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Zinc Deficiency Affects the Composition of the Rat Adrenal Gland[1] (42351)

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Abstract. The response of the adrenal gland to zinc deficiency was examined in male weanling rats. In comparison with decapsulated adrenals from *ad libitum* fed controls, glands from zinc deficient rats had greater relative weight (mg/g body wt), DNA concentration, and total lipid and cholesterol concentrations as well as a smaller protein/DNA ratio. Several of these differences (protein/DNA and cholesterol concentration) could be attributed to the inanition accompanying zinc deficiency, as zinc deficient values were similar to those of pair fed controls. Values for total DNA and protein concentration were similar for all groups. Electron micrographs of the zona fasciculata showed a small number of lipid droplets in the adrenals from *ad libitum* fed controls, an increase in lipid droplets from pair fed controls, and an even more striking increase in lipid droplets from the zinc deficient adrenals. The increased adrenal lipid composition in the zinc deficient group may be secondary to enhanced steroidogenesis or a zinc deficiency-induced defect of lipid metabolism.

Several studies have found that zinc deficiency alters the adrenal gland. Increases in adrenal weights relative to body weights have been reported for zinc deficient rats (1–5). Other reported alterations have included changes in cholesterol concentration: increases in weanling male rats (3), decreases in pregnant rats (1, 2), and greater concentration of corticosteroids in adrenals from zinc deficient weanling rats (3). Morphological alterations reported in response to zinc deficiency as compared to *ad libitum* fed controls include a marked increase in the number of lipid droplets in the rat adrenal cortex (6).

There are numerous unanswered questions about the nature of the adrenal response to zinc deficiency. The greater absolute or relative adrenal mass has been referred to as adrenal hypertrophy in response to stress (1, 2, 5), but it is unclear whether the greater mass is due to an increased number of cells or to increases or alterations in various cellular constituents. To examine these questions and to resolve differences due to inanition from effects of zinc deficiency, per se, we made weanling male rats zinc deficient and examined their adrenal morphology and decapsulated adrenal composition of total lipids, cholesterol, DNA, and protein. Results were compared with those from pair fed and *ad libitum* fed controls.

Materials and Methods. Male weanling Long Evans rats (Charles River Breeding Laboratories, Wilmington, Mass.) weighing 50–60 g were individually housed in stainless-steel, suspended cages in temperature and humidity controlled quarters. All rats were fed a purified diet[2] (modified AIN-76, outlined below) con-

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[2] The purified diet was based on the AIN-76 formulation and contained the following in grams/kilogram: egg white solids, 200; cornstarch, 150; sucrose, 501.815; cellulose,

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taining 20% egg white solids with biotin enrichment. The zinc content was 0.8 μg/g diet by analysis. Twenty-six rats were randomly assigned to one of three experimental groups. The zinc deficient (ZD) group (nine rats) was given purified diet and deionized water *ad libitum*. Individually pair fed control (PF) rats were fed diet equivalent in amount to that consumed by a corresponding ZD rat on the previous day. Eight *ad libitum* fed control (AL) rats were fed the diet *ad libitum*. Both control groups were given drinking water supplemented with zinc acetate at 25 μg Zn/ml. Food intakes and body weights of the rats were recorded daily for 45 days, a duration of dietary treatment previously shown to result in a maximal increase in lipid droplets in response to zinc deficiency (6).

On Day 45, the rats were anesthetized with pentobarbital sodium (Nembutal, 50 mg/ml) at a dose of 0.2 ml/100 g body wt. Two rats from each group were perfused through the heart with 3% glutaraldehyde (Ladd) solution in 0.1 *M* cacodylate buffer containing 0.02% $CaCl_2$ and 3% sucrose to fix the adrenal glands for electron microscopy (6). The adrenal glands and right femurs were removed from the remaining rats in each experimental group for biochemical analyses.

Prior to the biochemical determinations, the capsule was removed from each adrenal gland by dissection and each gland weighed individually. The left adrenal gland was homogenized in 2 ml of 0.05 *M* sodium phosphate in 2 *M* sodium chloride, pH 7.4, by using ground glass homogenizers and then sonicated for 2 min in a sonicating water bath. DNA determinations were performed by the method of Labarca and Paigen (7). Protein analysis was by the method of Lowry *et al.* (8). The right adrenal gland was homogenized in chloroform: methanol (2:1, v:v) to extract lipids (9). Aliquots of the extracts were taken for gravimetric determination of total lipids. Separate aliquots of the extracts were dried under nitrogen and saponified with alcoholic potassium hydroxide (10). After extraction into hexane, total cholesterol was determined by the method of Zak *et al.* (11).

The concentrations of zinc in the femur and in the diet were determined by atomic absorption spectrophotometry after wet digestion in Ultrex nitric acid (Baker Chemical Co.). Prior to analysis, the marrow was removed from bones with stainless steel instruments and the femurs were subjected to lipid extraction by refluxing in chloroform:methanol (2:1, v:v (9)). Samples of diet and extracted bones were dried by heating at 60°C overnight prior to weighing. Wet digestion was performed by the method of Clegg *et al.* (12). Absorbancies were determined using a Perkin Elmer Model 403 atomic absorption spectrophotometer and compared with those of standards in the range of 0.0 to 1.0 μg Zn/ml prepared from a commercial standard (Alfa Products, Danvers, Mass.).

Electron microscopy was performed as previously described (6). Briefly, after perfusion the adrenals were removed and placed in the perfusion medium for 30 min. The glands were then sliced several times and rinsed with cacodylate buffer prior to postfixation with 1% osmium tetroxide in cacodylate buffer. The tissues were then dehydrated in a graded series of ethanol and embedded in Epon 812. Thick sections were mounted for light microscopy with methylene blue and azure II (13). Thin sections were mounted on copper grids and stained with alcoholic uranyl acetate and lead citrate (14) prior to study in a Philips EM-300 electron microscope.

All data were statistically analyzed by a one-way analysis of variance. The results of this analysis were interpreted by subjecting them to the Neuman–Keuls test for the assessment of level of significance of the *F* ratios for pairs of data (15). Significance was assessed on the basis of *P* values less than 0.05.

Results. The rats in the ZD group in this study showed signs of anorexia within 4 days of being fed the zinc deficient diet and subsequently displayed a cyclical pattern of food intake similar to that described previously (17). Growth rates of the ZD and PF rats were severely impaired (Fig. 1). Although initially similar in body weights, PF rats were heavier than their ZD counterparts onward from Day

50; corn oil, 50; AIN-76 salt mix modified to delete the zinc, 36.172; AIN-76 vitamin mix, 10; choline bitartrate, 2; biotin, 0.0025; ethoxyquin, 0.01. Diet was obtained from Teklad, Inc. (Madison, Wisc.).

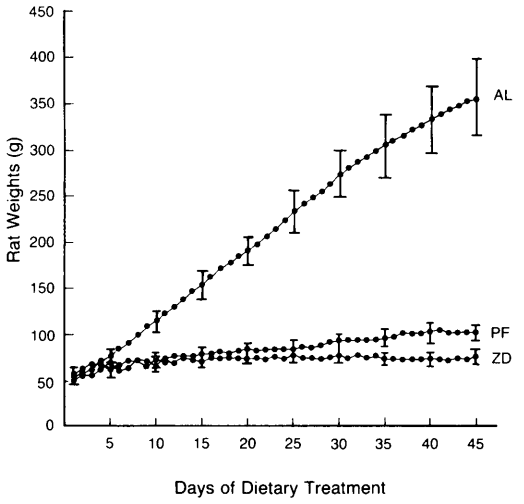

FIG. 1. Average rat weights from the three dietary treatment groups; zinc deficient (ZD), pair fed (PF), and *ad libitum* fed control (AL). Error bars represent standard deviations.

35 of the feeding regimen. Some of the other symptoms observed in the ZD rats included alopecia, dermal lesions, diarrhea, and priapism. Near the end of the experiment, two of the ZD rats died from undetermined causes.[3] The zinc status of the rats was confirmed by analysis of the zinc content of femurs (Table I). PF and AL animals showed much higher zinc concentrations, although PF animals had a lower concentration than did the AL animals, perhaps related to a lesser consumption of the supplemented drinking water.

Zinc deficiency and the inanition that accompanies it influenced both the weight and composition of the adrenal glands. Although the decapsulated adrenal glands from ZD and PF rats weighed less than those from AL controls, as a fraction of body weight, adrenals from the ZD and PF groups were much greater (Table II). In addition, ZD adrenals were heavier than those from PF controls and comprised a greater fraction of body weight. Zinc deficiency also affected several of the components of the adrenal gland (Table III). DNA concentration, the protein/DNA ratio, lipid

[3] As dietary intake data were available, pair fed controls were continued in the experiment to completion.

TABLE I. CONCENTRATIONS OF ZINC IN FEMURS FROM ZINC DEFICIENT, PAIR FED, AND *ad Libitum* FED CONTROLS

	n	Zinc concentration (μg/g)
Zinc deficient	5	81.6 ± 8.3[a]
Pair fed	7	312.5 ± 5.2[b]
Ad libitum fed	6	372.1 ± 16.2[c]

Note. Values are means for the right femurs ± SEM. Values not sharing a common superscript are statistically different at $P < 0.01$.

concentration, cholesterol concentration, and the cholesterol/total lipid ratio were all significantly different from values for the *ad libitum* fed controls. Several of these differences could be attributed to the inanition accompanying zinc deficiency, as values were similar between ZD and PF animals; this was so for the protein/DNA ratio, cholesterol concentration, and the cholesterol/total lipid ratio.

The morphology of the adrenal cortex was studied because a previous study reported alterations in structure as a result of zinc deficiency (6). The parenchymal cells of the zona fasciculata from AL rat adrenals contained oval to round nuclei, mitochondria with vesicular cristae, microvilli, and a small number of lipid droplets which abutted the mitochondria (Fig. 2). In contrast to the normal morphology of the AL rat adrenal zona fasciculata, the most consistent feature observed in the PF rat adrenals was the marked increase in lipid droplets in the zona fasciculata (Fig. 3). These

TABLE II. WEIGHTS OF ADRENAL GLANDS FROM ZINC DEFICIENT, PAIR FED, AND *ad Libitum* FED CONTROLS

	n	Wet weight (mg)	Relative wet weight (mg/100 g body wt)
Zinc deficient	5	14.6 ± 1.3[a]	20.2 ± 1.3[a]
Pair fed	7	12.8 ± 1.0[b]	12.0 ± 1.3[b]
Ad libitum fed	6	20.4 ± 3.8[c]	6.9 ± 0.8[c]

Note. Wet weight values are means for both adrenal glands without capsules ± SEM. Wet weight values not sharing a common superscript are statistically different at $P < 0.05$; relative wet weight values not sharing a common superscript are statistically different at $P < 0.01$.

TABLE III. PROTEIN, DNA, AND LIPID COMPOSITION OF ADRENAL GLANDS FROM ZINC DEFICIENT, PAIR FED, AND *ad Libitum* FED CONTROLS

	n	Protein concentration (mg/g)	DNA concentration (mg/g)	Total DNA (μg)	Protein/DNA	Lipid concentration (mg/g)	Cholesterol concentration (mg/g)	Cholesterol/ total lipid (%)
Zinc deficient	5	166.0 ± 7.6[a]	8.6 ± 0.5[a]	72.6 ± 4.3[a]	19.4 ± 1.6[a]	121.7 ± 12.6[a]	43.0 ± 5.3[a]	35.8 ± 3.7[a]
Pair fed	7	179.8 ± 8.2[a]	10.5 ± 1.2[b]	61.5 ± 6.8[a]	18.1 ± 1.5[a]	93.5 ± 7.2[b]	37.7 ± 3.9[a]	40.3 ± 2.9[a]
Ad libitum fed	6	177.3 ± 9.9[a]	6.5 ± 0.6[c]	61.9 ± 7.7[a]	28.0 ± 2.6[b]	61.6 ± 2.9[c]	9.4 ± 1.2[b]	15.5 ± 2.1[b]

Note. Values are means for one adrenal gland without capsule ± SEM. In each column, values not sharing a common superscript are statistically different at $P < 0.01$.

droplets tended to occur in clusters and abutted mitochondria. More observable lipid was present in the ZD than in the PF adrenals (Fig. 4). Lipid droplets and mitochondria almost filled the parenchymal cells. There were often differences in lipid electron density between ZD and PF adrenals which may reflect differences in functional states as well as in the quality of fixation and the degree of dissolution of the lipid.

Discussion. When male weanling rats were fed a zinc deficient diet for 45 days, the deficiency symptoms observed were similar to those reported previously (16), including anorexia, cyclic feeding, and growth retardation. Other symptoms generally consistent with reported manifestations of zinc deficiency included alopecia, dermal lesions, and priapism. Zinc status of the rats was evident in the concentration of zinc in femurs. Concentrations of zinc in the femurs from rats of the ZD group were significantly lower than those from PF and AL rats and comparable to those previously reported for zinc deficient animals (6). Zinc concentrations in femurs from PF rats, although much greater than for ZD rats, were also significantly lower than those from the AL rats. The reason for this finding is unclear, but may be attributed in part to differences in water intake. AL rats may have consumed more zinc supplemented water as they consumed more food. The ratio of food intake to water intake has been estimated at 1:1.9 (18). Thus, differences in zinc intake likely occurred; however, this cannot be stated with certainty because water intake was not quantitated.

Zinc deficient states produced by several different research designs have been reported to increase adrenal weights. Pregnant rats deprived of zinc during all or portions of gestation showed some degree of increased adrenal weight which was attributed to adrenal hypertrophy (1, 2, 5). This response was particularly evident when expressed as a fraction of body weight. Other studies using weanling male rats have also reported increased adrenal weights relative to body weights when zinc deficient animals were compared to pair fed controls (3, 4). In the present study, the weight of decapsulated adrenal glands from ZD weanling male rats relative to body weight was

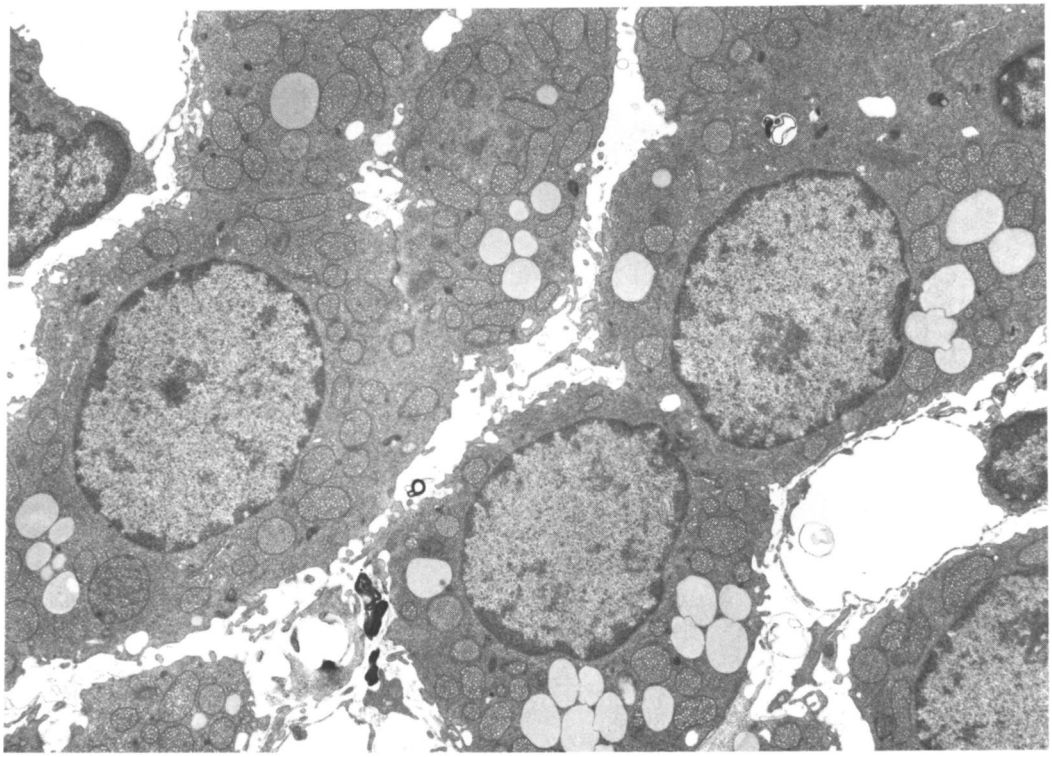

FIG. 2. Representative electron micrograph of the zona fasciculata of the adrenal gland from an AL rat. (×16,150)

greater than from pair fed and from *ad libitum* fed controls. The increased relative or absolute adrenal mass in zinc deficiency has been referred to as adrenal hypertrophy in response to stress (1, 2, 5). The present study is the first to our knowledge to analyze decapsulated adrenals for lipids, DNA and protein and to compare adrenocortical morphology of PF and ZD rats.

Increased adrenal mass could reflect either hypertrophy or hyperplasia, or perhaps a combination of the two. Assuming that the cells of the adrenal gland contain a constant amount of DNA per cell (19), total DNA content should be related directly to cell number. Neither zinc deficiency nor pair feeding had significant effects on the total DNA content of the decapsulated adrenal gland, indicating that the greater mass was not due to hyperplasia. Significant differences in DNA concentrations can be interpreted on the basis of constant cell number and variable gland weight. Although the concentrations of protein did not vary between groups, the ratio of protein to DNA was higher in the adrenals from AL rats than ZD or PF animals. This would seem to indicate that the adrenals from ZD and PF rats were composed of smaller cells, although lipid content of the cells could also influence cell size.

The decapsulated ZD adrenal gland had the highest concentration of total lipid; adrenals from pair fed controls also showed increased total lipid compared to *ad libitum* fed controls, albeit significantly less than that resulting from zinc deficiency. These findings are consistent with the electron microscopic observations of increased lipid droplets in ZD and PF animals (Figs. 2–4) and with published comparisons of ZD and AL animals (6). Although the nature of the lipid droplets has not been completely determined, a considerable portion of the droplet may be cholesterol. In this study, the higher concentration of total lipid observed in the adrenals from PF rats in comparison with AL controls could be accounted for by the increase in cholesterol concentration. This

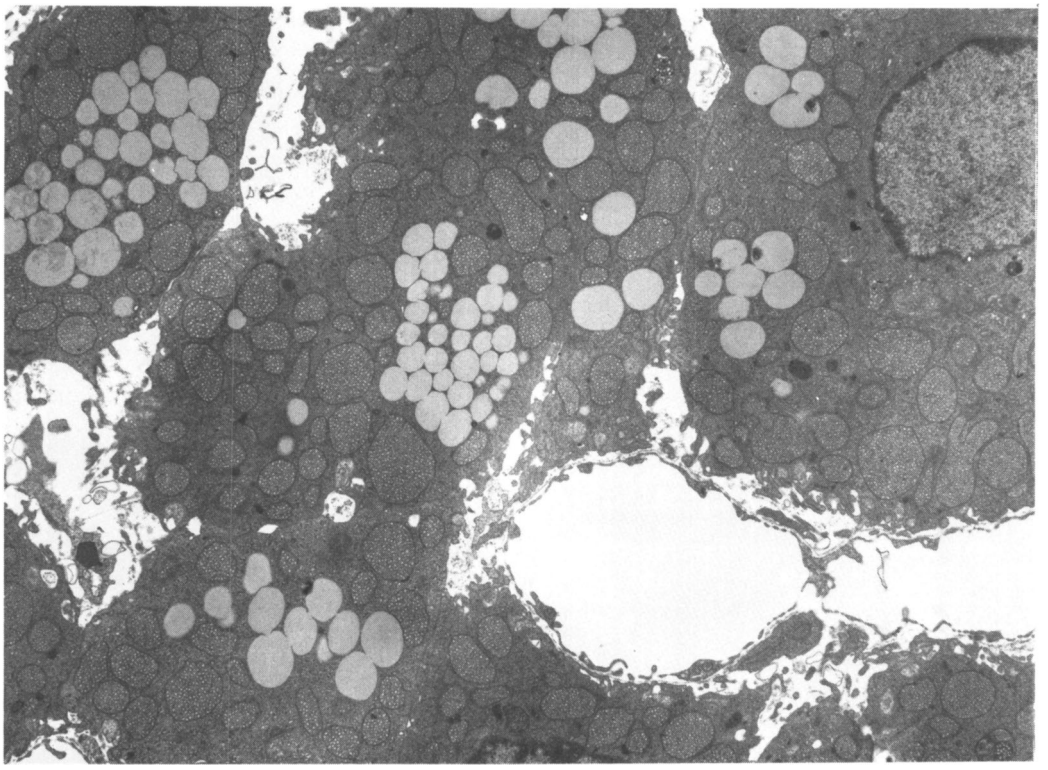

FIG. 3. Representative electron micrograph of the zona fasciculata of the adrenal gland from a PF rat. (×16,150)

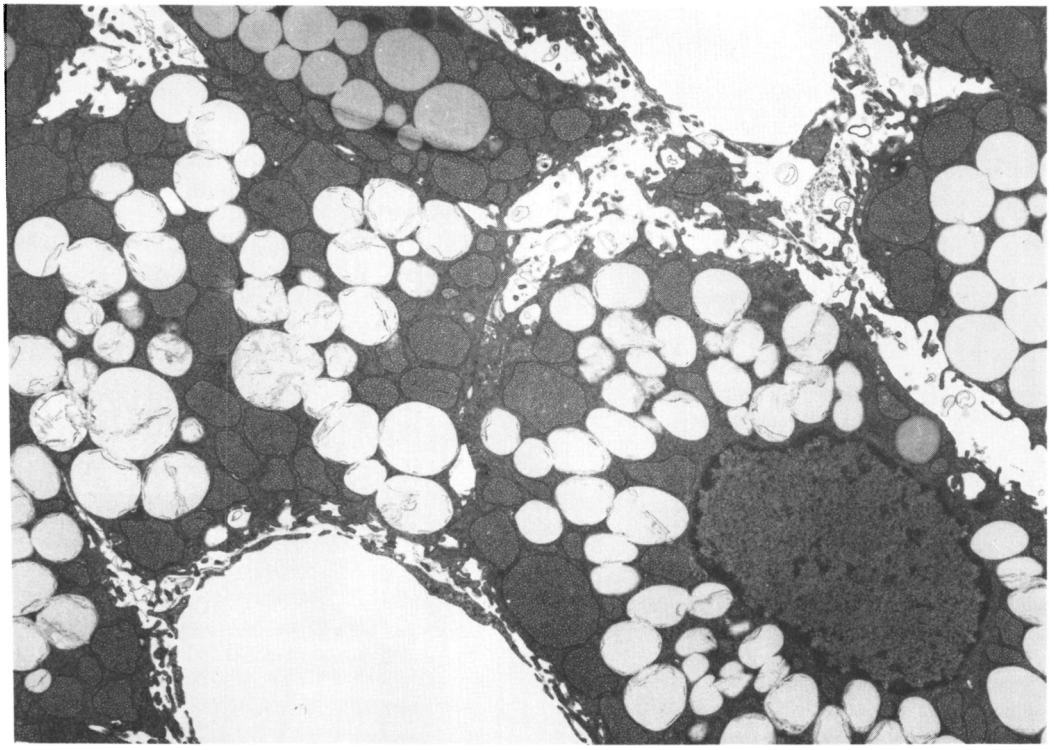

FIG. 4. Representative electron micrograph of the zona fasciculata of the adrenal from a ZD rat. Lipid droplets almost fill the cells. (×16,150)

was not the case for the ZD rats; although cholesterol concentrations were higher, they were not great enough to account for the striking increase in total lipid concentration in response to zinc deficiency. Previous studies have also reported changes in cholesterol concentration in response to zinc deficiency, although the nature of the change was not consistent. In agreement with the present study, Quarterman and Humphries (3) found increased cholesterol concentration in adrenals of zinc deficient male rats, whereas Apgar (1, 2) reported lower cholesterol concentrations in adrenals from zinc deficient pregnant rats. The reasons are unclear why zinc deficiency in the pregnant animal, but not in young male rats, resulted in reduced adrenal cholesterol concentration and reduced output of corticosterone (4) in response to a stressor. Endocrine differences between sexes, severity of stress, and duration of stress are likely important factors.

The remaining portion of the total lipid remains unidentified. Preliminary studies showed that phospholipid concentrations did not vary significantly between dietary treatment groups (20). Although it did not prove feasible to determine triglyceride concentrations, these compounds may have contributed to the elevated lipid concentration in zinc deficient rats. A greater sensitivity of the adrenal to ACTH has been reported for zinc deficient rats (3) and chronic ACTH administration has been shown to result in increased adrenal triglycerides (21). Corticosterone may also contribute to the increased lipid fraction observed in the zinc deficient animals. Corticosterone has been found in the lipid droplet fraction of decapsulated rat adrenal gland (22) and greater concentrations of corticosteroids in adrenals from zinc deficient than from pair fed controls have been reported (3). The increased levels of corticosteroids are thought not to be due to impairment of release as several studies have shown that release was normal or elevated in zinc deficient animals (3, 4, 23). Further research will be required to establish whether the increased lipid droplets seen in response to zinc deficiency represent a morphological manifestation of enhanced steroidogenic capacity of the adrenocortical cells of the zona fasciculata or a more generalized impairment of lipid metabolism secondary to impaired protein synthesis as has been reported for pancreatic acinar cells and for absorptive cells of the small intestine (24, 25).

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