THE REPRODUCTIVE SYSTEM OF A PREGNANT HERMAPHRODITE RABBIT (*ORYCTOLAGUS CUNICULUS*)

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The hermaphrodite rabbit described in this paper was a wild animal of medium size, purchased for class purposes early in February; it was trapped in the Llandovery district of Carmarthenshire. So far as I am aware no case of hermaphroditism in the rabbit has ever been recorded, and amongst rodents this phenomenon is extremely rare. The present specimen was particularly interesting because separate ovaries and testes, the latter descended into scrotal sacs, were present on each side of the body; at the same time it was functioning as a normal female individual, as shown by the presence of two foetuses in the right uterus. In the majority of mammalian intersexes the genital system consists of a mixture of male and female organs, usually abnormal and non-functional, together with imperfect external genitalia; Brambell & Hall (1936), however, have described an intersexual lesser shrew (*Sorex minutus*) which functioned as a typical male individual, in spite of the fact that it had well-developed Fallopian tubes, uteri and vagina; no ovaries were present. This case is analogous to the present specimen except that in the latter the opposite sex was the functional one, and degenerate testes were present as well as ovaries.

The tissues of the reproductive system were not specially fixed for histological investigation as the abnormality of the specimen was not discovered until it had been partially dissected. For the same reason certain anatomical details unfortunately could not be verified.

OBSERVATIONS

The appearance of the external genitalia was interesting because it showed a very definite male trend in development on which that of the female was superimposed. The opening of the vestibule was large and rather elongated; the margins of the 'labia minora' projected and were considerably folded; the vulva, which normally is formed from the labio-scrotal folds, was not represented as such but was differentiated in the form of scrotal sacs similar to those of a normal male.

Lying in the ventral wall of the vestibule was an enlarged clitoris which projected beyond the margin of the opening, and ended in an epithelial 'tag' (Fig. 1 D; and cf. that of a normal female, Fig. 1 C). The opening of the vestibule was closed by the folding inwards of the labia and the bending upwards of the clitoris (Fig. 1 E); the latter was stiffened by strong corpora cavernosa and controlled by well-developed retractor muscles.

The ovaries, each attached to the dorsal wall of the abdomen by the mesovarium, were normal in position and appearance; no epoöphoron or paroöphoron could be found on either side of the body, but this was to be expected since these bodies represent the vestiges of the epididymis and paradidymis respectively, and remain in close association with the Wolffian duct and the testis. The differentiation of the Müllerian ducts was complete; normal Fallopian tubes, uteri, and vagina were present (Fig. 1 A), and two foetuses were present in the right uterus; there was a considerable accumulation of fat in the broad ligaments.

The male organs were much less complete; the testes, each covered with a tough tunica albuginea, were descended into scrotal sacs, to which they were attached by gubernacula in the typical manner. Each testis (Fig. 1 B) was of normal length, but much more flattened and less bulky than that of a typical male. The Wolffian ducts and their derivatives had presumably undergone a process of degeneration under the influence of the female sex hormone, for no vas deferens or epididymis could be found on either side; the uterus masculinus and prostate were also absent.

The vascular system of the reproductive organs was a combination of the male and female arrangement; for, in addition to the typical vessels of the female, there were spermatic arteries and veins, passing to and from the testes in the spermatic cords. The connexion of these vessels with those of the main system could not be traced as the cords had been broken before this examination was carried out.

A histological examination was made of the ovaries, uterus, vagina and testes; the sections were stained with Ehrlich's haematoxylin and eosin. Sections of testis were also counterstained with Van Gieson and picro-indigo-carmine, the former being used in order to get a picture comparable with that obtained by Crew & Fell (1922, pl. 18). Frozen sections of the testis were stained for fat with Sudan IV.

Ovaries. These appeared to be perfectly normal; Graafian follicles in various stages of development were present. Some of the larger follicles seemed to be undergoing a process of absorption but, according to Hammond & Marshall (1925, p. 80), follicular atrophy is quite common in the rabbit,

especially during periods of pregnancy and lactation.

In a section through the middle of the right ovary there were three corpora lutea, each of which was formed of trabeculae of large luteal cells which converged to a central connective tissue strand containing the small remaining portion of the central cavity; in other words, the corpora lutea were almost fully formed, showing that copulation must

membrane described by Chipman (1903), Hammond & Marshall (1925) and others, were well defined; the two mesometrical or placental folds were the largest and were well supplied with blood vessels. In the connective tissue between the uterine glands in both the peri- and ob-placental folds, though more frequently in the latter, were large solitary decidual cells, the cytoplasm of which was granular and deeply stained with eosin.
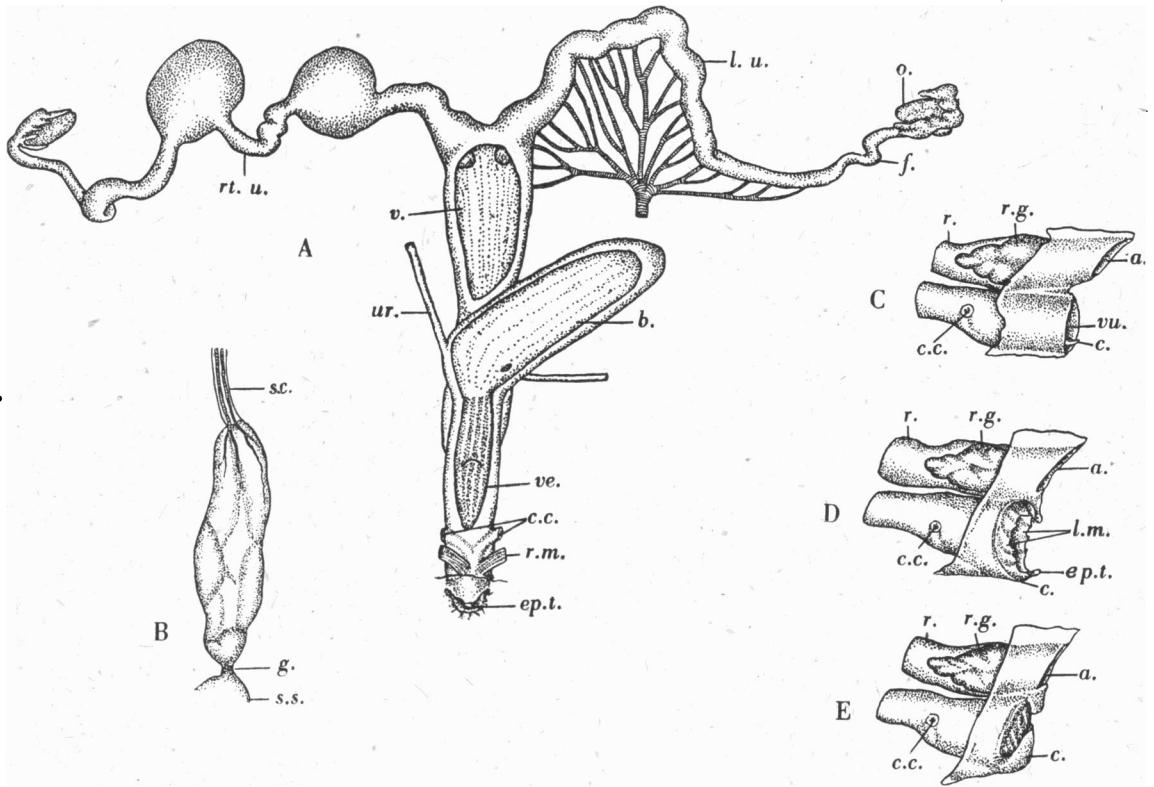

Fig. 1. A. Reproductive organs removed from the body and dissected from the ventral side. *o.* ovary, *f.* Fallopian tube, *rt.u.* and *l.u.* right and left uterus, *v.* vagina, *b.* bladder, *ve.* vestibule, *c.c.* corpora cavernosa, *r.m.* retractor muscle, *ur.* ureter, *ep.t.* epithelial tag. B. Testis withdrawn from its scrotal sac. *s.c.* spermatic cord, *g.* gubernaculum, *s.s.* remains of scrotal sac. C. Corresponding dissection of a normal female rabbit; the tip of the clitoris has been drawn outwards. *a.* anus, *r.* rectum, *r.g.* rectal gland, *vu.* vulva, *c.* clitoris, *c.c.* cut end of corpus cavernosum, *l.m.* labia minora. D and E. External genitalia seen from the left side after removal from the body. D. With the entrance to the vestibule open and E with it closed.

have taken place at least some ten days earlier. This assumption is based upon information obtained from statements made by Hammond & Marshall (1925, p. 61). A section of the left ovary showed the presence of a single corpus luteum undergoing atrophic changes.

Uterus. The wall of the uterus, as seen in transverse and longitudinal sections, was typical of that of a pregnant female rabbit; the muscular coat was hypertrophied and the six folds of the mucous

In the placental region the mucous membrane showed the structure typical of the normal pregnant uterus. The two blastocysts were not well preserved, and all that can be said about them is that they were firm and round, and measured about 20 mm. in breadth; they appeared to be undergoing normal development.

Vagina. The mucous membrane of the vagina was thrown into folds and its epithelial lining consisted of a single layer of columnar cells; the cyto-

plasm of these cells was vacuolated towards the base of the cell and granular towards the free surface; this appearance was very similar to that figured by Hammond & Marshall (1925, p. 90, pl. XI, fig. 3) for a pregnant rabbit with an accumulation of mucin at the base of the vaginal folds.

The result of this histological examination of the female organs of the hermaphrodite showed that the tissues did not differ from those of a typical female rabbit.

*Testis.** The structure of the testis was degenerate, but small lobules of seminiferous tubules, separated from each other by areas of adipose tissue, were discernible. The tubules were most abundant in the deeper regions around the blood vessels, while the peripheral areas consisted almost completely of fat separated into lobes by strands of fibrous connective tissue. A fairly large lymph nodule was present near the base of the testis.

The seminiferous tubules were much reduced in size, compressed and irregular in outline. White fibrous tissue, the elongated nuclei of which were clearly visible, was present between the basement membrane and the lining epithelium. Such a layer of tissue was observed by Crew & Fell (1922, p. 567) in the undescended testis of a rabbit; they suggested that it was similar to the intertubular fibrous tissue and was presumably formed by the cells of the basement membrane.

The tubules were not all in the same stage of degeneration, and some of the stages, mentioned by Crew & Fell in their paper quoted above, could be recognized; in no tubule was there any sign of active spermatogenesis and it seems unlikely that any had ever taken place.

In the less degenerate tubules the epithelium was represented by a single, often incomplete, layer of cells, the nuclei of which were quite distinct but the cytoplasm was fragmentary. Two types of cells, differing in the form of their nuclei, could be recognized; these, interpreted according to Crew & Fell, represented (1) cells of Sertoli with small ovoid and rather granular nuclei, and (2) spermatogonia in which the nuclei were larger and in many cases showed signs of degeneration; this was indicated by increased size, and the peripheral displacement of the chromatin in the form of granules.

Other tubules were more degenerate; in these most of the cells had broken away from the basement membrane and the lumina contained masses of cell detritus. In some of the tubules one or more spherical bodies, the outlines of which were clearly defined, were present in the cell debris; each consisted of a degenerate nucleus surrounded by a

* The sections of this tissue were submitted to Prof. F. W. R. Brambell for confirmatory examination, and he has expressed his opinion that it is probably testicular and not mesonephric.

concentric layer of vacuolated cytoplasm and resembled the ovum-like 'intra-tubular body' of Crew & Fell (1922, pp. 561–2 in the goat, and p. 569 in the rabbit). According to these authors the 'intra-tubular body' is produced as the result of colloid degeneration, either by the liquefaction of cell detritus around a degenerating nucleus or by the coalescence of several small 'colloid globules' in the midst of cell debris. The bodies were not as large or as deeply stained as those described by Crew & Fell, neither did they show any sign of calcification; it is probable that they represented an earlier stage in formation.

The intertubular tissue consisted almost entirely of metamorphosed interstitial cells, separated into lobules by strands of fibrous connective tissue. These cells appeared in one of three forms: (a) cells of oval or rounded shape, the outlines of which were very distinct, with granular cytoplasm and rounded, rather deeply stained, nuclei; these cells were present in the regions near the tubules and were few in number; (b) cells which were rather diffuse and vacuolated, with larger, less deeply stained, nuclei in which the chromatin appeared in the form of a reticulum; and (c) large cells almost entirely fitted with lipoids (as shown by the Sudan IV preparations), and in which the nuclei were small and eccentrically placed.

According to Crew & Fell (1922), who describe similar types of interstitial cells in a number of ectopic testes, these cells are not 'fat forming' but are hypertrophied by the accumulation within them of various fat globules and lipoids representing stored food material which normally would be used by the germ cells during spermatogenesis.

DISCUSSION

Hermaphroditism in certain mammals, especially in certain strains of pigs, is known to be fairly common; a number of cases has been described by various workers (Baker, 1925 a, b, 1926, 1928; Brambell, 1929; Crew, 1924; Pick, 1914). Amongst rodents, however, the abnormality is extremely rare, and, as Brambell (1930, p. 157) points out, no case in the mouse or rat is known, in spite of the fact that they are used in large quantities for laboratory work. Hermaphroditism in the rabbit has not been recorded previously although this animal is extensively used as a dissection type for teaching purposes.

The particular interest of the specimen described in this paper is not so much that it is unique amongst recorded abnormal rabbits in exhibiting hermaphroditism, but that it belongs to the rarest group of hermaphrodite types, namely, that in which a separate ovary and testis are present on each side of the body in their normal positions; the only other example of such a condition was

described by Walentowicz (1888), in a pig. Furthermore, the rabbit was pregnant and so was functioning as a normal female.

According to Brambell (1930, p. 163) and others, all vertebrates have the potentialities of both sexes during embryological development, but the decisive factor which actually determines the final development of the sex of the individual is the sex chromosome. The reproductive system in early development consists of paired indifferent gonads which, nevertheless, will have the chromosomal constitution of either the male or the female sex; at this stage the rudiments of both the Wolffian and the Müllerian ducts are present and the external genitalia are represented by the genital tubercle, etc. From this condition, which is exhibited by both sexes, either the male or the female type becomes organized.

It is well known that, in the development of the reproductive system, the gonad becomes differentiated first, then the external genitalia and lastly the ducts. Since the sex hormone is produced by the differentiating gonad, the external genitalia and the ducts will develop under its influence (male or female, as the case may be). It is also known that in mammals the differentiation of the gonad from the genital ridge commences with the proliferation of medullary cords in the cephalic part of the ridge and this finally extends along its entire length.

In man, and presumably in mammals generally, the medullary cords of the cephalic region of each ridge degenerate and the adult testis is formed from the caudal portion, the medullary cords of which give rise to the testicular tissue; at the same time, the connective tissue capsule thickens to form the tunica albuginea of the differentiated testis. The development of the ovary from the indifferent gonad takes place slightly later in time, from a second proliferation of the germinal epithelium which gives rise to the cortical cords. These grow inwards and replace the medullary cords which undergo retrogressive changes. In the male the production of the sex hormone by the testis results in the development of the penis and of the scrotal sacs, and the further growth and differentiation of the Wolffian ducts; at the same time it inhibits any further alteration in the Müllerian ducts which soon atrophy. In the female the reverse takes place, the sex hormone from the ovaries leads to the formation of the vulva and clitoris, the growth and differentiation of the Müllerian ducts, and the cessation of further changes in the Wolffian ducts which almost completely degenerate. Brambell (1930) and Crew (1924) both came to the conclusion that Goldschmidt's hypothesis can be applied to all known cases of mammalian intersexuality; this is based on his observations on intersexuality, resulting from certain racial crosses, exhibited by the offspring of the gipsy moth (*Lymantria dispar*). The hypothesis involves three fundamental ideas which are ex-

pressed very briefly by Brambell (1930, p. 651) as: '(1) The Mendelian factors determining sex are quantitative. (2) Racial variations occur in them. (3) A time factor is involved in their expression during development.' In the genotypic male therefore, during the developmental changes in the genital ridge, the male-differentiating substances will be in excess of the female-differentiating substances; in the genotypic female the opposite will be the case. If, for any reason, during the development of the reproductive system the action of the differentiating substances of the genotypic sex is delayed, then that of the opposite sex may produce some effect on the indifferent gonad before its action is overridden by that of the genotypic sex, and an intersexual individual will result. The amount of intersexuality exhibited will depend upon the time at which the overriding occurs.

Crew (1927, p. 95) explains this by assuming '(1) that in these cases, males genetically, the sex-determining gene-complex included "quickly elaborating" female-determining genes and "slowly elaborating" male-determining genes; and (2) that the differentiation of the gonads is not synchronous but consecutive, the left being affected before the right, and the cephalad pole before the caudad.' Crew considers that intersexuality usually occurs in the genetic male and points out that this is not surprising since the male is the digametic (XO) sex, and the sex complex is less stable in the digametic sex than in the monogametic (XX). Nevertheless, he agrees that there is evidence to show that intersexuality can occur in the genetic female, in which case the female-determining genes will be the 'slowly elaborating' genes, and the order of differentiation of the gonads will be from right to left, and from caudad to cephalad.

The present specimen can best be interpreted as a genotypic female in which the sex-determining gene complex included 'quickly elaborating' male genes and 'slowly elaborating' female genes, with the result that the minimum stimulus for gonad differentiation was reached by the male-determining genes first, and the caudal part of each genital ridge was laid down as a testis; thus differentiation started under the influence of the male-determining genes and resulted in the formation of testicular tissue and the consequent production of the male sex hormone: this sex hormone influenced the early differentiation of the external genitalia so that the urethral groove was of the male type.

At this stage the more 'slowly elaborating' female-determining genes exerted their influence and the ovaries became differentiated by the development of cortical cords produced by a second proliferation of the germinal epithelium (Fig. 2).

The formation of these cords started at the cephalic end of each genital ridge where, presumably, they replaced the already degenerating medullary

cords and extended backwards to the region occupied by the testis; here they were unable to penetrate the thickened tunica albuginea and proliferation ceased. Thus the stimulus of the female sex hormone, derived from the differentiating ovaries, was so much delayed that it was unable to affect the development of the caudal part of each ridge.

Measurements of the ovary, compared with those of a normal female, showed that the breadth was the same in both cases but that the ovary of the

into two parts, the anterior half of which is the functional ovary and the posterior part forms the so-called 'interstitial gland' which is said to be homologous with the testis.

These facts indicate the possibility that the ovary normally may be formed from the more anterior portion of the ridge, but this is masked by the speed at which differentiation occurs.

The influence of the female sex hormone of the hermaphrodite, overriding that of the male, re-
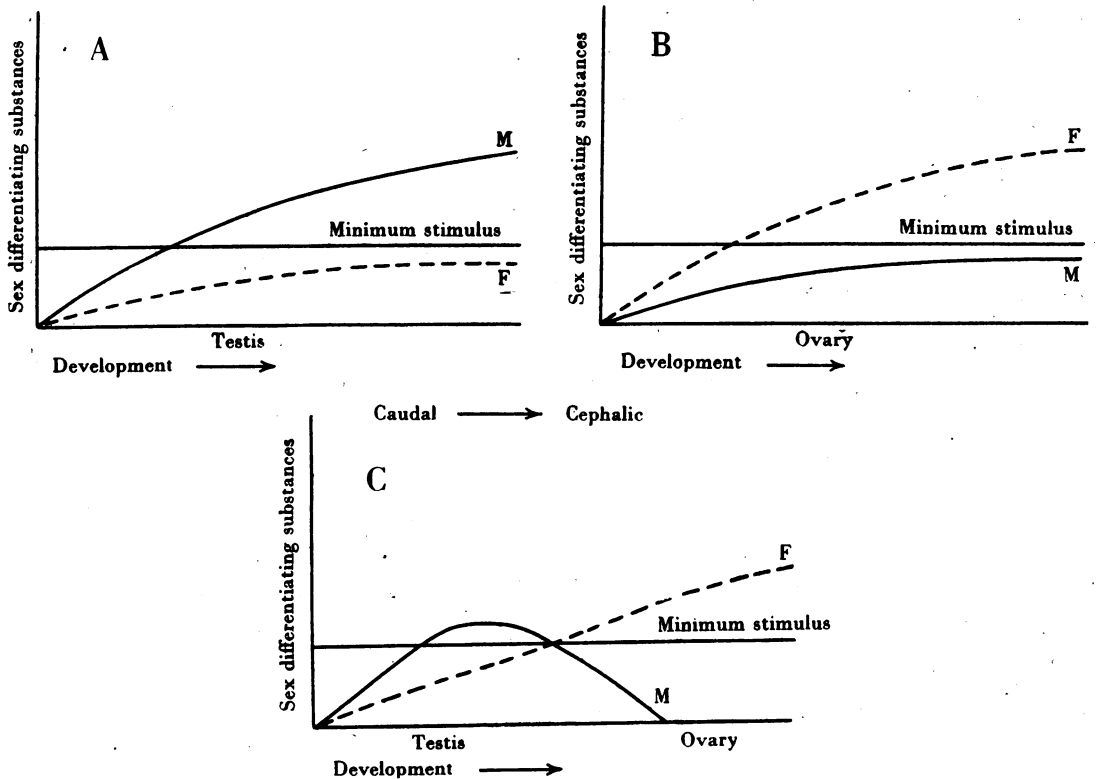

Fig. 2. Graphic representation of gonad differentiation. A. Normal genotypic male (after Crew) (male differentiating substances in excess throughout the period of gonad differentiation). B. Normal genotypic female (after Crew) (female differentiating substances in excess throughout the period of differentiation). C. Hermaphrodite rabbit. 'Quickly elaborating' male differentiating substances producing testes, later overridden by 'slowly elaborating' female differentiating substances producing ovaries. M, male-differentiating, and F, female-differentiating substances.

hermaphrodite was slightly shorter (10 mm. as compared with 12 mm.); this suggests that the proliferation of the cortical cords ceased sooner than it would have done in a normal female.

How much of the genital ridge is involved in the production of the normal adult ovary is uncertain, but in all cases of mammalian intersexuality in which an ovotestis is described, the ovarian tissue forms a cap over the anterior end of the testis; it is of interest to note that in the female mole, *Talpa europaea*, referred to below, the ovary is divided

sulted in the cessation of the male influence on the further development of the external genitalia and of the ducts which, owing to their normally later differentiation, were least affected by the male sex hormone. The effect on the external genitalia was to produce: (1) an intersexual condition in which the lips of the slit-like urinogenital opening failed to fuse (hypospadias) as they would have done had the male influence persisted; (2) an enlarged clitoris ending in an epithelial 'tag'; (3) abnormally prominent 'labia minora' (urethral folds of the in-

different stage); and (4) the absence of a normal vulva, since the labio-scrotal folds had already differentiated as the scrotal sacs.

The differentiation of the ducts must have proceeded completely under the influence of the female sex hormone, since the Müllerian ducts pursued their normal course in development, differentiating into Fallopian tubes, uteri, vagina and vestibule, whereas the Wolffian ducts and their derivatives, if they had begun to differentiate, had undergone a process of atrophy.

In this connexion it is interesting to note that the female mole, according to Matthews (1935), normally exhibits a form of intersexuality which he explains in the same way as the above. This animal is exceptional in having a male type of external genitalia, the clitoris is long and peniform, and the utero-vaginal canal has only an outlet to the exterior during the breeding season. The ovary is made up of two parts, an anterior functional ovary and a posterior so-called interstitial gland; these two parts undergo alternating periods of activity; the interstitial gland is regarded as homologous with the testis of the male, but it has no corresponding ducts. Matthews considers the female mole as a glandular hermaphrodite or female intersex during the period of anoestrus (when the interstitial gland is active), and as a functional female during the breeding season.

SUMMARY

1. A case of a pregnant hermaphrodite rabbit is recorded for the first time; there were a separate ovary and testis (the latter in a scrotal sac) on each side of the body.

2. The female organs were typical of those of a normal female, except for the external genitalia which were of an intersexual type; the male organs were represented by degenerate testes only.

3. Histological examination of the tissues of the reproductive system showed that the female organs were quite normal, but that the testes were undergoing fatty degeneration.

4. The type of intersexuality exhibited by the hermaphrodite is that of a genotypic female, in which the sex-determining gene complex includes 'quickly elaborating' male-determining genes, and 'slowly elaborating' female-determining genes.

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