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Response of Serum Corticosterone to ACTH and Stress in the Zinc-Deficient Rat[1] (39966)

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Introduction. There is evidence in the literature that zinc is involved in the function of adrenocorticotropin (ACTH). The work of Sandstead *et al.* (1) showed that more than one-half of a group of zinc-deficient human dwarfs responded with an abnormal delay in urinary output of 17-hydroxysteroids when given injections of ACTH. After periods of treatment with zinc these patients responded to ACTH by exhibiting a near-normal pattern of excretion of the urinary steroids. It is not clear, however, that the response was due directly to zinc since the prolonged treatment brought about general improvement in the health of the patients.

A direct approach to determine how zinc affects ACTH activity was taken by Flynn and co-workers (2). Rats, under hypotensive stress, were shown to have elevated levels of ACTH in the plasma. When these animals were relieved of the stress stimulus, ACTH returned to normal. Zinc levels in serum mimicked those of ACTH, suggesting a relationship between zinc and the function of the hormone. In subsequent studies this group (3) examined the *in vitro* effect of zinc on the stimulation of corticosterone synthesis by ACTH in isolated adrenal glands. It was shown that ACTH would not stimulate corticosterone synthesis when a zinc chelator was present in the incubation medium. Activity was restored when zinc was added in excess of that bound to the chelating agent. This observation suggests that ACTH is functionally dependent upon zinc.

The zinc concentration of extracellular fluid of intact rats depends greatly on dietary zinc and can be markedly reduced by feeding diets with low concentration of the metal. If ACTH activity in the intact animal is as dependent on extracellular zinc as it appears to be *in vitro,* there should be a reduction of corticosterone synthesis in the zinc-deficient rats. In this study the effect of zinc deficiency on corticosterone synthesis *in vivo* was examined under three conditions, stimulation by exogenous ACTH and two types of stress.

Materials and methods. Effect of ACTH administration on corticosterone synthesis in vivo. Experiment 1. Thirty male albino rats, weighing 117 ± 20 g, from our stock colony were randomly assigned to six groups. All were fed a purified diet based on EDTA-treated soybean protein which contained less than 1 ppm zinc (4). Groups 1 and 2 (Table I) were fed the basal diet supplemented with approximately 100 ppm zinc as $ZnCO_3$ in an amount corresponding to that eaten *ad libitum* by one (Group 3) of four groups that received the zinc-deficient diet. All animals were fed these regimens for a period of 23 days. On Day 24 two of the zinc-deficient groups (Groups 3 and 5) were given the zinc-supplemented diet for 2 days. All animals were then fasted for 18 hr and the experiment was terminated at 9:00 AM after ACTH was administered according to the schedule shown in Table I.

All animals were anesthetized with 5.0 mg of Nembutal/100 body weight and allowed to remain undisturbed at 30° for 1 hr. At timed intervals ACTH[2] in 0.85% NaCl or 0.85% NaCl alone was injected intraperitoneally. Previously, it had been determined that 200 mU of ACTH/100 g body weight would raise serum corticosterone levels to approximately one-half of the maximum response in 50 min. After this

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[2] ACTH was a generous gift from Dr. R. J. Schlueter, Armour Pharmaceutical Co., Kankakee, Illinois. Lot No. K600020D contained 1.08×10^{-3} μg of zinc/unit of ACTH.

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TABLE I. (A) EFFECT OF ZINC DEPLETION AND REPLETION ON BODY WEIGHT GAIN AND CONCENTRATION OF SERUM ZINC. (B) EFFECT OF ACTH INJECTIONS ON CONCENTRATION OF SERUM ZINC.

	(A) Days 1–21		Days 24–25	(B) Day 26	
Treatment group	Dietary Zn (ppm)	Gain (g)	Dietary Zn (ppm)	ACTH (mU)	Serum Zn (μg/100 ml)
1	100	42 ± 10*	100	200	126 ± 1.0*, [a]
2	100	47 ± 8	100	0	127 ± 3.2[a]
3	<1	6 ± 2	100	200	133 ± 11.1[a]
4	<1	6 ± 4	<1	0	82 ± 3.2[b]
5	<1	9 ± 2	100	0	129 ± 8.6[a]
6	<1	9 ± 1	<1	200	77 ± 4.8[b]

* Group mean ± SEM (five determinations per group). Any two means with different superscripts are significantly different at a P value of 0.05 or less.

period of time blood was collected from the trunk of each animal after the head was severed. Corticosterone concentration in the serum was determined by the method of Silber *et al.* (5) modified by Kitabachi and Kitchell (6). Zinc was determined in serum by atomic absorption spectrophotometry. Wet weights of the adrenal and pituitary glands were determined in Groups 2, 4, and 5.

Effect of stress on corticosterone synthesis in vivo. Experiment 2. Ten male rats were divided into two groups of five rats each. One group was fed the zinc-deficient diet as described above. The other group was fed each day the zinc-supplemented diet in an amount equal to that consumed *ad libitum* by the deficient group. Animals were not fasted at the termination of this experiment. After 28 days, at 9:00 AM, each rat was stressed by placing it for 2 min in air saturated with diethyl ether. It was allowed to recover for 20 min and again placed in the ether vapors until limp but alive. The head was quickly severed and trunk blood was collected. Corticosterone and zinc concentrations were determined in the serum.

Experiment 3. During early morning, pregnancy was determined in 200+-g rats by checking vaginal smears for sperm. The day sperm were found was designated as Day 0 of gestation. Starting on this day, one-half of the animals was given a zinc-deficient diet and the other half a diet with zinc added. The latter group was pair-fed to the first. At Day 19 of gestation, one-half of the group receiving the zinc-deficient diet was given adequate zinc for the duration of the experiment. The other half received no zinc through parturition. Four to six hours after parturition the dams were anesthesized with Nembutal and the blood pressure was recorded by exposing the carotid artery and inserting into it a small tube connected to a pressure transducer. Following approximately 5 min of pressure recordings the abdominal cavity was opened and blood was drawn from the descending aorta. Serum was separated for the determination of corticosterone. Blood pressure was recorded for another study and is mentioned here only to describe the acute stress situation under which all animals of these experiments were placed.

Results. Effect of ACTH administration on corticosterone synthesis in vivo. Experiment 1. Table I shows the effect of feeding the zinc-deficient diet on weight gain. Although the intake of animals fed the adequate-zinc diet was restricted to match that of the zinc-deficient group, their gain in body weight was greater. This suggests a better feed efficiency when zinc is present in the diet.

Figure 1 demonstrates the effect of feeding a low-zinc diet on serum corticosterone levels and the effect of exogenous ACTH on the biosynthesis and release of this steroid into the blood of intact animals. Neither dietary zinc level nor zinc status of the animal affected resting concentrations of serum corticosterone. Consequently, no effect was observed when zinc-depleted animals were refed zinc. Furthermore, depleting the animals of zinc had no effect on ACTH-induced corticosterone levels in serum. Animals consuming a diet adequate in zinc and those fed a diet low in zinc showed

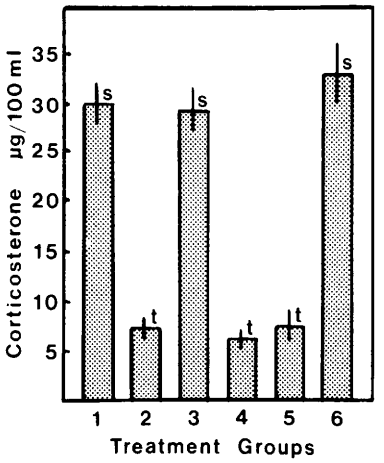

FIG. 1. The effect of zinc depletion and repletion and ACTH administration on serum levels of corticosterone in the rat. Mean ± SEM (five observations per group). Any two means with different superscripts are significantly different at a P value of 0.05 or less. See Table I for treatment schedule.

similar responses to ACTH. As might be expected, short-term supplementation of depleted animals did not affect serum corticosterone.

Mean zinc concentrations in the sera are shown in Table I. Feeding diets deficient in zinc had the expected effect of lowering zinc concentration in the serum. Refeeding zinc for only 2 days restored the concentration to normal. Administration of ACTH had no effect on zinc concentration whether or not the animal had an adequate intake of the element.

The wet weights of both the adrenal and pituitary glands, expressed as a function of body weight, were significantly increased in zinc-depleted animals (Table II). However, no significant differences were observed in the uncorrected weights.

Effect of stress on corticosterone synthesis in vivo. Experiment 2. The results of this experiment are shown in Table III. Stressing the animals in ether vapors increased corticosterone levels in the serum irrespective of dietary zinc concentration (compare to nonstressed animals in the previous study), but had no effect on serum zinc. The low intake of zinc caused a significant reduction in serum zinc indicative of a physiologically deficient status.

Experiment 3. This experiment involved stressing the animals at close intervals with different and severe stimuli. The first stress was that of gestation and parturition. In general, zinc depletion during gestation caused the dams to become moribund. The length of gestation was increased (23.2 ± 0.4 days vs 21.8 ± 0.1 days for controls). Once parturition began, the dams experienced profuse vaginal bleeding and had difficulty expelling the pups from the uterus. They became cold and lifeless to touch during and soon after delivery. None of these symptoms occurred in dams fed zinc.

Four to six hours after completion of parturition the dams were given an additional stress, i.e., surgical resection of the carotid artery and insertion of a catheter to measure blood pressure. Table IV shows the response of serum corticosterone to this treatment. Rats fed adequate zinc throughout gestation had serum corticosterone levels significantly higher than those not fed zinc. Animals fed zinc from Day 19 of gestation were in the midrange of the deficient and control group and not significantly different from them.

TABLE II. EFFECT OF ZINC DEPLETION ON WEIGHT OF ADRENAL AND PITUITARY GLANDS.

Experimental group	Wet weight	
	mg	mg/100 g body wt
Adrenal gland		
2	26.7 ± 2.7*,[a]	15.6 ± 1.0[a]
4	26.7 ± 1.0[a]	21.5 ± 1.2[b]
5	29.3 ± 2.4[b]	21.9 ± 1.0[b]
Pituitary gland		
2	3.8 ± 0.3[a]	2.3 ± 0.3[a]
4	4.0 ± 0.4[a]	3.3 ± 0.5[b]
5	4.4 ± 0.4[a]	3.3 ± 0.2[b]

* Group mean ± SEM (five determinations per group). Any two means with different superscripts are significantly different at a P value of 0.05 or less. See Table I for treatment schedule.

TABLE III. EFFECT OF ZINC DEPLETION ON SERUM ZINC AND CORTICOSTERONE DURING ETHER STRESS.

Dietary Zn (ppm)	Zinc (µg/100 ml)	Corticosterone (µg/100 ml)
100	112 ± 5.3*,[a]	41.0 ± 6.5[a]
<1	29 ± 2.1[b]	45.3 ± 7.4[a]

* Mean ± SEM (five observations per group). Any two means with different superscripts are significantly different at a P value of 0.05 or less.

TABLE IV. EFFECT OF ZINC DEPLETION AND REPLETION ON CORTICOSTERONE LEVELS IN SERUM OF POSTPARTURIENT RATS DURING SURGICAL STRESS.

Treatment	Corticosterone (μg/100 ml)
(A) −Zn Day 1 through parturition	24.8 ± 4.4*,[a] (5)
(B) +Zn paired-fed to (A)	48.3 ± 5.4[b] (4)
(C) −Zn Days 1–18 of gestation; +Zn Day 19 through parturition	36.8 ± 4.3[a,b] (5)
(D) +Zn pair-fed to (C)	47.3 ± 3.9[b] (4)

* Mean ± SEM. No. of observations per group is given in parentheses. Any two means with different superscripts are significantly different at a P value of 0.05 or less.

Discussion. Sandstead *et al.* (1) showed that zinc-deficient human dwarfs responded to ACTH treatment only if they were given dietary supplements of zinc. Flynn *et al.* (3) found that rat adrenal glands challenged *in vitro* with ACTH in the presence of diethyldithiocarbamate would not synthesize corticosterone. From these reports one might postulate that zinc deficiency prevents corticosterone synthesis and/or release when animals are stimulated with ACTH. The data presented here do not totally support this hypothesis. Zinc deficiency in young male rats had no effect on either the resting level or the ACTH-induced level of corticosterone in the serum. In both experiments there was a tendency, although not significant, for an overproduction of corticosterone in the zinc-deficient animals in response to exogenous or endogenous ACTH. Quarterman (7) made a similar observation and suggested that zinc-deficient rats were hypersensitive to ACTH. In fact some signs of zinc deficiency may be the result of adrenal hyperfunction.

In support of the hypothesis that zinc deficiency affects corticosterone synthesis it was observed in this study that postparturient rats depleted of zinc produced significantly less serum corticosterone when given an additional stress a few hours after parturition. Zinc-depleted dams given zinc on Day 19 of gestation were able to partially respond to the second stress by producing serum corticosterone levels midway between the totally depleted and adequate groups. Apgar (8) observed low cholesterol in the adrenal tissue of zinc-depleted postparturient rats, indicating that the process of delivery was more traumatic to the depleted females than the controls. This might be expected since, as reported here, dams deprived of zinc throughout gestation exhibited profuse bleeding, low body temperature, and difficulty expelling the fetuses. Apgar also observed an increase in adrenal cholesterol at term in zinc-depleted pregnant rats which were zinc supplemented on Day 19 of gestation. Neither in Apgar's experiments nor in those reported here did the supplemented group show the signs prevalent in the zinc-depleted group. It is not clear why zinc deficiency in pregnant but not male rats causes a reduced output of corticosterone in response to a stress stimulus. The defect in the females could be failure of pituitary release of ACTH or failure of the adrenals to respond.

Apgar (8) reported a 20% increase in adrenal weights in zinc-deficient female rats post partum. In the present experiments there was an increase in adrenal weights of zinc-deficient young male rats relative to body weight, but not in absolute weight. Thus the difference may only exemplify the loss of fat, protein, and water caused by inanition.

Flynn *et al.* (2) made a point of the observation that under their experimental conditions, i.e., oligemic hypotension, the plasma zinc concentration was elevated along with that of ACTH. This suggested a functional relationship between zinc and ACTH, but no other type of stress was tested. Stressing zinc-deficient animals with air saturated with diethyl ether in the present study gave the same response to corticosterone production as in the zinc-supplemented controls. This stress situation did not increase the concentration of serum zinc in either group, suggesting that the increase in serum zinc observed by Flynn *et al.* (2) was initiated by a factor other than the release of ACTH.

Summary. The effect of zinc deficiency on *in vivo* synthesis and release of corticosterone was examined under three conditions, stimulation by exogenous ACTH and two types of physiological stress. The serum

concentrations of corticosterone were elevated by intraperitoneal injections of ACTH, but were not influenced by the zinc status of young male rats. Zinc-deficient male rats given an acute stress stimulus, exposure to diethyl ether vapors, responded with elevated levels of serum corticosterone that were not different from those of controls. Pregnant rats fed zinc-deficient diets throughout gestation and then subjected to an acute stress stimulus, surgery under anesthesia, after parturition had serum corticosterone concentrations 50% lower than control rats treated similarly. Zinc-deficient pregnant rats given zinc on the 19th day of gestation had corticosterone levels midway between the deficient and control rats. It was concluded that young zinc-deficient male rats respond with the normal release of corticosterone when challenged with exogenous ACTH or a simple physiological stress. Zinc-deficient female rats subjected to the severe stress of pregnancy, parturition, and surgery show a subnormal response.

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