preciable production of (dialysable) hexosamine containing oligosaccharides. Intrinsic viscosity of the acid mucopolysaccharides produced varied from one case to another but kept rather constant from week to week.

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Relationship of Steroid and Pituitary Hormones to Myocardial Calcification in the Mouse.* (23948)

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Calcification of the myocardium can readily be induced in the mouse with hydrocortisone(1), and increases in blood calcium to toxic levels have been demonstrated in the dog following administration of the steroid (2). Furthermore, it is recognized that, in the human, hyperfunction of the adrenal glands is often accompanied by a negative calcium balance; if excessive production of corticoids is maintained for a prolonged period of time, as occurs in Cushing's syndrome, myocardial damage, calcific deposition, and osteoporosis may result(3). In an earlier study with intact C3H mice, it was established that the mineral deposit associated with the cardiac lesion is a calcium salt and that the degree of calcification is related to the dosage of hydrocortisone administered(1). The participation of other steroid and pituitary hormones in this degenerative process is described here.

Procedure. Female mice of the C3H strain were used throughout the study, with the exception of a few males that were included in order to ascertain whether or not endogenous androgen would afford some protection to the heart. The mice were injected subcutaneously once daily with the hormones for 15 days, beginning at 50 days of age. Hypophysectomies were performed at 35 days of age, ovariectomies and adrenalectomies at 42 days of age. The adrenalectomized animals were given free access to 1% sodium chloride water and to tap water. The animals were fed a modified McCollum's Diet I, *ad libitum*(4), except for those whose sodium or potassium intake was restricted. At autopsy, the heart was inspected for grossly visible calcific deposits and the intensity of the calcification was estimated on an arbitrary scale of 0 to 5. In addition, the weights of the adrenals, heart and right kidney were recorded. The hydrocortisone, corticosterone, desoxycorticosterone (DOCA), testosterone, and estradiol were administered as

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TABLE I. Effect of Hydrocortisone, Corticosterone or Desoxycorticosterone on Myocardial Calcification in Female C3H Mice.

*	Treatment†	Daily dose, mg	No. of mice	Body wt, g		Organ wt, mg			Calcification‡
				Onset	Autopsy	Adrenals	R. kidney	Heart	
N	None	.0	6	18.5	20.5	3.7 ± .1§	113 ± 4	89 ± 2	0
A	"	.0	4	17.5	19		102 ± 9	89 ± 9	0
H	"	.0	4	12.5	13	1.5 ± .1	61 ± 3	50 ± 2	0
N	Hydrocortisone	.3	5	20	20	2.7 ± .1	176 ± 8	133 ± 5	1½
A	"	.3	6	18	19		153 ±10	127 ± 4	½
H	"	.5	5	12	12	1.8 ± .2		53 ± 2	+ in 1 of 5
H	Corticosterone	2.0	5	12	15	1.7 ± .2	65 ± 3	47 ± 3	¼
A	Desoxycorticosterone	.3	6	17.5	22		131 ± 7	114 ± 5	0
A	Hydrocortisone + Desoxycorticosterone	.3 + .3	6	17.5	19		162 ± 4	121 ± 7	¼

* Operative condition: N = normal mice; A = mice adrenalectomized at 42 days; H = mice hypophysectomized at 35 days.

† Single daily subcut. inj. were begun at 50 days of age and continued for a period of 15 days, except in the case of corticosterone where they were continued for 28 days.

‡ This estimate represents extent of calcification observed grossly in the heart at autopsy. + in 1 of 5 indicates that a minute deposit was observed in one of the 5 experimental animals.

§ Mean ± stand. error.

saline suspensions.‡ The growth hormone was prepared from bovine pituitaries by the procedure previously described(5). The purified adrenocorticotropic hormone fraction (designated as ACTH-F, and having an activity of 40 I.U. per mg) was prepared from sheep pituitaries and the α-corticotropin (α-ACTH, with an activity of 100-150 I.U. per mg) was isolated by a further purification of this material(6); ACTH preparations were administered in 16% gelatin. The Dowex 50-4X resin was prepared by being decanted and recycled in 2 N HCl and 0.5 N KOH.

Results. Calcification of the myocardium was consistently induced in intact female mice at daily dose levels of hydrocortisone above 0.25 mg. The intraventricular septum was the preferential site for deposition although as the calcification became more extensive, the right ventricle was also affected; only rarely was the left ventricle involved. The participation of an adrenal hormone other than hydrocortisone was not a prerequisite for the calcific deposition, for removal of the adrenal glands prior to injection of hydrocortisone did not prevent the appearance of the myocardial lesions. Moreover, simultaneous administration of 0.50 mg of DOCA did not aggravate the condition (Table I).

In the absence of the pituitary gland, both hydrocortisone and ACTH were almost entirely ineffective in inducing calcification of the myocardium. In accord, however, with earlier observations that administration of growth hormone along with hydrocortisone or ACTH aggravates calcification in the intact mouse(1), it was demonstrated in this study that a similar enhancement is produced by growth hormone in the hypophysectomized animal (Table II). Contrariwise, lactogenic hormone given at a daily dose level of 0.10 mg along with ACTH gave results similar to those obtained with ACTH alone.

In the intact animal, hormones other than pituitary growth hormone, *e.g.*, the sex hormones, are probably operating to modify the cardiac calcification induced by hydrocortisone. The non-operated male was much less affected than the female; however, removal of the testes abolished this protective effect so that the myocardial condition in the castrated male was comparable to that in the intact female. In the female, the calcification was alleviated by administration of 0.50 mg of tes-

‡ Hydrocortisone acetate and corticosterone were obtained from Merck & Co.; testosterone, estradiol and desoxycorticosterone acetate were products of Schering Corp.

TABLE II. Effect of ACTH plus Growth Hormone or Lactogenic Hormone on Myocardial Calcification in Hypophysectomized Female C3H Mice.*

Treatment	Daily dose, mg	No. of mice	Body wt, g		Organ wt, mg			Calcification†
			Onset	Autopsy	Adrenals	R. kidney	Heart	
None	.0	4	12.5	13	1.5 ± .1‡	61 ± 3	50 ± 2	0
ACTH-F	.8	4	12	15	6.2 ± .2	99 ± 4	60 ± 2	+ in 4 of 4
α-cortocotropin	.1	5	13	15	6.5 ± .2	92 ± 2	52 ± 1	+ in 1 of 5
Growth hormone	.1	10	12.5	19	2.2 ± .2			0
Idem	1.0	7	13	19	2.8 ± .3	100 ± 3	78 ± 4	0
Lactogenic hormone	.1	5	11.5	12	1.5 ± .2	58 ± 5	52 ± 5	0
ACTH-F + Growth hormone	.8 + .1	5	13	16	10 ± .4	144 ± 5	81 ± 2	¾
Idem	.8 + 1.0	6	13	17	8.6 ± .5	160 ± 7	94 ± 2	½
ACTH-F + Lactogenic hormone	.8 + .1	6	12	15.5	8.1 ± .5	123 ± 5	68 ± 3	+ in 2 of 6

* All animals were hypophysectomized at 35 days of age. Single daily subcut. inj. were begun at 50 days of age and continued for a period of 15 days.

† This estimate represents the extent of calcification observed grossly in the heart at autopsy. + in 4 of 4 means that a minute deposit was observed in 4 of the 4 experimental animals, etc.

‡ Mean ± stand. error.

tosterone in conjunction with the hydrocortisone (Table III). On the other hand, administration of estradiol did not aggravate the condition, and ovariectomy was ineffective in preventing the deposition.

Inasmuch as an excess of hydrocortisone would be expected to promote sodium retention along with potassium excretion, and since calcified lesions of the myocardium and other tissues have been observed in animals maintained on various abnormal diets including those low in potassium (7,8,9), an attempt was made to ascertain whether the condition would be alleviated by increasing the potassium uptake of the animals or by decreasing their sodium intake. Four groups of mice were placed on special diets for the period of 15 days during which they received the hormone; however, the animals derived no benefit from any of the dietary modifications (Table IV). Those given a diet deficient in both sodium and potassium lost 40% of their original body weight and were in poor condition by the time of autopsy. The animals given free access to additional potassium in the form of 0.5% KCl H_2O or as the potassium form of the Dowex 50 resin exhibited no signs of toxicity; however, the cardiac calcification was nonetheless aggravated. Similar effects were noted with the sodium-deficient diet.

Hypertrophy of the kidney and of the heart was obtained with hydrocortisone in the intact, the ovariectomized and the adrenalectomized animals. Hypertrophy of the kidney was likewise observed in the hypophysectomized animals with ACTH as well as with growth hormone, the increases in the weight of the organ being additive when the two hormones were injected concurrently. Cardiac enlargement, however, did not parallel the kidney enlargement and an increase in the weight of the heart was observed only in the hypophysectomized animals treated with growth hormone, not in those treated with α-corticotropin, hydrocortisone or corticosterone. It can also be seen from Table II that the weights of the adrenal glands were considerably larger in the hypophysectomized mice injected with ACTH plus growth hormone than in those injected with ACTH alone. Thus, in this instance there is a synergism operative between the two hormones, in contrast to the simple additive effect on the kidney noted above.

TABLE III. Effect of Hydrocortisone plus Testosterone or Estradiol on Myocardial Calcification in C3H Mice.

Treatment*	Daily dose, mg	No. of mice and sex	Body wt, g		Organ wt, mg			Calcification†
			Onset	Autopsy	Adrenals	R. kidney	Heart	
Hydrocortisone	.3	5 ♂	23.5	24	1.7 ± .1‡	227 ± 14	126 ± 5	+ in 4 of 5
"	.3	5 ♀	20	20	2.7 ± .1	176 ± 8	133 ± 5	1½
"	.3	5 ♀ §	19	23	2.6 ± .2	179 ± 9	134 ± 6	+ in 5 of 5
"	.5	4 ♂	22.5	20	2.1 ± .1	209 ± 10	144 ± 6	¼
"	.5	5 ♂ §	20	16.5	2.2 ± .1	190 ± 9	132 ± 4	2½
"	.5	4 ♀ §	19	18	2.6 ± .3	195 ± 4	134 ± 7	2½
Hydrocortisone + Testosterone	.3 + .5	6 ♂	23.5	24.5	1.8 ± .1	238 ± 9	128 ± 4	+ in 5 of 6
Idem	.3 + .1	3 ♀	20	21	2.2 ± .2	151 ± 7	119 ± 2	1½
"	.3 + .5	5 ♀	20	18	2.1 ± .1	192 ± 11	114 ± 9	¼
"	.3 + .5	5 ♀ §	19	21.5	2.2 ± .2	243 ± 16	140 ± 5	+ in 3 of 5
Hydrocortisone + Estradiol	.3 + .002	8 ♀	20.5	20.5	4.3 ± .2	173 ± 2	117 ± 3	2
Idem	.5 + .01	4 ♀ §	19	21	3.9 ± .1	183 ± 7	141 ± 7	2

* Single daily subcut. inj. begun at 50 days of age and continued for 15 days.

† This estimate represents extent of calcification observed grossly in heart at autopsy. + in 4 of 5 indicates that a minute deposit was observed in 4 of 5 experimental animals, etc.

‡ Mean ± stand. error.

§ These animals were gonadectomized at 42 days of age, 8 days prior to first inj.

Discussion. Hydrocortisone administered for a period of 15 days consistently induced calcification of the myocardium in normal female mice. Neither experimental renal damage(10) nor unilateral nephrectomy(11) was resorted to in these studies. However, hypertrophy of the kidneys did result from the treatment and some derangement in kidney function undoubtedly was involved in the degenerative process. Similar results were evident in the hypophysectomized animals, in whom enlargement of the kidneys was observed following administration of either ACTH or growth hormone.

The anterior pituitary appears to influence the course of the myocardial calcification in other ways besides secreting the ACTH that stimulates the release of corticoids from the adrenal. Thus, the administration of growth hormone aggravates the cardiac condition in both the hypophysectomized and the intact mouse. On the other hand, the gonadotropins must contribute to some alleviation of the condition, inasmuch as the presence of the testes in the male as well as the concurrent administration of testosterone along with hydrocortisone to the female, afforded considerable protection to the heart. Selye has likewise found that calcification resulting from the administration of dihydrotachysterol (AT-10) is reduced by concurrent treatment with methyltestosterone(12). There was no suggestion that the ovarian hormones are involved in the process whereby calcific deposition is induced by the action of hydrocortisone; ovariectomy of the female did not prevent the occurrence of the deposits and the injection of estradiol had no effect whatsoever on the condition.

Attempts made in the present investigation to prevent the cardiac damage induced with hydrocortisone by increasing the potassium intake or by decreasing the sodium intake, were unsuccessful. On the other hand, en-

TABLE IV. Effect of Alterations in Sodium-Potassium Content of Diet on Myocardial Calcification Produced in Female C3H Mice with Hydrocortisone.*

Diet	Daily dose, mg	No. of mice	Body wt, g		Organ wt, mg			Calcification†
			Onset	Autopsy	Adrenals	R. kidney	Heart	
Stock	.3	5	20	20.5	4.0 ± .1‡	186 ± 5	124 ± 2	1½
"	.5	9	17.5	19	2.5 ± .1	164 ± 5	119 ± 3	3½
Stock + .5% KCl H_2O	.5	10	16.5	18.5	2.8 ± .1	166 ± 6	138 ± 6	4
Stock + .3% resin§	.5	10	17	19.5	2.7 ± .1	163 ± 3	123 ± 5	4
Sodium-deficient‖	.3	9	21.5	16	2.9 ± .2	124 ± 4	100 ± 3	4
Sodium-potassium-deficient‖	.3	6	20	11.5	2.5 ± .3	136 ± 7	69 ± 3	1¼

* Single daily subcut. inj. of hydrocortisone were begun at 50 days of age and continued for a period of 15 days.

† This estimate represents extent of calcification observed grossly in the heart at autopsy.

‡ Mean ± stand. error.

§ 100 mg of Dowex 50 resin (50 mg K form, 50 mg H form) distributed in 30 g of stock diet—approximately 45 mg resin/70 g body wt/day.

‖ Special diets purchased from General Biochemicals, Inc., Chagrin Falls, Ohio.

larged adrenal glands were observed in the earlier studies in which the animals had been given a potassium-deficient diet(13,14) and it may well be that an excess of the adrenal corticoids, rather than a deficiency of the K ion *per se*, was largely responsible for the production of the necrotic and calcareous lesions.

Summary. Hydrocortisone administered at daily levels above 0.25 mg for 15 days consistently induced calcification of the myocardium in adolescent female mice. The male was less affected than the female and the concurrent administration of testosterone to the female alleviated the condition; ovariectomy afforded no protection to the heart. Both hydrocortisone and ACTH were quite ineffective in inducing the deposition of calcium in the heart of the hypophysectomized mouse. Moreover, the administration of growth hormone with hydrocortisone or ACTH aggravated this condition. Lactogenic hormone, DOCA and estradiol had no effect.

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