Influence of the Pituitary-Adrenal Axis on Development of the Rat Foetal Thymus.* (31109)

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The importance of the rat foetal pituitary in the normal development of the foetal adrenal was reported by Wells(1,2) and Jost (3). Similar findings in the rabbit have been described by Jost(4) and Bearn(5).

The first indication that the foetal pituitary-adrenal axis plays any role in development was demonstrated by Jost and Jacquot (6). In a series of experiments they showed that this system is essential for the normal deposition of glycogen in the foetal liver. Glycogen begins to accumulate in the liver on the 25th day, after which it increases rapidly up to birth. When adrenal function is inhibited by foetal hypophysectomy by decapitation, this accumulation fails to occur.

A further function of the foetal adrenal was reported by Bearn(5,7) who demonstrated that the foetal adrenal in the rabbit was essential for normal development of the thymus. He showed that when the function of the foetal adrenal is inhibited by foetal decapitation, the thymus increases in size. This effect was prevented by injecting depot ACTH into the foetus at the time of the hypophysectomy. These experiments were interpreted as indicating that the foetal adrenal exerts an inhibiting influence on the developing thymus before birth.

Experiments of a similar nature have been performed in the rat foetus and are now reported.

Materials and methods. Foetuses from Wistar rats were used for these experiments. A male rat was left overnight with the females, and the onset of pregnancy established by the presence of a vaginal plug the following morning. The operations were performed on the foetuses *in utero* after 18½ days of pregnancy under open ether anaesthesia. The uterus was exposed through a midline abdominal incision and a segment containing one foetus was delivered through the wound.

Hypophysectomy by decapitation was performed by extracting the head of the foetus through a small hole cut in the uterine wall, and removing the head with a ligature. The hole in the uterine wall was closed with fine sutures, leaving the now headless foetus to continue development within the uterine cavity. A further series of foetuses were injected with ACTH at the time of their decapitation. The injection was given subcutaneously into the interscapular region before removing the head. 0.2 ml of ACTH in gelatin, Acthar Gel, was given as a depot to obtain slow release of the hormone into the foetal circulation.

The experimental foetuses, and litter mates for controls were recovered by Caesarian section on the 21st day of pregnancy after a further 72 hours of development. The thymus was removed from the mediastinum under a dissecting microscope and weighed to the nearest 1/10 mg on a single pan microbalance. The results were expressed in mg per 100 g of headless body weight.

In addition to the studies on the thymus, the abdominal cavity of all experimental and control foetuses was explored and the adrenal glands were observed under a dissecting microscope. The size and vascularity of the adrenals from the experimental and control foetuses were compared.

Results. 1. *Thymus.* The results are summarized in Table I. The thymuses of the 14 decapitated foetuses were found to be significantly larger than the 24 litter mate controls, 311 mg per 100 g headless body weight as opposed to 246 mg per 100 g. The thymuses of the foetuses which were decapitated and injected with depot ACTH were found to be smaller than the controls, 202 mg per 100 g.

2. *Adrenals.* The size of the adrenals of experimental and control foetuses was compared under a dissecting microscope at a magnifica-

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TABLE I. Weights of Thymuses from 21½ Day Rat Foetuses Subjected to Hypophysectomy by Decapitation, at 18½ Days, Either With or Without Injection of Depot ACTH.

	No.	Age at operation, days	Age at study, days	Thymus, mg/100 g	S.E. of mean
Decapitated foetuses	14	18½	21½	311[1]	12.8
Decapitated foetuses inj with ACTH	16	18½	21½	202[2]	13.7
Litter mate control foetuses	24		21½	246[3]	6.5

The difference between 1 and 3 is significant. $P < .001$
The difference between 2 and 3 is significant. $P < .001$

tion of × 6 and it was seen that the adrenals of the decapitated foetuses were obviously smaller than those of the litter mate controls.

The adrenals of foetuses subjected to decapitation and injection of ACTH were larger than the controls, and also showed a great increase in vascularity.

Discussion. These results provide evidence that the pituitary-adrenal axis of the rat foetus is exerting an inhibitory influence on the development of the foetal thymus. When adrenal function is depressed by foetal hypophysectomy by decapitation, the thymus hypertrophies. Since this effect is prevented by injection of depot ACTH into the foetus at time of decapitation, it suggests that the hypertrophy of the thymus is secondary to the reduction in adrenal function following the decapitation rather than to the direct effect of the loss of the pituitary. This view is further supported by the observations on the adrenals, which are clearly reduced in size in the foetuses subjected to decapitation.

Those foetuses decapitated and then injected with ACTH show well marked hypertrophy of the adrenals, which are both larger and more vascular than the adrenals from the controls. The dose of ACTH is clearly in excess of the amount of hormone secreted physiologically by the foetal pituitary. In these foetuses, the thymus is smaller than in the controls, and this hypoplasia of the thymus is likely to be due to excessive production of hormones from the hypertrophied adrenals.

The foetal adrenal is thus part of a homeostatic mechanism providing an inhibitory regulatory control on thymus growth. This inhibition fluctuates with adrenal activity; increased adrenal activity, as follows for example the injection of an overdose of ACTH into the foetus, resulting in thymus hypoplasia; and decreased adrenal activity, as follows for example foetal hypophysectomy, resulting in hyperplasia of the thymus.

This work in the rat is in agreement with earlier work by Bearn(5,8) in the rabbit showing that foetal hypophysectomy by total decapitation resulted in a reduction in the development of the adrenals, and a hypertrophy of the thymus. Bearn(7) also showed that this effect could be abolished by injecting ACTH into the foetal rabbit at time of decapitation.

These findings provide an explanation for some of the endocrine abnormalities present in human anencephalic foetuses. The pituitary in this abnormality is small or absent, and the adrenals markedly hypoplastic(9). Various explanations for these observations have been put forward in the past but in view of these experimental results in the rat and rabbit foetus the small adrenal of the anencephalic is probably due to a lack of foetal pituitary ACTH. The large thymus found in these foetuses(10) can now be interpreted in the light of the results reported here, and is likely to be secondary to the interference with the adrenal control of thymus growth.

The relationship between the development of the foetal thymus and adrenal function is of interest in view of the recent understanding of the role of the neonatal thymus in immunological competence. Neonatal thymectomy results in hypoplasia of the lymphoid system, immunological incompetence and retarded body growth. These effects do not occur when the thymectomy is performed later than the neonatal period, indicating that its functional importance occurs towards the end of foetal life(11).

Earlier work on the role of the foetal pituitary in the development of functional activity in the foetal adrenal of the rat has been

reported by Kitchell and Wells(12), Yakaitis and Wells(13) and Coetzee and Wells(14), and has demonstrated clearly a reciprocal functional interrelationship between the foetal pituitary and the adrenal cortex.

The effect of adrenal cortical hormones on lymphatic tissue in adult animals has been studied extensively and the subject has been reviewed by Dougherty(15) and Dougherty *et al*(16). In the adult the adreno-cortical hormones are part of a homeostatic mechanism regulating lymphatic organ mass, and the work reported here indicates that a similar homeostatic mechanism is active during foetal life in the rat and rabbit regulating thymus growth.

Summary. Rat foetuses were subjected to hypophysectomy by decapitation at 18½ days of development. At 21½ days the experimental foetuses showed a hypertrophy of the thymus and a decrease in adrenal size compared with the litter mate controls When depot ACTH was injected into the foetuses at time of decapitation, this hypertrophy of the thymus was inhibited, and the experimental foetuses showed a hypoplasia of the thymus, and a hypertrophy of the adrenals. These results are interpreted as indicating that the foetal pituitary acting through the foetal adrenals is part of a homeostatic mechanism providing an inhibitory regulatory control on thymus growth. These experiments also suggest that the thymus hypertrophy found in human anencephalic foetuses is secondary to the hypoplasia of the foetal adrenals found in this condition.

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Histobacteriology of the Genus *Pasteurella* II. Specificity and Stability Of the Various Species of *Pasteurella*. (31110)

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The use of immunofluorescence for identification of microorganisms has received general acceptance since its introduction by Coons *et al*(1). The refinements of fluorescent antibody technique and its broad application in the fields of microbiology, experimental pathology, and immunology have been reviewed by Cherry and Moody(2), Beutner (3), Nairn(4), Smith *et al*(5), and others.

Study of the interrelationship between invading parasites and their host tissues is dependent upon the specific identification of the organism or its products *in situ*. Immunofluorescence has been used to determine the

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