Comparative Effects of ACTH, Cortisone, Corticosterone, Desoxycorticosterone, Pregnenolone on Growth and Development of Infant Rats. (18728)

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In a previous paper(1), it was reported that desoxycorticosterone (DCA) injected into newborn rats in daily doses of 0.25 mg did not affect body growth but stimulated eye opening and eruption of teeth. This hastening of development was apparently not mediated through the thyroid gland because the phenomenon occurred despite depression of the gland by means of thiouracil(2). Aqueous adrenal cortical extracts had an effect similar to DCA indicating that the active principle was not solely related to the "salt and water" hormone.

The present report deals with the effects of more recently available hormones on growth and development of infant rats.

Method. Over 200 newborn albino rats of mixed strain were used. Wherever possible, the individual litters were divided so that there were 4 or 5 rats receiving the test drug and an equal number of litter-mates of the same sex distribution acting as controls. Unless otherwise stated, two or more litters were used for each experimental dose. After marking the rats for identification, the experimental and control rats were returned to the mother so that environmental and nursing conditions were identical. Injections using non-sterile technic were given subcutaneously and were usually begun when the rats were approximately 24 hours old; their weight at that time was 6 or 7 g. The control rats received injections of the solvent or suspending medium of the experimental drug. Observations were made once or twice daily on body weight, eruption of incisor teeth, development of the gingivae, and opening of the eyelids.

Results. Adrenocorticotropic hormone (ACTH) was injected in doses of 0.1 mg to 0.75 mg (of LA1-A Standard) per day usually in 2 or 3 divided doses for 8-12 days. With doses up to 0.4 mg per day, little or no effect was noted on body weight or eyelid and tooth development. When the dose was increased to 0.75 mg per day, a 15-20% decrease in body weight as compared to litter-mate controls was noted after 7 days. Upon cessation of injections, there occurred a rapid gain in weight so that by the 14th to 18th day of life, the weight was equal to that of the controls. There was only a questionable effect on development with very slight effect on eye opening or tooth eruption. Higher dose experiments are planned now that the supply of ACTH is greater.

Corticosterone was injected into 5 rats in single daily doses of 0.2 mg for 8 days. No demonstrable effect was noted on body weight but a definite hastening of teeth eruption and eye opening occurred. The experimental animals had erupted incisor teeth and showed opening of the eyelids 1 to 1½ days before their controls.

Cortisone. A single injection of 0.25 mg of cortisone at 24 hours of age resulted in a failure to gain weight normally for several days. By two weeks of age, the rats had recovered sufficiently to approach the weight of their litter-mate controls. Two consecutive daily injections of 0.25 mg beginning on the second day of life resulted in a similar effect on body weight but by the second week, they still weighed several grams less than their controls. Three or more consecutive daily injections of 0.2 mg beginning at 24 hours of age resulted in death of most of the animals. Apparently 0.6 mg or over of cortisone given in the first few days of life is a lethal dose.

* New York City. ACTH, Armour Lab.; Cortisone, Merck & Co.; Corticosterone, courtesy of Dr. E. C. Kendall; DCA and Pregnenolone, Schering Corp.

1. Mulinos, M. G., and Parmer, L. G., *Science*, 1942, v95, 484.

2. Parmer, L. G., PROC. SOC. EXP. BIOL. AND MED., 1947, v66, 574.

This indicates that the baby rat is markedly sensitive to cortisone as compared with other adrenal hormones studied. Five daily injections of 0.1 mg of cortisone beginning at 24 hours of age resulted in marked inhibition of growth with stimulation of development as judged by eruption of teeth, separation of the lips from the dental gingiva, and opening of eyelids. The teeth erupted on the average of 2.5 days sooner and the eyes 2.8 days sooner than litter-mate controls. A similar dose begun after 48 hours of age had slightly less effect on growth inhibition as well as developmental changes (1.6 days difference in teeth eruption and 1.8 days for eye opening). Injection of 0.15 mg of cortisone for 5 days produced results similar to the 0.1 mg dose except that body weight gain was slightly more retarded. With either dose, the inhibition of body growth and the failure to regain weight equal to that of the controls persisted for the duration of the experiment (90 days) at which time some of the cortisone-treated rats weighed 10 to 15% less than the controls.

FIG. 1. Effect of cortisone on growth. Rats are litter-mate males, age 16 days. The one on right weighing 15 g received subcutan. over back area 0.1 mg cortisone/day for 5 days during first week of life. Note incidental infection at injection site. The other rat weighing 23 g received corresponding volume of suspending medium as control injection.

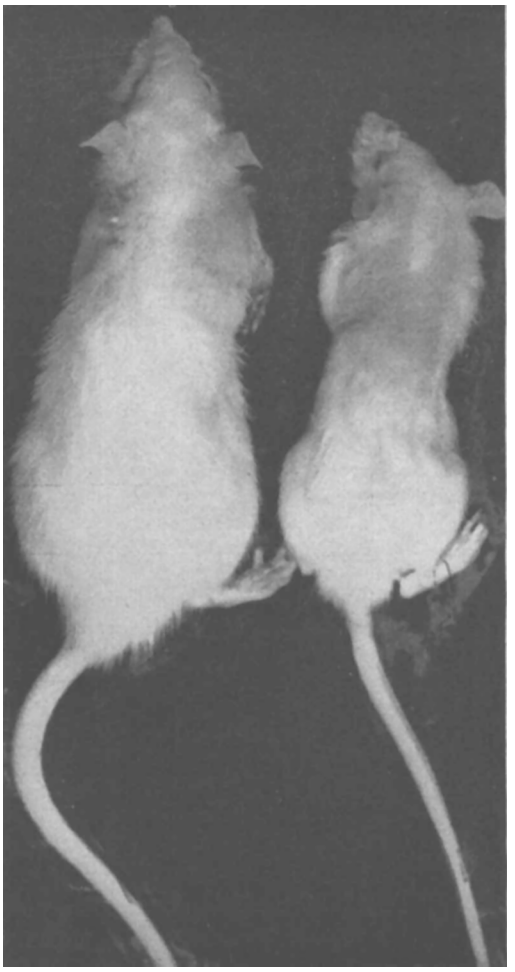

FIG. 2. The same rats 6 weeks later. This cortisone-treated rat which was the most severely stunted in its group weighed half as much as its litter-mate control.

It was noted that many of the cortisone-treated rats were more susceptible to infection as judged by the occurrence of subcutaneous infections at the injection site and the development of diarrhea with loose yellow stools. It was not clear whether or not this was nonspecific and related to their general ill health. Hair growth was also found to be somewhat retarded in cortisone-treated rats. As an incidental finding, it was observed that 0.1 mg of cortisone per day for 3 days hastened the death of infant rats that were malnourished. This occurred in one litter where the mother became temporarily ill, and could not nurse

well. All 5 of the experimental rats died on the average of 2 days before the controls began to succumb. Three controls died and 2 survived. It would appear that this particular dose of cortisone is detrimental to infant rats during a period of stress due to malnutrition.

Toxicity to cortisone in the infant rat is mainly demonstrated by failure to gain weight. (Fig. 1, 2). At no time was edema seen such as is reported for man(3). On the contrary, the higher the dose, the more dehydrated the animal became. This may have been a reflection of a failure to nurse well plus the fact that a nursing rat is on a relatively low sodium intake.

Desoxycorticosterone was injected in doses of 0.25 mg per day for 10-14 days. The results of a previous report were confirmed(1). Even with a higher dose of 0.5 mg per day, no marked effect on the growth curve was elicited in most cases. Eruption of incisor teeth and opening of eyelids occurred about 1 to 1½ days before controls.

Pregnenolone was given to one group of rats in a dose of 5 mg per day for 13 days. Although a total dose of 65 mg was injected, no discernible effect was noted on body weight, teeth eruption or eye opening. No evidence of toxicity was apparent. At autopsy at 18 days of age, there was a considerable amount of unabsorbed drug at the injection site but without signs of inflammation.

Discussion. The infant rat lends itself readily for studies on growth and development. Adequate litter-mate controls on an identical dietary and environmental status are available. The animal is a rapidly growing one and its steep growth curve can act as a sensitive measure of factors affecting body weight. The newborn rat is markedly undeveloped with no teeth or hair, sealed eyelids and fusion of the lips to the gingivae. These externally visible characteristics serve as an indicator of at least one phase of development. Normally, all animals in a litter develop at about the same rate. Therefore, differences can readily be detected by comparing treated animals to their untreated litter-mates. Another advantage of infant rats is that both males and females grow at the same rate for the first few weeks of life. If older animals are used for growth experiments, scrupulous attention must be paid to the sexes because the male rat rapidly outgrows the female. Finally, with scarce hormones and drugs, less material is needed for study when infant rats are employed.

The effect of cortisone on growth has been known for many years. Wells and Kendall(4) reported that feeding of 1 mg of cortisone daily to rats weighing 30 to 40 g resulted in marked suppression of growth. They weighed about 30 g less than their controls at the end of only 10 days of treatment. A similar experiment with corticosterone resulted in growth inhibition to a much lesser degree. Recently, Winter *et al.*(5) injected daily doses of 3 mg of cortisone into rats weighing 200 g with complete cessation of growth during the 40-day injection period. Fifty days later, although their growth had resumed, the rats were still markedly underweight. It is of interest that the cortisone-treated animals consumed much more food than control animals whose food intake was restricted in order to maintain the same body weight as the injected animals. Li has reported that ACTH also retards body growth probably by means of increasing nitrogen excretion(6). When rats were hypophysectomized and injected with both ACTH and growth hormone, the effect of each compound was nullified by the action of the other.

The present experiment extends the observations on older rats to newborn rats. Both cortisone and ACTH are potent growth inhibitors. However, on a strictly drug weight basis, cortisone is the more effective growth inhibitor for the infant rat. Table I shows the lack of correlation between growth inhibition and precocious eruption of teeth and opening of eyelids by means of various sub-

3. Hench, P. S., Kendall, E. C., Slocumb, C. H., and Polley, H. F., *Proc. Staff Meet.*, Mayo Clinic, 1949, v24, 181.

4. Wells, B. B., and Kendall, E. C., *Proc. Staff Meet., Mayo Clinic,* 1940, v15, 324.

5. Winter, C. A., Silber, R. H., and Stoerk, H. C., *Endocrinology,* 1950, v47, 60.

6. Li, H. C., *Growth Symposium,* 1948, v12, 47.

TABLE I. Comparative Effects of Various Substances on Infant Rats. Injections Begun at One Day of Age.

Substance	Daily dose	Days treated	Total dose	Retardation of growth	Hastening of tooth eruption and eye opening
Cortisone	.1 mg	5	.5 mg	4+	4+
Aqueous adrenal extr.(1)	.2 ml	10	2 ml	0	3+
Desoxycorticosterone	.25 mg	14	3.5 mg	0	3+
Corticosterone	.2 "	8	1.6 "	0	2+
ACTH	.75 "	8	6 "	2+	±
Pregnenolone	5. "	13	65 "	0	0

stances. It also reflects the fact that the infant rat can withstand much larger doses of DCA than cortisone. This is the reverse of what occurs in man where relatively small doses of DCA are toxic as compared with the dose of cortisone. The dose of DCA that can be administered to man is limited by virtue of its marked effect on electrolyte and water balance. Apparently, this sign of toxicity is not manifested in the infant rat. The present experiments do not shed light on the mechanism of the precocious changes. Both the adrenal hormones affecting carbohydrate metabolism and those affecting electrolyte metabolism produce these changes. It does not appear to be due to a non-specific effect of the steroids because pregnenolone which structurally resembles the other substances is ineffective.

Summary. 1. ACTH, cortisone, corticosterone, desoxycorticosterone and pregnenolone were injected into newborn rats in varying dosage. Cortisone was found to be the most toxic with a total of 0.6 mg in the first few days of life being a lethal dose, as contrasted with the lack of toxicity of 0.25 mg of desoxycorticosterone given daily for 14 days. Cortisone was the most potent growth inhibitor and also the most potent stimulus for eruption of teeth, opening of eyelids and development of the gingivae. ACTH, although employed in larger doses than cortisone had a lesser effect on growth inhibition and little or no effect on development. Larger doses of ACTH are yet to be used. Corticosterone in the dose employed did not affect growth but did stimulate development. Pregnenolone in huge doses had no effect on either growth or development of teeth and eyelids. 2. Cortisone in a total dose of 0.5 mg (sub-lethal) given to newborn rats in the first week of life resulted in long-term damage to the animal as evidenced by failure to regain normal body weight 3 months after cessation of injections. The younger the animal at the time of the first injection, the more marked was the growth failure.

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Content of Radioactive Vitamin B_{12} in the Feces of Rats Fed Co^{60} and Aureomycin.* (18729)

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The importance of trace elements such as cobalt for optimal growth and normal health was long recognized by McCollum(1). Gall (2) demonstrated that the oral administration

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1. McCollum, E. V., Orent-Keiles, E., and Day, H. G., *The Newer Knowledge of Nutrition,* New York, 1944, The Macmillan Co.