**Effect of Peruvosid (CD412) on excitability and functional refractory period of atrial and ventricular tissues in cardiomyopathy caused by *Trypanosoma cruzi***

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Attempts were made to produce myocarditis by *Trypanosoma cruzi* inoculation in healthy dog puppies 6 to 8 weeks old. Significant electrocardiographic abnormalities were produced, coinciding with interstitial inflammatory processes in the cardiac tissue and with degenerative changes in the myocardial fibres. In puppies showing these changes, profound changes in the excitability and the functional refractory period of the atrial and ventricular muscular tissue were observed.

The administration of Peruvosid in doses of 0.024 to 0.0647 mg./kg. tended to diminish the excitability, previously increased by the inflammatory process, at the same time increasing the functional refractory period duration which had previously been shortened. The fact that Peruvosid corrects these fundamental factors in the genesis of cardiac arrhythmias suggests that the drug may be useful in the treatment of cardiac insufficiency produced by Chagas' cardiomyopathy, in which arrhythmias are one of the basic characteristics.

Chronic inflammatory processes in the myocardium, caused by natural or experimental infections induced by *Trypanosoma cruzi*, produce important changes in cardiac rhythm; it is this that provoked from Carlos Chagas (1928) the statement that 'arrhythmia constitutes the most important symptom at this stage of the illness'.

Chagas and Villela (1922) related arrhythmias to changes in the main functions of the cardiac muscle; they considered that extrasytoles were due to changes in the excitability, an opinion also held by Cossio (1943).

Recent studies (Pifano et al., 1962; Rodriguez, 1963) have shown that the smaller interval between two propagated responses, the functional refractory period (Rosenblueth, Alanis, and Mandoki, 1949), is shortened and the velocity of propagation of the impulse is diminished in the atrial and ventricular tissue in Chagas' myocarditis. The change occurring in these properties of the myocardium is greater during the chronic than during the acute phase of the illness, and explains atrial and ventricular extrasystole, atrial flutter and fibrillation, and sudden death from ventricular fibrillation, which were first reported by Chagas and Villela (1922).

Treatment of cardiac arrhythmias in Chagas' chronic myocarditis is still a problem. Preparations available to date have not produced encouraging results. This paper examines the effect of a new glucoside preparation (Peruvosid) on some of the fundamental electrophysiological properties of the myocardium of the dog, in which an inflammatory process had been induced previously by the inoculation of *T. cruzi*.

**Materials and methods**

Forty-nine healthy puppies, between 6 and 8 weeks of age, were subjected to clinical and electrocardiographic tests before experimental inoculation with *T. cruzi*. The inoculations were made subcutaneously and intraperitoneally with the 'Bertoldo strain' of *T. cruzi*, maintained in highly homozygotic Carworth Farms Webster white mice. The mice were initially obtained from an inbred stock developed by Carworth farms from the Webster-Rockefeller Swiss mouse strain (Law, 1948). Each puppy was inoculated with a 1/2 ml. solution consisting of 1 ml. mouse blood in 3 ml. sodium citrate 3:8 per cent. The blood used for preparing the inoculation showed approximately 20 parasites per field. Six puppies died, with a
picture of paralysis of the hind-quarters; in 10, no electrocardiographic changes were observed in a series of tests made after the infection, and histological examination showed no changes in the myocardium. Eight puppies died immediately after anaesthesia, owing to profound changes in the myocardium.

Artificial respiration was applied through a tracheal cannula with an aspiring-impelling pump of the Harvard type, for recording the intensity-duration curves and the functional refractory period of atrial and ventricular tissue. The heart was exposed by mid-section of the sternum. Pentobarbitone sodium was used for the anaesthesia in doses of 35 mg./kg. An electroencephalograph, Schwarzer model E-502 with six channels, was used as the recording apparatus. Rectangular pulses of variable intensity and duration, generated by Grass stimulators model S4E, were used for stimulating the tissues. The drug was administered with a Harvard pump, model 600-900, through one of the saphenous veins, in a solution of 0·6 mg. of Peruvosid dissolved in 50 ml. of 5 per cent glucose solution, at an infusion speed of 0·382 ml./minute.

The histological examination of the heart was carried out by the usual technique: fixation in formol 10 per cent; embedding in paraffin; sections of 5–7 μ. The pigments used were haematoxylin-eosin, PAS, Gomori's trichrome, and Wilder's reticulin.

Results

Of the 25 dogs infected with T. cruzi, 7 were used to study the ventricular functional refractory period and atrial functional refractory period, 3 for atrial excitability, and 4 for ventricular excitability. We were unable to elicit responses to strong stimulation in 6 dogs; this was explained by the conspicuous histological alterations in the atrial and ventricular myocardium.

Atrial and ventricular excitability Intensity-duration curves were plotted using a cartesian co-ordinates system in which fixed voltages were represented on the ordinate, and the required time to obtain a response for that given voltage was represented on the abscissa. The minimum voltage necessary to elicit a response with infinite time is the rheobase.

With the object of comparing the changes produced by T. cruzi cardiomyopathy in atrial and ventricular excitability, the intensity-duration curves were studied in 12 healthy dogs; the averages, which are normal values, are shown in Fig. 1.

Fig. 2 shows the curve of a dog (LCH4D4) with Chagas' infection of eight weeks' duration. Electrocardiograms during the working period showed, in comparison with the con-
Effect of Peruvosid (CD412) in cardiomyopathy

FIG. 3 (A) Dog LCH4D4: Infiltration with monocytes. (H. and E. x 176.) (B) Dog LCH4D4: Extensive infiltration with monocytes that dissociate the fans of cardiac muscular fibres. Some of the fibres show moderate degenerative changes. (H. and E. x 305.) (C) Dog LCH4D5: Cross-section of muscular fibres showing small foci of monocyte infiltration, slight oedema, and atrophy of some muscular fibres. (H. and E. x 305.) (D) Dog LCH4D5: Conspicuous infiltration of monocytes with necrotic areas in some fibres. (H. and E. x 305.)

trol, a complete right bundle-branch block with an accentuated deviation of the electrical axis towards the left and primary changes in the T wave in the left praecordial leads. These changes pointed to histological changes of the kind seen in Chagas' myocarditis (Anselmi et al., 1964). Histological examination showed (Fig. 3A and B) a dense and diffuse infiltration of monocytes cells at the atrial and ventricular level, and slight degenerative changes of myocardial fibres. The control curve of atrial intensity-duration shows variations, as compared to normal dogs. In fact, it was seen that though the rheobase remained normal, i.e. for long-duration stimuli the required voltage was the same as in normal dogs (Fig. 1), for short-duration stimuli the voltage required to elicit a response was less than that in normal dogs (15 V per 0.02 msec.). An infusion of 0.096 and 0.0252 mg. Peruvosid increased the values of the rheobase and of the voltage required to produce responses with short duration stimuli.

Fig. 4 shows the atrial intensity-duration curves of dog LCH4D5, obtained 8 weeks after infection with T. cruzi. The electrocardiographic abnormalities, suggesting myocarditis, consisted of complete right bundle-branch block, with accentuated deviation of the electrical axis towards the left (Laranja, Pellegrino, and Dias, 1949; Pellegrino, 1946); the morphology of the right ventricle in all the praecordials suggested dilatation of these cavities (Fig. 5). Histological examination (Fig. 3C and D) showed dense and diffuse lymphoplasmohistiocytic infiltrations and degenerative processes in myocardial fibres.

The control curve of the intensity-duration shows important changes, consistent with the accentuated decrease of the rheobase, as well
as the reduction of the short stimulus intensity (10 V for stimuli of 0.02 msec.). A dose of 0.096 mg. CD412 corrects the atrial excitability curve, increasing the values of the rheobase and the voltage required for obtaining short duration stimuli. A lethal dose of 0.420 mg. does not modify significantly the results obtained by the previously mentioned dose.

Fig. 5 shows the ventricular intensity-duration curve after the appearance of the electrocardiographic changes described above. There is an important reduction in the intensity of the short-term stimuli voltage (15 V per stimulus of 0.02 msec.), a dose of 0.144 mg. CD412 diminishing the ventricular excitability and a lethal dose of 0.324 mg. producing no significant changes.

**Atrial and ventricular functional refractory period** The functional refractory period was obtained with the method described by Méndez and Méndez (1953).

Fig. 6 shows the changes in the absolute functional refractory period in atrial (continuous line) and ventricular (dashed line) tissue with increased doses of Peruvosid.

The study of atrial and ventricular functional refractory period presented difficulties because of the profound histological changes in the tissues, the dog having died after the first control curves were completed. Fig. 7 shows the result of the study of the atrial functional refractory period in a dog, histological examination of which showed small changes consisting of inflammatory infiltrations of slight density and diffusion. The cont-

**FIG. 4** The atrial intensity-duration curve of dog LCH4D6. The control curve shows a distortion and an important reduction of the rheobase and the voltage intensity for stimulation of short duration. The administration of Peruvosid resulted in a normal trace.

**FIG. 5** The changes in dog LCH4D6. The electrocardiogram 8 weeks after inoculation with T. cruzi shows complete right bundle-branch block with conspicuous left axis deviation of the QRS. There is a right ventricular pattern in all the chest leads. An important reduction in voltage intensity is seen as a response to short duration stimulation in the ventricle intensity-duration curve. An increase in this value is seen after the administration of CD412.

**FIG. 6** Absolute functional refractory period in the ventricular tissue of 7 dogs and absolute functional refractory period in the atrial tissue of 5 dogs.
Fracto
trol curve is superimposed on the normal values. An infusion of Peruvosid produced a progressive increase in tissue functional refractory period.

Fig. 7 The changes in atrial functional refractory period curve after progressive administration of Peruvosid. There is a linear relation between drug dosage and increase in functional refractory period.

Discussion
Infection of puppies with a highly virulent strain of *T. cruzi* produces violent reactions on a level with the heart, a fact that has been fully confirmed by various investigators (Pifano et al., 1962; Taquini, 1941; Anselmi et al., 1967). Recent studies have shown that a considerable reduction in the atrial and ventricular functional refractory period in these muscles is caused by Chagas' myocarditis (Pifano et al., 1962; Rodriguez, 1963; Rodriguez et al., 1964). Atrial and ventricular conduction decreases in its transmission velocity when the impulse passes through tissues showing inflammatory processes and degenerate myocardial tissues (Rodriguez and Anselmi, 1963; Anselmi et al., 1965). The change in these two fundamental properties explains, according to Wiener and Rosenblueth (1946), the high frequency of atrial fibrillation in this type of cardiomyopathy (Rosenbaum and Alvarez, 1953; Tejada and Castro, 1958).

Doses of 0.0470 mg./kg. of Peruvosid produced considerable increases in the values of atrial functional refractory period. In the ventricle, the increase in the functional refractory period was produced with doses of 0.0240 mg./kg. The effect of the drug, in increasing the duration of the functional refractory period, consists in correcting a most important factor which, according to the circular movement theory, maintains atrial flutter and fibrillation. The effect of CD412, in increasing the functional refractory period of the atrial and ventricular muscles in myocarditis induced by *T. cruzi*, is similar to the effect reported by Méndez and Méndez (1953) in dogs with cardiac insufficiency produced by digitoxin and ouabain. In this connexion, we must note that of the 12 puppies with myocarditis in which the functional refractory period was studied, 11 showed global dilatation of all the cavities and clinical signs of heart failure, all showed dense and extensive inflammatory infiltrations, and only in one case (LCH1D1) were the inflammatory processes mild and the cardiac dilatation slight.

The atrial and ventricular intensity-duration curve showed significant modifications in myocarditis induced by *T. cruzi*, as much in the rheobase values and in the intensity of stimuli of short duration as in the distortions of the shape of the curve itself. Comparing the intensity-duration curves of normal dogs with those of dogs with electrocardiographic and histological changes compatible with Chagas' myocarditis, we found that in the latter the voltage and time required to elicit a response was less than in the former. These changes
show that the dogs with Chagas' myocarditis have an increased excitability in both atrial and ventricular tissues. This fact might be the cause of the arrhythmias so often found in this disease. The 7 puppies in which excitability was studied showed that there was a close connexion between the changes in the intensity-duration curves and the histological changes produced by the inflammation. Doses of 0.0147 mg./kg. of Peruvosid produced an increase in the values of the rheobase, and doses of 0.0647 mg. raised the threshold of short-duration stimuli, making the values of the intensity-duration curve approximate to the normal.

References


