Summary. Cecectomized rabbits (removal of appendix and all of cecum except proximal 3 segments) were challenged intravenously with highly purified staphylococcal enterotoxin. Diarrhea was induced in cecectomized rabbits in doses ineffective for normal rabbits. Average latency was 1¾ hours with recovery from diarrhea within 24 hours. Successive weekly injections of enterotoxin resulted in development of resistance. Pretreatment of cecectomized rabbits with atropine or pyribenzamine gave definite but incomplete protection against enterotoxin-induced diarrhea.

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Relationship of Food Intake to the Effect of Cortisone Acetate on Skin Wound Healing. (29511)

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Glucocorticoids have been reported to inhibit wound healing in laboratory animals(1-6) by suppressing formation of granulation tissue(7,8), collagen(2,9,10) and acid mucopolysaccharides(11-13). The effect of cortisone acetate may not be immediate but may follow a "lag" period(14). Some investigators have been unable to demonstrate histologically faulty wound healing, fibroblastic proliferation(15) or differences in granulation collagen(8).

Much of the confusion may have developed from use of incomparable dose levels of steroids and from failure to control obvious anorexic effects of glucocorticoids. Accordingly, effects of graded doses of cortisone acetate on skin wound tensile strength were reevaluated utilizing the technique of pair-feeding to control food consumption.

Materials and methods. One hundred and forty-four male Wistar rats weighing 170 ± 10 g were individually housed and arranged in 9 groups consisting of 16 animals each as indicated in the Tables. Groups A, B-1, C-1, D-1, E-1 received Rockland ground food and tap water *ad libitum.* Group A was the absolute control; whereas the other groups received respectively, 1, 2, 4, 8 mg of cortisone acetate s.c. per rat per day. Each cortisone treated animal had a corresponding pair-fed control (Groups B-2, C-2, D-2, E-2).

On day 1 the rats were anesthetized with ether, the skin of the dorso-lumbar region was shaved with electrical clippers and a wound approximately 1 inch in length was incised. The wound was closed with 3 Michel wound clips which were removed 48 hours later. Eight rats from each group were killed 3 days and the remaining eight 9 days after wounding.

Body weights were recorded on days 1, 3, 6, 9. Food consumption of cortisone acetate treated animals was recorded daily. Skin wound tensile strength was measured on day 3 and day 9.

The apparatus used to measure the wound tensile strength consisted of 2 alligator clips, a ball-bearing pulley system, plastic volumetric cylinders and a constant rate of flow water source. After the rat was killed one clip was attached to one side of the wound

TABLE I. Effect of Cortisone Acetate on 3rd Day After Wounding.

Group	Treatment, s.c.	Dose, mg/rat	No. of animals	Body wt, g Initial	Body wt, g Final	Body wt change, g	Skin wound tensile strength* (g) ± S.E.
A	Absolute control	—	8	164	172	+ 8	103 ± 6
B–1	Cortisone acetate	1	8	178	181	+ 3	94 ± 11
–2	Pair-fed control	—	8	177	176	- 1	104 ± 9
C–1	Cortisone acetate	2	8	172	172	0	88 ± 6
–2	Pair-fed control	—	8	170	169	- 1	96 ± 10
D–1	Cortisone acetate	4	8	177	170	- 7	84 ± 10
–2	Pair-fed control	—	8	177	172	- 5	109 ± 9
E–1	Cortisone acetate	8	8	185	172	-13	101 ± 13
–2	Pair-fed control	—	8	182	179	- 3	117 ± 8

* Significance of differences between groups: none.

and to a stationary post; the other clip was attached to the opposite side of the wound and to the volumetric cylinder via a ball-bearing pulley system. A constant flow of water was allowed to fill the cylinder until the wound was disrupted. The volume (ml) of water and/or weight (g) was recorded.

Results and discussion. On the 3rd day after wounding, tensile strength of healing skin wounds was similar in cortisone acetate-treated rats, their pair-fed controls and *ad libitum* fed controls (Table I). On the 9th day, wound tensile strength (W.T.S.) was significantly lower in all cortisone-treated and pair-fed control groups than in controls with free access to food (Table II). However, W.T.S. of rats treated with 1 or 2 mg cortisone acetate daily did not differ from that of their pair-fed controls (Table II). Daily injections of 4 or 8 mg cortisone acetate were associated with a W.T.S. significantly lower than that induced by pair-feeding (Table II). In assessing these results, the following should be considered: increasing levels of cortisone acetate induced roughly dose proportional reductions in food intake, body weight and W.T.S. (Tables I-III). The effect of steroid on food consumption was evident on day 2 and the effect on body weight on day 3. W.T.S. was not adversely affected by cortisone on day 3, supporting the concept of a "lag period" in steroid interference with wound healing(14). On day 9, the apparent effects on W.T.S. and body

TABLE II. Effect of Cortisone Acetate on 9th Day After Wounding.

Group	Treatment, s.c.	Dose, mg/rat	No. of animals	Body wt (g) on day: 1	3	6	9	Body wt change, g	Skin wound tensile strength* (g) ± S.E.
A	Absolute control	—	8	185	192	224	238	+53	357 ± 13
B–1	Cortisone acetate	1	8	177	182	191	205	+28	278 ± 16
–2	Pair-fed control	—	8	177	174	182	193	+16	312 ± 13
C–1	Cortisone acetate	2	8	183	182	184	191	+ 7	295 ± 11
–2	Pair-fed control	—	8	183	179	183	193	+10	304 ± 20
D–1	Cortisone acetate	4	8	179	169	159	148	-31	230 ± 16
–2	Pair-fed control	—	8	183	184	170	184	+ 1	292 ± 9
E–1	Cortisone acetate	8	8	176	155	144	126	-50	198 ± 14
–2	Pair-fed control	—	8	176	169	159	179	+ 3	271 ± 10

* Significance of differences between groups:

A & B–1, $P < .01$
A & B–2, $P < .05$
A & C–1, $P < .01$
A & C–2, $P < .05$
A & D–1, $P < .001$
A & D–2, $P < .01$
A & E–1, $P < .001$
A & E–2, $P < .001$
B–1 & B–2, $.1 < P < .2$
C–1 & C–2, $P > .6$
D–1 & D–2, $P < .01$
E–1 & E–2, $P < .001$

TABLE III. Food Consumed by Cortisone-Treated Animals and Offered to Their Respective Pair-Fed Controls.

Groups*	Avg food consumption (g/rat) per day: 1	2	3	4	5	6	7	8	9	Avg food consumption (g/rat) over 9 days period
A	23.9	20.0	24.7	19.9	23.4	25.4	20.0	21.0	20.7	22.1 ± .8
B–1 & –2	24.9	14.9	21.1	15.5	11.4	22.3	15.6	19.0	16.7	17.9 ± 1.5
C–1 & –2	20.3	16.3	16.9	15.0	9.3	20.5	14.3	13.0	17.3	15.9 ± 1.2
D–1 & –2	19.3	14.4	15.3	10.4	5.6	16.3	12.3	11.6	12.0	13.0 ± 1.3
E–1 & –2	20.3	13.2	13.1	10.9	6.3	16.6	11.0	9.7	10.7	12.4 ± 1.3

* See Table II for treatments.

weight gain of 1 or 2 mg daily doses of cortisone acetate were roughly mimicked by restricting food to the amount consumed by steroid-treated rats (72-81% of *ad libitum* food intake). Reduced W.T.S. at these doses of cortisone was thus largely ascribable to the anorexic effects of the drug, as was reduced body weight gain. Since intact animals were used, one cannot exclude the effects of an adrenal-cortical stimulation in response to the stress of starvation. With the larger doses of cortisone acetate (4 or 8 mg/day), a drastic reduction in W.T.S. and loss of body weight occurred. Food restriction alone (56-59% of the *ad libitum* food intake) could not account for the adverse effects on W.T.S. or the loss of body weight. Effects of high doses of cortisone on W.T.S. resemble those obtained with low protein diets(16) and are therefore likely ascribable to direct steroidal interference with protein (collagen?) synthesis(2,9,10). Cortisone acetate thus appears to influence wound healing in 2 ways: first, indirectly by exerting an anorexic effect observable at low dose levels and second, by direct interference with anabolic processes when given at toxic dose levels.

Summary. Healing of standardized skin wounds was studied in rats treated with graded doses of cortisone acetate. The anorexic effects of the drug were controlled by pair-feeding of control groups.

On the 3rd day after wounding, cortisone treatment or pair-feeding did not significantly alter the tensile strength of the skin wounds. However, on the 9th day both the cortisone-treated rats and their pair-fed controls demonstrated significant reductions in skin wound tensile strength when compared to controls on unrestricted food intake. On the other hand, if the cortisone-treated animals were compared to their respective pair-fed controls, significant reductions in healing were observed only with high doses of steroid. Low doses of cortisone thus interfere with wound healing by inducing a mild anorexia; this effect is duplicated by food restriction without drugs. Large doses of steroid impair wound healing and also induce a drastic body weight loss; neither of these effects is duplicated by food restriction alone.

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Mammary Lobulo-Alveolar Growth in Adreno-Ovariectomized Rats Following Transplantation of "Mammotropic" Pituitary Tumor.* (29512)

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The ovarian hormones, estrogen and progesterone, have been shown to induce mammary lobulo-alveolar (L-A) growth in intact or ovariectomized rats and to synergize with prolactin and growth hormone (STH) in adreno-ovariectomized-hypophysectomized rats(1). Ovarian hormones themselves have little or no effect on rat mammary L-A development in absence of the anterior pituitary (AP), whereas our laboratory has recently reported that AP hormones (STH and prolactin) alone induced mammary L-A growth in the absence of the ovaries, adrenals and pituitary(2). This suggested that STH and prolactin are of primary importance for mammary L-A growth in the rat.

Furth and his co-workers(3,4) have developed many types of transplantable pituitary tumors. One of these is a monomorphous pituitary "mammotropic" tumor, strain F_4 ($MtT.F_4$), originally induced by estrogen(5). This tumor secretes prolactin, STH and corticotropin (ACTH) but no FSH-LH or TSH in the host animal(6-10). The tumor provides a continuous source of prolactin, STH and ACTH, which are secreted in increasing amounts as the tumor grows in the host. It was of interest, therefore, to determine the effect of a $MtT.F_4$ transplant on mammary growth in the rat in which the sources of estrogen and progesterone have been eliminated by removal of the ovaries and adrenals.

Methods. Highly inbred, virgin female Fischer rats of the CDF strain (Charles River Breeding Laboratories, Brookline, Mass.), 3 months old, were fed *ad libitum* on Wayne Lab Blox pellets, supplemented with canned dog food (Dash) and slices of oranges. Rats in Groups 1 and 2 (Table I) were sham-operated, while those in Groups 3 and 4 were adreno-ovariectomized. Following each operation, the rats were injected once subcutaneously with 30,000 units of penicillin G. The completeness of adrenalectomy was assessed in 2 ways: (a) after killing the rats at the end of experiment, they were carefully examined with a magnifying lens for adrenal remnants, and (b) 9 rats of the same strain, age, and weight were adreno-ovariectomized and given saline (1% NaCl) in their drinking water for 7 days. From the 8th day onward, the rats were given distilled water instead of saline. The day on which each rat died, after saline withdrawal, was recorded.

Seven days after the operation, rats in Groups 2 and 4 were transplanted subcutaneously with a Furth pituitary "mammotropic" tumor ($MtT.F_4$, passage 42) in the dorsal neck region. Each $MtT.F_4$ was removed aseptically, cut into small pieces with iris scissors, and the tumor mince was suspended in an equal volume of medium 199 (pH 7.4, Difco Laboratories, Detroit, Mich.). This was injected in a 0.1 ml volume. The tumors attained palpable size within 4-5 weeks. Vaginal smears were taken from the adreno-ovariectomized rats to test for estrogenic activity.

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