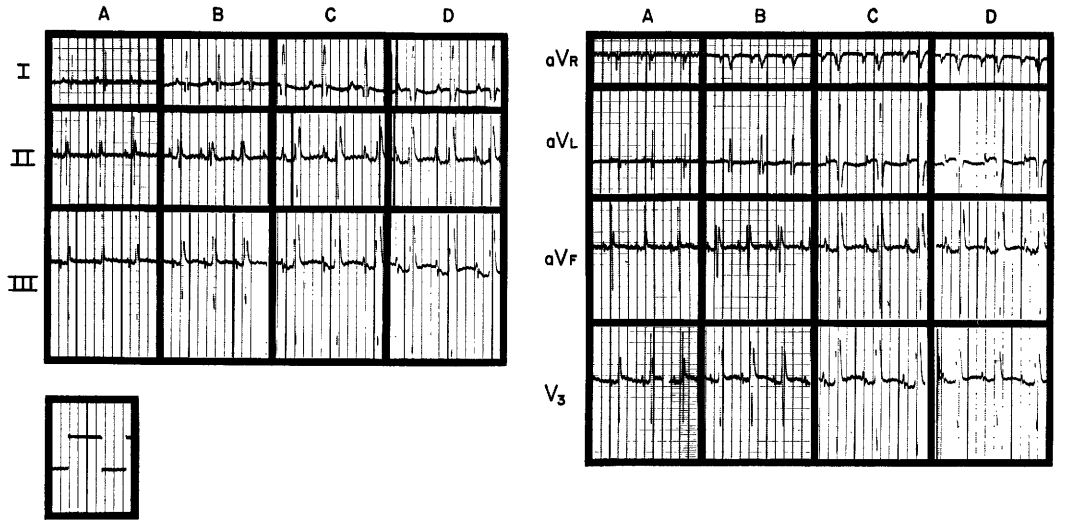

FIG. 1. Electrocardiographic tracings obtained from a virus-infected mouse before and after the glucose–insulin infusion: (A) at the time the animal was admitted to the study; (B) 7 days after inoculation of the test virus, immediately after glucose–insulin infusion; (C) 30 min after glucose–insulin infusion; (D) 60 min after glucose–insulin infusion. There was significant prolongation of the P–R interval, increase in the amplitudes of the P wave and the QRS complex after the infusion.

tion. In addition there would be alterations in amplitude of both the depolarization and repolarization process, as well as changes in the conduction time (9).

Grodums and Dempster (10) and Kilbourne and his associates (11) have shown that Coxsackie virus infection in adult mice may cause myocarditis. Using this experience, we have studied the cardiac response of normal mice following infection with a strain of Coxsackie B_1 virus before and after the infusion of glucose and insulin. The disadvantage inherent in study of the electrocardiogram of the mouse is the rapid heart rate (300–600/min) which makes analysis of the T wave and the Q–T interval virtually impossible (12). Therefore, only the P wave, the P–R interval, and the QRS voltage could be studied in detail. We are aware that significant changes should have affected the repolarization process as well; however, the experimental animal and our technique limited the analysis of this portion of the electrocardiogram.

In the study groups presented, a very significant prolongation in the P–R interval was noted in virus-infected mice after the infusion of glucose and insulin. A similar effect, which was not statistically significant, was noted in control animals following the same infusion. Even the normal animal given glucose and insulin experiences a shift of potassium from extracellular spaces to the cell; therefore, the lengthened P–R interval apparently reflected this ionic shift in the normal cell (4, 13, 14). However, the change in the virus-infected animals was far greater, because the ionic changes that occurred with the glucose–insulin infusion were probably magnified by the virus-related cell membrane damage. To support this theory, we have presented data which indicates that the experimental animals developed the prolonged P–R interval at the time the test virus could be recovered from blood specimens (Expt. 1). The effect was intensified during convalescence (Expt. 3) when sera from the virus-infected mice had developed measurable levels of Coxsackie B_1 neutralizing antibodies. The appearance of these antibodies indicates recent infection with the test virus.

Of interest are the results obtained in Expt. 2. The P–R interval was not sufficiently prolonged at 30 min postinfusion among the virus-infected animals 72 hr after inoculation. The reason for this difference may be

that this particular group of mice received 10 rather than the 100 $TCID_{50}$ given to their experimental mates (Expts. 1 and 3) and had no detectable viremia. The dose factor, therefore, is probably of great importance in determining the likelihood of myocardial infection.

It is possible that the human subject with viral myocarditis may, like the mouse, show an exaggerated response to the infusion of glucose and insulin. Early minimal changes in myocardial function not detectable by routine electrocardiographic studies might be demonstrable following this infusion test. It is possible, therefore, that this technique could be utilized to confirm the presence of damage early in the devleopment of viral myocardial disease.

Summary. The electrocardiogram was used to study the myocardial effects of viral infection in mice. Thirty-seven adult mice were infected with a cardiotropic Coxsackie B_1 virus. The tracings of the infected animals were not unlike those obtained from uninfected controls, however, following the infusion of glucose and insulin, the virus infected animals exhibited significant prolongation in the P–R interval as compared with that of 38 uninfected controls ($p < 0.005$). The calculated P–R index 60 min after infusion was greater than 1.30 (mean P–R index: 1.01 $\pm$ 0.128 SD) in more than 60% of the virus-infected mice as compared to less than 15% in the uninfected controls. The difference observed was probably due to cell membrane damage related to viral infection which was exaggerated by the glucose–insulin infusion.

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