there exists an AAA trough at 8 days in the manipulated rat and a rise in AAA at 12 days; for the nonmanipulated animal, this pattern is present at 10 and 14 days of age. Thus we find here, as in the case of other developmental parameters, an accelerated pattern of development as a function of infantile stimulation.

What is unique about the AAA maturational pattern is the initial high values declining to a period of low values and the return to what appear to be the adult values. The nature of this decline is difficult to evaluate. It would be of interest to determine whether resting levels of corticosterone show a corresponding elevation at this time.

It appears that a transitional period is under way in the adrenal and, hypothetically, in the central nervous system during this period. This transition may be related to the maturation of the neuroendocrine regulation of the stress response.

In one sense, the analysis of the data concerning resting levels of AAA supports the position taken by Ader, since the presentation of depletion scores masks the presence of maturational differences in the nonstressed animals. Future analyses based on AAA levels should always include a presentation of the actual values as well as depletion scores. However, the presentation of the original nonstressed and stressed values does not alter the interpretation based on depletion scores. It might have been argued that at 12 days of age the nonmanipulated subjects fail to show a significant AAA depletion to cold because of the very low resting levels of AAA. However, at 14 days of age, when there exists in both groups of subjects what would appear to be sufficiently high values of AAA, the nonmanipulated subjects still fail to show significant depletion. Also in Shapiro's study, like the 8-day-old nonmanipulated animals in our present study, infant animals with high AAA values fail to show either depletion of AAA or elevation of corticosterone to stress. Shapiro calls this the "stress non responsive period."

Shapiro does, however, report a significant AAA depletion in response to electric shock, along with an elevation of adrenal corticosterone as early as 8 days of age. In contrast, the earliest adrenal response to cold occurs at 12 days of age. This difference is probably due to the nature of the stress. The response to cold is dependent upon other mechanisms, such as temperature regulation, in addition to the neuroendocrine mechanisms controlling ACTH. The correlation of the onset of thermoregulatory mechanisms and other endocrine responses is another of the many problems to be investigated in this area (*9*).

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Antagonistic Relationship between Dietary Cadmium and Zinc

Abstract. *The growth of chicks decreased, and specific abnormalities of hocks and feathers increased, when cadmium was added to a zinc-deficient diet. Supplementation of the diet with zinc prevented the adverse effect of cadmium on hock and feather development and partially offset the effect on growth. Changes in the gizzard lining, also resulting from cadmium ingestion, were partially prevented by increase in the zinc intake.*

In previous limited tests with young turkeys in this laboratory (*1*), the addition of cadmium to a zinc-deficient diet was found to accentuate symptoms of zinc deficiency. This adverse effect of cadmium was reduced by increasing the zinc content of the diet, a result which suggested a reversible antagonistic relationship between the two elements. Because of the implications of the results in relation both to the problem of cadmium toxicity and to studies of the nutritional role of zinc, additional experiments were conducted with chicks.

The tests were carried out in galvanized steel brooders with tap water supplied in stainless steel containers. Under these conditions, chicks fed a basal diet containing no supplementary zinc (*2*) grow at a subnormal rate and exhibit a moderate incidence of hock and feather abnormalities characteristic of zinc deficiency (Fig. 1), whereas growth, hock development, and feather development are normal when the diet is adequately supplemented with zinc.

Results of three tests in which the basal diet was supplemented with zinc and cadmium, singly and in combination, are given in Table 1. Supplementation with zinc alone increased growth significantly over that obtained with the basal diet and eliminated the hock and feather abnormalities. In contrast, the addition of cadmium to the basal diet increased the abnormalities and progressively lowered the growth rate. The data of experiments 1 and 3 suggest that abnormal development of the hocks and feathers is relatively reduced, or is less definitely identifiable on visual inspection, when growth is very severely reduced. When cadmium treatment was coupled with adequate zinc supplementation, the hocks and feathers developed normally. The growth-depressing effect of cadmium was markedly reduced but not eliminated by zinc supplementation, the degree of irreversibility of the effect on growth being greatest at the highest level of cadmium used (80 parts per million).

Additional but less consistent evidence of an antagonistic relationship between zinc and cadmium was obtained after the observation, in preliminary work, that cadmium feeding produced marked changes in the gizzards of chicks. Typically, these changes consisted of a bleaching of the gizzard lining from its normally yellow or yellowish brown color to, in extreme cases, an ivory white; roughening of the entire surface of the lining; and development of ulcer-like erosions similar to those frequently seen even in chicks fed conventional rations but more extensive and severe. Occasionally the lining adjacent to severe erosions separated from the underlying tissue. All these gizzard effects tended to be reduced by increased intake of zinc, though the degree of protection was variable, lessening of the effects being in some instances quite obvious, in others scarcely detectable, and never complete.

The extent to which the effects observed in these tests reflect direct and variably reversible inhibition by cad-

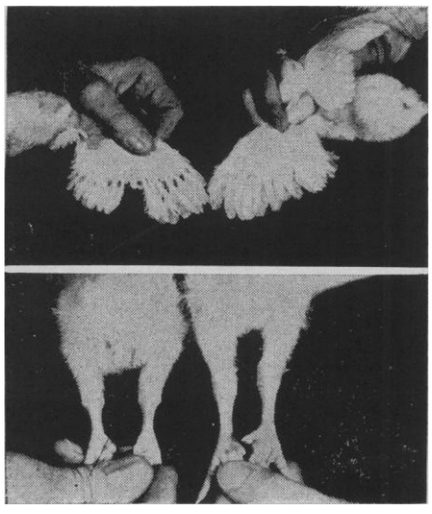

Fig. 1. Hock and feather abnormalities characteristic of zinc-deficient chicks. Wing (top left) shows abnormal thinning of wing feathers; wing (top right) is normal. Legs (bottom left) show knobby enlargement of hocks; legs (bottom right) are normally tapered. Both abnormalities, as illustrated, are relatively mild.

mium of essential zinc-dependent reactions remains to be established, but there is good basis for believing it to be considerable. Parizek (*3*) suggested interference with zinc function as the mechanism of a cadmium-induced pathology, after his observation that testicular degeneration produced in rats by the injection of a small dose of Cd^{++} was prevented if a large excess of Zn^{++} was administered simultaneously with the cadmium. Antagonistic zinc-cadmium effects in relation to testicular function also have been described by Kar *et al.* (*4*) and by Gunn and his co-workers (*5*). Cotzias *et al.*, in tracer studies with rabbits and mice, obtained sufficient evidence of interchange between zinc and cadmium (*6*) and of lack of discrimination between the two elements by certain cell fractions (*7*) to propose that cadmium may be a very subtle poison, competing with zinc and partially replacing it in some organelles and at certain cellular binding sites.

Table 1. Weight gains and incidence of hock and feather abnormalities in chicks fed cadmium or zinc, or both. Day-old Arbor Acre male chicks were used in all the tests.

Added Cd (ppm)	Added Zn (ppm)	Gain (g)	Abnormalities*	
			Hocks	Feathers
Experiment 1 (14 days)				
0	0	165	2/7	2/7
0	20	177	0/8	0/8
0	200	204	0/8	0/8
20	0	114	8/8	7/8
20	20	177	1/8	2/8
20	200	180	0/8	0/8
40	0	103	4/8	5/8
40	20	156	4/8	1/8
40	200	175	0/8	1/8
Experiment 2 (15 days)				
0	0	185	2/8	1/8
0	200	230	0/7	0/7
40	0	107	7/8	8/8
40	200	183	0/8	0/8
40	400	168	0/8	0/8
Experiment 3 (14 days)				
0	0	147	0/14	4/14
0	200	188	0/14	0/14
40	0	111	8/14	8/14
40	200	152	0/14	0/14
40	400	156	0/14	0/14
80	0	62	2/14	6/14
80	200	131	2/14	0/14
80	400	114	0/14	0/14

* Ratio of surviving birds with abnormalities to total surviving birds.

Whatever the specific mechanisms involved, the findings described in this report indicate that abnormal zinc metabolism contributes significantly to the toxicity syndrome resulting from chronic cadmium ingestion. The results suggest, also, that cadmium feeding might prove useful in studies in vivo of the physiological functions of zinc (*11*).

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2. The basal diet, in percentages, is as follows: 31, C-1 Assay Protein (Archer Daniels–Midland Co.); 57, glucose monohydrate (Cerelose); 5, corn oil (Mazola); 0.6, DL-methionine; 0.75, glycine; 0.02, antioxidant (ethoxyquin); 0.017, antibiotic (25 percent oleandomycin mix, Pfizer); 0.025, $FeSO_4 \cdot 7H_2O$; 0.003, $CuSO_4 \cdot 5H_2O$; 0.03, $MnSO_4 \cdot H_2O$; 0.0002, $CoSO_4 \cdot 7H_2O$; 0.002, KIO_3; 0.001, H_3BO_3; 0.02, $KAl(SO_4)_2 \cdot 12H_2O$; 0.001, $Na_2MoO_4 \cdot 2H_2O$; 0.005, $Na_2SiO_3 \cdot 9H_2O$; 0.002, NaBr; 0.00006, Na_2SeO_3; 0.3, NaCl; 0.77, KCl; 0.73, Na_2HPO_4; 3.21, $Ca_3(PO_4)_2$; 0.25, $MgSO_4 \cdot 7H_2O$; 0.23, $CaCO_3$; 0.48, vitamin mixture. The vitamin mixture supplies components (in milligrams per 100 g of diet) as follows: 200, choline chloride; 100, inositol; 0.6, menadione sodium bisulfite; 0.003, vitamin B_{12}; 2, ascorbic acid; 0.5, *p*-aminobenzoic acid; 1, folic acid; 0.08, biotin; 3, pyridoxine HCl; 7, niacin; 4, calcium pantothenate; 2, riboflavin; 2, thiamin HCl; 3.2, vitamin A concentrate (325,000 I.U./g), 12.5, vitamin D concentrate (15,000 U.S.P. unit/g); 140, vitamin E concentrate (44 I.U./g). The diet contains adventitious zinc in the amount of 15 parts per million. Supplementary zinc was added in the form of $ZnCO_3$; cadmium, as a 1.63-percent aqueous solution of $CdCl_2$.
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Chromosome Fibers from an Interphase Nucleus

Abstract. *Red blood cells of the newt,* Triturus, *were spread on a water surface and picked up with carbon-coated grids for examination in the electron microscope. The identifiable nuclear material consists entirely of long fibers having a diameter of approximately 400 to 600 Å. Similar fibers have been seen in human and grasshopper chromosomes prepared in the same manner.*

Conventional sectioning techniques have proved to be of limited value in nuclei studies with electron microscopy. Whole chromosome mounts and interphase nuclei have been examined but they are generally too thick for high resolution studies. Two technical problems must be overcome in developing a successful "squash" or "spread" technique for electron microscopy: (i) the nuclear material must be spread into a very thin layer, and (ii) it must be dried without undue destruction.

A solution to the first problem is suggested by the recent work of Kleinschmidt and Lang (*1*), who have obtained striking preparations of fibers by spreading bacteria and viruses on a liquid surface. Drying in air may be adequate in some cases, but the critical point method of Anderson (*2*) should be more generally useful.