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Intraocular Grafts of Embryonic Sexual Ducts of the Rat.* (24554)

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The guiding hypothesis behind much of the investigation pertaining to differentiation of sexual ducts in vertebrates calls for a response of the ducts to secretions provided by the developing gonads. Accordingly, developing testes provide materials which selectively promote elaboration of wolffian ducts while suppressing the oviducts; developing ovaries do the converse. Generally speaking, this 'modus operandi' appears to prevail, yet some workers(1,2,3) incline to the view that in mammals only the testes are concerned in duct differentiation while another(4,5) feels development of the reproductive tract is not controlled by the gonads at all. With these alternatives in mind, it is believed that a report of some observations on behavior of gonads and sexual ducts of the embryo rat interplanted to the anterior chamber of the eye may be of interest. The observations derive from 230 grafts prepared for earlier studies on gonads and rete tubules(8,9). The general procedures in preparing this material have been described. It should be added, however, that not all two hundred odd grafts included the primordial sex ducts; thus, this report is based on a lesser group of 67 relevant cases selected from the total pool. Specific details as to ages of donor embryos, constitution and survival of grafts and other pertinent data will be given as these cases are described. A complete series of "normal stages" of rat embryos has been available as standard of reference. A necessary first step towards interpretation of the grafts is a brief review of the time-table of normal embryogeny of the parts concerned (6,7). The wolffian ducts split directly off the top of the nephrogenic ridge, beginning 10th day postcoitum, then progressively project themselves caudad by free terminal growth. They contact the wall of the cloaca during 11th day and 1 day later open thereto. By the 19th day, in females, wolffian ducts have disappeared. The first mesonephric tubules appear early on 11th day. A maximum number of 15 to 18 pairs of tubules is reached near the end of 12th day. Of these, all disappear by the end of 16th day except the 3 most anterior pairs which are retained as epigonadal elements. In contrast to the early appearing wolffian ducts, the oviducts do not arise until the latter half of 13th day and do not open to cloaca until 2 days later. They disappear in males by the end of 19th day. As for the gonads, those destined to be testes begin to assume their characteristic structure towards the close of 13th day; prospective ovaries do not express themselves until the 16th day. It follows from this listing that embryos of 14 days, plus or minus a few hours, present prospective ducts and epigonadal elements for both sexes and gonads of one sexual prospect or the other. Moreover, the presence of the opposite elements in grafts of a given sex residing in the eye chamber for periods which would make them equivalent to term or postpartum ages is indicative of departure from their normal fate. The grafts to be considered all derived from embryos ranging in age from 13½ to 14¼ days postcoitum and are of 2 types. Fig. 1 is intended to portray these. In one series, the urogenital

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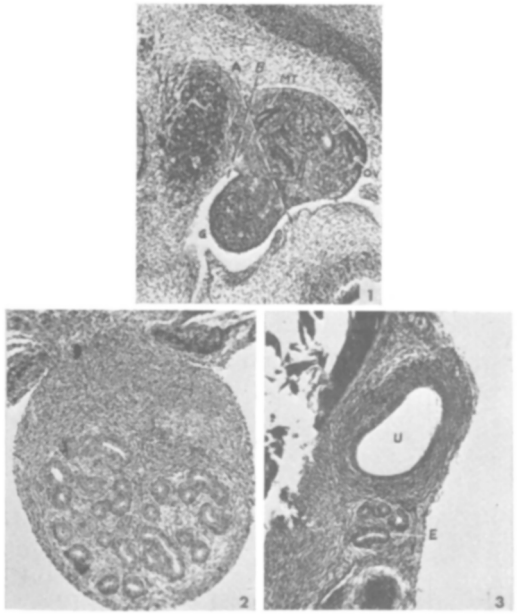

FIG. 1. Urogenital system of 14-day rat embryo. Line "A" shows plane of separation of mesonephric ridge; line "B" of urogenital ridge. MT, mesonephric tubules; WD, wolffian duct; OV, oviduct; G, gonad. ×35.

FIG. 2. Epididymis in graft of mesonephros. Theoretical postpartum age, 22 days. ×38.

FIG. 3. Graft of mesonephros at theoretical postpartum age of 10 days. E, epididymis; U, uterus. ×38.

area was split along the line "A" to provide a strip of tissue consisting solely of mesonephros and adjacent wolffian duct and oviduct. In the other series the entire urogenital primordium was detached along line "B". In other words, the 2 series provide tests of differentiating capacities of the oviduct and the wolffian duct with and without the gonads. The hosts were both male and female and ranged in age from 2 months to a year. Performance of the grafts was in no way correlated with age and sex of hosts, a point which will be considered elsewhere.

Results. Grafts of mesonephric ridge. Seventeen grafts comprise this series. Seven of them remained in the eye chamber until they reached an age equivalent to postpartum age of 3½ days. The remaining 10 were permitted to attain theoretical postpartum ages ranging from 10 to 22 days, averaging 12+ days. Of the 17 interplants, 12 (70%) survived to produce an assortment of structures; the remaining 5 regressed to a vascular stroma. In all 12 surviving grafts, vas deferens and vasa efferentia (epididymis) appeared to a variable degree (Fig. 2). The number of cases is too small to provide any basis for calculation of the genetic sex of the embryos which provided the grafts. It is conceivable that all 12 were prospective males and thus the presence of male ducts is only a manifestation of normal prospects. There are two related circumstances to be noted. Firstly, in 5 of these 12 cases the host animals were females. In fact, the particularly favorable case chosen for illustration (Fig. 2) is a graft which reached a theoretical postpartum age of 22 days in the eye chamber of a female host and in so doing produced a male duct system fully as elaborate as that of a normal male of this age. Secondly, in 3 grafts a well developed oviduct and uterus also appear (Fig. 3). Two of these developed in male hosts, the other in a female. The case illustrated involved a male host and has a theoretical postpartum age of 10½ days. The epididymis is less elaborate than in some other grafts, but still well formed; the uterus has a diameter approaching that in a normal female, but differs from the normal in being devoid of glands.

These results suggest that in the absence of associated gonads, both male and female ducts are capable of differentiation. Keeping in mind that the grafts acquire a blood supply from the irises of the hosts, it is obvious that the hormonal milieu provided by the host exercises no selective influence on the ducts. As to the lesser frequency of appearance of female ducts, these possible explanations have to be considered. The 3 instances of female ducts may indicate these were the only ones originating from genetically female embryos. If so, then one must account for simultaneous occurrence of male elements. Conversely, as already suggested, all grafts may have been male in origin which calls for an accounting for the presence of any female parts at all. It is even conceivable that all grafts may have been genetic females or distributed between male and female in some other proportion. Since the number of cases is too small for any

analysis of probabilities here, one can only point to the fact that the intraocular environment appears to favor differentiation towards maleness(9). But further information is provided by the second series of grafts.

Grafts of the urogenital ridge include fifty grafts. Of these, 6 were resorbed and 7 were lost through infection or death of host, leaving 37 for analysis. Theoretical postpartum ages ranged from 6 to 59 days with an average of 20+ days. The bulk lay in 12 to 22 day range, with fewer at extremes. An analysis of these grafts (in conjunction with others of a different type) with respect to performance of gonads has been reported(9). It need only be recorded here that of the 37 grafts, 4 consisted solely of vascular stroma and 5 presented stroma and various non-gonadal tissues only. The remaining 28 showed gonadal differentiation as follows: 26 testes, 1 ovary and 1 ovotestis. The 26 cases of testes in turn fall into 2 groups: 18 conventional testes and 8 possessing structures which have been termed "converted medullary tubules." Evidence for the conclusion that the latter should be interpreted as having been zygotic female in origin has been given(9). If one assumes that the 9 cases wherein only stroma and/or non-gonadal tissues appeared were female, by no means a necessary assumption, and adds these plus the 2 cases of ovary and ovotestis to the 8 cases of converted medullary tubules, there results a total of 19 judged zygotic female alongside 18 judged male.

This census of the status of the gonads in the grafts has been recorded to provide a reference base for interpretation of such sex ducts as also appeared. As in the previous series, both male ducts (vas deferens and vasa efferentia) and female ducts (oviduct and uterus) appeared. The following summarizes their occurrence with reference to status of the gonad.

Male ducts + testis	4
Female ducts + testis	4
Male ducts + ovary or ovotestis	2
Female ducts + ovary or ovotestis	0
Male ducts + converted medullary tubules	4
Female ducts + converted medullary tubules	0
Male and female ducts + converted medullary tubules	1
Male ducts without gonads	1
Female ducts without gonads	1
Testis without ducts	10
Converted medullary tubules without ducts	3
Total	30

The above summary accounts for 30 of the 37 recovered grafts. It has already been noted that 4 consisted of stroma alone; the remaining 3 presented no reproductive structures of any kind, but did contain adrenal cortex and cartilage. Of 30 cases, the obvious point to be noted is that sex ducts of one kind or another appear in only 17 of them. This is in striking contrast to their more frequent appearance, percentagewise, in grafts of the mesonephric ridge alone. One might expect them to survive and differentiate at least as well in association with gonads, yet that they do not, can only be a matter for speculation. It is possible, of course, that presence of gonads is actually deleterious to their development, and the 2 cases of duct development in the absence of the gonads would bear this out. Yet where the ducts do occur in the company of gonads, their development is just as complete as in the absence of gonads; in normal ontogeny, ducts and gonads accompany each other.

A second general point is that there is no correlation between presence or absence or type of duct and sex of host. Of the 4 instances of male ducts + testis, 3 were in male hosts, the other in a female; of 4 female ducts + testis, all 4 were in male hosts; of 4 male ducts + converted tubules, 2 were in males and 2 in females; the ovary + male ducts was in a male, the ovotestis + male ducts in a female; and in the case of male and female ducts together + converted tubules, the host was male.

The very randomness of association between duct types and gonad types has its positive and negative aspects. On the negative side, there is no consistency or pattern of performance exhibited by the ducts. Female ducts appear in conjunction with testes as often as male ducts. While it is true, with the exception of the case of simultaneous appearance of both ducts, that only male ducts ap-

pear in conjunction with converted tubules, which would suggest an influence towards maleness emanating from this type of testis, it also happens that only male ducts appear on the rare occasions of the differntiation of ovarian tissues, the reverse of what one would expect if the gonads are really exercising any influence. There is, of course, no evidence as to whether the gonads in these grafts produce endocrine substances at all. If they do, there is no consistency of endocrine effect upon the ducts; if they do not, the independence of the ducts is self-evident. On the positive side, this randomness of differentiation of the ducts coupled with the fact that good gonad differentiation as often as not fails to elicit or support any duct differentiation whatsoever suggests the essential independence of the ducts.

Conclusion. Reviews(11,12) of the vast literature on gonadal control of embryonic differentiation of sexual ducts in mammals reveal 3 general viewpoints: (a) that the developing testes and ovaries produce sex hormones and that each has 2 opposing actions, stimulation of ducts of corresponding sex and inhibition of ducts of opposite six; (b) that the testes produce hormone which stimulates male parts while suppressing the female, but the ovaries do not influence sexual differentiation; (c) that sexual differentiation is not governed by gonad hormones. The results here submitted, namely, absence of correlation between duct development and hormonal environment provided by host and the similar lack of correlation between ducts and accompanying gonads, support the third view. This conclusion conforms in general to that reached by Bronski(10).

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Release of Norepinephrine from the Isolated Heart.*† (24555)

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Hoffmann *et al.*(1) reported the presence of an epinephrine-like substance in the perfusate of isolated mammalian heart treated with acetylcholine or nicotine. Similar observations were made by McDowell(2). More recently Paasonen and Krayer noted a marked decrease of norepinephrine content of mammalian heart treated with reserpine(3). These observations were based on bioassay technics. The present report concerns chemical identification and assay of biologically active agents liberated from the isolated heart during the interval of stimulation following administration of acetylcholine.

Methods and materials. Isolated rabbit hearts were perfused by means of a modified Langendorff apparatus with oxygenated

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