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THE EFFECT OF PROGESTERONE ON BRAIN AND BODY GROWTH OF CHICK EMBRYOS[1]

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It has been suggested that in the embryo hormonal steroids may act also as control factors for the growth of neural systems. In the present work progesterone was introduced onto the chorioallantoic membrane of the chick embryo on day 7 or days 7 and 10 of incubation. The embryo, dissected at day 10, showed significant increases in body weight and cerebral hemispheres weight. The response at day 13 was less pronounced; male embryos responded to progesterone more than the female embryos. Progesterone is a precursor to other corticosteroids, but corticosterone itself had a significant harmful effect on embryonal growth. Several possible explanations of these results have been offered. It appears that progesterone itself promotes the growth of the early embryo, but the effect depends on its age and sex.

INDEX WORDS: Brain growth; brain, chick embryo; progesterone, effect on brain growth.

INTRODUCTION

It has been suggested that hormonal steroids may also act as control factors for the growth of specific neural systems during early brain development (Vernadakis, 1971). According to previous reports, in the chick embryo progesterone itself had either no effect (Vernadakis, 1971) or was weakly toxic or teratogenic (Piotrowski, 1966). On the other hand, we have recently reported (van Marthens and Zamenhof, 1979) that the administration of progesterone to rats malnourished *in utero* significantly improves development of fetal brain and body on days 16 and 20, as compared to nontreated malnourished fetuses.

Progesterone can be detected in adrenals of chick embryo as early as on 9th day of incubation, possibly as a precursor to other corticosteroids (Kalliecharan and Hall, 1974, 1976).

Recently, evidence was obtained that biogenic amines, in addition to their role as neurotransmitters after birth or hatching, may also act as growth promoting or regulating factors for embryonal cerebrum (Vernadakis, 1973; Ahmad and Zamenhof, 1978).

In view of all the above, in the present work we investigated the possibility that progesterone, in addition to its later role, is also a growth promoting or regulating factor for the embryo. The evidence obtained suggests that this may indeed be the case, but the effect depends on the age and possibly also on the sex of the embryo.

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Materials and Methods

Chicken eggs and their handling were essentially as described in our previous work (Zamenhof, 1976), except that all eggs were incubated at 37.5°C ± 0.1°C.

Progesterone or corticosterone (Sigma Chemical Co., St. Louis, Missouri) was suspended in olive oil and the suspension was introduced onto the portion of chorio-allantoic membrane directly over the developing embryo, on the 7th only or 7th and 10th day of incubation; the controls received the corresponding volumes of olive oil alone. To introduce the oil, the egg was placed on its side to allow the embryo to float to the upper surface. A small hole was punched in the shell over the embryo and another one over the natural air space of the eggs. Using suction, the air was then removed from the natural air space creating a new air space directly over the embryo. Into this new air space 10 μl (in the case of progesterone; 1 mg/ml) or 100 μl (in the case of corticosterone; 200 μg/ml) or oil suspension or of oil alone, 10 μl or 100 μl, respectively (controls) were introduced with syringe, and the holes closed with paraffin. The eggs were then placed in the incubator and not turned again.

After incubation for the indicated length of time, the eggs were opened, the embryos weighed and their sex determined as previously described (Lillie, 1930; Romanoff, 1960). The embryos were then decapitated, their cerebral hemispheres (without olfactory and optic lobes) were dissected out and weighed.

Student t-test was used for statistical analysis to calculate probability values.

Results and Discussion

The results of progesterone treatment on day 7 or days 7 and 10, on embryonic development at day 10 or 13 are represented in Tables 1 and 2. It can be seen that the treatment on day 7 produced highly significant increases in body weight and cerebral hemispheres weight on day 10; this corresponds to the active period of neuronal proliferation which in chick embryo cerebral hemispheres terminates on or before day 12 of incubation (Nurnberger, 1958). The increases caused by a single treatment on day 7 ceased to be significant at day 13; however, two treatments (days 7th and 10th), produced on day 13 an increase which was on the borderline of significance in male embryos, but was not significant in the female embryos (Table 2).

Several mechanisms could account for these results. It could be that the effect of progesterone is transient and disappears as progesterone concentration decreases, possibly due to the uptake by receptors (see below). Another possibility is that older embryos produce enough of their own progesterone (Kalliecharan and Hall, 1974; 1976) to minimize the effect of progesterone supplied exogenously.

Table 1. The effect of progesterone and corticosterone administered on day 7th on chick embryo development on day 10th (**sexes** combined)

	Treatment						
	Control	Progesterone (10 µg/egg)			Corticosterone (20 µg/egg)		
	Mean ± S.D.	Mean ± S.D.	Δ	P	Mean ± S.D.	Δ	P
Body weight (g)	2.02 ± 0.22	2.27 ± 0.17	+12.3	<0.005			
	2.18 ± 0.21				1.91 ± 0.13	-13	<0.005
Cerebral hemispheres weight (mg)	53.9 ± 4.0	58.9 ± 4.35	+7.8	<0.005			
	52.3 ± 4.3				47.8 ± 3.61	-9%	<0.005

Δ, Difference to its control, in % of control

P, Probability (student t-test).

Number of animals in each group: 15 to 18.

Alternatively, it could be that progesterone is a growth promoting or regulating factor only for young embryos, during the active period of neuronal proliferation. Such age-dependence was also observed in the growth-promoting action of serotonin (Ahmad and Zamenhof, 1978). The lower effect on day 13, even with two doses of progesterone (Table 2), is consistent with any of these explanations.

The basis for the differential response to progesterone of male and female embryos is at present not clear. Although progesterone has been demonstrated in chick embryos as early as on the 9th day of incubation (Kalliecharan and Hall, 1974), separate studies for the two sexes have not been made. Progesterone could have been converted to testosterone in male embryos. Estrogen-treated female chick oviducts contain progesterone receptor (Socher *et al.*, 1976) which binds progesterone and presumably lowers its concentration; in female chick embryos, however, this has not been investigated.

In pregnant rats progesterone has been reported to decrease the incidence of resorptions caused by malnutrition and faulty placental

Table 2. The effect of progesterone administered on days 7th and 10th[a] on chick embryo development on day 13 (**sexes** separately)

	Treatment							
	Control (Mean ± S.D.)		Progesterone					
	♂	♀	♂ Mean ± S.D.	Δ	P	♀ Mean ± S.D.	Δ	P
Body weight (g)	4.46 ± 0.36		4.86 ± 0.54	+9.0	<0.025			
		4.21 ± 0.34				4.48 ± 0.63	+6.4	N.S.[b]
Cerebral hemispheres weight (mg)	102.0 ± 8.9		108.4 ± 9.3	+6.3	<0.05			
		98.7 ± 8.9				104.9 ± 14.7	+6.3	N.S.

a Each dose 10 µg/egg;

b N.S. - not significant

Other symbols and details as in Table 1

development (review in Zamenhof *et al.*, 1971). It is not clear whether any similarity can exist also in chick embryos, but it is of interest that in our work, single treatment with progesterone at day 7 decreased mortality of the embryos by 32% at day 10 and by 40% at days 13 and 16.

Since progesterone is a precursor to other steroid hormones, such as corticosteroids (Kalliecharan and Hall, 1976) one might postulate that the stimulatory action of progesterone as demonstrated in this work, may be merely secondary, *viz.* to supply this precursor. The results in Table 1 do not support this suggestion: corticosterone has a highly significant harmful effect on embryonal growth. Thus, the action of progesterone on embryos appears to be different from that of corticosteroids.

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