BIOLOGICAL DETERMINATION OF ANDROGEN CONTENT IN GONADS OF GUINEA PIG EMBRYOS

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The androgen content in the gonads of guinea pig embryos of both sexes was determined by measuring the height of the epithelium around a 5-day graft into the acinus of the seminal vesicles of a castrated rat. The presence of androgens in the testes was observed at all stages of intrauterine development studied, starting from the age of 24 days. The hormone content varied from 0.125 to 9.5 μg. At some stages of development, the presence of substances with androgenic properties was found in the ovaries, but their level was much lower than in the testes at the same stages of development.

Before the role of hormone production by the embryonic gonads can be understood, the physiological level of male sex hormone in embryos required for normal development of the reproductive tract must be established.

In this investigation an attempt was made to determine the androgen content in the gonads of guinea pig embryos by a biological method. There is no information in the literature on this subject.

The accessory glands (seminal vesicles and prostate) are the target organs for male sex hormone. The epithelium lining the acini of these glands react specifically to injection of testosterone. Castration causes a decrease in height of the epithelium, while injection of testosterone in physiological or excessive doses leads to normalization or hypertrophy of the cells, respectively. Furthermore, the embryonic testis, if grafted into the acinus of a seminal vesicle of a castrated adult rat, causes local stimulation of the epithelium [5, 8], while the testis of a guinea pig embryo can maintain the normal histological structure of the rat prostate in organ cultures [11]. This ability of the epithelium of the accessory glands to react to male sex hormone has been used as a test for determining the content of hormone with androgenic properties in embryonic gonads.

EXPERIMENTAL

Male rats (247), each weighing 100 g, were castrated. To test whether the above-mentioned reaction of the epithelium can be used as a specific test for androgen, and to obtain standard measurements related to the dose of injected hormone, equal pieces of various tissues (muscles, skin, lung) were grafted into the acinus of the seminal vesicle one month after castration. Some of these pieces of tissue were dried with acetone to enable them to soak up pure peach oil more easily, because an oily solution of testosterone propionate was used in the experiments. The weight of the dried piece of tissue was 1 mg. On the 5th day after grafting the rats were sacrificed and the seminal vesicles removed and treated histologically. Sections (8 μ) from the region of the graft were stained by Heidenhain's azan method. By means of an ocular micrometer 100 measurements of the height of the epithelium of the seminal vesicle were made in each case. The doses of hormone used during grafting were 0.25-100 μ g. The results were subjected to statistical analysis. To determine the content of androgens in the gonads of the guinea pig embryos at different stages of development

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TABLE 1. Changes in Height (in μ) of Epithelium of Seminal Vesicles of Castrated Rats depending on Injection of Different Doses of Testosterone Propionate

No. of animals	Character of experiment	Height of epithelium, M ± m
3	Normal adult rats	15,8±0,74
7	Castration	6,42±0,38
10	Castration + graft. of embryonic tissue	6,72±0,24
11	The same + graft. of tissue soaked in peach oil	7,53±0,15
	Injection of testosterone propionate (μg)	
6	0,25	9,83±0,51
5	0,5	11,03±0,51
20	1—4	12,54±0,29
5	5	13,39±0,04
5	9,5	14,78±0,45
5	24	16,53±1,49
15	50	17,63±0,79
5	100	15,65±1,25

TABLE 2. Content of Androgens (in μg) in Gonads of Guinea Pig Embryos

Sex of embryo	Age of donor embryo (in days)	No. of grafts	Content of androgens,* min-max	
			per gland	per mg gland tissue
Not determined	24—25	8	0,0—0,125	0,0—1,25
Male	26	4	0,5	
	27—29	5	0,125—0,25	0,22—0,45
	29,5—30	4	5—9,5	8,3—15,8
	30,5	2	0,125	0,17
	31—32	4	0,5	0,5—0,6
	33	4	0,25—0,5	0,25—0,5
	34—35	12	1—5	0,8—4,0
	36—37	8	0,25	0,14
	38	5	1—5	0,7—3,6
	39	1	0,25—0,5	
	40	4	1,5	0,8—4,0
	41—42	5	0,25—0,5	0,1—0,2
	43	1	5—9,5	1,9—3,7
	44—46	7	0,5—1	0,1—0,2
	47	4	5—9,5	0,9—1,7
	49—54	6	0,25—0,5	0,03—0,06
	55—56	17	1—5	0,14—0,7
	63— neonates	6	0,25—1	0,007—0,03
Female	26	1	0,0—0,125	
	28 и 30	4	0,125—0,5	0,31—0,6
	31—36	6	0,0	0,0
	37—63	9	0,125—0,25	0,08—0,16

*Content of androgen calculated from data in Table 1, allowing for significant difference.

(from the 24th day until birth), 139 blasts of gonads into the seminal vesicles of castrated rats were carried out by a similar technique. The quantity of tissue in each experiment varied from $2\frac{1}{2}$ glands to 1/8 of a gland depending on its activity. The gonads were grafted 0.5-2 h after removal of the fetus from the uterus by caesarian section. The age of the embryos was counted from the time when a copulation plug or spermatozoa were found in the female genital tract.

EXPERIMENTAL RESULTS

Castration of the male led to a sharp decrease in height of the epithelium in the seminal vesicles (Table 1). The grafted embryonic tissue had no effect on its height, but during soaking of the tissue with peach oil, a slight nonspecific increase in height of the epithelium was observed. Testosterone propionate, in doses of 0.25, 0.5, 1.5, and 9.5 μg, caused a significant increase in height of the epithelium. No significant difference was found between the action of doses of 2-4 μg and 24-100 μg.

The accessory glands of the castrated adult rat thus respond to administration of androgen in doses of between 0.25 and 1 μg by a change in height of the epithelium surrounding the graft. This reaction is specific, for tissues not changing androgens were ineffective.

After grafting of embryonic gonads into the seminal vesicles of castrated rats (Table 2), it was discovered that they secrete androgens which cause local stimulation of the epithelium. Traces of androgens, not exceeding 0.125 μg per gland, were found in the gonads of 24- and 25-day embryos, the sex of which could not be determined histologically. The content of androgens in the testis of embryos starting from the age of 26 days and until birth varied with age from 0.125 to 9.5 μg. On the 30th, 35th, 38th, and 43rd days, an increase in the level of androgens was found. This agrees with previous histochemical findings showing the presence of lipids [2] but only after the 27th day. Price and co-workers [9] found Δ^5-3β-ol-hydroxysteroid dehydrogenase, essential for steroid synthesis, only on the 29th day. In 5-day organ cultures, these workers found that the guinea pig testis had a hormonal effect on the rat prostate starting from the stage of 22 days [10, 11]. The increase in androgen content observed at some stages always preceeds intensive morphogenetic processes in the male genitalia [2], and it evidently reflects the content of true sex hormone essential for normal organogenesis of the genitalia of the male embryo. The discovery of such a very small amount (0.1-9.5 μ) of androgens, yet sufficient for normal development of the reproductive apparatus, can account for the paradoxical effect in development of the genitalia observed by some workers after injection of large doses of hormone, much in excess of physiological, into the mother or fetus [3, 4].

The question of the ovarian function of the embryo has not been finally settled, despite reports of steroidogenesis in the ovary (7, 12). Transplantation of the ovaries led to the discovery of substances with androgenic properties in them at some stages (Table 2). However, their content was very small compared with that in the testis at the same stages, except on the 28th day, when the level of androgen in testis and ovary was similar. This phenomenon can evidently be explained either by steroidogenesis, because androgens and estrogens share common precursors during synthesis, or by the assumption that the medullary layer of the as yet undifferentiated ovary can produce a certain quantity of androgen. Jost [6], who injected benzyl alcohol into a female rabbit fetus, observed atrophy of the cortical layer of the ovary and regression of the Müllerian ducts as the result of the possible effect of the embryonic medullary layer.

However, the test used in this investigation cannot determine conclusively whether a definite quantity of androgen is present at the time of transplantation or whether the tissue of the embryonic glands survives and is able not only to secrete, but also to produce further quantities of hormones. Although the histological sections did not reveal any progress in differentiation of the gland, the possibility cannot be ruled out that the tissue of the embryonic gonad can nevertheless produce a certain quantity of hormone, perhaps under the influence of an excess of gonadotropins in the castrated animal [1]. A sharp decrease in the level of androgens almost to their complete absence was discovered in testes transplanted after storage for 6-12 h in a refrigerator, by comparison with the contralateral glands transplanted immediately after removal of the fetus from the mother, presumably because of the inability of this cooled tissue to secrete hormone.

Hence, when transplanted for a period of 5 days into the seminal vesicles of castrated rats, the testes secreted androgens at all studied stages of intrauterine development of the embryonic guinea pig. At some stages of development, androgen appeared in the ovaries, but only in small traces compared with the level of androgens in the testis at the same stages of development.

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