flected a higher renewal rate of the muscle proteins in Vit. E-deficiency. However, the rapid change of non-protein radioactivity demonstrated in the present study raises doubts as to the significance of free amino acid specific activity determinations made 24 hours after injection.

Statistical analysis shows that the difference between the S.A. of proteins of control and of dystrophic animals at 4 hours after injection was significant in all 4 subcellular fractions ($P<0.05$). The possibility that the difference between non-protein radioactivity of control and of dystrophic muscle at 4 hours after injection was due to chance cannot be excluded ($P = 0.1$).

Summary. Normal and Vit. E-deficient rabbits were injected with glycine-1-C^{14} and sacrificed at time intervals ranging from 0.5 to 12 hours. Homogenates of skeletal muscle were fractionated by differential centrifugation, and specific activity of the subcellular protein fractions determined. In all subcellular fractions and at all time intervals the proteins of Vit. E-deficient animals had a much higher specific activity. The effect of Vit. E-deficiency on incorporation of glycine-C^{14} was approximately the same in all subcellular protein fractions. Compared with control animals the radioactivity of the non-protein supernatant fraction rose and fell faster in muscle of Vit. E-deficient animals.

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Pituitary Inhibitory Effects of Digitoxin and Hydrocortisone.* (27086)

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It is well known that adrenal cortical steroids depress the secretion of ACTH by the anterior pituitary gland(1,2,3). In addition, numerous other steroids, for example testosterone(4) and the synthetic corticosteroids (5) have been shown to have similar pituitary inhibitory properties. It has often been suggested that the cardiac glycosides, owing to their structural similarity to the adrenal corticosteroids might possess similar pharmacological properties. Since the cardiotonic structure-activity relationships of adrenal corticoids have been studied extensively in recent years(6,7,8), it became of interest to compare the effects of digitoxin and hydrocortisone on the pituitary-adrenal axis. The experiments described here demonstrate that chronic administration of digitoxin significantly inhibits both synthesis and stress-induced release of pituitary ACTH.

Materials and methods. The experiments were performed on male albino Wistar rats fed on a diet of commercial rat chow and water *ad lib.* and maintained at a constant temperature of 24°C.

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Digitoxin (Lilly) and hydrocortisone free alcohol (Upjohn) were suspended in normal saline. All drugs were administered subcutaneously in a total volume of 0.1 ml/100 g body weight.

(1). Forty-eight rats, weighing from 120-150 g, were divided into 4 groups. One group served as controls, the rats in this group receiving daily injections of normal saline. Two groups received daily doses of 0.5 or 1.0 mg hydrocortisone per 100 g body weight and the remaining animals 0.5 mg digitoxin per 100 g per day. The animals were weighed frequently and after 35 days treatment were decapitated and their adrenals, testes, thyroid and pituitary glands removed, dissected free from adhering tissue and weighed quickly on a torsion balance to minimize loss of moisture.

The excised pituitary glands were transferred to tubes containing 0.5 ml N/10 hydrochloric acid per pituitary gland. The glands were ground well with the aid of a glass rod and a little sand. Six pituitaries were pooled and extracted for every determination. The extracts were stored in the refrigerator for 24 hr. They were diluted with normal saline immediately before injection into the assay animals and assayed for corticotropin content in hydrocortisone-treated male rats, as described by Cox, *et al.*(9). A preparation of ACTH (Armour) containing 25 U per mg was used as the standard. A conventional 2 + 2 assay design was employed and the results and their fiducial limits ($P = 0.95$) were calculated by standard methods. The results were expressed as milli-units (mu) ACTH/pituitary gland and mu ACTH/mg pituitary tissue.

(2). Groups of 6 male rats, weighing 120-150 g, were given a single subcutaneous injection of normal saline or of 0.5 mg digitoxin/100 g body weight. The animals were anaesthetized with ether and sham adrenalectomized(10) 2, 4 or 8 hr after receiving the injections. They were allowed to recover from the operation and were killed one hour later. Control rats were killed 4 hr after receiving the injections without any previous surgical interference. The adrenal glands were removed, dissected free from periadrenal tissue, weighed rapidly and transferred to 4% trichloracetic acid solution for estimation of their ascorbic acid contents by the method of Roe and Kuether(11).

In another experiment, rats which had received daily injections of digitoxin (0.5 mg/100 g/day), hydrocortisone (0.5 mg/100 g/day) or normal saline (0.1 ml/100 g/day) for 30 days were anaesthetized with ether. The left adrenals were removed and served as a control. One hour later, the animals were killed and the right adrenal glands were removed. Concentration of ascorbic acid in these glands was determined as described above. The difference in ascorbic acid concentration between the left and right adrenals was a measure of the ascorbic acid depletion elicited by the stress of unilateral adrenalectomy under ether anaesthesia.

In all chronic experiments, any rats which developed tremors or other evidence of central nervous system toxicity from digitoxin were excluded from this study. With the dose used, this occurred infrequently (20%).

Results. Digitoxin, in doses of 0.5 mg/100 g/day, produced no significant alteration of growth from that of the saline controls. The same dose of hydrocortisone depressed the growth of rats slightly only after it had been administered for 20 days. However, a 2-fold increase in the dose of hydrocortisone produced a marked inhibition of growth as shown in Fig. 1.

Both drugs caused adrenal atrophy (Table I). The effect of hydrocortisone was more marked than that of digitoxin. In contrast, those digitoxin-treated rats that showed tremors had clearly hypertrophied adrenals at the end of the experimental period (mean left adrenal weight (mg $\pm$ S.E.) 26.2 $\pm$ 0.6; mean right adrenal weight (mg $\pm$ S.E.) 24.3 $\pm$ 0.8). There was no significant difference ($P>0.05$) in the absolute weights of the testes, thyroid or anterior pituitary glands between the digitoxin, hydrocortisone or saline treated rats.

Subcutaneous injections of suspensions of digitoxin or hydrocortisone resulted in no changes in adrenal ascorbic acid concentration. The stress of sham adrenalectomy or unilateral adrenalectomy under ether anaes-

TABLE I. Adrenal, Testis, Thyroid and Pituitary Weights (± S.E.) in Rats after 35 Days Treatment with Digitoxin and Hydrocortisone.

Treatment	No. of rats	Adrenal wt (mg) Left	Adrenal wt (mg) Right	Testis wt (g) Left	Testis wt (g) Right	Thyroid wt (mg)	Pituitary wt (mg)
Digitoxin, .5 mg/100 g/day	12	*16.7 ± .5	*15.9 ±.6	1.65 ±.7	1.65 ±.08	19.8 ± 1.0	10.2 ±.3
Hydrocortisone, .5 mg/100 g/day	6	*12.6 ±1.0	*11.4 ±.5	1.57 ±.03	1.55 ±.04	19.9 ± .5	8.7 ±.4
Hydrocortisone, 1 mg/100 g/day	6	* 9.6 ± .4	* 9.0 ±.8	1.52 ±.09	1.53 ±.09	19.6 ± .9	8.2 ±.3
Saline, .1 ml/100 g/day	10	21.7 ± .9	20.8 ±.7	1.53 ±.05	1.55 ±.4	21.1 ± 1.8	9.9 ±.8

* The difference from saline controls is statistically significant ($P < .05$).

thesia produced marked adrenal ascorbic acid depletion in the rats which had been pretreated with normal saline only. A single dose of digitoxin administered 2, 4 or 8 hours previously did not affect the fall in adrenal ascorbic acid concentration normally caused by the operation (Table II, A). However, chronic treatment with either digitoxin or hydrocortisone for 30 days completely prevented the fall in adrenal ascorbic acid caused by unilateral adrenalectomy (Table II, B).

To determine the cause of the adrenal effects described above, the effect of chronic digitoxin administration on pituitary ACTH content was measured. Table III shows that chronic administration of digitoxin reduces the pituitary content of ACTH to an extent approximately equivalent to the effect of twice the dose of hydrocortisone.

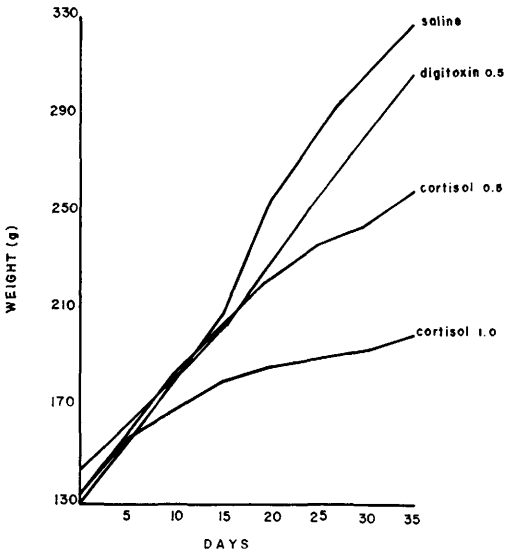

FIG. 1. Growth rate of rats receiving daily subcutaneous injections of digitoxin, hydrocortisone or normal saline. (Doses indicated in mg/100 g/day.)

Discussion. It has been demonstrated in many laboratories that prolonged administration of natural or synthetic corticosteroids as well as other steroid substances causes marked adrenal atrophy, and depresses the growth rate of rats(12,13,4,5). This is attributable to inhibition of ACTH synthesis and release(14.15). Digitoxin produces a similar effect and the results described above indicate that it is almost as potent as hydrocortisone in causing adrenal atrophy but less potent in inhibiting growth. On the other

TABLE II. Adrenal Ascorbic Acid Depletion Caused by Stress in Rats at Various Time Intervals after Single or Repeated Subcutaneous Injections of Digitoxin. Adrenal ascorbic acid concentration in uninjected controls was 524 ± 11 mg/100 g adrenal tissue. No. of animals given in parentheses. Unstressed saline and digitoxin controls (II A) were killed 4 hr after injection.

	Treatment	Adrenal ascorbic acid depletion, mg/100 g adrenal tissue ± S.E.
A.	Saline, .1 ml/100 g	11 ± 11 (6)
	Idem, stressed 4 hr later	167 ± 6 (6)
	Digitoxin, .5 mg/100 g	−13 ± 4 (6)
	Idem, stressed 2 hr later	148 ± 12 (6)
	" " 4 " "	152 ± 14 (6)
	" " 8 " "	151 ± 7 (6)
B.	Saline, .1 ml/100 g/day for 30 days stressed	166 ± 14 (6)
	Digitoxin, .5 mg/100 g/day for 30 days stressed	22 ± 11 (6)
	Hydrocortisone, .5 mg/100 g/day for 30 days and stressed	23 ± 4 (6)

TABLE III. Concentrations of Adrenocorticotropic Hormone in Anterior Pituitary Glands of Rats Receiving Daily Injections of Digitoxin, Hydrocortisone or Normal Saline for 35 Days. Fiducial limits (P = .95) given in parentheses.

Treatment	No. of rats	Mean rat wt (g) ± S.E.	Mean ant. pit. wt (fresh) (mg) ± S.E.	No. of assay rats	Corticotropin conc. mu/pituitary gland	Corticotropin conc. mu/mg pituitary tissue	Corticotropin conc. % control content
Saline controls, .1 ml/100 g/day	6	326 ± 6	9.9 ± .8	12	84 (50-142)	8.5	—
Digitoxin, .5 mg/100 g/day	6	305 ± 7	10.2 ± .3	12	33 (18-62)	3.3	40
Hydrocortisone, 1 mg/100 g/day	6	199 ± 4	8.2 ± .3	12	27 (12-62)	3.3	31

hand, some investigators have suggested that digitalis glycosides have a stimulating effect on the adrenal cortex. Pyorala and Eranko (16) have found that chronic treatment with large doses of digitoxin causes an increase in adrenal weight. However, they observed that these animals showed transient paralysis of the hind legs. This is in agreement with the present finding that digitoxin-treated rats which exhibited tremors showed adrenal hypertrophy. It appears therefore that the stimulation of the adrenal cortex in their experiments was due to the central nervous system stimulation produced by the toxic dosage of glycosides used.

There is little doubt that the adrenal cortical hormones depress the secretion of corticosteroids by interfering mainly with the release of ACTH from the adenohypophysis rather than by acting directly on the adrenal cortex. The results of the experiments described here indicate that digitoxin produces a similar effect. The inability of a single dose of digitoxin to prevent the stress-induced release of ACTH can be attributed to the limitations in the dosage that can be administered without producing toxic symptoms. However, chronic administration of digitoxin blocked the fall in adrenal ascorbic acid caused by stress and markedly depressed the ACTH content of the anterior pituitary gland. These data are consistent with the findings that digitalis glycosides increase the performance of animals subjected to stress, prevent the adrenal hypertrophy caused by stress(17,18), and prolong the life of adrenalectomized rats, mice and cats(19). Thus, it appears that digitoxin can affect both the release and the synthesis of ACTH from the pituitary gland in a manner quantitatively similar to that of the corticosteroids.

Bornstein and Trewhella(20) reported that patients with congestive heart failure had high levels of circulating ACTH which could be controlled by treatment with digitalis. The possible therapeutic significance of the pituitary inhibitory properties of the digitalis glycosides is an intriguing idea which has yet to be proven.

Summary. Digitoxin and hydrocortisone administered in a dose of 0.5 mg/100 g body weight daily did not significantly affect the growth rate of rats, although 1 mg/100 g of hydrocortisone markedly inhibited growth. Digitoxin and hydrocortisone caused atrophy of the adrenal glands but had no effect on the absolute weights of the testes, thyroid and anterior pituitary glands. Pretreatment of rats with a single dose of digitoxin did not prevent the stress-induced fall in adrenal ascorbic acid although chronic treatment completely prevented this effect in a manner quantitatively similar to hydrocortisone. Daily treatment with digitoxin or hydrocortisone for 30 days reduced the ACTH content of the anterior pituitary gland to 30-40% that found in control glands.

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Effects of a High Cholesterol Diet on Synthesis of Plasma Low Density Lipoprotein.* (27087)

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When diets high in fat and cholesterol are fed to animals, plasma cholesterol levels are elevated. In some species, such as the rabbit, the levels reached are very high and atherosclerosis readily develops(1). Under these circumstances, the low density or β lipoprotein levels in plasma are particularly increased. It is difficult to elevate the plasma low density lipoproteins in the rat to this degree by feeding such diets, but there is no doubt that elevation of plasma and liver cholesterol content can be achieved(2). Can such diets affect the rate of hepatic synthesis of the low density lipoproteins in the rat? We have recently succeeded in measuring the net synthesis of plasma low density lipoproteins in the perfused rat liver by an immunochemical method(3) and have now applied this technic to an investigation of the effects of a high fat-high cholesterol diet. Our results indicate that, in the rat, such a diet does not lead to an increased synthesis of the protein moiety of the low density lipoproteins but does increase the amount of cholesterol present in the lipoprotein molecule released by the liver during perfusion *in vitro*.

Materials and methods. The rats used were of the Wistar strain, weighing between 270 and 300 g, and were fed for 3 weeks on a commercial "cholesterol-free" diet (Nutritional Biochemicals Corp.) containing either 15% of added Wesson oil or 15% of Wesson oil plus 5% of cholesterol. The livers were perfused for 1 hour at 37° with a suspension of rat red cells in Krebs-Ringer-bicarbonate medium as previously described(3,4) and the amount of low-density lipoprotein measured by means of the quantitative precipitin reaction with an antiserum prepared in the goat. The immune precipitate was analyzed for its cholesterol(5) and protein(6) content, thus affording a measure of the synthesis of both cholesterol and protein moieties.

Results and discussion. The results shown in Table I indicate that addition of 5% of cholesterol to the diet resulted in a 3-fold increase in amount of cholesterol in low density lipoprotein appearing in the perfusion fluid with no significant increase in amount of the protein moiety. The high cholesterol diet caused a 3-fold increase in liver cholesterol

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