Prolonged Administration of Hydrochlorothiazide on Cardiac Function in Beagle Dogs (37715)

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Clinical efficacy of thiazides in the management of hypertension and congestive heart disease has been established by several workers (1–4). Despite widespread and prolonged use of these compounds, most of the available information on the pharmacology of thiazides is based on acute and subacute studies (usually for a period of a few weeks), and few reports if any dealt with chronic effects of these compounds on the cardiovascular system. Previous studies on chronic effects of reserpine and hydrochlorothiazide from this laboratory revealed certain pharmacological properties and mechanisms, which were not evident in acute studies (5–7). The present report is part of an overall investigation on the effects of oral administration of hydrochlorothiazide to beagle dogs for 12 months and deals with the effects of this compound on cardiac function. In addition, an attempt is made to determine whether physiological effects observed following chronic administration of relatively low doses of hydrochlorothiazide for 12 months could be simulated or reproduced by administering large doses for relatively shorter time periods.

Methods. Twelve pure bred beagle dogs weighing between 8 and 12 kg were divided into two groups (treated and control) consisting of an equal number of both sexes. Capsules of hydrochlorothiazide (HCT) (10 mg/kg, po) or lactose (placebo) were administered daily for 12 months. During this period, the following tests were obtained in these dogs. (a) Measurement of plasma and blood volumes with indocyanine green (Cardio-Green) (8); 1.25 mg of indocyanine green was injected into a brachial vein, and blood samples were obtained from the contralateral brachial vein. The accuracy of this method was initially verified by using the ^{131}I serum albumin method (9). (b) Measurement of total body extracellular fluid by using $Na_2{}^{35}SO_4$ as described by Walser *et al.* (10). (c) Estimation of serum sodium, potassium, calcium, and magnesium; a Perkin–Elmer atomic absorption spectrophotometer (Model 303) was utilized to determine the serum concentration of these cations. This determination was also performed on left myocardial ventricular tissue obtained at the time of sacrifice.

At the end of the treatment period, 6 treated and 6 control dogs were anesthetized with sodium pentobarbital, 35 mg/kg iv, and prepared for the recording of blood pressure from a catheterized femoral artery using a Statham pressure transducer (P23AC). A Grass Tachograph (7P4D) was used to monitor heart rate. The trachea was intubated, and the animal was placed on intermittent positive pressure respiration by means of a Bird respirator (Mark 7). Thoracotomy was performed at the fourth intercostal space. The pericardium was incised to expose the heart, the ascending aorta was freed from surrounding tissue, and a 12-mm Statham electromagnetic flow probe was placed around the aorta to monitor cardiac output by means of a Statham electromagnetic flow meter (M-

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4001). A polyvinyl catheter was introduced into the left atrium via the apex to monitor left atrial pressure, utilizing a Statham venous pressure transducer (P23BC). All parameters were recorded on a Grass polygraph (Model 7). Stroke volume, stroke work, and total peripheral resistance were mathematically computed.[3]

In order to evaluate the ability of the myocardium to handle external volume loads (11–13), left ventricular function curves were obtained in the following manner. A large catheter (3/16 in. i. d.) was placed into the right atrium via the left jugular vein. This catheter was connected to an elevated reservoir (draining by siphon action) of Tyrode's solution kept at 37°. The animals were infused at rapid rate (10–12 ml/sec) with this solution until the cardiac output reached a peak level (usually in 60–90 sec). The mean left atrial pressure, heart rate, aortic blood pressure, and cardiac output were monitored throughout the infusion. The relationships between stroke work and left atrial pressure were plotted as described by Sarnoff and Berglund (14).

After completion of the above investigation, an acute study was designed to ascertain whether the cardiovascular alterations induced by chronic HCT administration could be observed with a ten times larger dose of HCT (100 mg/kg, po) administered daily for 2 days.

The data are expressed as a mean ± SEM. Significant differences between the two means were evaluated by using the Student's t test. In the present study, a p value of less than 0.05 was considered to indicate a significant difference between compared values.

Results. Body weight, blood and plasma volumes, hematocrit extracellular fluid volumes, and serum and myocardial tissue electrolytes were not significantly affected by chronic oral administration of hydrochlorothiazide (HCT) for a period of 1 year (Fig. 1, Tables I and II). Similarly, the mean aortic blood presure and heart rate obtained at the end of the treatment under pentobarbital anesthesia were not altered by HCT. However, in the HCT-treated dogs, there was a clear attenuation of stroke volume, stroke work, and cardiac output evidently accompanied by an elevation of total peripheral resistance (Fig. 2). These cardiovascular alterations were almost entirely reproduced by administering HCT in a larger dose (100 mg/kg, po) for a 2-day period (Fig. 4).

When the myocardium was subjected to volume loading, the elevation in left atrial pressure was accompanied by a marked increase in left ventricular stroke work. The relationship of stroke work to atrial pressure is referred to as left ventricular function curve. Chronic administration of HCT caused a significant diminution in the capacity of the myocardium to perform external work as indicated by the shift toward the right in the ventricular function curves (Fig. 3). This effect was independent of heart rate since there was no difference in cardiac rate responses to loading between the HCT- and placebo-treated dogs. The failure to observe marked positive chronotropic effect in response to loading in anesthetized animals has been previously described by several investigators (15, 16). Acute oral administration of HCT (100 mg/kg/day) for 2 days also resulted in a depression of the myocardial function (Fig. 5) similar to that observed following chronic HCT.

Discussion. Prolonged administration of relatively small doses of HCT for a period of 12 months failed to produce any significant changes in blood pressure and heart rate of beagle dogs, while the cardiac output, stroke volume, and stroke work were decreased significantly. An elevation of total peripheral

[3] Total Peripheral Resistance (dynes·sec/cm^5) =

$$\frac{\text{Mean Blood Pressure (mm Hg)} - \text{R.A.P. (mm Hg)} \times 1332}{\text{Cardiac Output (ml/sec)}}.$$

Stroke Volume (ml/beat) =

$$\frac{\text{Cardiac Output (ml/min)}}{\text{Heart Rate (beats/min)}}.$$

Stroke Work (gram·meter) =

$$\frac{\text{Stroke Volume (ml)} \times [\text{Mean Blood Pressure (mm Hg)} - \text{Left Atrial Pressure (mm Hg)}] \times 13.6}{1000}.$$

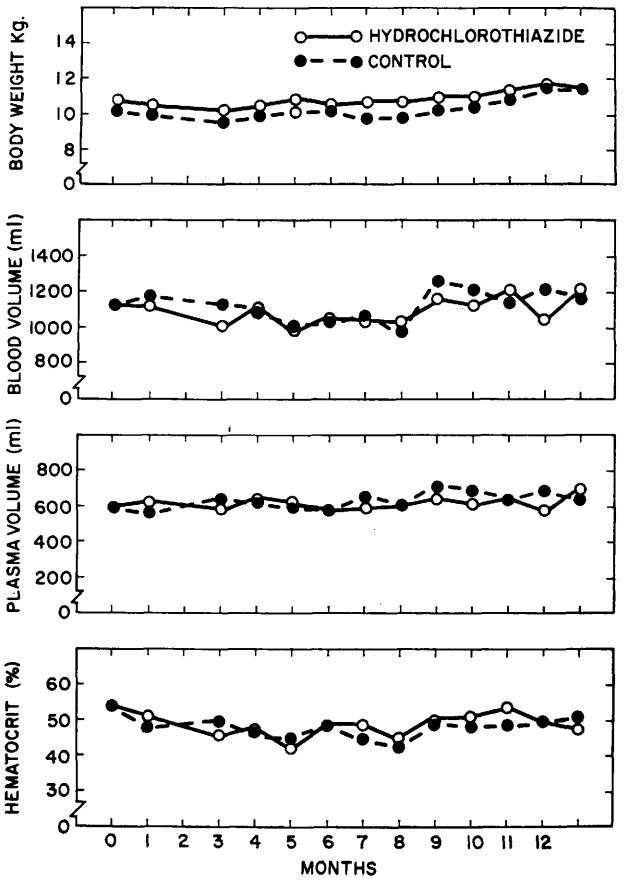

FIG. 1. Effects of chronic hydrochlorothiazide (10 mg/kg/day for 12 months) on body weight, blood volume, plasma volume, and hematocrit of beagle dogs.

resistance (TPR) appeared to maintain the arterial blood pressure. The decrease in cardiac output in the treated dogs cannot be explained on the basis of a decrease in blood volume or heart rate since no such changes occurred. This reduction was apparently due to a decrease in stroke volume which may have resulted from a decreased venous return and ventricular filling or due to an attenuation of contractility of the myocardium. Alterations in the contractility or pumping ability of the myocardium of the HCT-treated and control dogs can be compared if the heart was subjected to external load under similar conditions. In order to evaluate the effects of HCT on cardiac performance, ventricular function curves were obtained by rapidly infusing the Tyrode's solution and recording corresponding alterations in the cardiovascular system. Validity and the usefulness of the ventricular function curves has been demonstratetd by various investigators (14–17). Sarnoff and Berglund (14) pop-

TABLE I. Effects of HCT Administration (10 mg/kg, po, for 12 months) on Whole Body Extracellular Fluid Volume (ECF).

Time after treatment	Animal group	N	ECF mean ± SE (% body weight)
0	Treated	6	18.4 ± 1.2
	Control	6	18.2 ± 1.3
6 months	Treated	6	18.9 ± 1.3
	Control	6	18.1 ± 1.1
11 months	Treated	6	18.3 ± 1.6
	Control	6	18.4 ± 1.3
12 months	Treated	6	16.9 ± 1.2
	Control	6	16.2 ± 1.3

TABLE II. Effects of Chronic Hydrochlorothiazide Administration (10 mg/kg, po, for 12 months) on Serum and Heart Electrolytes of Beagle Dogs.

		Electrolytes ($\bar{X} \pm$ SE)			
	Group	Na^+	K^+	Mg^{2+}	Ca^{2+}
Heart (mEq/kg fat-free wet wt)	Treated ($N = 6$)	18.9 ± 1.8	68.4 ± 2.6	20.2 ± 0.6	0.9 ± 0.1
	Placebo ($N = 6$)	19.0 ± 1.0	64.1 ± 3.0	20.9 ± 0.5	0.9 ± 0.1
Serum (mEq/liter)	Treated ($N = 6$)	145.7 ± 1.83	4.31 ± 0.14	1.48 ± 0.04	5.86 ± 0.13
	Placebo ($N = 6$)	145.0 ± 1.50	4.82 ± 0.20	1.47 ± 0.06	5.73 ± 0.13

ularized the concept of a family of ventricular function curves for a given heart. From their plots of stroke work vs mean left atrial pressure, they could evaluate alterations in contractility. They further demonstrated that "in any given circulatory state, there is a consistent relationship between atrial pressure and stroke work." Thus, the stroke work, which is a function of stroke volume and aortic pressure, is an index of the ability of the ventricle to eject blood against certain peripheral resistance at a given end diastolic fiber length. It was shown that administration of catecholamines or sympathetic nerve stimulation shifted the ventricular function curves to the left, indicating an enhancement of the

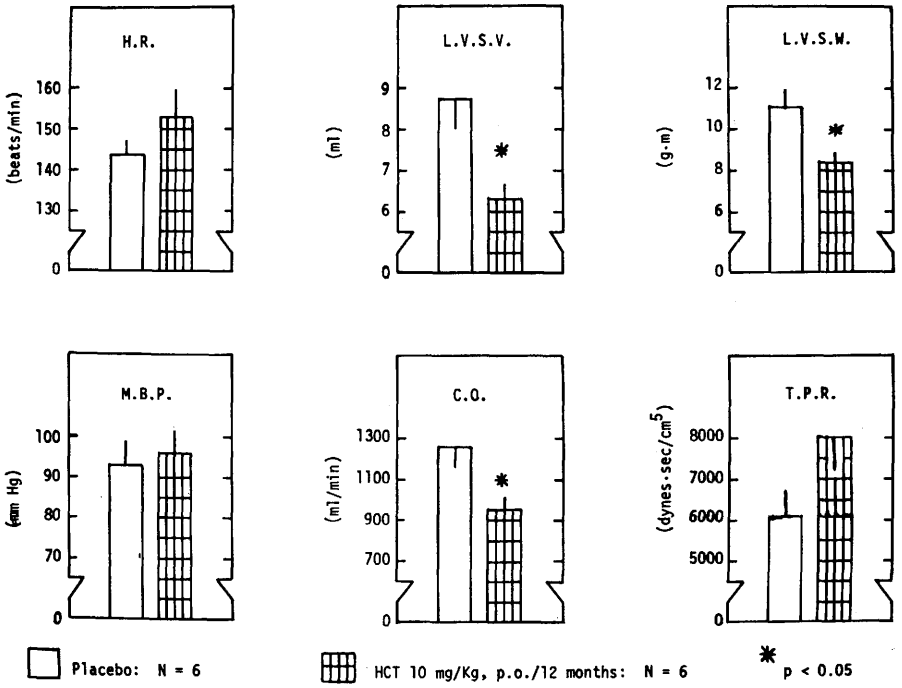

FIG. 2. Effects of chronic hydrochlorothiazide (10 mg/kg/day for 12 months) on heart rate (H. R.), left ventricular stroke volume (L. V. S. V.), left ventricular stroke work (L. V. S. W.), mean blood pressure (M. B. P.), cardiac output (C. O.), and total peripheral resistance (T. P. R.) of anesthetized beagle dogs.

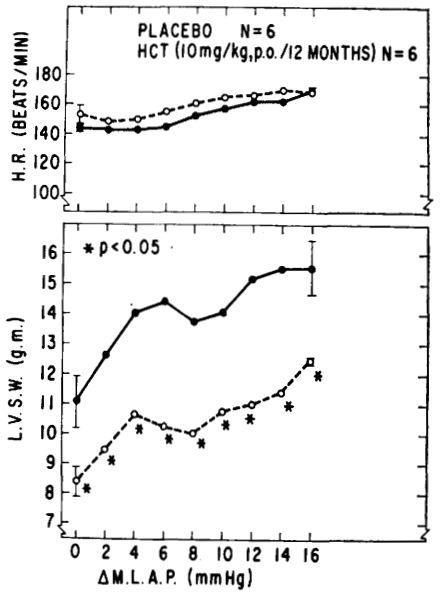

FIG. 3. Effects of chronic hydrochlorothiazide (10 mg/kg/day for 12 months) on ventricular function of anesthetized beagle dogs. Treated (O-O), placebo (●—●).

capacity of the myocardium to perform external work (14–18). However, different conditions such as heat (19), anoxia (19), ventricular distension (20), or halothane (21) displaced ventricular function curves to the right, suggesting diminution in contractility.

In the present study, chronic administration of HCT for 12 months shifted ventricular function curves to the right, indicating diminished contractility and capacity to handle external loads. During rapid infusion of the Tyrode's solution, stroke work of the treated dogs was consistently lower and significantly different from the values of untreated dogs at several left atrial pressure levels. Significant attenuation of contractility of the myocardium could be reproduced by administering 100 mg/kg of HCT for 2 days. These results lend strong support to the hypothesis that the significant decrease in stroke volume and cardiac output noted in the chronic and acute studies was at least in part due to

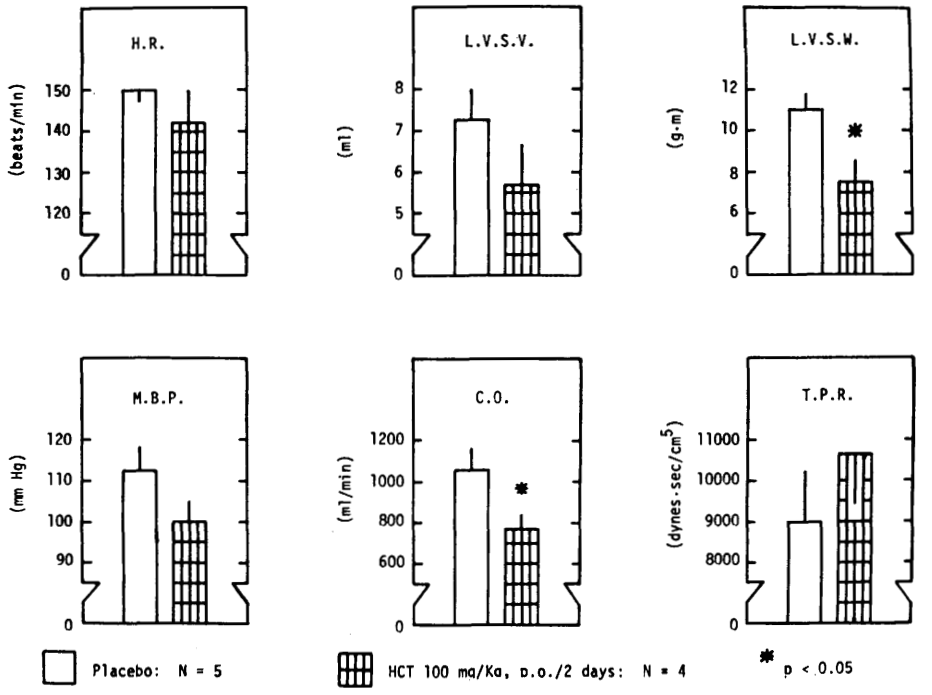

FIG. 4. Effects of acute hydrochlorothiazide (100 mg/kg/day for 2 days) on heart rate (H. R.), left ventricular stroke volume (L. V. S. V.), left ventricular stroke work (L. V. S. W.), mean blood pressure (M. B. P.), cardiac output (C. O.), and total peripheral resistance (T. P. R.) of anesthetized beagle dogs.

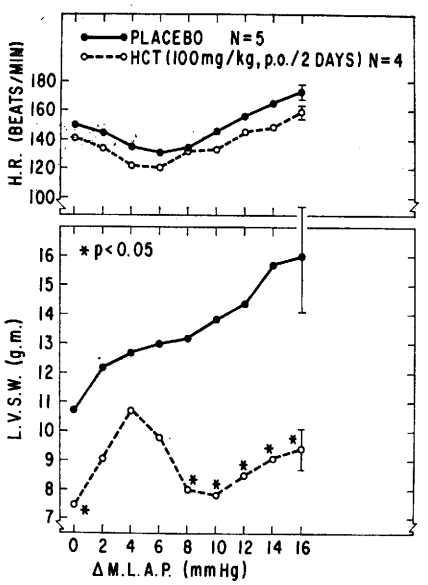

FIG. 5. Effects of acute hydrochlorothiazide (100 mg/kg/day for 2 days) on ventricular function of anesthetized beagle dogs. Treated (O-O), placebo (●—●).

diminished contractility of the myocardium.

In 1959, Barrett *et al.* (22) reported that small doses of HCT, then a newly discovered diuretic, possessed negative inotropic properties in isolated heart preparation. Later, Preziosi *et al.* observed similar effects with higher doses of chlorothiazide (23). Herbert and Buxton (24) warned against the "danger of sensitization" of the myocardium to the action of digitalis in obstetrical patients receiving concurrent treatment with HCT. Such toxicity was related to hypokalemia induced by HCT, to the presence of cardiac decompensation, and to postpartum diuresis. Daniel (25) administered HCT to DOCA hypertensive rats and found marked changes in the composition of the left ventricle, indicating possible alterations in left ventricular function and suggested that more attention should be paid to the action of HCT on myocardium. More recently, Naylor and coworkers (26) demonstrated that diazoxide, a nondiuretic thiazide, "displaced left ventricular function curves to the right in dogs indicating a diminution in the capacity of the left ventricle for doing external work."

Several other clinical as well as experimental investigations suggest depletion of total body potassium secondary to HCT might be responsible for certain toxic effects of HCT (27–29). However, in the present investigation, prolonged administration of HCT for 12 months did not produce any significant alterations in the serum and myocardial tissue content of Na^+, K^+, Ca^{2+}, and Mg^{2+} and did not significantly affect total extracellular fluid volume. Further, in another aspect of this study, 12-month treatment of HCT (10 mg/kg, po) did not affect peripheral adrenergic neuronal or receptor functions, indicating alteration in contractility was not in any way related to this mechanism (6). Thus, it appears that the depression of cardiac function to chronic administration of HCT was due to a direct effect of the compound on the myocardium via a mechanism yet to be identified. Similar alterations in cardic function could also be demonstrated when large doses of HCT were administered for shorter periods.

Conclusions. Chronic administration of hydrochlorothiazide (10 mg/kg, po for 12 months) to beagle dogs failed to produce any alterations in blood pressure and heart rate, despite a significant reduction in cardiac output, stroke volume, and stroke work. Analysis of ventricular function curves indicates a diminution in contractility of the myocardium in the treated animals. It is concluded from this investigation that the adverse effects of HCT on the myocardium in the face of increased total peripheral resistance noted in this study may warrant careful reevaluation of this agent in cardiovascular therapy.

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