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The behavior of embryonic chick gonads when transplanted to embryonic chick hosts.

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Experiments were begun in the spring of 1924 for the purpose of analyzing the sex potentialities of developing chick gonads by transplanting them to the vascular chorio-allantoic membranes of host chick embryos. In these experiments the circulations of the host embryo and the sex graft are related in a manner which is essentially similar to the relations existing between twin embryos in cattle.[1] In this analysis several important problems arise. It may be possible to determine first how early in development the sex potencies of the gonads and their laterality are fixed; secondly, whether these original sex potencies may be modified or inverted by transplanting the gonadal primordia during the morphologically indifferent stage to host embryos of opposite sexes; thirdly, the behavior of sexually differentiated right and left ovaries and testes when implanted to male and female host embryos.

The method employed was essentially the same as previously reported.[2] The Wolffian body with its associated sex gland and adrenal was isolated with the aid of a binocular microscope from a donor embryo at various periods of incubation as indicated below. This isolated complex was then implanted to the chorio-allantoic membrane of a host chick embryo. In this manner the right and left gonads were implanted to male and female host embryos. The transplantations were made in all cases upon host embryos during the ninth day of incubation, and permitted to grow for an average period of nine days. The grafts were then removed and prepared for histological examination.

The sexual glands used in transplanting may be grouped into two stages of development or differentiation: (1) before sexual

[1] Lillie, F. R., *J. Exper. Zool.*, 1917, xxiii, 371-452.

[2] Willier, B. H., *Am. J. Anat.*, 1924, xxxiii, 67-103.

differentiation, that is, during the morphologically "indifferent stage"; and (2) after sexual differentiation.

I. *Grafts obtained from transplantations of gonads in a morphologically "indifferent stage".* During this stage sex cannot be determined by the structural appearance of the gonad. This indifferent stage extends from the origin of the gonad on approximately the fourth day of incubation until the time of sex-differentiation at approximately the seventh day of incubation.[3, 4, 5] The gonads for implanting were taken from embryos incubated 4, 5, 6½ and 6¾ days. During the 4th and 5th days the gonad is in an early stage of formation, *i. e.,* the genital ridge stage; while between the 6th and 7th days the gonad is near or in the process of sexual differentiation.

After a growth period of 9 days on the chorio-allantoic membrane of the host embryo, the indifferent gonads, regardless of their ages, underwent the following differentiations. The right indifferent gonad when grown upon a male host embryo differentiates into either a testis (4 grafts) or a right ovary (3 grafts), no gonad (1 graft); when grown upon a female host embryo differentiates also into either a testis (2 grafts) or a right ovary (4 grafts). The left indifferent gonad when grown upon a male host embryo differentiates into either a testis (2 grafts) or a left ovary (1 graft), no gonad formed (1 graft); when grown upon a female host embryo it differentiates also into either a testis (2 grafts) or a left ovary (1 graft).

It thus appears from an examination of these data from 21 grafts of indifferent gonads, that the right indifferent gonad differentiates into either a testis or a *right ovary,* while the left indifferent gonad differentiates into either a testis or a *left ovary.* These differentiations take place irrespective of the sex of the host embryo.

The conclusion is therefore reached that the gonad-forming tissue is already specific and differentiated as to sex and laterality during the so-called "indifferent stage" of gonad development. Apparently, the right indifferent ovary has the potencies of a right ovary only, and the left indifferent ovary has the potencies of a left ovary only. This interpretation with respect to sex and

[3] Firket, Jean, *Arch. de Biologie,* 1914, xxix, 201-351.

[4] Firket, Jean, *Arch. de Biologie,* 1920, xxx, 393-516.

[5] Swift, C. H., *Am. J. Anat.,* 1915, xviii, 441-470.

laterality is possible since the right and left grafted ovaries show the same differences in size and structure as normal right and left ovaries (the right and left grafted testes like the normals cannot be distinguished histologically). Thus it is apparent that the sexual potencies are determined in the indifferent gonad before morphological evidences for sexual differentiation appear. In other words, the morphologically indifferent gonad is not physiologically indifferent.

How early in the development of the embryo the specific organization of the gonad with respect to sex and laterality appears is an interesting question. Such an organization is already established in the indifferent gonad at the approximate time of its origin in a 4-day embryo (this statement is supported so far by only three grafts). As yet this is the earliest stage that I have attempted to transplant. Whether or not this specific organization of the gonad is established before the fourth day may possibly be analyzed by transplanting the right and left primordia of the Wolffian bodies so as to include the immediately surrounding peritoneum from chicks incubated less than four days.

The primordia of the right and left gonads apparently have the capacity for independent growth and differentiation, for they differentiate according to their specific organizations, and are not modified by the sex of the host embryo. This is demonstrated (1) by the differentiation of the right indifferent gonad, whether implanted to a male or a female host embryo, into either a testis or a *right ovary,* and (2) by the differentiation of the left indifferent gonad, whether grown upon a male or a female host embryo, into either a testis or a *left ovary.* These results prove that hormonic sex-differentiating factors are either absent in the host, or, if present, they are ineffective in the modification of the sex graft in the developing chick.

The indifferent gonads, therefore, seem, except for dependence for nourishment from the blood stream of the host embryo, independent of the host surroundings in the attainment of specific differentiation. Whether or not the differentiation of the indifferent gonad is independent of the associated adrenal and Wolffian body of the graft is an open question. This may be tested by implanting an indifferent gonad which has been completely isolated from its associated organs (this ought to be possible on at least the fifth day of incubation).

II. *Grafts obtained from transplantations of sexual glands after the occurrence of sexual differentiation.* From the end of the seventh day of incubation the sex of the gonads may be distinguished with certainty by differences in size and particularly form.[6] The ovaries and testes for implanting were obtained from embryos incubated 7, 9, 10, 11, 12 and 13 days respectively. After a growth period of 9 days on the chorio-allantoic membrane of the host embryo, the graft was removed and examined histologically. To date 46 such grafts have been examined.

Right ovarian grafts. The right ovary when grown upon either a male (4 grafts) or a female (10 grafts) host embryo is unmodified in its essential structures. It is histologically similar to the right ovary of a normal chick of late embryonic stages. So far no cortex (second set of sexual cords) has been distinguished. In the majority of grafts the gonad consists of a distinct ovarian medulla. This medulla is composed of the usual structural components of an ovarian medulla, such as the epithelial or medullary cords (often in the form of small epithelial groups or as isolated cells in some grafts) and a connective tissue stroma.

There are considerable variations in the form, the size and the texture of the implanted right ovary. These variations, however, could not be associated with the sex of the host embryo, but are apparently conditioned by the exigencies of the experimental procedure of grafting.

Left ovarian grafts. The left ovary retains its essential structures when grown upon either a male (6 grafts) or a female (3 grafts) host. The engrafted left ovary shows a distinct cortex and medulla, which are histologically similar to these regions in a normal left ovary of the later stages of embryonic life. The cortex consists of groups of epithelial cells including primordial germ cells. The medulla has the same components as described for the right ovary. The implanted left ovary shows variations in form and size, in the thickness of the cortex, and particularly in the texture of the medullary region. These variations of the medulla are of the same sort as occur in the engrafted right ovary, and are apparently similar to the "Phénomènes dégénératifs des cordons médullaires" of the right and left ovaries described by

[6] Swift, C. H., *Am. J. Anat.*, 1916, xx, 375-410.

Firket.[3, 4] These variations of both cortex and medulla cannot be correlated, so far as at present ascertained, with the sex of the host embryos but are more probably associated with the isolation and implantation of the graft.

Right and left testicular grafts. The right testis was grown upon either male (7 grafts) or female (5 grafts) hosts; the left testis upon either male (3 grafts) or female (5 grafts) hosts. An examination of these testicular grafts shows that in all cases the essential testicular structures such as sexual cords (potential seminiferous tubules) containing supporting epithelial cells and primordial sex cells, intercordial tissue, and a more or less well-developed *tunica albuginea,* are present. These structures are similar to those of normal testes of the late embryonic stages. The engrafted testes vary in form, size and topographical relations to the Wolffian body. It is quite evident that these variations from the normal, as in the grafts of right and left ovaries, are not casually related to the sex of the host embryo, but rather are due to the operative conditions.

Finally the question is raised as to whether the engrafted gonads modify the development of the sex organs and ducts of the host embryo. These host embryos occasionally show such atypical features as (1) variations in the size and form of the sex glands, (2) the persistence of a complete right Müllerian duct in a female, and (3) the presence of both right and left Müllerian ducts in a male embryo. These atypical structures, however, are not specifically related to the sex of the engrafted gonad, and they appear in control embryos on which grafts of various endocrine glands from adult chickens have been growing.