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Hormonal Differentiation of the Developing Central Nervous System with Respect to Patterns of Endocrine Function [and Discussion]

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Hormonal differentiation of the developing central nervous system with respect to patterns of endocrine function

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Contents

Three types of sexual cycles are found in mammals. First, the life-cycle—puberty, adulthood, senility; secondly, the breeding season; and thirdly, the oestrous or menstrual cycles. The first two types of cycle are to be found in both male and female mammals, while the *third type is found in females alone*. Since the early embryo is sexually undifferentiated, it is of interest to see what factors are responsible for bringing about the development of the short and rhythmic oestrous and menstrual cycles in the female mammal, but the steady-state, arhythmic pattern of activity in the male mammal. Relatively recent work has shown that: (*a*) the oestrous and menstrual rhythm is essentially dependent on some neural mechanism in the brain, probably situated in the hypothalamic-preoptic region, and (*b*) that the factors responsible for the development of this neural mechanism in the brain of the female, but not in the male, are basically similar to the factors that bring about the development of the Müllerian duct system in the genetic female and the Wolffian duct system in the genetic male.

Sexual differentiation of the reproductive tract

The freemartin has been known for some hundreds of years as a sexually abnormal calf, born as a twin to a normal male. The internal anatomy of this intersex female was described by John Hunter in the eighteenth century. Keller & Tandler (1916) and Lillie (1916, 1917) independently pointed out that the placentae of the normal male and female intersex twin were united by vascular anastomoses and proposed that the abnormalities in the developing female were brought about by hormones carried to it from the male. More recent views on the aetiology of the freemartin have suggested that the condition is due to primitive germ-cells from the male being carried to the female and settling in the female gonadal ridge (see Moore & Owen 1965). However, with respect to the factors controlling the development of the reproductive tract and genitalia, it may be said that in either case it is male testicular hormone (whether from the testis of the male or the ovotestis of the female) which results in masculinization of structure in the female.

Bouin & Ancel (1903) first proposed that the reproductive structures of the embryo are under the differentiating action of hormones produced by the developing gonads, on the grounds that the testes of pig embryos showed great numbers of interstitial cells during the stage of sexual differentiation. This, together with the observations of Keller & Tandler, and Lillie, stimulated much work on the influence of hormones on genital tract development. Many of the early studies were carried out on amphibians and birds, in which the embryo is freely available for experiment as free-swimming larvae or as eggs. From about 1940 experiments have been conducted on mammalian embryos (see Dantschakoff 1936; Greene & Ivy 1937; Raynaud 1937, 1942; Burns 1942; Jost 1947). The general conclusion from a great deal of work on many forms is that the differentiation of the reproductive tract and external genitalia is under the influence of hormones secreted by the gonads. In mammals at least, it is the developing testis which seems to be the important factor—the ovary playing no part. If the testis is present then male organs develop. If the testis (or indeed ovary) is absent then female organs develop. The position is aptly summarized by the statement of Wells (1962) that the evidence would seem '. . . to lead to the notion that only the developing testes prevent an hypothetical state of affairs in which every mammalian embryo would develop into an individual with a female type of reproductive tract'. This subject is fully discussed by Jost (see p. 119, this volume).

Sexual differentiation of the brain

Work over the last ten years gives strong indication that the embryonic brain is sexually differentiated in a manner very similar to that of the reproductive tract. The critical period during which gonadal hormones exert an inductive influence on neural mechanisms occurs later than the critical period during which the tract is influenced. This may be related to the fact that the reproductive tract is affected by a local diffusion of hormones from the developing gonad, while the central nervous system has to await the formation of efficient general and local circulatory systems for the effects to be mediated.

The 'sexual differentiation of the brain' may be assessed in the laboratory by means of two criteria: (*a*) The hypothalamic region of the brain is responsible for regulating the secretion of gonadotrophic hormones in a rhythmical fashion in the female mammal, and this in turn results in the oestrous or menstrual cycles. In the male no such neural mechanism or cycles exist. And (*b*) the hypothalamic region of the brain is also responsible for integrating the pattern of sexual behaviour which is typical of the female or male. Dr R. Goy's paper deals with evidence relating to this latter criterion, while the account presented below is concerned with evidence relating to the establishment of rhythmic or arhythmic patterns of reproductive activity. The most complete evidence in this latter report is based on studies on the rat since this animal is born, relative to many other mammals, in an immature state. The newborn rat may be equated in terms of development with the human foetus at the third month of pregnancy, and it is during the first few days of life that the hormonal inductive influences are exerting their effects on the rat's central nervous system. Thus the newborn rat is a convenient free-living animal for experiments in this field.

A key point in the normal oestrous or menstrual cycle is the sudden surge of secretion of luteinizing hormone (LH), which is neurally controlled, and which evokes ovulation and the formation of corpora lutea in the ovaries. Many years ago it was known that ovarian transplants placed into male rats, castrated when adult, failed to show ovulation or the formation of corpora

lutea (Goodman 1934), although ovarian transplants placed into ovariectomized females showed rhythmic formation of corpora lutea and maintained normal reproductive cycles. In 1936, a classical paper by Dr C. A. Pfeiffer described the effects of gonadectomy, and gonadal transplantation, in newborn rats on the secretion pattern of gonadotrophic hormones seen when these animals became adult. He showed, among other findings, that:

(1) Male rats castrated at birth, and transplanted with an ovary when adult, showed the capacity to form corpora lutea in this ovarian tissue.

(2) Female rats ovariectomized at birth, and implanted with an ovary when adult, showed normal oestrous cycles and corpora lutea formation.

(3) Male rats in which the testes were transplanted into the neck region at birth, and which were implanted with an ovary when adult, showed no capacity to form corpora lutea in the ovarian tissue.

(4) Many female rats into which testes were transplanted at birth failed to show any sign of oestrous cycles when they became adult, but entered a state of constant vaginal oestrus and failed to show the formation of corpora lutea in the ovaries.

Pfeiffer drew the conclusion that both male and female rats were born with a sexually undifferentiated anterior pituitary gland which had the capacity to secrete gonadotrophins in a cyclical fashion. If newborn animals of either sex were exposed to the secretion of the testis then differentiation of the pituitary occurred into that of the male type in which the power of rhythmic secretion of gonadotrophin is lost. He commented: '. . . the sex difference in the hypophysis is not genetic but is secondary and dependent upon the presence of differentiated sex glands'. The facts recorded by Pfeiffer have been well established, but the conclusions he drew have needed modification in the light of further data. Harris & Jacobsohn (1952), having established a method for obtaining active secretory anterior pituitary transplants, showed that *male* pituitary glands transplanted into the hypophysectomized female were fully capable of maintaining normal *female* sexual functions. Confirmation of these results came from the studies of Martinez & Bittner (1956). These results show that anterior pituitary tissue does not become sexually differentiated as supposed by Pfeiffer, but remains plastic and pluripotential in nature. Pfeiffer's results were then reinterpreted in terms of the central nervous system. Both Everett, Sawyer & Markee (1949) and Harris (1955) expressed the view that sexual differentiation of the nervous system (probably hypothalamus) occurs during foetal or neonatal life (according to species) under the action of male gonadal hormones.

Studies on female rats given testicular hormone at birth

A great technical simplification was achieved when it was found that injection of exogenous steroids into newborn mice and rats gives similar results to Pfeiffer's gonadal transplantation techniques on the eventual sex rhythms shown by the animals. Mazer & Mazer (1939), Bradbury (1941) and Huffman (1941) observed that injection of prepubertal female rats with androgen resulted in subsequent infertility. Barraclough & Leathem (1954) found that single injections of testosterone propionate to 5-day-old mice also resulted in permanent sterility, but that injections into mice 20 days of age was without effect. Subsequently, Segal & Johnson (1959) and Barraclough (1961) analysed the effects more completely in rats. Barraclough found that injection of 1.25 mg of testosterone into 5-day-old, in some 10-day-old, but not in 20-day-old, female rats results in permanent sterility, lack of ovulation and of corpora lutea formation, and an

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increased growth rate. These results are similar to those of Pfeiffer, in which newborn female rats received testis transplants, and lead to a similar conclusion: that the presence of testosterone in the early neonatal period of the female rat may masculinize the central nervous system, thus resulting in acyclic release of gonadotrophin when the animal is adult.

Harris & Levine (1962, 1965) confirmed and extended the original observations of Barraclough and others. It may now be said that a single injection of testosterone into a newborn female rat results in the following abnormalities: (1) increased growth rate; (2) early puberty (breakdown of vaginal membrane); (3) a state of 'constant oestrus' from puberty onwards—loss of oestrous cycles and lack of ovulation and corpora lutea development in the ovaries; (4) a loss of the normal female sexual behaviour pattern (even when ovariectomized and primed with oestrogen and progesterone), but a marked enhancement of the male pattern of sexual behaviour (when ovariectomized and primed with testosterone).

The increased growth rate of the testosterone-injected female rat was noted by Barraclough (1961), Harris & Levine (1962), and studied in detail by Swanson & van der Werff ten Bosch (1963). Barraclough (1961) noted the protein anabolic effect of the androgen could hardly be the sole cause of the increased growth, since a similar injection after ten days of age did not have the same effect. It has been suggested (Harris 1964) that these results may be attributable to a sexual differentiation of some hypothalamic mechanism which regulates the secretion of growth hormone.

The earlier date of puberty of testosterone-treated neonatal female rats was seen by Segal & Johnson (1959). They found that the testosterone-treated females showed a precocious opening of the vagina some 7 to 10 days before normal females; an observation confirmed by Harris & Levine (1962).

The 'constant-oestrus' state that supervenes after puberty has been seen by many workers. Daily vaginal smears taken from these animals show no sign of the usual 4- to 6-day cycles, and consistently show the presence of cornified squamous cells. The ovaries are small and white in colour, showing the development of follicles but no sign of ovulation and no corpora lutea. The uterus is not distended with fluid (as in the normal rat at oestrus), and the adrenals, thyroid and pituitary appear normal.

The efficiency of the ovarian feed-back mechanism has been tested in these neonatally testosterone-treated females. Harris & Levine (1962, 1965) found that administration of oestradiol to these animals, when adult, resulted in ovarian atrophy; and after ovariectomy castration cells appeared in the pituitary gland. However, the results indicated that the tissues acted upon by oestrogens (anterior pituitary gland and uterus) were less sensitive than normal to the hormone. Barraclough (1963) observed that unilateral ovariectomy in these animals was followed by an equal degree of compensatory hypertrophy of the remaining ovary, as in normal animals, and concluded that the hypothalamo-hypophysial axis of the anovular rat can increase FSH secretion in response to a decrease in blood oestrogen level.

Various parameters of the initial injection of testosterone into neonatal rats have been compared with the ultimate results by Barraclough and his co-workers, and others. The timing of the injection, the dose and the specificity of the response have all been studied. As mentioned above, testosterone injection into 2- and 5-day-old rats showed a consistent effect, into 20-day-old animals was ineffective and into 10-day-old rats was effective in about half the animals. From this it appears that the nervous system is passing through a plastic and undifferentiated stage up to about 10 days of age, but from then on becomes fixed in its form. The question may

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be asked as to whether the critical period of differentiation of the nervous system of the rat exists before birth. Swanson & van der Werff ten Bosch (1964, 1965) found that large doses of testosterone propionate (10 to 25 mg) given to pregnant rats in the late stages of pregnancy could induce the early androgen syndrome (Caesarian section and foster mothers were used). Injection of 20 or 100 μg given directly into the foetus 1 to 4 days before delivery also resulted in masculinization of the females and the anovular syndrome when adult. The conclusions are drawn that the apparent low sensitivity of the foetus to testosterone propionate injected into the mother is due to poor transmission of the steroid from the maternal into the foetal body; that the external genitalia of the foetus are more sensitive than the hypothalamo-hypophysial complex to the action of testosterone; and that the critical period of the nervous system does in fact begin in the later days of foetal life. Somewhat similar results have been obtained by Gerall & Ward (1966) who studied the sexual behaviour pattern of female rats androgenized prenatally (1, 2 or 5 mg testosterone propionate injected into the mother during the last week of pregnancy).

The dose of testosterone propionate necessary to elicit the early androgen syndrome has been investigated by Barraclough & Gorski (1962) and Gorski & Barraclough (1963). These workers injected 1, 5 or 10 μg dissolved in oil, subcutaneously, into 5-day-old animals, and found that injection of 1 μg induced the syndrome in 30% of cases, 5 μg in 44% and 10 μg in 71%. Injection of 1.25 mg induced the syndrome in 99.8%. It is of some interest that the injections of 10 μg had not greatly impaired the sexual behaviour of these animals though it impaired the gonadotrophin control mechanism. It is therefore likely that the neural substrata of sexual behaviour and gonadotrophin control are distinct and have a different threshold in their differentiation to testosterone. The ultimate effect on the gonadotrophin-regulating centre in the hypothalamus appears to be dependent on the dose of androgen given in the postnatal days. For example, if low doses of testosterone propionate (5 or 10 μg) are given to female rats on the fourth day of life, they may ovulate for a few weeks after puberty and later become anovular (Swanson & van der Werff ten Bosch 1964; Gorski 1966). Animals given larger doses pass into a state of constant vaginal oestrus at puberty and never ovulate. Gorski (1966) compared these data with the results observed after transplanting testes subcutaneously into 2-day-old female rats and concluded that a single testis from a newborn rat produces the equivalent of a single injection of approximately 30 μg testosterone propionate or more. He concludes: 'The fact that the rat androgenized by 10 μg TP can ovulate for a time probably reflects a subthreshold or incomplete masculinization . . . It is possible, therefore, that with the lower dosage some capacity for the cyclic release of LH remains. With the passage of time, however, this capacity may be lost, due possibly to exhaustion of the neural system . . .' It is of interest to compare the approximate figure of the secretion of one newborn testis, given by Gorski ($\equiv$ 30 μg testosterone propionate injected subcutaneously) with the measurements of Resko, Feder & Goy (1968) in which the plasma concentration of testosterone (μg/100 ml plasma) found at various postnatal time periods were: day 1 (0.027), 5 (not detectable), 10 (not detectable), 30 (0.018), 90 (0.202), 120 (0.118). These workers also gave figures for the testosterone concentration in the testes (μg/g tissue): day 1 (0.463), 5 (0.134), 10 (0.122), 30 (0.005), 60 (0.014). K. Brown-Grant, A. Munck & F. Naftolin (1969 personal communication) and Alklint & Norgren (1969), however, have found that injections of testosterone are less effective than injections of testosterone propionate, in equivalent doses, in exerting a masculinizing action in the neonatal female rat. Thus the figure given above by Gorski (1966) may not be comparable in any way with those given by Resko *et al.* (1968).

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The site of action of testosterone administered to newborn female rats, in producing its masculinizing effects, was at first debatable. Three possible sites of action—the ovary, pituitary gland and central nervous system—were apparent. Transplantation experiments have clarified the situation. To see whether the anovular ovaries of testosterone-treated females had the potentiality of normal function, they were transplanted into normal adult females (ovariectomized) and found to ovulate with a normal rhythm, to form corpora lutea and to maintain normal oestrous cycles (Bradbury 1941; Harris 1964; Harris & Levine 1965). By similar methods Segal & Johnson (1959), and Adams Smith & Peng (1966) showed that pituitary glands transplanted from androgenized and anovular females into normal (hypophysectomized) females are capable of maintaining normal oestrous cycles with rhythmic ovulation. Thus there is no reason to believe that the original injection of testosterone has a direct action on ovarian or pituitary tissue which is responsible for the development of the 'androgen-anovular' rat. It is now generally accepted that the structure affected by the neonatal injection of testosterone resides in the central nervous system, and probably in the hypothalamic area. This view is reinforced by the observations of a number of workers that various central nervous depressant drugs can block the masculinizing action of testosterone. These drugs include reserpine, chlorpromazine, pentobarbital and phenobarbital (Kikuyama 1961, 1962; Arai & Gorski 1968*a*, *b*). The protective action of pentobarbital is said to be eliminated by simultaneous injection of metrazol (Arai & Gorski 1968*a*). Kawashima (1964) found that newborn male rats that received daily injections of reserpine for the first 10 days of life, showed a female pattern of secretion of gonadotrophin at 80 days of age. According to this evidence then, reserpine can block the action of endogenous testicular hormone. It should be mentioned, however, that Zucker & Feder (1966) found that reserpine treatment did not protect neonatal female rats against the masculinizing action of exogenous oestradiol. Progesterone has also been found to block the masculinizing action of testosterone administration as judged by the presence of corpora lutea in 45-day-old female rats (Kincl & Maqueo 1965). This may be an example of competitive binding at the target tissues. Further evidence that the hypothalamus or preoptic region is the site of action of testosterone in the rat's neonatal period is the data which have accrued from studies using electrical stimulation or lesions in these areas. This evidence has been fully discussed by Barraclough (1966). Barraclough and his co-workers proposed that the ventromedial-arcuate nuclei region of the hypothalamus regulates a *tonic* secretion of gonadotrophin from the pituitary which is sufficient to maintain oestrogen secretion from the ovary. The preoptic area, they suggest, regulates the cyclic ovulatory surge of gonadotrophin responsible for ovulation, and acting via the ventromedial-arcuate region affects the anterior pituitary. If this view is correct, then testosterone in the neonatal rat would be expected to exert at least one of its actions on preoptic neurones. It has been argued that if testosterone, or some testosterone-like hormone, acts in an inductive way to develop a male neural mechanism in the anterior hypothalamic–preoptic region, then this differentiation might be reflected in biochemical changes in the properties of the neurones (greater or lesser ability to accumulate isotopically labelled gonadal steroids from the blood; a change in RNA or protein synthesis), and anatomical (maybe ultrastructural) changes. There is some evidence that the nuclear and nucleolar size of nerve cells in the anterior hypothalamic–preoptic region is different in normal male and female rats and in experimentally treated animals (see Döcke & Kolocyek 1966; Pfaff 1966; Arai & Kusama 1968; Dörner & Staudt 1968). It would appear that the nuclear size of nerve cells in this area of the brain is less in normal male rats than females. Male rats castrated on the first day of life are reported to

show a significant increase of such nuclear size which approximates to the size of that in female animals. These findings are of such importance that independent studies on other species are anxiously awaited.

The specificity of testosterone as the physiological hormone involved in masculinization of the male has been debated and much discussed. In 1962, Takewaki reviewed the evidence that oestrogen, progesterone, desoxycorticosterone acetate, and even cholesterol could all have similar effects after injection into the newborn female rat. Recent studies have substantiated the curious fact that oestrogen can have a masculinizing action in the neonatal female rat (Wilson 1943; Gorski 1963; Arai 1964; Whalen & Nadler 1965; Feder 1967), but the effects claimed by Takewaki for some of the other steroids are now a little doubtful. Gorski (1966) summarizes much evidence in this respect and states that of the steroids, only testosterone and oestradiol were routinely effective and of the non-steroids, diethyl stilboestrol and hexoestrol were also active. However, the interesting fact remains that the threshold dose of testosterone propionate seems to be in the range of 10 μg (injected in oily solvent, s.c.) and of oestradiol benzoate 5 μg (similarly injected). In other words, the female hormone seems to exert a stronger masculinizing action than the male hormone. However, it may well be that the physiological hormone secreted by the testis and involved in this masculinizing action on the hypothalamus is not testosterone itself but some closely related androgen. Much interest, at the moment, centres on dihydro-testosterone.

Studies on male rats deprived of testicular secretion at birth

Ovarian tissue transplanted into male rats, *castrated when they are adult*, does not show the processes of ovulation and formation of corpora lutea. Ripe follicles develop and some thecal luteinization of the follicular walls may occur (as discussed by Harris 1964), but the evidence is strong that such luteinization is not consequent upon previous ovulation. The interpretation may be made that the hypothalamic control of the surge in the release of luteinizing hormone, which occurs at 4- or 5-day intervals, is absent in the normal male rat.

Studies on male rats castrated at birth, however, gave different results. As mentioned previously, Pfeiffer (1936) was the first to report the basic finding that castration of the neonatal male, followed by transplantation of ovarian tissue after puberty, is followed by cycles of LH release with the consequent development of many large corpora lutea in the ovaries. From these results and those he obtained on female rats, Pfeiffer concluded that at birth both male and female rats possess an undifferentiated mechanism which has the potentiality of regulating LH release in a cyclical fashion. In the normal female, or in the male castrate at birth, this mechanism becomes fixed or set in this basic pattern. In the normal male the testicular secretion acts on this mechanism in the critical few days after birth to differentiate it into an acyclic control mechanism so that swings or cycles do not occur in gonadotrophin secretion in the normal adult male. From Pfeiffer's results it was clear that neonatal castration is followed by luteal formation in ovarian transplants, but it was not clear whether this formation of corpora lutea occurred at regular intervals with a rhythm equating to that of the oestrous pattern of the female. That such was indeed occurring has recently been shown by Yazaki (1960). Yazaki transplanted both ovarian and vaginal tissue into various experimental groups of male rats. If the transplants were placed into males castrated when adult, then persistent cornification of the vagina was observed even though a few 'corpora lutea' were found in the ovarian

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transplant (presumably thecal in origin). This persistent state of vaginal cornification could not be interpreted by procedures thought to influence gonadotrophic secretion in the normal female, such as application of silver nitrate to the nasal mucosa, injections of oxytocic hormone or repeated copulation. In another series of experiments, Yazaki castrated male rats at 0, 3, 5, 10, 20 and 40 days after birth and transplanted ovarian and vaginal tissue side by side under the abdominal skin. In thirteen out of fifty animals castrated on days 0 and 3, vaginal cycles of approximately 4 days in length were observed to occur for periods of up to 2 months. The males that were castrated after 3 days of age showed persistent vaginal cornification. Yazaki concludes that in the rat '. . . the testis exerts an influence on the hypothalamus within 3 days after birth and induces the male type functional differentiation in the hypothalamus'.

Recent studies from my own laboratory (published in preliminary form—Harris 1964) have extended the observations of Pfeiffer and of Yazaki. Since these results will be submitted for full publication by the Royal Society in the near future, only a summary is presented here. Up to the moment 175 rats have been gonadectomized at various stages of life and the rhythm of gonadotrophin secretion studied by making transplants of ovarian tissue into the anterior chamber of the eye. Sixty-one male rats were castrated within the first 24 h of life and 58 of these showed the capacity to form many large corpora lutea in the ovarian transplants. In animals castrated between 24 and 72 h of birth, only seven out of 33 showed a similar capacity, and none out of 33 castrated after 72 h showed this response. Likewise, none out of 15 males sham-operated during the first 24 h of life showed the ability to form corpora lutea in ovarian transplants.

The cyclical nature of luteal formation and endocrine activity in ovarian eye transplants was also investigated. For comparison with the males, ovarian transplants were placed in the eyes of 16 ovariectomized (but otherwise normal) female rats and the vaginal cycles were followed. In all cases corpora lutea developed in the transplants, and regular vaginal cycles of normal duration were observed. The possibility that a cycle of about 5 days' duration occurs in the ovulation process and endocrine function of the ovarian transplants made in the newborn male castrate has been investigated in three ways. First, daily colour photographs have been taken of the transplants over a period of several months and an attempt made to ascertain whether new batches of corpora lutea appear rhythmically. Even by careful study of the eye daily through a binocular microscope and by scrutinizing the daily photographs, it is difficult to plot the cycle of ovulation clearly. The first cycle of ovulation that occurs in a transplanted ovary may be clearly observed, since the appearance of the ovarian tissue changes so dramatically. This usually occurs between the 10th and 14th day after implanting in the eye. Often the second cycle of ovulation may be observed, but at later dates the transplant becomes such a complex mass of follicles and corpora lutea of different ages that identification of the dates of follicular rupture becomes uncertain. Secondly, vaginal transplants have been placed subcutaneously beneath the abdominal skin according to the method of Yazaki (1959). In male rats castrated during the first 24 h of life and in which ovarian transplants showed the development of many large corpora lutea, typical cycles of vaginal oestrus of about 5 days' duration were observed. The smears of dioestrus, pro-oestrus, oestrus and metoestrus were similar to those seen in normal female rats. Thirdly, activity cycles have been followed in many rats—normal females and males, and males castrated at various times of life. It is well known from the work of Richter (1933) and Richter & Hartman (1934), and others, that if a rotating drum is made

available to rats they will enter and run varying amounts each day. Normal male rats show a low and irregularly fluctuating amount of running, whereas females show high peaks of activity at oestrus and lower levels of activity during other phases of the sex cycle. It was found that newborn castrated males, implanted with ovarian and vaginal tissue when adult, showed female patterns of running with peaks of activity at the 'oestrous' stage of their sexual cycles. These cycles did not occur in males sham-operated at birth.

It would thus appear that male rats, castrated within the first 24 h of life, have the neural mechanism for controlling LH secretion according to the normal female pattern.

Evidence derived from other forms

Natural sex reversal is found to occur spontaneously in the lower vertebrates (such as in some teleost fishes) and has been discussed in the paper by Dr S. T. H. Chan (see p. 59). Complete sex reversal may be obtained in amphibians by grafting experiments or by administration of the sex steroid hormones (see reviews by Burns 1961; Wolff 1962; Wells 1962). In some cases it has been found possible to obtain fertile matings between a normal male and a sex-reversed male (both with ZZ chromosomes) and in these instances the offspring are all males (with ZZ chromosomes). Similarly, fertile matings between two genetic female amphibians has been achieved. In mammals, however, transformation of gonads into that of the opposite sex is much more difficult. Burns (1955) found it possible almost completely to transform the gonads of newborn male opossums (born after a gestation of 12 days) into ovaries by daily injections of oestradiol dipropionate. Turner & Asakawa (1964) found it possible to transform a foetal mouse ovary into an ovotestis by transplanting it, in close proximity to a foetal testis, under the kidney capsule of a castrated adult male mouse. Thus in mammals, while striking examples of sex reversal in the developing reproductive tracts have been achieved, there are few examples of transformation of the gonads.

The work discussed above on the sexual differentiation of the central nervous system was concerned largely with the rat. There is good evidence, however, that other mammals fit into the general pattern indicated by this work. Some of the early work in this field was performed on *mice*. Barraclough & Leathem (1954) and Barraclough (1955) gave groups of female mice injections of testosterone propionate at 2, 5, 10 or 20 days of age and killed them at 10-day intervals until 60 days of age. The ovaries of all animals treated with a single injection of 1.25 mg testosterone propionate at 2 or 5 days of age showed an absence of corpora lutea. Treatment with androgen at 10 days of age delayed the appearance of corpora lutea by approximately 10 days (from 50 to 60 days of age); at 60 days of age 85 % of the ovaries contained corpora lutea as compared with 90 % of the litter-mate controls. All ovaries of mice treated with androgen at 20 days of age were normal at autopsy. Following these early studies on mice, and the more recent and more extensive studies on rats, other species have also been investigated. Swanson (1966) and Alleva, Alleva & Umberger (1969) have studied the effects of early androgen treatment on *hamsters*. It seems clear that animals treated at 2 days of age are eventually acyclic and their ovaries do not develop corpora lutea. At maturity, the animals given testosterone propionate at 4 days of age displayed normal 4-day vaginal, oestrous and ovulatory cyclicity. Apparently the female cyclic type of hypothalamic control over pituitary gonadotrophin secretion is normally determined on the third or fourth day of age in this animal in spite of the relatively short duration of pregnancy, as compared with other species.

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Results obtained on the *guinea-pig* are of much interest, since this animal normally develops functioning corpora lutea each oestrous cycle, as does the human. The long duration of pregnancy in the guinea-pig leads to the conclusion that the newborn rat equates in development with (about) the mid-term guinea-pig foetus. In a brief abstract Tedford & Young (1960) reported abnormalities of corpora lutea function and, in a few cases, complete absence of luteal tissue in the ovaries of animals born to mothers treated with testosterone propionate from day 10 or day 24 of pregnancy up to day 65. These animals were killed on day 60 or day 90 of postnatal life. A more recent and detailed investigation by Brown-Grant (1969) gives more precise data regarding the critical period for the production of the anovular syndrome: when testosterone propionate was administered to pregnant guinea-pigs over a short period (days 33 to 37 of pregnancy) a high proportion of the female offspring developed the anovular syndrome when adult. Some masculinization of the external genitalia was observed, the masses of the uteri and ovaries were increased over those of the control animals, and histological examination of the ovaries showed the presence of antral follicles but no luteal tissue. Thus it may be concluded that the guinea-pig conforms in its response to early androgen treatment with the mouse, rat and hamster.

In the above description of work in forms other than the rat, no mention has been made of concurrent studies on the sexual behaviour patterns shown by the treated animals. This subject is discussed in detail in the paper by Dr Goy (this volume, p. 149). However it may be mentioned here that extensive studies show that masculinized female rats and guinea-pigs both tend to show masculine patterns of sexual responses, and that feminized male rats show marked female type behavioural responses. Experimentally masculinized female monkeys also show male patterns of behaviour (again see Dr Goy's paper), and human males suffering from the 'testicular feminizing syndrome' are female in bodily appearance, and also show female patterns of behaviour in their married life and toward adopted children (see paper by Professor Polani). There are strong indications then that the same principles of sexual differentiation apply to a number of forms—mice, rats, hamsters, guinea-pigs, monkeys and man.

It may be argued on the above evidence that the gonad of the sexually undifferentiated embryo is first transformed, under genetic influences which may involve a chemical step, into a testis or an ovary. If a testis is formed, then normally a sequence of events occurs which results in the development of a male foetus and offspring. If a testis is not formed then a female results. The sequence of events in the male line involves, first, the hormonal differentiation of internal male ducts and accessory glands, secondly the formation of male external genitalia, and thirdly the inductive influence of hormones on the central nervous system with its repercussions on the patterns of gonadotrophic secretion and of sexual behaviour in the final adult animal. It is clear that there are various steps in this sequence at which errors of development could occur. As a speculative indulgence, such errors could be listed as follows:

(*a*) In the initial stage—gonadal differentiation may result in the development of an ovotestis, or an ovary on one side with a testis on the other.

(*b*) In the second stage—if testes develop but there is an enzymic block to the action of the testis hormone at the tissue level, then the sequence of future development could be along the female line, and the end result might be similar to the 'testicular feminizing syndrome' seen clinically.

(*c*) In the third stage—failure of action of the testis or its hormones might result in an individual with a male internal duct and gland system, but with feminized external genitalia.

(*d*) And finally, if the inductive error occurred in the last stage, so that only the central

nervous system was unaffected by the male hormone, then the organism would be expected to show the full anatomy of the male but to have neural mechanisms controlling gonadotrophin secretion and sexual behaviour of the female type. In other words, one form of male homosexual (or female) could be accounted for on this hypothesis.

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Discussion on paper by G. W. Harris, p. 165

A. Muncк (*Department of Physiology, Dartmouth Medical School, Hanover, New Hampshire, U.S.A.*): While testosterone is perhaps the physiological androgenizing hormone, there are a number of difficulties with this assumption. First, the lack of specificity is a problem; similar effects can be produced with similar doses of oestrogens. Secondly, localization of a sensitive region in the brain using testosterone propionate is relatively poor. Furthermore, with such implants rather high doses are required for activity, so high indeed as to be effective subcutaneously to some extent. Thirdly, the levels of testosterone that have been found by Goy, Feder and Resko in early postnatal and young rats are much lower than in adult rats; if a burst of testosterone is necessary for masculinization then one might expect the opposite.

With Drs Exley and Naftolin we have carried out some experiments which also cast doubt on the role of testosterone. We have found that a dose of testosterone propionate as low as 10 μg, given subcutaneously to female 5-day-old rats, results in levels of testosterone in the blood which are initially ten times greater than those in male rats of that age, and which persist above the male rat levels for 3 days. We are currently testing testes extracts, and also dihydrotestosterone and androstanediol on the working hypothesis that there may be substances other than testosterone coming out of the testes and causing androgenization.

R. W. Goy: Two discrepancies exist between the data on rats and monkeys. Short periods of injection into rats led to either delayed or immediate anovulation and persistent oestrus, whereas large injections over a long period of time into rhesus monkeys have not produced psuedo-hermaphroditic females that fail to ovulate. From our observations on eight monkeys, there is clear evidence that three have ovulated. The remaining five may yet be too young to do so.

The second discrepancy is that puberty is advanced in the rat but delayed in the rhesus monkey. A delay in puberty could be considered as a masculinization effect because puberty in the male rhesus and in human occurs at a later chronological age than in the female. Could the mass of the rat offspring be the factor behind this discrepancy? We have never found any differences between the growth curves of treated female monkey and normal females, whereas androgenized rats gain mass or grow more rapidly than normal females. In guinea-pigs there is a closer correlation between body mass and the first oestrus than between chronological age and the first oestrus.

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G. W. Harris: It is difficult to explain why many pseudo-hermaphroditic monkeys have menstrual cycles. At a critical stage of development insufficient testosterone might cross the placenta to virilize the central nervous system, although enough to virilize external genitalia. Gorski and others have shown that in rats 10 μg of testosterone propionate is the lower threshold dose to completely virilize the c.n.s., and at this level some females will show oestrus cycles immediately after puberty and then revert to a constant oestrus by 90 days of age. Monkeys may respond more immediately to a lower dose, although masculinization is incomplete. Radioactive testosterone had not demonstrated any specific site of localization on the hypothalamus, perhaps because this was not the physiological hormone. This approach was difficult as compared with the localization of radioactive steroids in other tissues, e.g. progesterone in the uterus. There may be no requirement for the concentration of testosterone in the hypothalamus, and the analysis of metabolic changes in the c.n.s. may be a more successful approach, e.g. by measuring effects on DNA, RNA and protein metabolism.

H. Peters: We have repeated the experiment of Barraclough and Harris on the administration of testosterone to mice in order to analyse ovarian development in the infant period. There is a direct effect on the ovary. The number of oocytes is reduced immediately, followed by other effects. When the treated females were paired with males vaginal plugs were found on average 5 days later than in normal mice. The treated animals became pregnant but failed to implant the embryos during early maturity. This might be due to a metaplasia of the endometrium which is often seen in young adult mice that have been given androgens.

G. W. Harris: When vaginal tissue from mice in constant oestrus is transplanted into a cyclic mouse, the transplant remains cornified, again indicating an action on epithelium as Peters has described for the endometrium.

D. Jacobsohn: It is necessary to be clear if testosterone or testosterone propionate is used in various experiments. Testosterone propionate has a longer period of action than testosterone when given in the same dose and in the same solvent. For example, testosterone propionate has a duration of action on the mammary gland of 10 days, whereas the action of testosterone is much briefer.

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