Comparison of Degree of Susceptibility of Hyperthyroid and Euthyroid Animals to Cardiac Glycoside-Induced Arrhythmias

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Abstract □ The present studies were performed to determine the arrhythmogenic dose of ouabain in euthyroid and hyperthyroid guinea pigs and rabbits. The animals were made hyperthyroid by daily injection of thyroxine 100 mcg./kg. i.p. for 10–14 days. Cardiovascular responses, including heart rate from limb lead II electrocardiogram and arterial blood pressure, were monitored. By the method of analysis of variance, no significant difference was shown between the arrhythmogenic dose of ouabain in euthyroid and hyperthyroid guinea pigs. However, this difference was statistically significant in rabbits. An increase in extracellular serum calcium-ion concentration did not change the responsiveness of the myocardium to ouabain toxicity in either the euthyroid or the hyperthyroid animal. In addition, the β-adrenergic receptor blocking drug practolol (10 mg./kg.) was ineffective in completely abolishing the resting tachycardia in hyperthyroid rabbits. These studies do not support the idea of decreased responsiveness to cardiac glycosides due to the hyperthyroid state.

Keyphrases □ Ouabain-induced arrhythmias in hyperthyroid and euthyroid guinea pigs and rabbits—species differences, effect of extracellular calcium and practolol □ Cardiac glycoside-induced arrhythmias in hyperthyroid and euthyroid guinea pigs and rabbits—species differences, effect of extracellular calcium and practolol □ Arrhythmogenic response to ouabain—comparison between euthyroid and hyperthyroid guinea pigs and rabbits □ Hyperthyroid and euthyroid guinea pigs and rabbits—degree of susceptibility to ouabain-induced arrhythmias compared

There is now abundant evidence (1, 2) that the hyperthyroid state is usually associated with increased sensitivity of the myocardium to various drugs. In addition to sympathomimetic amines, these drugs include histamine, cocaine, endotoxin, monoamine oxidase (MAO) inhibitors, and barbiturates (3). Evidence also exists that thyroid hormones influence the excitability of the myocardial conducting system. For instance, feeding rabbits with thyroid extract made the rabbit hearts more susceptible to epinephrine arrhythmias, such as atrial fibrillation and ventricular tachycardia (4). Other studies (5–7) showed that hyperthyroidism is a common cause of atrial fibrillation and other supraventricular arrhythmias, whereas irregularities of cardiac rhythm are rare in myxedema.

However, the findings of other investigators do not support this view of increased sensitivity of the myocardium in hyperthyroid conditions to certain drugs. In experimentally induced hyperthyroidism in dogs, hyperthyroid animals were more resistant to the development of ouabain-induced ventricular arrhythmias than were hypothyroid and euthyroid dogs (8) and no significant difference was found, between the arrhythmogenic doses of ouabain in hypothyroid and euthyroid animals.

The difference in response to digitalis glycosides demonstrated in experimental animals (8, 9) and clinically (1, 10) has made the question of why the thyroid state modifies the effectiveness of digitalis glycoside action an increasingly important one from the standpoint of both therapy and the potential susceptibility of the myocardium to cardiac glycoside-induced arrhythmias. The action of thyroxine in sensitizing the myocardium to biogenic amines and other drugs has been described (3) as being nonspecific.

Although it has been implied (11, 12) that intact catecholamine stores are necessary for the increased sympathetic activity and sometimes the arrhythmias observed in hyperthyroid animals, it is still equivocal whether the increased sympathetic activity seen in conditions of hyperthyroidism plays a role in the arrhythmogenic toxicity of cardiac glycosides. If such arrhythmias were dependent upon increased levels of sympathetic activity brought about by the hyperthyroid state, then these arrhythmias would occur much more readily and at lower dose levels of the cardiac glycoside in the experimentally induced hyperthyroid state than in the euthyroid state. Furthermore, this would imply an increased rather than a decreased sensitivity to the cardiac glycoside as suggested by earlier investigators (8, 9). Since digitalis toxicity is associated with myocardial potassium loss, it is also postulated (13–15) that ionic imbalance such as the acute loss of potassium from myocardial cells caused by rapid digitalization or an increase in calcium plasma levels will cause the precipitation of cardiac arrhythmias in hyperthyroid states sooner than in euthyroid conditions.

The aims of this study were: (*a*) to elucidate further the influence of excessive thyroxine on ouabain-induced changes of spontaneous heart rate and arterial blood pressure in hyperthyroid and euthyroid guinea pigs and rabbits, and (*b*) to determine if there is a difference in the arrhythmogenic dose of ouabain under such conditions.

EXPERIMENTAL

Albino guinea pigs, weighing 200–300 g., and New Zealand White rabbits, weighing 2.5–3.5 kg., of either sex, fed on a standard diet[1] and water *ad libitum* were used in these experiments. A condition of hyperthyroidism was induced by daily injection of 100 mcg./kg. i.p. of sodium levothyroxine[2] for 10–14 days. Euthyroid animals were given a daily intraperitoneal injection of normal saline in amounts equivalent to the volume of the thyroxine dose employed. Prior to each experiment, each animal was anesthetized with sodium pentobarbital[3]; the guinea pigs received 25 mg./kg. i.p. and the rabbits received an identical dose intravenously. Supplemental doses of 0.2–0.5 ml. were given as needed.

The trachea of each animal was cannulated to ensure uninterrupted respiration. A jugular vein was exposed and cannulated for

[1] Purina.
[2] Synthroid, 100 mg./ml., Flint Laboratories.
[3] Nembutal, 50 mg./ml., Abbott Laboratories.

Table I—Comparison of Arrhythmogenic Dose of Ouabain in Euthyroid and Hyperthyroid Animals (Mean ± *SE*)

Type of Drug Treatment	Animal Species	Number of Animals	Total Cumulative Ouabain Dose Inducing Arrhythmias, mcg./kg.	
			Euthyroid State	Hyperthyroid State
Saline-treated animals	Guinea pigs	8	87.0 ± 5.4	90.0 ± 8.6 $p > 0.9$
	Rabbits	6	110.0 ± 5.6	87.5 ± 4.5 $p < 0.025$
Reserpinized animals	Guinea pigs	6	150.0 ± 9.1	178.0 ± 16.4 $p > 0.9$
	Rabbits	8	130.6 ± 10.9	72.5 ± 3.9 $p < 0.0025$
Continuous calcium chloride infusion	Guinea pigs	9	82.7 ± 6.8	82.7 ± 4.6 $p = 1.0$
	Rabbits	6	59.2 ± 4.1	65.0 ± 3.7 $p > 0.8$

intravenous administration of drugs, and a cannulated common carotid artery was connected by means of a polyethylene catheter to a pressure transducer[4] for monitoring arterial blood pressure. Heparinized saline (sodium heparin, 10 mg./ml.) was used routinely for flushing both arterial and venous catheters. Heart rate was measured from an electrocardiogram (ECG) obtained with limb lead II needle electrodes inserted under the skin. ECG changes as well as arterial blood pressure were recorded on an ink-writing oscillograph[5]. In those experiments in which a 2 m*M* concentration of calcium chloride was administered, the solution was infused by means of an infusion pump[6] at the rate of 5.5 ml./hr. After the surgical procedure, 15–20 min. was allowed for stabilization before baseline heart rate and blood pressure were measured. Control readings were taken prior to any drug administration.

The following drugs were used: sodium levothyroxine (100 mg./ml.), sodium pentobarbital (50 mg./ml.), reserpine (25 mg./ml.), ouabain (0.5 g./ml.), calcium chloride (2 m*M* solution), and practolol (10 mg./ml.).

The values obtained from these experiments were expressed as the mean ± *SE* of six or more observations. Statistical analyses of the data were performed using conventional formulas; differences between means were tested with the Student *t* test and analysis of variance described in Goldstein (16).

Control Experiments with Hyperthyroid and Euthyroid Animals—The guinea pigs and rabbits used in this study were divided into groups of euthyroids and hyperthyroids. These animals received daily injections of normal saline and thyroxine, respectively, for a total of 14 days. Heart rate, determined from limb lead II ECG's, and arterial blood pressure were monitored every 10 min.

Following stabilization of the monitored variables, a loading dose of 25 mcg./kg. i.v. of ouabain was administered followed by 15 mcg./kg. 15 min. later and a maintenance dose of 10 mcg./kg. given every 10 min. therafter until the first signs of rhythm disturbance occurred. The end-point was taken as the appearance of three or more premature ventricular contractions or extrasystoles. The total dose of ouabain was then noted, and the means of the values for the total number of animals in the series for both euthyroid and hyperthyroid animals were compared for significance.

Experiments with Reserpine-Treated Animals—Euthyroid and hyperthyroid guinea pigs and rabbits were pretreated with 0.5–1.0 mg./kg. i.p. of reserpine for 2 consecutive days prior to the day of challenge with ouabain.

Heart rate, arterial blood pressure, and ECG changes were monitored in all animals. The total amount of intravenously administered ouabain that evoked arrhythmia was noted for each animal.

Experiments Involving Calcium Infusion—Both hyperthyroid and euthyroid rabbits and guinea pigs received calcium chloride by infusion. Prior to the ouabain administration, a continuous infusion of a 2 m*M* solution of calcium chloride *via* the cannulated jugular vein was commenced at the rate of 5.5 ml./hr. Infusion was continued for 10–20 min. before the initial dose of ouabain. The calcium chloride infusion was continued until the first signs of cardiac irregularities appeared; it was then stopped and the total amount of ouabain evoking the arrhythmias was noted.

Experiments with β-Adrenergic Receptor Blocking Drugs—The β-adrenergic receptor blocking agent practolol[7], reported (17) to have a selective cardiac blocking action, was used to determine the effect of adrenergic blockade on the increased sympathetic activity in hyperthyroid rabbits. Anesthetized animals breathing room air spontaneously were used. Doses of 1, 2, 5, and 10 mg./kg. i.v. of the blocking drug were administered on a cumulative basis at 10-min. intervals. Control readings were taken in all instances prior to practolol administration.

[4] Narco-Biosystems.
[5] Narco-Biosystems physiograph model 4-A.
[6] Sage model 240.
[7] AY-21011.

RESULTS

Control Experiments with Euthyroid and Hyperthyroid Animals—*Guinea Pigs*—The apparent difference in the arrhythmogenic dose of ouabain in the euthyroid and hyperthyroid states was not statistically significant (Table I). In eight animals, prior to ouabain administration the mean heart rate response was found to be 248 ± 7 beats/min. in the euthyroid state as compared to 318 ± 12 beats/min. in the hyperthyroid state (Table II). However, serial administration of ouabain intravenously produced a progressive increase in spontaneous heart rate. The arterial blood pressure responses in the resting state and during ouabain administration in the guinea pigs (Table III) were higher in hyperthyroid than in euthyroid states. Injection of ouabain caused a progressive rise in both systolic and diastolic pressures in the hyperthyroid state, while euthyroid animals demonstrated a nonuniform response.

Rabbits—With rabbits not pretreated with either reserpine or calcium, the average resting heart rate values for six animals prior to ouabain injection were 372 ± 19 beats/min. in hyperthyroid rabbits and 297 ± 8 beats/min. in euthyroid rabbits. This difference in spontaneous heart rate values between hyperthyroid and euthyroid states was statistically significant ($p < 0.01$). Unlike guinea pigs, spontaneous heart rate in euthyroid and hyperthyroid rabbits did not increase following ouabain administration.

The average total dose of ouabain evoking arrhythmias (Table I) in these two groups of rabbits was significantly different ($p < 0.025$) in both euthyroid and hyperthyroid rabbits. Arterial blood pressure values at the start of the experiment were 230 ± 8/206 ± 6 mm. Hg in hyperthyroid and 193 ± 9/173 ± 11 mm. Hg in euthyroid rabbits. Ouabain administration resulted in a slight but progressive increase in the systolic pressure of euthyroid animals. In hyperthyroid rabbits, both systolic and diastolic pressures decreased with time (Table III).

Reserpine-Treated Animals—*Guinea Pigs*—Reserpinization of guinea pigs for 2 days caused a reduction in the resting spontaneous heart rate in both euthyroid and hyperthyroid animals. These values were 204 ± 9 beats/min. and 227 ± 13 beats/min., respectively. There was an observed difference in the average values of the resting spontaneous heart rate of nonreserpinized and reserpinized euthyroid guinea pigs. Heart rate was higher in the nonreserpine-pretreated animals (248 ± 7 beats/min.) than in the pretreated group of animals (204 ± 9 beats/min.) (Table II). Similarly, the resting spontaneous heart rate value was higher in nonreserpinized hyperthyroid guinea pigs (318 ± 12 beats/min.) than in the reserpinized hyperthyroid group (227 ± 13 beats/min.). The reduction in heart rate seen in hyperthyroid guinea pigs due to reserpine pretreatment was significantly different from that of the nonreserpinized hyperthyroid animals ($p < 0.01$ analysis of variance).

The initial arterial blood pressure of 121 ± 6/96 ± 6 mm. Hg in euthyroid reserpinized guinea pigs was low compared with 151 ± 14/119 ± 19 mm. Hg pressure in the nonreserpinized animals. In hyperthyroid guinea pigs, the increased diastolic blood pressure response to ouabain injection was much more gradual than the systolic component (Table II). Although there was an apparent difference in the total dose of ouabain evoking arrhythmias in euthyroid and hyperthyroid guinea pigs, this difference was not statistically significant (Table I).

Table II—Spontaneous Heart Rate in Guinea Pigs and Rabbits following Ouabain Administration[a]

Animal Treatment	Experimental Condition	Heart Rate, beats/min.[b] 0 min.	10 min.	30 min.	50 min.	70 min.	90 min.
Control guinea pigs	Euthyroid	248 ± 7 (8)	246 ± 6 (8)	264 ± 4 (7)	282 ± 9 (4)	303 ± 9 (3)	
	Hyperthyroid	318 ± 12[c] (8)	314 ± 11[c] (8)	324 ± 6[c] (7)	332 ± 9[c] (4)	333 ± 17 (3)	
Reserpinized guinea pigs	Euthyroid	204 ± 9 (6)	202 ± 9 (6)	210 ± 9 (6)	237 ± 10 (6)	248 ± 16 (4)	256 ± 16 (3)
	Hyperthyroid	227 ± 13 (6)	215 ± 11 (6)	221 ± 9 (6)	245 ± 11 (6)	242 ± 21 (5)	—
Calcium-infused guinea pigs	Euthyroid	242 ± 5 (9)	259 ± 5 (9)	271 ± 9 (8)	278 ± 5 (6)	262 ± 10 (4)	-
	Hyperthyroid	329 ± 14[c] (9)	324 ± 13[c] (9)	329 ± 9[c] (9)	339 ± 5[c] (9)	330 ± 6[c] (3)	-
Control rabbits	Euthyroid	297 ± 8 (6)	297 ± 7 (6)	285 ± 7 (6)	267 ± 8 (6)	260 ± 8 (6)	253 ± 9 (4)
	Hyperthyroid	372 ± 19[c] (6)	372 ± 16[c] (6)	358 ± 26[c] (6)	322 ± 36 (6)	332 ± 30[d] (5)	308 ± 22 (3)
Reserpinized rabbits	Euthyroid	231 ± 9 (7)	217 ± 9 (7)	231 ± 11 (7)	239 ± 12 (7)	242 ± 21 (4)	250 ± 25 (3)
	Hyperthyroid	280 ± 31[d] (8)	276 ± 29[d] (8)	254 ± 24[d] (8)	230 ± 14[d] (8)	208 ± 31[d] (5)	215 ± 1[d] (2)
Calcium-infused rabbits	Euthyroid	283 ± 8 (6)	283 ± 12 (6)	260 ± 10 (6)	278 ± 20 (5)	300 ± 1 (1)	300 ± 1 (1)
	Hyperthyroid	342 ± 26 (6)	326 ± 23 (6)	292 ± 25 (6)	292 ± 22 (5)	320 ± 26 (3)	—

[a] Numbers in parentheses refer to number of animals tested in each group. [b] Heart rate values (mean ± *SE*). [c] p significant at 0.01% level. [d] p significant at 0.05% level.

Rabbits—Pretreatment of rabbits with reserpine also caused a reduction in the resting spontaneous heart rate in both euthyroid and hyperthyroid animals. The heart rate values were 231 ± 9 beats/min. and 280 ± 31 beats/min., respectively (Table II). Serial administration of ouabain did not change these values substantially; however, only a slight progressive increase in spontaneous heart rate occurred with time in the reserpinized hyperthyroid rabbits. The difference in the arrhythmogenic dose of ouabain between the reserpinized euthyroid rabbits and the reserpinized hyperthyroid rabbits was highly significant (Table I).

In reserpinized euthyroid rabbits, both systolic and diastolic arterial pressures declined progressively with time following ouabain administration (Table IV). However, reserpinized hyperthyroid rabbits showed an apparently consistent level of both systolic and diastolic arterial pressures.

Calcium Chloride Infusion in Animals—*Guinea Pigs*—The infusion of calcium chloride solution into euthyroid and hyperthyroid guinea pigs did not seem to affect either the spontaneous heart rate or the arrhythmogenic dose of ouabain. A comparison of the data of noncalcium-treated and calcium-treated euthyroid guinea pigs shows that there was no significant difference in the resting heart rate ($p > 0.1$). The spontaneous heart rate values were 248 ± 7 beats/min. and 242 ± 5 beats/min. in the untreated and treated animals, respectively. There was no appreciable difference in the time of onset of arrhythmia, and the arrhythmogenic doses of ouabain were numerically identical.

In both the euthyroid and hyperthyroid states, diastolic blood pressure responses were severely suppressed. This effect was more pronounced in the hyperthyroid state. The levels of arterial pressure of both calcium-infused euthyroid and hyperthyroid guinea pigs 90 min. following ouabain injection were 135 ± 10/92 ± 7 mm. Hg and 123 ± 8/75 ± 1 mm. Hg, respectively (Table II).

Rabbits—Infusion of calcium chloride in euthyroid and hyperthyroid rabbits did not appreciably increase the spontaneous heart rate response over 90 min. following ouabain injection. However, a comparison of these data with those of noncalcium-treated euthyroid and hyperthyroid rabbits (Table II) indicates only a slight increase in spontaneous heart rate due to ouabain injection. The heart rates were 318 ± 12 beats/min. in noncalcium-treated hyperthyroid compared to 342 ± 26 beats/min. in calcium-treated hyperthyroid animals ($p > 0.1$).

Calcium chloride (2 m*M* solution) infusion shortened the time required for the onset of ouabain-induced arrhythmias in euthyroid rabbits. Ventricular extrasystoles appeared early following repeated injections of ouabain in four out of six euthyroid rabbits tested. These extrasystoles occurred 20 min. later in five out of six hyperthyroid rabbits.

In both calcium-treated euthyroid and calcium-treated hyperthyroid animals, systolic blood pressure remained nearly constant whereas diastolic pressure showed a progressive decline.

Effects of β-Adrenergic Receptor Blockade in Hyperthyroid Rabbits—The resulting changes in heart rate and arterial blood pressure caused by administration of cumulative doses of practolol, a β-adrenergic receptor blocking agent, are depicted graphically in Fig. 1. Practolol produced a progressive reduction of spontaneous heart rate. However, the resting tachycardia resulting from the hyperthyroid state was decreased, but not abolished, by β-adrenergic blockade. The spontaneous heart rate prior to administration of 1 mg./kg. practolol was 363 ± 28 beats/min., and it was 299 ± 29 beats/min. after a total cumulative dose of 10 mg./kg. This reduction in heart rate was not statistically significant ($p > 0.05$).

DISCUSSION

These studies indicate that there was a varying degree of sensitivity to the arrhythmogenic dose of ouabain between the euthyroid group and hyperthyroid group of animals tested. In rabbits, the arrhythmia-evoking doses of ouabain were found to be significantly different between euthyroid and hyperthyroid groups of animals not premedicated with either reserpine or calcium as well as the reserpine-pretreated animals. However, in both calcium-treated euthyroid and hyperthyroid rabbits, this dose of ouabain was identical (Table I). Therefore, these findings suggest that the level of sensitivity to ouabain toxicity had not been altered in the guinea pig by the experimentally induced hyperthyroid state but was significantly modified in rabbits. It further suggests that experimental hyperthyroidism may modify the tolerance level to cardiac glycosides in one species of animal without significant effects in others. This modification usually results in an increase in the amount of glycoside required to attain the arrhythmia end-point. The present findings are to the contrary.

Although observations in laboratory animals suggested a relationship between many of the manifestations of thyroxine-induced hyperthyroidism and the activity of the sympathetic nervous system, the existence of such a relationship is still equivocal. The data from this study do not wholly support the assumption that intact catecholamine stores are necessary for some of the toxic effects of cardiac glycosides on the heart in hyperthyroid states. The mean arrhythmogenic dose of ouabain was slightly higher in the reserpinized guinea

Table III—Arterial Blood Pressure Responses in Guinea Pigs following Ouabain Administration[a]

		Arterial Blood Pressure, mm. Hg[b]					
		0 min. S/D[c]	10 min. S/D	30 min. S/D	50 min. S/D	70 min. S/D	90 min. S/D
Control guinea pigs	E[d]	151 ± 14/119 ± 19 (8)	155 ± 18/124 ± 20 (8)	162 ± 19/127 ± 22 (8)	134 ± 17/95 ± 23 (4)	133 ± 11/90 ± 28 (3)	144 ± 24/82 ± 17 (2)
	H[e]	164 ± 17[f]/132 ± 18[g] (8)	171 ± 17[f]/136 ± 18[g] (8)	179 ± 19[f]/142 ± 20[g] (8)	176 ± 17[g]/141 ± 19[g] (8)	178 ± 30/154 ± 35 (4)	210 ± 1/200 ± 1 (1)
Reserpinized guinea pigs	E	121 ± 6/96 ± 6 (6)	127 ± 8/106 ± 8 (6)	146 ± 8/123 ± 7 (6)	143 ± 7/122 ± 7 (6)	146 ± 13/128 ± 9 (4)	139 ± 18/123 ± 14 (3)
	H	79 ± 20[f]/55 ± 22[g] (6)	88 ± 17[f]/58 ± 21[g] (6)	93 ± 19[f]/62 ± 20[g] (6)	103 ± 19[g]/67 ± 20[g] (6)	108 ± 35/85 ± 35 (4)	93 ± 23/50 ± 15 (2)
Calcium-treated guinea pigs	E	108 ± 12/85 ± 12 (9)	126 ± 10/87 ± 16 (9)	140 ± 18/83 ± 4 (8)	144 ± 24/103 ± 15 (6)	122 ± 5/84 ± 4 (4)	135 ± 10/92 ± 7 (2)
	H	129 ± 4/75 ± 2 (9)	134 ± 7/79 ± 4 (9)	143 ± 6/83 ± 4 (9)	136 ± 6/78 ± 4 (9)	125 ± 14/75 ± 13 (3)	123 ± 8/75 ± 10 (2)

[a] Numbers in parentheses refer to number of animals per group. [b] Arterial blood pressure responses (mean ±*SE*). [c] Systolic over diastolic arterial pressures. [d] Euthyroid animals. [e] Hyperthyroid animals. [f] *p* significant at 0.01% level. [g] *p* significant at 0.05% level.

Table IV—Arterial Blood Pressure Responses in Rabbits following Ouabain Administration[a]

		Arterial Blood Pressure, mm. Hg[b]					
		0 min. S/D[c]	10 min. S/D	30 min. S/D	50 min. S/D	70 min. S/D	90 min. S/D
Control rabbits	E[d]	193 ± 9[e]/173 ± 11[e] (6)	195 ± 9[e]/172 ± 11[e] (6)	195 ± 9[e]/175 ± 10[e] (6)	199 ± 11/170 ± 14[e] (6)	207 ± 10[g]/175 ± 9[g] (6)	200 ± 14[g]/166 ± 12[g] (4)
	H[f]	230 ± 8[g]/206 ± 6[g] (6)	227 ± 7[g]/197 ± 5[g] (6)	234 ± 7[g]/207 ± 7[g] (6)	195 ± 20[g]/167 ± 17[g] (6)	218 ± 13[g]/181 ± 3[g] (6)	207 ± 7[g]/177 ± 9[g] (4)
Reserpinized rabbits	E	130 ± 26[e]/93 ± 25[e] (6)	128 ± 24[e]/100 ± 24[e] (6)	128 ± 24[e]/97 ± 26[e] (6)	136 ± 29/85 ± 30[e] (6)	73 ± 23[e]/36 ± 29[e] (3)	70 ± 10[g]/25 ± 15[g] (2)
	H	128 ± 7[g]/100 ± 4[g] (6)	123 ± 2[g]/81 ± 20[g] (6)	121 ± 4[g]/90 ± 7[g] (6)	93 ± 20[g]/78 ± 9 (6)[g]	119 ± 7[g]/88 ± 11 (3)[g]	113 ± 13[g]/44 ± 21 (2)[g]
Calcium-treated rabbits	E	170 ± 22/149 ± 20 (5)	170 ± 21/151 ± 20 (5)	174 ± 22/149 ± 21 (5)	159 ± 15/131 ± 14 (5)	193 ± 28/165 ± 40 (2)	—
	H	170 ± 10/143 ± 9 (5)	171 ± 10/141 ± 9 (5)	168 ± 8/139 ± 8 (5)	165 ± 23/135 ± 21 (4)	175 ± 36/143 ± 29 (4)	163 ± 8/124 ± 1 (2)

[a] Numbers in parentheses refer to number of animals per group. [b] Arterial blood pressure responses (mean ± *SE*). [c] Systolic over diastolic arterial pressures. [d] Euthyroid animals. [e] *p* significant at 0.05% level. [f] Hyperthyroid animals. [g] *p* significant at 0.01% level.

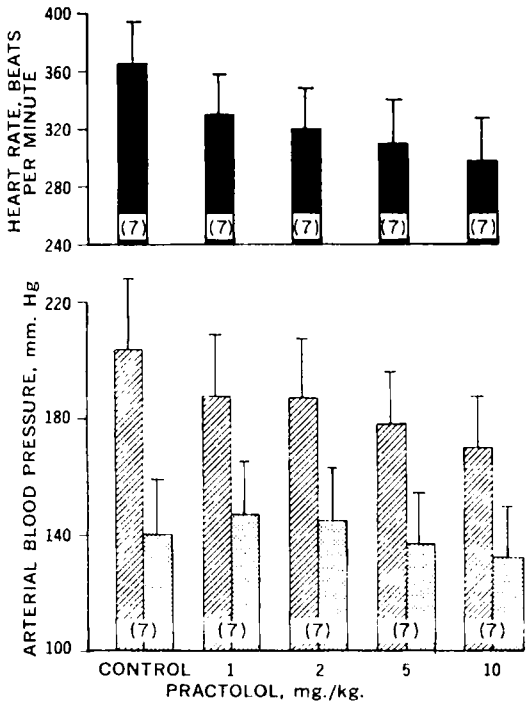

Figure 1 —*(Top) Effects of different doses of practolol (a β-adrenergic receptor blocking agent) on spontaneous heart rate in hyperthyroid rabbits. Number of observations* (n) = 7. *(Bottom) Corresponding arterial blood pressure response to the doses of practolol* (n = 7). *Key:* ▨, *systolic pressure of hyperthyroid rabbits;* ▩, *diastolic pressure of hyperthyroid rabbits; and* I, SEM.

pigs in the hyperthyroid state than in the reserpinized euthyroid condition. This suggests a change in responsiveness due partly to a depletion of catecholamine stores and partly to a predominance of parasympathetic activity produced by reserpine. This view is in agreement with that expressed by Cairoli and Crout (18), who found that the rate-normalizing effect of reserpine as seen by induced bradycardia in both euthyroid and thyroxine-treated unanesthetized rats could be blocked with atropine.

In rabbits, the arrhythmogenic dose of ouabain was significantly lower in the reserpinized hyperthyroid animals than in the reserpinized euthyroid animals. This observation, however, does not support those of Roberts *et al.* (19), who reported that reserpine pretreatment increased the amount of acetylstrophanthidin required to induce ventricular arrhythmias in the dog. This difference in response to ouabain in both reserpinized rabbits and guinea pigs in the hyperthyroid state still leaves an equivocal answer to the question of the role of sympathetic activity in cardiac glycoside toxicity in experimental hyperthyroidism. The present data imply that intact catecholamine stores are essential as mediators of the toxic manifestation of glycosides in hyperthyroidism in certain animal species but not in others.

Although it is known that cardiac glycosides can cause a loss of potassium ions from myocardial cells, thereby rendering the heart sensitive to increased concentrations of serum calcium, the present study shows that an increase of extracellular calcium ions, induced by an infusion of a 2 m*M* solution of calcium chloride, did not seem to make a difference in the degree of sensitivity of the heart to the arrhythmogenic dose of ouabain in either euthyroid or hyperthyroid animals.

The β-adrenergic receptor blocking agent practolol, which is known to possess a selective blocking action on the heart, failed to abolish the resting tachycardia of hyperthyroidism. This observation is explained partly on the basis of the fact that the many manifestations of hyperthyroidism are not mediated entirely by increased sympathetic activity and partly because practolol lacks a depressant activity on the myocardium. As a β-blocker, it is less potent than its congener propranolol, which had been used in the treatment of tachycardia in thyrotoxic patients (20–22). The reduction of the resting tachycardia from control values by different doses of practolol was not statistically significant at all dose levels ($p > 0.05$). These observations suggest either that the blockade was incomplete or that nonadrenergic factors continue to sustain cardiac hyperactivity in the hyperthyroid state.

SUMMARY AND CONCLUSIONS

The present investigation permitted the further elucidation of the relationship of the thyroid state and increased sympathetic activity and their contribution to any altered sensitivity of the myocardium to cardiac glycosides. Of particular interest was the finding that an increase in extracellular serum calcium ions did not change the responsiveness of the myocardium to ouabain toxicity in either euthyroid or hyperthyroid animals.

Furthermore, euthyroid and hyperthyroid guinea pigs responded differently from rabbits in certain drug treatments. This observation may partly explain the contrasting findings of Goodkind *et al.* (23), who found that myocardial norepinephrine concentration in guinea pig ventricles increased in hyperthyroidism, and those of Kurland *et al.* (24), who showed that the norepinephrine concentration in rabbit ventricles was decreased in hyperthyroidism and increased in hypothyroidism.

The β-adrenergic receptor blocking drug practolol was ineffective in normalizing resting tachycardia in hyperthyroid rabbits at the dose levels used in these studies. Although these studies have pointed out definite differences in some cardiovascular responses in the euthyroid and hyperthyroid states, the findings with regard to the arrhythmia threshold in euthyroid and hyperthyroid animals, in reserpinized animals as well as in calcium-treated animals, only help to confirm further the observations of earlier investigators and to point out that the factor or factors responsible for this change in myocardial response to cardiac glycosides in hyperthyroidism are still unknown.

The answers to these questions will most likely be found in the elucidation of other mechanisms at the molecular level since the direct effects of excessive blood level of thyroxine on myocardial cells are still obscure.

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Coumarins XII: Synthesis of (±)-*cis*- and *trans*-3′,4′-Dihydroxy-3′,4′-dihydroxanthyletin and Their Diesters

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Abstract □ (±)-*cis*-3′,4′-Dihydroxy-3′,4′-dihydroxanthyletin was synthesized from xanthyletin by the use of osmium tetroxide. It was also obtained, unexpectedly, by acid or base hydrolysis of (±)-*trans*-3′-acetoxy-4′-(*m*-chlorobenzoyloxy)-3′,4′-dihydroxanthyletin and, spontaneously, by recrystallization of the product from epoxidation of xanthyletin with trifluoroperacetic acid. (±)-*trans*-3′,4′-Dihydroxy-3′,4′-dihydroxanthyletin was obtained together with the (±)-*trans*-3′-hydroxy-4′-acetoxy derivative by peracetic acid treatment of xanthyletin. The diacetates, dipropionates, and di-*n*-butyrates of both the *cis*- and *trans*-diols were prepared together with the *trans*-3′-propionoxy-4′-acetoxy- and 3′-*n*-butyroxy-4′-acetoxy-3′,4′-dihydroxanthyletins. NMR studies of the diols and the diesters were carried out to establish chemical shifts of specific groups, with special emphasis on the 2′-gem-dimethyls together with the *J* values for the 3′- and 4′-protons as diagnostic features for the *cis*- or *trans*-configuration.

Keyphrases □ Xanthyletin derivatives—synthesis and structure determination of (±)-*cis*- and *trans*-3′,4′-dihydroxy-3′,4′-dihydroxanthyletin and diesters □ Dihydroxy-3′,4′-dihydroxanthyletin, (±)-*cis* and *trans*, and diesters—synthesis and structure determination □ Coumarin derivatives—synthesis and structure determination of (±)-*cis*- and *trans*-3′,4′-dihydroxy-3′,4′-dihydroxanthyletin and diesters

Naturally occurring pyranocoumarins, also known as chromeno-α-pyrones, have been known since the initial isolation of xanthoxyletin (I*b*) by Staples (1) in 1829, although its structure was not established until 1936 (2). Subsequent isolations and structural studies revealed three different basic ring fusions which may be said to be derived from xanthyletin (I*a*), alloxanthyletin (II*a*), and seselin (III*a*). The xanthyletin type is comprised of xanthyletin (I*a*), xanthoxyletin (I*b*), and luvangetin (I*c*). In the alloxanthyletin series, the known compounds are alloxanthoxyletin (II*b*), calophyllolide (II*c*), and inophyllolide (II*d*). Only two representatives of the seselin type are presently known: seselin (III*a*) and braylin (III*b*).

It was not until 1957 that the now well-known 3′,4′-diesters derived from 3′,4′-dihydroseselin were isolated and structurally characterized by Bencze *et al.* (3) when they described samidin (IV*a*) and visnadin (IV*b*). Their work was quickly followed by that of Smith *et al.* (4) who, in addition, characterized dihydrosamidin (IV*c*). Since then, a number of these diesters have been isolated and structurally elucidated (*e.g.*, 5–7) and their widespread presence in umbelliferous plants has been established. In addition, following the isolation and structural elucidation of lomatin (3′-hydroxy-3′,4′-dihydroseselin) (IV*d*) (8), esters of this coumarinic alcohol were reported (9, 10).

With respect to the corresponding esters related to the linear dihydro-I*a*, the first discoveries were the 3′-monoesters, namely, decursin (V*a*) (11) and the corresponding angelate (V*b*) (12). The present work was initiated in the hope that, eventually, the 3′,4′-diesters derived from 3′,4′-dihydroxy-3′,4′-dihydroxanthyletin would be isolated and that knowledge gained from the synthesis of known derivatives would be useful for characterization of such compounds. Indeed, during these studies the first such compound, xanthalin (V*c*), was reported from *Xanthogalum purpurascens* Lall. by Sokolova *et al.* (13) and provided an excellent corroboration of other findings (14). Since then Zheleva and coworkers (15–17) assigned similar structures to the coumarins of *Peucedanum arenarium*, and configurational assignments (17) have relied on the present work.

DISCUSSION

Xanthyletin (I*a*) was utilized as the starting point in the present work. This material is readily obtainable in a relatively pure form through the petroleum ether extraction procedure of King *et al.* (18) from the heartwood of East Indian satinwood, *Chloroxylon swietenia*. Recrystallization from warm methanol according to the procedure of Lemmich *et al.* (12) provided a means of separating xanthyletin from the accompanying xanthoxyletin in a form sufficiently pure for synthetic work. The xanthyletin thus obtained, when subjected to TLC on silica gel, showed only traces of xanthoxyletin when examined in the dark under UV light, both being highly fluorescent. The identity of xanthyletin was established by direct