head with tightly closed blowhole, inside head with open blowhole, and at blowhole slit) suggests that these animals can use each of several parts of the nose for these whistles, rather than just a single area (*4, 6, 7, 9, 13, 14*).

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31 October 1962

Developmental Pattern of Adrenal Ascorbic Acid in the Rat

Abstract. *Adrenal ascorbic acid was determined in nonstressed, newborn rats. The maturational pattern of the acid consists of high resting levels early in development, a marked decrease during a period between 8 and 12 days, and a subsequent return to high levels. The onset of the maturational pattern is accelerated by previous manipulation of the newborn rat.*

In a recent letter in this journal, Ader (*1*), in response to a paper of Schaefer *et al.* (*2*), discussed the use of ascorbic acid depletion scores as follows: "The main difficulty, however, lies in the fact that only *depletion* scores are presented. In this the authors are apparently following an unfortunate precedent [(*3*)], but a depletion score is a poor substitute for the actual values observed in stressed and nonstressed animals, since the same depletion score may be a resultant of a variety of actual values." In view of these comments, we reanalyzed the data from previous studies concerned with ascorbic acid depletion in response to cold stress in infant rats that had been manipulated from birth and in controls which had received no experimental treatment. What proved interesting from this analysis was the pattern of resting (nonstressed) values of ascorbic acid as a function of development and the differences in this maturational pattern with respect to infantile stimulation.

Previous research on the effects of infantile experiences on development have indicated that, in general, animals that have received some form of stimulation during infancy appear to exhibit an overall acceleration of growth and development. Adrenal ascorbic acid (AAA) depletion in response to cold stress (*3*) and the opening of the eyes (*4*) appear 3 to 4 days earlier in stimulated rats. Brain myelination also occurs earlier in stimulated rats (*5*), and there are differences in body weight, with the stimulated rats showing significantly heavier body weights both at weaning and in adulthood. The data presented here also indicate a difference in maturation with early experience and further demonstrate an interesting maturational pattern with respect to AAA. Some of these data are derived from previous studies.

One hundred and eighty-four infant Sprague-Dawley Holtzman rats were used as subjects. Approximately half (48 ♂, 47 ♀) the rats were subjected to a standardized manipulation procedure which consisted of removing the pups from the nest, placing them in a small compartment for 3 minutes, and returning them to the nest. The procedure was followed once daily until the infant was sacrificed. The remaining infants (48 ♂, 41 ♀) received no treatment and were not manipulated in any manner until the time they were sacrificed. Animals were sacrificed at 8, 10, 12, 14, and 16 days of age. At each of these ages, the pups were removed from their cages, killed quickly by cervical spinal separation, and weighed. Their adrenals were removed, weighed on a 25-mg Roller Smith balance, and analyzed for ascorbic acid content. Adrenal ascorbic acid was assayed by a modification of the technique used by Glick *et al.* (*6*). This procedure has been described in detail in a previous publication (*7*).

The results are presented in Fig. 1. The initial analysis of sex differences

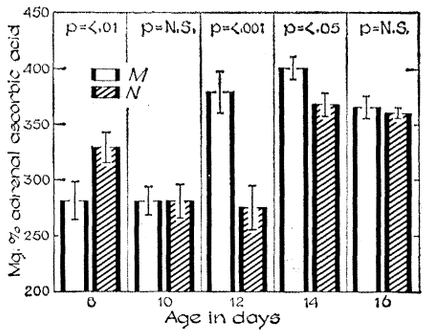

Fig. 1. Mean values of adrenal ascorbic acid at various ages. The open bars represent the values for the manipulated animals. The striped bars represent the values for the nonmanipulated animals. Small bars above and below the mean are the standard error of the mean.

proved insignificant. The data presented include both sexes and indicate that in the nonmanipulated rats the resting levels of AAA are high at 8 days of age, drop markedly at days 10 and 12, and then show a rapid rise at day 14, with an apparent leveling off after that period. In contrast, lower resting levels of AAA are apparent at 8 and 10 days of age for the manipulated animals, with rapid elevation occurring at 12 days of age and high AAA values thereafter. On the basis of these data alone, it would be difficult to plot a developmental curve of AAA values. However, Shapiro *et al.* (*8*) have recently presented values of AAA for the ages of 1 through 4 days. These data indicate that during the early periods of development AAA continues to be high. Figure 2 presents the maturational curve for AAA development in both manipulated and nonmanipulated rats. It should be noted that although the shape of the curve is essentially the same for both groups, there is a general displacement by 2 days, so that

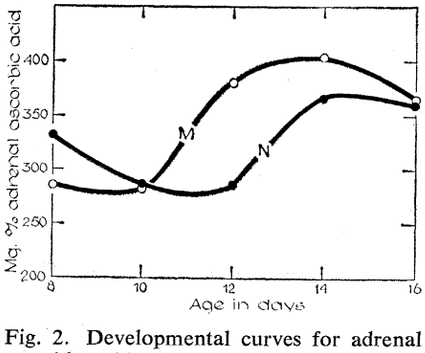

Fig. 2. Developmental curves for adrenal ascorbic acid. *M* represents the curve for manipulated infant rats, and *N* the curve for the nonmanipulated infant animals.

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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13 November 1962

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-