coefficients of self-diffusion and viscosity, whereby his efforts on these problems, covering 231 pages of his *Collected Works,* would have borne fruit.

[1] L. Boltzmann, *Collected Works* (1872), **1**, 361.

[2] D. Hilbert, *Grundzüge einer allgemeinen Theorie der linearen Integralgleichungen* (New York: Chelsea Publishing Co., 1953), p. 267.

[3] S. Chapman and T. G. Cowling, *The Mathematical Theory of Non-uniform Gases* (Cambridge: At the University Press, 1952).

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[6] S. Chapman, *Phil. Trans. Roy. Soc.*, **A216**, 279, 1916; **A217**, 115, 1917.

[7] D. Enskog, dissertation, Uppsala, 1917.

[8] Chapman and Cowling, *op. cit.*, pp. 85 and 139.

[9] *Ibid.*, p. 198.

[10] Pidduck, *op. cit.*, p. 99.

[11] Boltzmann, *op. cit.*, **3**, 48.

[12] *Ibid.*, eq. (100) where $y$ is proportional to $\Psi$.

[13] *Ibid.*, **2**, 388–556.

[14] *Ibid.*, p. 541.

[15] See a forthcoming publication by the author.

[16] L. Boltzmann and J. Nabl, *Encyklopedie math. Wiss.*, vol. V, Part 1, p. 541, 1905.

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## *EXPERIMENTAL REVERSAL OF SEX IN THE GONADS OF THE OPOSSUM DIDELPHIS VIRGINIANA*

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Since steroid sex hormones in pure form became available for experimental purposes some twenty years ago, much work has been devoted to the study of their effects on the developing genital system in the embryo and the problem of the role of sex hormones in the normal differentiation of sex. In many species of amphibia, and in bird embryos, sex hormones administered during the period of sexual differentiation readily control the development of the accessory sex structures; at the same time they also transform the gonads histologically by reversing the normal balance (as genetically determined) between the medullary and cortical components.

In the case of mammalian embryos, however, a serious discrepancy has long existed in the apparent failure of sex hormones to induce significant histological changes in the gonads, even in cases in which the accessory sex structures are strongly transformed. This was the situation encountered in several species of placental mammal (rat, mouse, rabbit, guinea pig, hamster, and rhesus monkey) and in a marsupial—the North American opossum (*Didelphis virginiana* Kerr).[1] This conspicuous failure to influence the differentiation of mammalian gonads lent weight to the argument that the steroid sex hormones are probably not similar, either chemically or physiologically, to the sex-differentiating substances elaborated by the embryonic gonads and have no proper role in the normal processes of sex differentiation.

However, there seem to be no obvious reasons for supposing that mammalian gonads, as a class, are inherently incapable of reacting to sex hormones. On the contrary, indirect evidence indicates that—as in other vertebrates—the embryonic gonad of mammals is potentially bisexual in its constitution. Spontaneously occurring hermaphroditism, characterized by the development of ovotestes (hermaphroditismus verus), is a familiar anomaly in many mammals. Also, embryonic gonads which develop as grafts in strange environments frequently give rise to both ovarian and testicular tissues, through the unregulated development of both primordial sex components.[2] Finally, the modified ovaries of freemartins commonly develop testicular structure to a high degree.[3] There is, then, no reason to doubt that in the early stages of development mammalian gonads possess the requisite structural basis and are potentially capable of undergoing sex transformation. Their failure to react to sex hormones administered experimentally thus posed a problem.

The question remained in this state until a few years ago when a number of cases of well-developed ovotestes appeared in a litter of young opossums which had been treated from birth with a rather low dosage of the female hormone *estradiol dipropionate*.[4] In every male of this litter (seven in all) a well-defined cortical layer had developed over the surface of the testis, external to the tunica albuginea. The character of the induced cortex, and the state of preservation of the testicular region of these gonads, varied considerably in individual cases according to the length of treatment. The history of this experiment revealed that the litter had been born at an earlier stage than usual (this point will be considered later) and presumably had received the first hormone treatment earlier than in any previous experiment. Since at the time of birth the gonads of young opossums are just at the verge of histological sex differentiation, it seemed likely that the condition of the gonads when first exposed to the hormone might be the determining factor. However, the dosage was much lower than in previous experiments (1–2 $\mu$g. per day), and it was felt that this also might be important.

A histological study of the intersexual gonads showed that the first step in the differentiation of the cortical zone consists in a persistence of the *germinal epithelium* over the surface of the embryonic testis long beyond the stage when it normally disappears. This observation recalled certain cases noted occasionally in some of the earlier experiments on opossums, in which this phenomenon had occurred to a limited extent. Such cases were infrequent and were found only in subjects which were sacrificed (or had died) comparatively early; in older animals the germinal epithelium was absent or was so vestigial in character as to seem insignificant. It was now evident, however, that survival of any considerable area of germinal epithelium on a testis for an appreciable length of time represents, potentially at least, the initial stage in the development of a cortex. Thus these earlier cases were put in a new light.

This unexpected result, and the circumstances under which it appeared, suggested that previous failures—in the opposum at least—might perhaps be due to experimental conditions rather than to an innate incapacity of the embryonic gonads to react to sex hormones. If the embryos could be exposed to the hormone in the proper dosage and at a sufficiently early stage in development, transformation of the gonads might be obtained regularly. Further success seemed to hinge on

securing the young at the earliest possible moment after birth and, particularly, on the occasional appearance of litters born somewhat prematurely.

It is well established that young opossums are born after an unusually brief gestation of from 12.5 to 13 days. The evidence on this point has been reviewed by McCrady.[5] According to the study of McCrady, birth normally occurs at about 12 days and 19 hours, and in his series of normal stages, based on criteria of external form, the stage of birth is designated as *stage 35*. It is readily distinguished from *stage 34* (which precedes it by about 12 hours) by the form and proportions of the head, the structure of the hind limb, and especially by the *absence of the oral shield*—a peculiar plaque-like, epidermal structure surrounding the mouth which characterizes stage 34. In the case of the experimental litter which yielded the group of ovotestes referred to above, it is known (from specimens preserved and photographed when the litter was first seen) that the young were born at stage 34; hence they were probably treated some 12 hours earlier (approximately) than in previous experiments.

Accordingly, an effort has been made during the past three years (1) to secure embryos for treatment at the earliest possible moment after birth and (2) to determine how much variability may exist as to the stage at which viable embryos are born, since presumably only those born earlier than usual (at or near stage 34?) would provide favorable material for the purpose at hand. The effort has been rewarded to the extent that four other litters of this age have been obtained and used experimentally with success. The development of ovotestes in males after treatment with *estradiol dipropionate* has been confirmed. In a number of cases, moreover, the reversal process has gone much further than in the original group, the transformed gonads closely resembling ovaries in their structure and appearance. At the present time a total of twenty-three cases, representing every male in each of five litters, are available for study and are listed in Table 1.

TABLE 1

| Litter | Length of Treatment (Days) | Av. Dose per Day (μg.) | Condition of the Cortex | No. of Cases |
|---|---|---|---|---|
| I | 30 | 1–2 | Well developed but *sterile;* persistent germinal epithelium | 7 |
| II | 20 | 5 | Thin to well developed but *sterile;* persistent germinal epithelium | 6 |
| III | 10 | 0.3 | Persistant germinal eipthelium; incipient cortical cords; *germ cells* | 3 |
| IV | 10 | 0.2 | Persistent germinal epithelium; incipient cortical cords; *germ cells* | 2 |
| V | 20 | 0.15 | Thick and well developed, with *germ cells;* persistent germinal epithelium; primordial follicles with growing ovocytes | 5 |
| | | | Total | 23 |

The process of sex reversal in these gonads, as seen histologically, involves the gradual development of a cortical layer over the surface of an embryonic gonad which (as revealed by certain peculiarities of its later structure) has already undergone a certain amount of differentiation as a testis. The development of the cortical zone in an ovotestis is based, histologically, on the normal mechanism of sex differentiation. It is a well-established principle of sex differentiation in verte-

brates that up to a point in development the embryonic gonad—irrespective of its future sex as genetically determined—is potentially capable of becoming either an ovary or a testis. During the "sexually indifferent" phase of development this dual potentiality finds expression in the presence of two distinct histological elements (Fig. 1, *B*). The *primary sex cords*, which have originated somewhat earlier as masses of cells proliferated from the inner surface of the *germinal epithelium*, represent the male element of the indifferent gonad and are strictly male in potentiality. In the development of a testis they eventually form the *seminiferous*

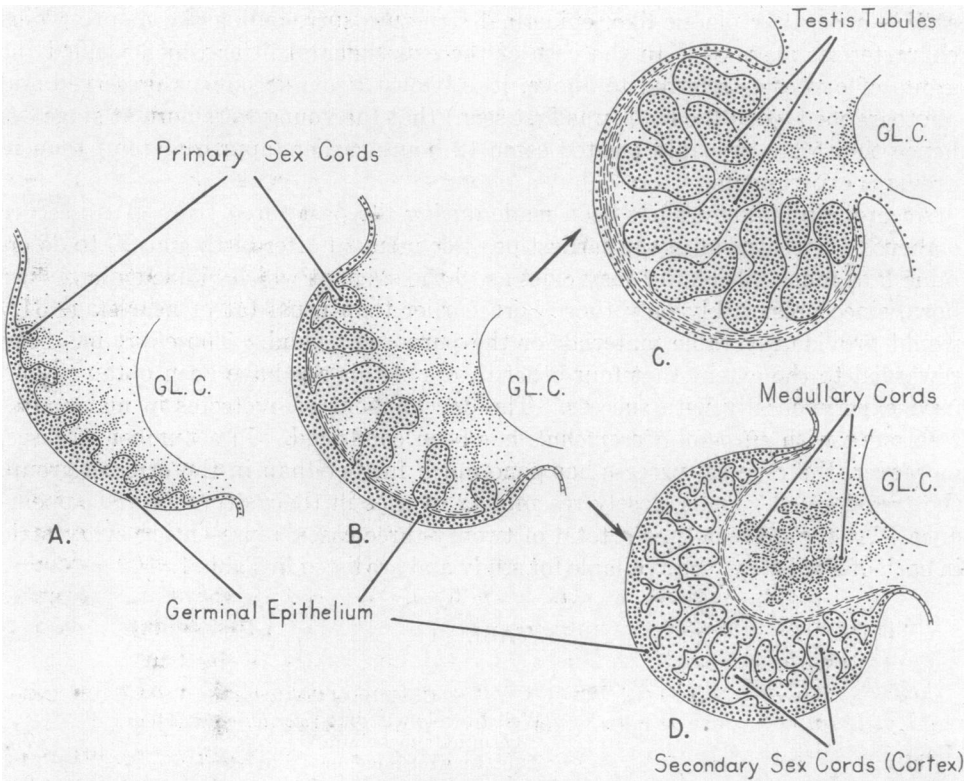



FIG. 1.—The process of sex differentiation in the gonads of mammalian embryos, illustrated semi-diagrammatically. *A:* The origin of the primary sex cords from the *germinal epithelium*. *B:* The *indifferent stage* of sex differentiation, in which the male, or testicular, component of the gonad is represented by the *primary sex cords;* the *germinal epithelium*, with its potentiality for producing *secondary sex cords*, represents the female component. *C:* The development of a testis—the *primary sex cords* become the testis tubules, while the germinal epithelium undergoes involution. *D:* The development of an ovary—*secondary sex cords* arise from the germinal epithelium form the cortex, while the primary sex cords retrogress. (In each drawing *GL. C.* is the cavity of a glomerular capsule of the mesonephros.)

*tubules*, while the germinal epithelium undergoes involution (Fig. 1, *C*). Female potentiality in the sexually indifferent phase resides in the germinal epithelium itself, which at this stage is present and well developed in both sexes. In prospective ovaries (Fig. 1, *D*) the germinal epithelium persists and continues to proliferate cells which give rise to the *secondary sex cords* and the *cortex* of the ovary. The primary sex cords (male component) regress and ultimately either disappear or survive in a more or less vestigial condition, their behavior varying somewhat in different species.

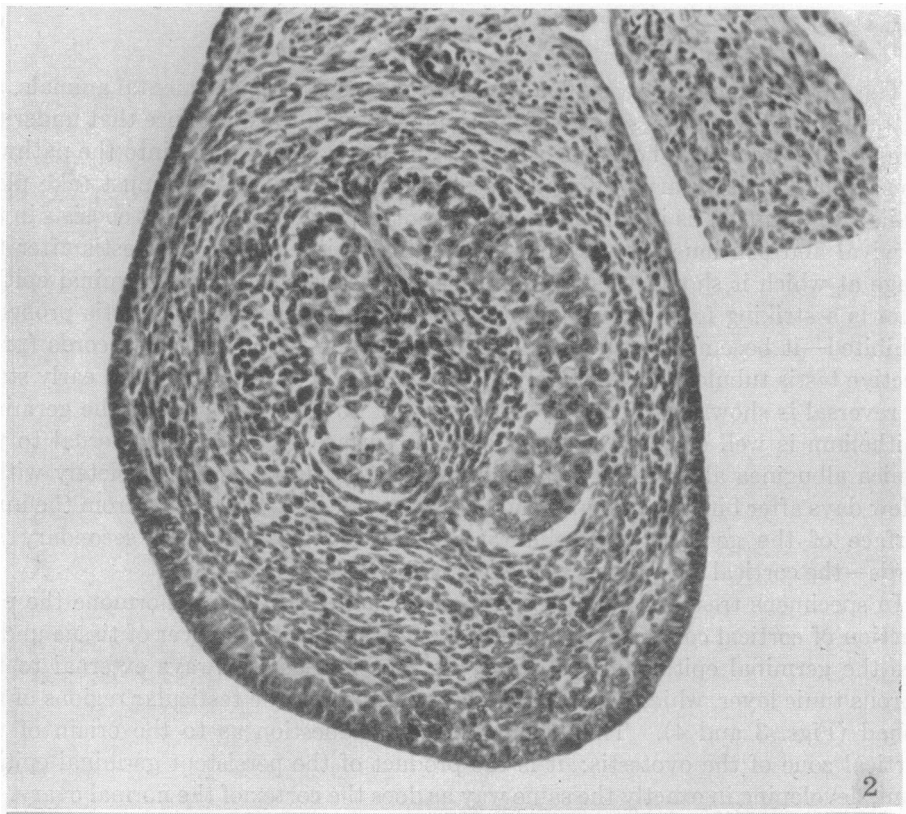
The various stages of reversal, as seen in the gonads of experimental animals, are patterned closely on this scheme of development, with the difference that under the influence of the female hormone a prospective testis is redirected into the pathway prescribed for the ovary. The first step in the reversal process must take place while the yount testis is still in the indifferent stage (Fig. 1, *B*). It consists in the survival and continued growth of the germinal epithelium of the testis after the stage at which it should normally disappear. Persistence of the germinal epithelium is a striking feature in all ovotestes. At the same time the testis proper is inhibited—it becomes greatly reduced in size, while the primary sex cords (prospective testis tubules) are distinctly atrophic. An ovotestis in such an early stage of reversal is shown in Figure 2. At an age of 20 days *post partum* the germinal epithelium is well developed over the entire surface of the testis external to the tunica albuginea although it would normally have disappeared completely within a few days after birth. In many places clusters of cells proliferating from the inner surface of the germinal epithelium represent the beginnings of secondary sex cords—the cortical component of the ovotestis (Figs. 2 and 3).

In specimens treated for a longer time (30 days) with female hormone the production of cortical cords continues until a thick and compact layer of tissue underlies the germinal epithelium. The cortex thus formed is always external to the fibrous tunic layer, which separates it everywhere from the testicular regions of the gonad (Figs. 3 and 4). Thus there can be no question as to the origin of the cortical zone of the ovotestis: it is the product of the persistent germinal epithelium, developing in exactly the same way as does the cortex of the normal ovary.

Many different stages in the development of the cortex, intermediate between the conditions shown in Figures 3 and 4, are found. In such a series, parallel stages in the atrophy and transformation of the primary sex cords are encountered, until, in cases with a well-developed cortex like that in Figure 4, little evidence remains of tubular structure. They are finally reduced to the status of pale-staining, epithelial strands with no trace of a lumen and in this condition closely resemble their homologues—the so-called "medullary cords" of the ovary.

At this point an important feature of these intersexual gonads must be emphasized—the total absence of germ cells in the induced cortex (compare Figs. 4 and 5). Complete sterility of the cortex was found in all ovotestes of the first two litters of Table 1. The reason remains uncertain, but is is possibly related to the dosage. In the later experimental groups, in which considerably lower dosages were used, it did not occur, although the number of germ cells present was much lower than that in the cortex of a normal ovary. Cortical sterility, then, is not a necessary condition in transformed testes.

By far the most complete and convincing examples of histological transformation are found in the males of the last three experimental litters, which received the lowest dosages of the hormone yet administered (Table 1). In these animals the internal organs of sex were all strongly transformed, and the copulatory organs were of female type and greatly hypertrophied. Obviously, from the stand point of the accessory sex structures, the dosages in these experiments are still well above the level required to elicit a strong reaction. The gonads of these males can no longer be properly called ovotestes; indeed, in most respects they approach very closely the structure of ovaries. The development of the cortex is remarkable, far exceeding anything previously encountered (Fig. 5). The germinal epithelium



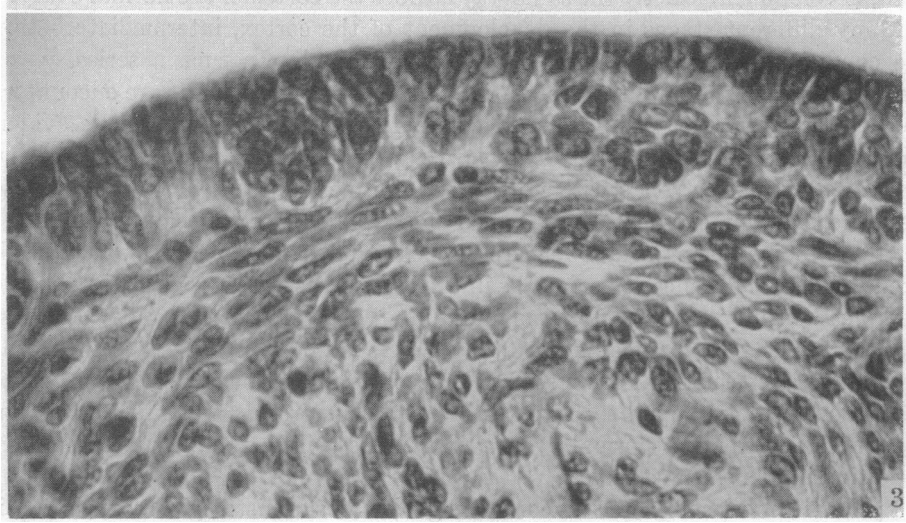
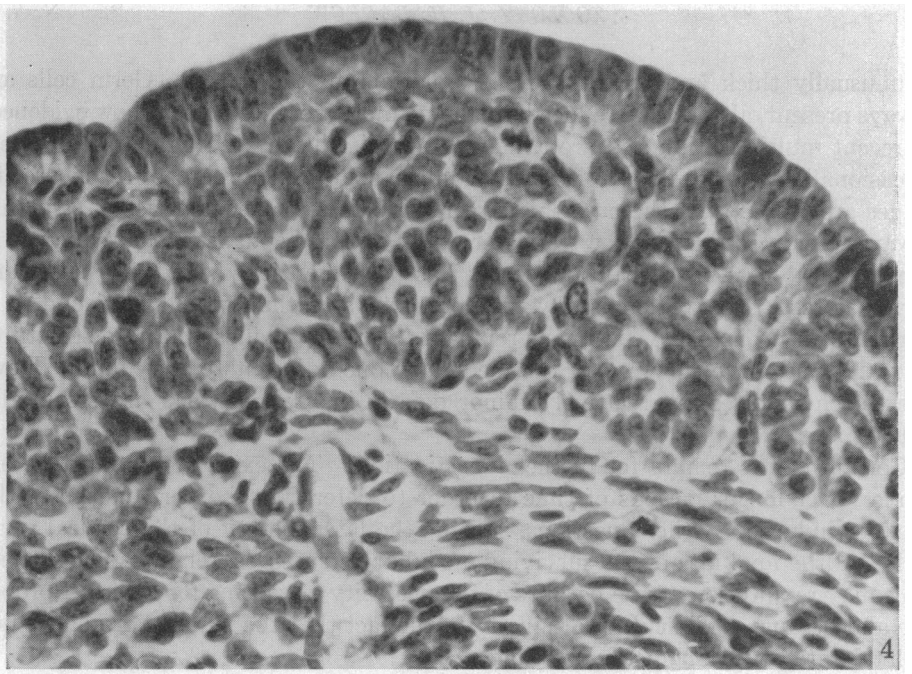




PLATE I

FIG. 2.—Photomicrograph of an ovotestis in a young male opossum (litter II, Table 1) produced by treatment with female hormone for a period of 20 days. The testis cords (*primary sex cords*) are greatly reduced in size and show evidences of degeneration. The persistent *germinal epithelium* covers the entire surface of the gonad and shows marked thickening over the distal region (*below*), where *secondary sex cords* are beginning to form. The incipient cortex is everywhere separated from the testicular part of the gonad by a thick fibrous layer, the *tunica albuginea*. ×200.

FIG. 3.—Photomicrograph, at higher power, of the incipient cortex of the ovotestis shown in Figure 2. Prominent clusters of cells, proliferating from the undersurface of the *germinal epithelium*, represent the *secondary sex cords*. The underlying layer of flattened cells is the *tunica albuginea*. ×600.



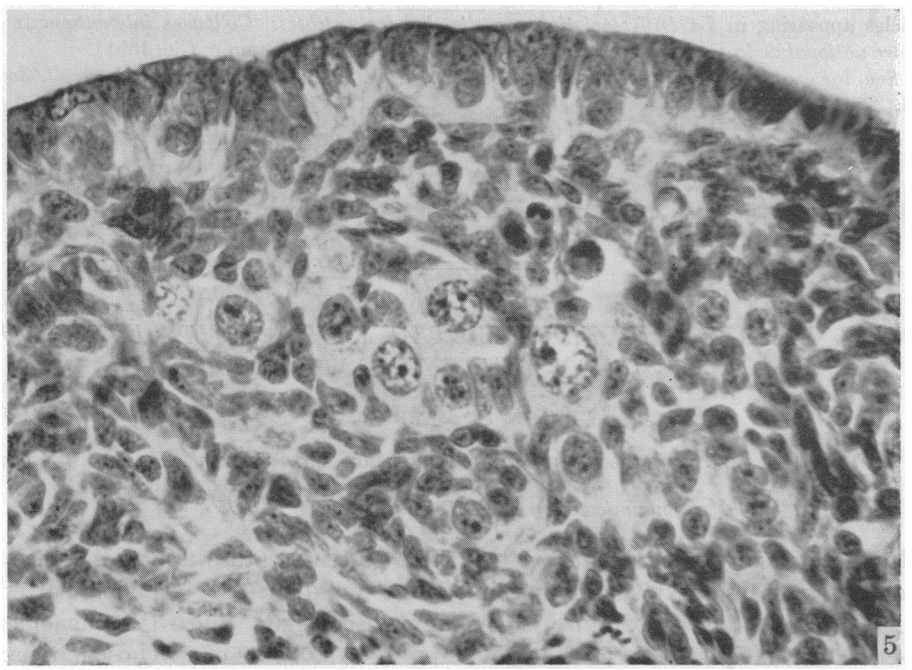




PLATE II

FIG. 4.—The cortical zone and the underlying *tunica albuginea* in an ovotestis, after treatment with female hormone for 30 days (litter I, Table 1). The cortex is composed of numerous cortical cords, compactly arranged, external to the *tunica albuginea* however, no germ cells appear, either in the cords or in the *germinal epithelium*. Compare with Figure 5. ×500.

FIG. 5.—Photomicrograph showing the cortical region of a transformed testis in a member of litter V. The structure is typically ovarian. Germ cells are present throughout the cortex, but in greatly reduced numbers as compared with a normal ovary. ×600.

is unusually thick and covers the entire surface of the gonad. Germ cells are always present, although in reduced numbers; moreover, they often show evidences of recent multiplication and are rather evenly distributed throughout the cortex. Occasionally, well-developed primordial follicles containing ovocytes in early growth stages are found. On the other hand, certain defects or peculiarities of structure always remain to indicate the testicular origin of these gonads. The reduction in the number of germ cells in the cortex has been mentioned, and the medullary (original testicular) region is always more extensive than would be found in a normal ovary. In some specimens remnants of testis tubules, and rete structures of male type, have survived in the medullary one adjacent to the hilum. However, such features are relatively minor defects in gonads which are essentially ovaries.

Certain points remain for further investigation. The experiments must be extended over longer periods of time in order to determine the permanence of the changes induced. Other questions, such as the later history of the cortical germ cells and the final fate of the surviving medullary structures, remain to be settled. This may require some time because of the necessity of waiting for litters of proper age and because of difficulties arising from the fact that the female hormone is not well tolerated over long periods of time.

[1] The results for most of the species mentioned are discussed and summarized in a series of articles appearing in *La Différenciation sexuelle chez les vertébrs: Colloques internationaux du Centre national de la Recherche scientifique*, Vol. XXXI (Paris: Masson & Cie, 1951).

[2] See, for example, A. Buyse, *J. Exptl. Zool.*, **70**, 1–41, 1935; and T. Torrey, *J. Exptl. Zool.*, **115**, 37–58, 1950.

[3] B. H. Willier, *J. Exptl. Zool.*, **33**, 63–127, 1921.

[4] R. K. Burns, *Arch. anat. microscop. et morphol. exptl.*, **39**, 467–483, 1950.

[5] E. McCrady, Jr., *The Embryology of the Opossum* ("American Anatomical Memoirs, No. 16 Philadelphia: Wistar Institute of Anatomy and Biology, 1938).

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## *SOCIAL BEHAVIOR IN FRESH-WATER FISH AND ITS EFFECT ON RESISTANCE TO TRYPANOSOMES*

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In the course of experiments on the effect of temperature upon resistance of fresh-water fishes to trypanosome infection, unanticipated behavior patterns which modified the temperature effects were discovered. Since the phenomena observed are likely to be of considerable interest to students of psychosomatic and social aspects of resistance, these experiments are reported here in advance of a large body of data on fish trypanosomiasis obtained at the same time.

The fish in these experiments were kept in the constant-temperature apparatus of the zoölogy department of Cambridge University[1] in aquaria measuring 24 × 12 × 12 inches. The temperatures maintained were 5°, 10°, 15°, and 20° C., with aeration and a constant slow flow of the water. The fish were fed *Daphnia, Enchy-*