A CONTRIBUTION to the STUDY of SEX-DETERMINATION in the ANURA.

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by

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TABLE of CONTENTS.

TABLE of CONTENTS (Contd.) PAGE.

TABLE of CONTENTS (Contd.) PAGE.

INTRODUCTION.

The problem of sex-determination possesses a peculiar fascination for the student of animate Nature and the fact that it remains as yet unsolved, permits one to set out on the great adventure of its solution with high hopes and, if one is young, with confidence.

But with the experience of the difficulties and complexities of the subject comes a chastened mind, and at the journey's end one is left with just the hope that in the results of the work which has enslaved by its all-absorbing fascination, there may be found some suggestion as to the direction in which the truth of the matter really lies.

In this study the question of sex-determination is approached through a consideration of the abnormalities of the reproductive system which have been recorded in Zoological literature. Often an appreciation of the abnormal can illumine the mechanism of the normal.

The typical sexual characters are first briefly described, so as to furnish standards to which the abnormalities may be referred. The abnormalities/

abnormalities are then tabulated and analysed. A brief consideration of the embryology of the reproductive system naturally follows and the investigation is brought to a close by a review of what is known of the sex-ratio. An account is given of certain experiments.

It is intended to demonstrate that the abnormalities obviously suggest that in the case of the frog, sex is not irreversible; that the facts of embryology lend support to this view; and that a study of the variations of the sex-ratio supplies conclusive proof that sex-reversal can and does occur. The graduated degrees of abnormality as portrayed, are shown to provide a clear view of the process by which sex-reversal is effected.

A CONTRIBUTION to the STUDY of SEX-DETERMINATION in the ANURA.

I.

THE SEXUAL CHARACTERS in the ANURA.

It is convenient to describe those of the frog and then to contrast them with those of other members of the Order. In the frog, an examination of the sexual characters, grouped for purposes of description into primary and secondary, demonstrates the morphological differences which distinguish male from female. The primary sexual characters consist of the primary sex-glands or gonads, and of the accessory sexual apparatus; while the secondary sexual characters include all other characters which are peculiar to one sex.

The primary sex-glands are arranged in the same manner in the two sexes, the testes of the male corresponding in position with the ovaries of the female, being bilaterally symmetrical and placed upon the ventral surface of the kidneys, and, save at the hilus, - the area where the vessels of the gonad enter and leave its substance, - each is completely ensheathed in peritoneum which is extended from the hilus as a double-layered suspensory membrane which passes/

passes over the ventral surface of the kidney to become continuous with the general coelomic lining upon the dorsal wall of the body-cavity. This suspensory membrane is called the mesovarium in the female, the mesorchium in the male, and between its layers the vessels of the gonad pass. Each gonad presents for examination two surfaces, two borders, and two poles. The ventral surface, distinctly convex in outline abuts into the abdominal cavity while the dorsal surface lies against the ventral surface of the kidney. The outer border is free, rounded, and markedly convex in outline, that of the ovary being deeply cleft by the divisions which separate lobule from lobule, whereas the inner border is straight and fixed, for from it the suspensory membrane runs to the body-wall, and upon it is the hilus. The posterior pole is rounded and clearly defined, but the anterior pole is hidden by the fat-body, more firmly attached to a testis than to an ovary, which has the form of a crown of vividly yellow finger-like processes.

The testes vary widely in shape, size, and colour, on the two sides of the same individual, in different individuals, in different species, and with the seasons. In Rana esculenta the testis is rounded or ovoid in shape; in Rana temporaria, it is typically longer than broad, and ovoid, but pear-shaped, cone-shaped/

shaped, or even dumbbell-shaped testes are not uncommon. Its surface is not smooth but is pockmarked with the outlines of its constituent tubules. The testis of Rana esculenta is typically heavily covered with fat and its colour in consequence, is a vivid yellow, but the testis of Rana temporaria is frequently of a deep black colour, especially towards its outer border. This pigment is identical with that widely present upon the serous membranes of the body generally, and viewed under a hand-lens, is seen to be distributed upon the surface of the testis in the form of an intricate reticulum, the lines of which map out the boundaries of the seminal tubules. The pigment, which consists of branched chromatophores, is restricted to the connective-tissue of the peritoneal investment of the gonad and when this is stripped from off the testis, all the pigment is removed, leaving the whitish-yellow but otherwise non-pigmented gland, quite free from any black colour. Under certain conditions these chromatophores may appear rounded, as pointed out by LIM; moreover, superficial sections convey the impression that the pigment invades the substance of the gonad along the lines of the intertubular connective-tissue. This is not so and the suggestion is due to the fact that the pigment lies in the surface depressions of the testis./

testis. Actually the pigment is entirely superficial and consists of branched chromatophores which are not rounded but elongated. In many instances the surfaces of the inner third of the testis are non-pigmented, even when the rest of the gonad is covered with a dense black coat, and observations of two years have shown that at the time of the breeding season, that is, at the time of the greatest metabolic activity, a greater percentage of pigmented testes will be found.

Beneath the peritoneal investment there is a thin tunica albuginea of connective tissue which contains the essential spermatic elements of the gonad, sending in towards the centre trabeculae which enclose the separate lobules of seminal tubules. On section, each tubule is seen to consist of an outer membrana propria and an inner lining layer of cells of various shapes, those nearer the periphery being rounded with large rounded nuclei, while others, elongated and with oval nuclei, radiate from the central lumen of the tubule to the periphery. These cells include the flattened cells described by BERTACHINI, follicle-cells, and the various stages in the formation of the spermatozoa.

There is no definite correlation between the size and weight of the individual and the size and/

and weight of the testes. Accurate data have been collected from many hundreds of frogs but nothing more exact than this conclusion has been reached. In no case were the measurements of the two gonads of an individual identical, one testis was invariably larger and heavier than its fellow and in the majority of specimens the larger testis was the right one. Certain authorities have recorded a seasonal variation in the size of the testis. NUSSBAUM and PLOETZ record that the testes of Rana fusca are smallest in May after they have discharged their spermatozoa, and are largest in August, after which they decrease throughout the winter. In Rana esculenta, according to PLOETZ, the testes vary but slightly with the seasons, and this would seem to be the case also in Rana temporaria. An ovary when fully developed has the form of a lobulated sac, the internal cavity of which is sub-divided by thin septa into a number of complete chambers filled with fluid. Each chamber corresponds to a lobule externally and the number of these lobules is subject to considerable variation. The wall of the sac consists of three coats, an external peritoneal coat, which may be the seat of branched chromatophores, as in the case of the testis, and which has ciliated cells upon its surface/

surface: an internal single layer of flattened epithelial cells; and between these, the stratum medium which is composed mainly of ova in various stages of development.

The appearance of an ovary varies with its age and with the seasons. Two months after the metamorphosis, it is a solid organ, very much like a testis in general appearance, save that it is larger and has a notched outline. As it approaches maturity, its surface becomes studded with projecting ova, its colour is bright yellow, and the typical lobulated sac-like form becomes apparent. As the ova become mature, they increase greatly in size and the grape-like clusters become more and more deeply pigmented. A pigmented ovum is a mature ovum and the pigment is not as that of the testis, for it is intrinsic and a product of cell-metabolism and takes the form of polygonal masses which coat the animal pole of the ovum. As the time for egg-laying approaches, the ovaries undergo an enormous increase in size, and come to occupy the greater part of the body-cavity, displacing the other organs: and the intense black colouration of the ova is visible through the stretched abdominal wall. The relatively large size of the ovaries give to the female her characteristic conformation, for the trunk of a female is typically ovoid as contrasted with/

with the square, thick-walled body of the male. When mature, the ova break through the outer wall of the ovary and are carried into the mouth of one or the other oviduct by the motion of the cilia of the coelomic epithelium which, according to NUSSBAUM, drive in a forward direction any small bodies lying free in the coelum, to pass along into the uterine segment where they await the time of mating. The uterine segments of the oviducts become greatly distended as the mature ova accumulate therein and the ovaries themselves gradually become reduced to relatively small wrinkled bodies containing the crops of minute ova of the future years, and in colour a whitish-yellow, [illegible].

There is never much difference between the size of the two ovaries of an individual although the two are never quite equal. On the other hand, the two uterine segments are never equally distended, one always contains more ova than its fellow, during the period immediately preceding the mating. It was found that the left is more often the larger one, and that the two are evacuated in succession, for if a female whose cloaca is filled with ova is examined it will be found that the contents of the cloaca are continuous with the ova in one uterine segment, and not with these in the other.

The size and general appearance of the fat-bodies/

bodies varies with the seasons and with the conditions in which the individual has been living. PLOETZ and FUNKE found that in Rana temporaria and R.esculenta the fat-body changed but little during the winter months, but underwent a marked diminution in size before and during the time of the breeding season in the late spring, after which there was a gradual increase until the autumn when it attained its maximum.

THE ACCESSORY SEXUAL APPARATUS.

In the Anura it is convenient to restrict this term to the vasa efferentia, the urino-genital ducts, and the expanded portions of these, the seminal vesicles, in the case of the male, and in the case of the female, to the paired oviducts. These ducts, which bear along their course local enlargements, are the ducts which convey the products of the gonads to the place of fertilisation.

The vasa efferentia connect the hilus of the testis to the inner border of the adjacent kidney and their number is subject to considerable variation, not only in different specimens but also on the two sides of the same individual. In some cases these ducts combine to form a network, in others they run separately,/

separately, but usually each bifurcates at an acute angle just before it enters the kidney, though a few end blindly in the mesorchium. They are lined with a short columnar epithelium. According to MARSHALL, in Rana temporaria, "as the Malpighian bodies developed on the tubules of the Wollfian body, those lying nearest to the genital ridge give off hollow tubular diverticula, which arising from the capsules of the Malpighian bodies, grow towards the median plane and into the substance of the genital ridge where they form the so-called tubuliferous tissue. In the ovary of the female the tubules of this tissue expand very greatly and give rise to the large axial cavities of the ovary; in the male the outgrowths from the Malpighian capsules acquire still more intimate relations with the reproductive organs than in the female and become the vasa efferentia of the adult. The Malpighian bodies with which the vasa efferentia are connected, are, at first, perfectly normal, but later on, they undergo retrogressive changes, and by the end of the first year their glomeruli have disappeared, and the Malpighian bodies themselves merely remain as slight ampulliform enlargements of the tubules. These tubules do not join those of the other parts of the kidney, but open at/

at once into the terminal or collecting tubules of the Wollfian body". In the case of Rana esculenta, on the other hand, GRAHAM KERR describes the vas efferens as opening into an otherwise normal Malpighian body which contains its glomerulus and as being continued into a functional renal tubule.

Though the development of vasa efferentia is typically a male character, yet an irregular development of the tubuliferous tissue occasionally produces a system of exactly similar vessels in the female.

The urino-genital ducts of the male are the paired ureters which serve as ureters and vasa deferentia combined. These are straight tubes which run from alongside the lower part of the outer border of the kidney of either side to open into the cloaca posteriorly. Each is developed from the Wollfian duct, and each presents an expanded enlargement, called the receptaculum seminis, in its posterior half. Along its inner and shorter border, each seminal vesicle is continuous with the urino-genital duct while its outer free border is strongly convex, thick, and often deeply pigmented in a fashion similar to the testis. A suggestion by TARCHANOFF to the effect that in the frog, a filling of the seminal vesicles serves to excite sexual feeling in the male during the/

the breeding season, is not supported by the observation that the seminal vesicles of a male during the first days of the nuptial embrace are usually found to be empty. The size of the seminal vesicles varies in different species and with the seasons. They are large and complex in R.fusca, and poorly developed in R.pipiens and R.catesbiana, and in those species in which it is well-defined, it is largest at the time of the breeding season. There is no indication of any such localised enlargement of the ureter in the female in whom this duct is firmly and intimately bound by connective tissue to the adjacent oviduct.

The Müllerian ducts arise in both sexes apparently from a modified strip of peritoneal epithelium which runs parallel to the Wollfian duct on either side of the body. In the male, the development of these ducts ceases when these bands of epithelium have become tubular and have acquired posteriorly independent openings into the cloaca, but in the female, their development is continued until they have attained the form and size typical of the oviducts of the adult female. Each oviduct is a tube which has an anterior fimbriated patent extremity situated near the root of the lung; a relatively narrow neck region succeeds the tip which bears the opening called the ostium/

ostium abdominale, and this is succeeded in turn by the median much convoluted portion of even calibre which passes into a posterior thin-walled, pigmented, expanded portion, called the uterus or uterine segment. Posteriorly the uterine segment communicates with the cloaca by an opening which is separate and distinct from that of the ureter. The median convoluted portion has a thick, glandular wall, the inner surface of which is thrown into longitudinal ridges covered with ciliated epithelium. At the bottom of the grooves between these ridges are the openings of the glands which secrete the gelatinous coat of the eggs, which GIACOSA found to consist of pure mucin. The glands are mostly of the simple tubular type, and are lined with a single layer of cylindrical secreting cells. During the breeding-season, the oviducts exhibit a remarkable capacity for absorbing water, but at other times this capacity becomes greatly reduced. In the young female the oviducts are quite straight, thin-walled, and of small calibre.

In the typical adult male, the Müllerian ducts are merely straight thin strands of tissue, which are found with difficulty along the outer side of the urino-genital ducts. But in the normal female they are subject to considerable variation in size in the different species and with the seasons. They are best/

best developed at the time of the breeding-season, when the convoluted portions are actively manufacturing the albuminous material which coats the ova as they pass along, and late in this season the uterine segments become expanded to their fullest extent as the mature ova accumulate therein. There is also a specific variation in the design of the posterior ends of these ducts in the female; among the Anura generally, each oviduct has its separate opening into the cloaca, so that there are four openings thereinto, but in Bufo and Alytes the posterior portions of the two ducts are confluent, and in Hyla there is but one unpaired opening. In the male frequently considerable remnants of these ducts are encountered in Bufo and occasionally also in Rana. In R.pipiens they a are well developed and possess posterior enlargements equivalent to the uterine segments of the female; in R.catesbiana they are absent; in Bombinator they are incomplete with only the anterior portions remaining; and in Discoglossus and Alytes all traces of these ducts have vanished.

THE/

THE SECONDARY SEXUAL CHARACTERS.

The secondary sexual characters among the Anura are not conspicuous, and in some few species it is practically impossible to distinguish male from female by an examination of the external features. There are ways of identifying a female of R.esculenta, but they are often not very obvious, and it was found that the surest way during the time of the breeding season of R.temporaria was to put into the tank containing the edible frogs a male of R.temporaria, for he immediately 'smelt out' the females without ever making a mistake as to the sex of his chosen mate. Often the only observable structural difference between the sexes is, that the male is rather smaller than the female as is the case in Bufo lentiginosus. But sexual dimorphism does exist, and for purposes of description the differences can be grouped into three sets, cutaneous, muscular and skeletal.

CUTANEOUS SEXUAL DIFFERENCES.

Colouration itself is no guide as to the sex of an individual, for only in Mantella madagascariensis is the male distinctly different in colour, although the males of R.temporaria, R.arvalis, and R. fusca/

fusca assume at the time of the breeding season a blue colouration which is best seen on the ventral aspect of the body and especially about the throat, while the females of R.fusca assume a general reddish brown colouration and a bluish tint at the throat. Many species possess the power of changing their colour in harmony with their surroundings, so that colouration is no sure guide to the sexes. There is a greater development of the web between the toes in the males of R.temporaria, R.fusca and R.arvalis, while in R.catesbiana the tympanum is larger in the male than in the female. The skin of the male of R. fusca at the time of the breeding period becomes swollen to hang down at the sides and to assume what LEYDIG describes as a 'quammig-quappiges Ansehen', while the female of R.temporaria develops a characteristic wartiness of the skin of the back and flanks, as described by HUBER, at this time.

Other cutaneous features typically female are the three fleshy flaps which protect the vent of the female Xenopus laevis: the dorsal pouch developed by the females of the genus Nototrema, and the pouch-like cavities in the dorsal skin of Pipa americanus.

The male commonly possesses vocal sacs, but the degree of their development varies with the different/

different species. R.agilis and Pelobates have none; in the majority of cases the sacs are internal, but in some dozen species, including R.esculenta, R.rugosa, R.tigrina, R. hexadactylo, and R.glandulosa, these sacs have become external. In the female of R.esculenta there are some traces of these typical male organs seen as slit-like folds of the outer skin below the angle of the lower jaw, In the male of Rhinoderma darwinii, the internal sac extends over almost the entire ventral surface of the animal and it is in this that the eggs are incubated .

At the time of the breeding-season, the males of many species develop nuptial excrescences upon their forelimbs. These attain their greatest size in R.liebigi, but are very well defined also in Bufo calamita, Hyla coerulea and Pelobates punctatus. The male of Bufo viridis develops a prominent callosity upon the inner side of the first finger; the male of Leptodactylus ocellatus has a sharp black spur upon the inner carpal edge and another upon the rudiment of the thumb; the male of Pelobates fuscus has an oval gland upon the upper surface of the upper arm; and the male of Rana temporaria has a pair of swollen pads upon the inner fingers which become coated with brilliantly black horny spicules.

MUSCULAR/

MUSCULAR DIFFERENCES.

The male of the Common Frog can be distinguished from the female by the more massive build of the arms and by the stoutness of the muscles of the abdominal wall. These differences result from the greater development in the male of the rut-muscle - m.rectus abdominis and of that division of this which forms the portio abdominalis of the m.pectoralis; the m.flexor carpi radialis; and of the abductor indicis longus. The results of this difference in degree of development of these muscles in the two sexes are strikingly depicted in the contrasted attitudes assumed by the males and the females in rigor mortis, as was described by the present writer.

SKELETAL DIFFERENCES.

The greater development of the above muscles demands larger bony attachments, and so the crista medialis humeri clearly denotes a male skeleton. In Hyla infrafrenata the male has a sharp bony process in the middle of the chest and the male of Hyla humeralis has a bony spine upon the inner aspect of the forearms.

In consequence of these characters, the male possesses a stouter build than the female. He looks more muscular, more sturdy, and more powerful, whereas the female is relatively more slender. During the breeding/

breeding-season there is usually no difficulty in recognising a female for her abdomen is tensely distended with ova, so that she has an oval outline as compared with the waisted square trunk of the male.

These secondary sexual characters, where they exist, play their part in the exercise of the function of reproduction. Some, such as the vocal sacs of the male, are effectively used in the wooing of the female, for though both sexes have voices, the characteristic croaking is essentially a male accomplishment.

CUMMINS has shown that sex recognition can be attributed to differential behaviour of the two sexes when clasped. A male clasped by a male croaks, struggles violently, and is soon released.

Some are concerned in the actual nuptial embrace, for example, the wartiness of the skin of the female and the nuptial excrescences of the male, Others are employed in the rearing of the young, as is the case in Rhinoderma darwinii, where the vocal sac of the male is used as a brood-chamber, and in Pipa americanus and in the genus Nototrema in which the eggs are incubated within skin-pouches.

A consideration of the male and female accessory reproductory apparatus and of their secondary/

secondary sexual characters, leads to the firm conviction that the sexual differences in these are nothing more than the results of differences and divergences in the development of tissues commonly possessed by both sexes.

II.

ABNORMALITIES of the REPRODUCTIVE SYSTEM of the ADULT.

The abnormalities which have been recorded can be so tabulated that the first case most nearly approximates the normal female and the last the typical male, with respect to the nature of both primary and secondary sexual characters. Thus arranged, it is seen that the cases furnish an almost complete series of graduations which ranges from an individual which is almost completely female, to one which is almost completely male, and that the conditions found readily appear to be merely graded stages of a single process.

A. RANA.

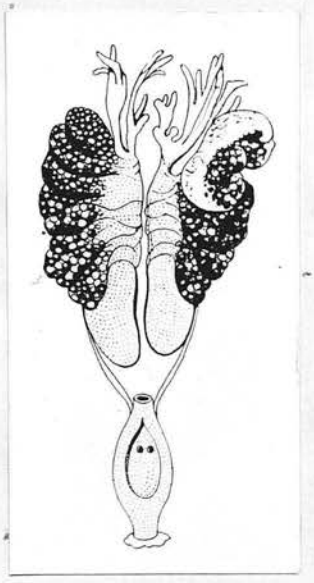
DIVISION I.

Those cases in which the secondary sexual characters were not typically male.

GROUP A.

Those cases in which, on inspection, one gonad was an ovary and the other an ovo-testis.

I./



Bourne

Youngman

SUB-GROUP I. The ovary was normal in appearance

1. BOURNE. R.temporaria. Adult. Secondary sexual characters were not described.

Right gonad. An ovary.

Left gonad. An ovo-testis. The larger ovary-portion bore along the anterior half of its inner border the smaller testis portion.

Vasa efferentia could not be observed.

Seminal vesicles not present.

Müllerian ducts well-developed.

On section. Ovary-portion of the Ovotestis contained well-developed ova; testis portion contained motile spermatozoa; and there was no distinct line of separation between ovary- and testis-portions.

2. YOUNGMAN. R.temporaria. Adult. Killed June. Secondary sexual characters male but poorly developed.

Right gonad. An ovo-testis. An ovoid non-pigmented testis-portion suspended from the ventral surface of a small but apparently normal ovary.

Left gonad. An ovary of normal appearance.

VASA efferentia. "There appears no means of exit to the exterior for the spermatozoa/

spermatozoa, unless they burst into the coelom, for I can make out neither macroscopically nor microscopically by sections, any trace of vasa efferentia, and sections of the ureter do not show the presence of spermatozoa therein".

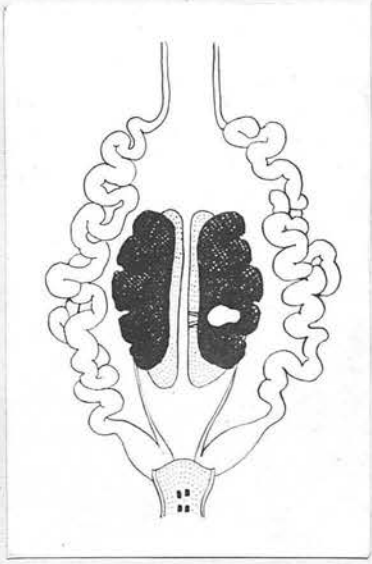
Seminal vesicles not present.

Müllerian ducts exceedingly well-developed with eggs within the convoluted portions of each and with uterine segments crammed to distension.

On section. Left ovary and the dorsal ovary portion of the right gonad showed the following structure. Numerous normal ova were present, but there were also cavities among the ordinary egg-containing chambers, and of the same size, which were filled with black pigment ("representing perhaps degenerate eggs".) The ventral testis-portion of the ovo-testis showed testicular structure, and contained immature and mature spermatozoa, and in addition, peculiar eosinophilous bodies.

SUB-GROUP II. The ovary was abnormal in appearance.

3. HUXLEY. (I). R. temporaria. Adult. Killed April. Secondary sexual characters as/



Huxley (1)

as those of a well-developed female. No male characters were present.

Right gonad., An ovary of usual size but more deeply pigmented than usual.

Left gonad. An ovo-testis consisting of an ovary of unexceptional size, which bore upon its ventral surface a small nodule of testis material.

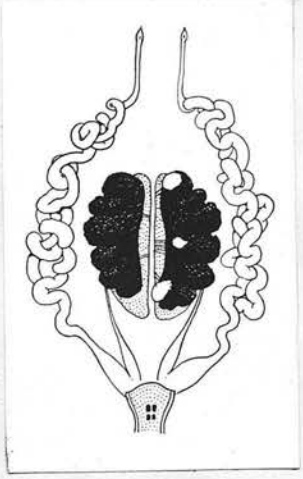
Vasa efferentia were not present in connection with the right gonad but there were two in association with the testis-portion of the ovo-testis.

Seminal vesicles were present, being very small and spindle-shaped.

Müllerian ducts were well-developed and ova were contained therein.

On section, the right gonad was seen to be entirely ovarian in structure, the fibrous tissue of the ovarian wall was everywhere hyperplastic and there was excess of pigment.

The left gonad consisted of two portions. The smaller was composed of spermatic and the larger of ovarian tissue similar in structure to the ovary of the opposite side. The spermatic tissues were healthily/



Crew(1)

healthily normal for the most part, but ova were found amid them, within and between the seminal tubules. An ovum within a tubule lay among spermatozoa which were deformed and degenerate, a consequence possibly of the pressure exerted by the ovum. The seminal tubules in the neighbourhood of an ovum which lay between the seminal tubules were contorted and misshapen and the intertubular connective-tissue in this area was hyperplastic.

There was ciliated epithelium upon the peritoneum and the ova within the Müllerian ducts were all degenerate.

4. CREW. (I) R.temporaria. Adult. Killed April. Secondary sexual characters male but imperfectly developed.

Right gonad. An ovary, somewhat firmer and more strongly pigmented than usual.

Left gonad. An ovo-testis, having the appearance of an ovary rather more deeply pigmented and firmer to the touch than usual, which bore along its inner border three small nodules of testis-substance.

Vasa efferentia, - few in number-were present/

present on either side.

Seminal vesicles were present, being small and fusiform.

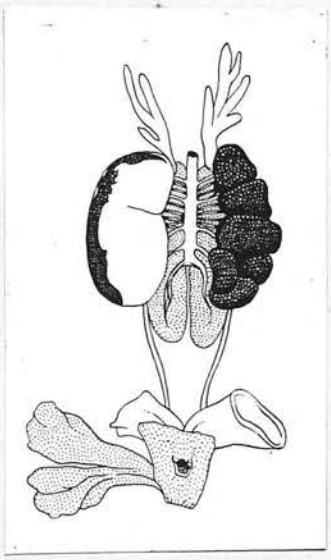
Müllerian ducts very well-developed, Ova within uterine segments and convoluted portions.

On section. Right ovary and ovary-portion of the left gonad entirely ovarian in structure, but practically all of the more mature ova showed signs of degenerative changes. There was widespread increase of the pigment normally present in an ovary and the connective-tissue was distinctly hyperplastic. The three nodules upon the inner border of the left gonad had the structure of normal spermatic tissue.

There were ciliated cells upon the peritoneum. The Müllerian ducts contained only degenerate and spurious ova.

5. MARSHALL. (D). R.Temporaria. Adult. Secondary sexual characters not described.

Right gonad. An ovo-testis. The larger testis-portion had the ovarian tissue disposed along its outer border. The body of the testis-portion presented upon its/



Marshall (D)

its ventral surface a very deep transverse groove. At the anterior end of the ovary-portion was a deeply pigmented lobule which was bent back over the dorsal surface of the gonad.

Left gonad. An ovary more strongly pigmented than usual and divided into six lobes by well-marked constrictions.

Vasa efferentia on the right side as those of the normal male; two or three thin-walled tubules connected the left ovary to its kidney.

Seminal vesicles not present.

Müllerian ducts well-developed.

On section, the greater part of the right gonad had the structure of normal testis. The pigmented band which ran along its outer border, extended inwards about a quarter of the way through the substance of the testis, and consisted of masses of pigment which followed the lines of the intertubular connective tissue, this being hyperplastic in these areas. The polygonal masses of pigment were, in most cases, entirely outside the seminal tubules/

tubules which had become crushed together and obliterated by the increase of the intertubular connective-tissue, and only in the last stages of atrophy of the tubules were pigment found within them. The dorsal lobe of the ovo-testis and the left gonad showed exactly similar structure, that of ovary with hyperplastic connective tissue and great abundance of pigment. Few ova were found which were in a thoroughly healthy condition and the great majority were in various stages of degeneration, the protoplasm having shrunk from the follicle wall, the nuclei being of small size, and the follicle in some cases invaded by pigment.

"It is worthy of note that while the male portion of the essential glands is absolutely normal throughout, the female portion, though more bulky, forming the whole of the left gland and part of the right one as well, is not normal in any part, the majority of the ova showing more or less well-marked signs of degeneration, and the whole structure exhibiting very obvious sclerotic induration, which must be regarded as pathological".

Hooker

GROUP B. THOSE CASES in which, on INSPECTION BOTH GONADS were OVO-TESTES.

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6. HOOKER. (B). R. fusca. Adult. Killed October. Secondary sexual characters imperfectly developed male, but in addition the skin of the back was warty.

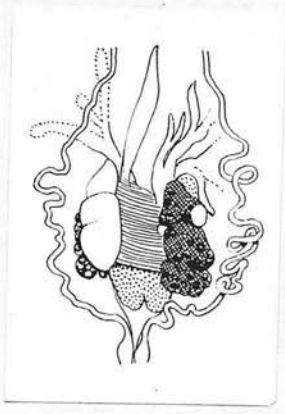
Right gonad. An ovotestis. To the outer border of the larger testis-portion the smaller ovary-portion was attached.

Left gonad. An ovo-testis, having the form of a normal sized six-lobed ovary which had a small nodule of spermatic tissue attached to the middle of its inner border.

Vasa efferentia. (of the usual male pattern?)
Seminal vesicles present, being small and spindle-shaped.

Müllerian ducts well-developed.

On section of the left gonad, (it was assumed that the right was similar), the testis-portion, discrete from the ovary-portion, showed the structure of normal testis; the ovary-portion contained large numbers of ova, some immature, some mature, and many showing various stages of degeneration, being associated with black pigment.



Smith

7. SMITH. R.temporaria. Adult. Secondary sexual characters male but imperfectly developed. (The skin below the throat was lighter on the left-side; the typically male pad was present on the index finger of the right side only.)

Right gonad. An ovo-testis. The larger testis-portion presented several constrictions upon its surface and bore along the outer border the smaller ovary-portion, on the surface of which the outlines of ova could be distinguished.

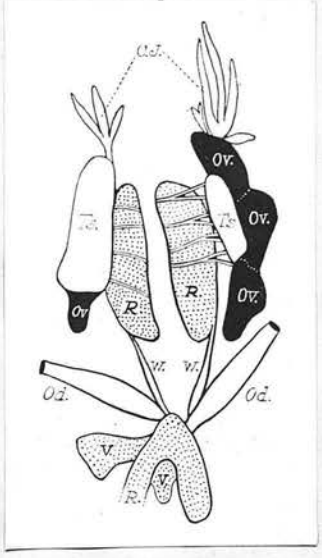
Left gonad. An ovo-testis consisting of a larger ovary-portion bearing upon its inner border a nodule of spermatic tissue. There was a second nodule of this tissue upon the outer border.

Vasa efferentia.(of the usual male pattern?)
Seminal vesicles not present.

Müllerian ducts - right one moderately, left one well-developed.

No sections made.

GROUP/



Boulangé

GROUP C. THOSE CASES in which ONE GONAD was an OVO-TESTIS & the OTHER A TESTIS. - Nil.

GROUP D. THOSE CASES in which BOTH GONADS were TESTES - Nil.

DIVISION II.

THOSE CASES in which the SECONDARY SEXUAL CHARACTERS were typically MALE.

GROUP A. THOSE CASES in which ONE GONAD was an OVARY and the other an OVO-TESTIS - Nil.

GROUP B. THOSE CASES in which, on inspection, both GONADS were OVO-TESTES.

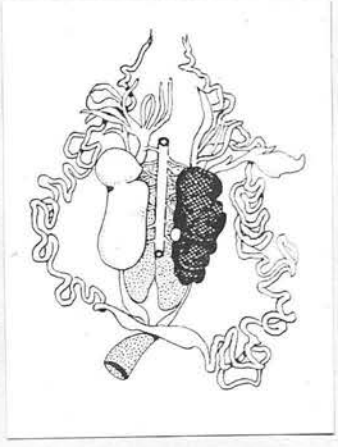
8. BOULANGÉ. R.fusca. Adult. Killed December Secondary sexual characters typically male.

Right gonad. An ovo-testis of which the testis-portion constituted ⅔ of the whole gonad and was situated anteriorly.

Left gonad. An ovo-testis of which the three-lobed ovary-portion formed 4/5ths of the whole and encompassed the testis-portion which lay upon its inner border.

Vasa efferentia were present in association with the testis-portions of both gonads.

Seminal/



Punnett

Seminal vesicles were not present.

Müllerian ducts well-developed.

On section, the ovarian portions showed the structure of normal immature ovaries: the testis-portions, that of normal testis.

9. PUNNETT. R. temporaria. Adult. Killed February. Secondary sexual characters male.

Right gonad. An ovo-testis with a very large testis-portion bearing a deep constriction upon its inner border. The ovary-portion was merely a patch of ovarian tissue situated upon the outer border of the testis-portion exactly opposite the constriction upon the inner border, and it lay within a deep angle of testicular material.

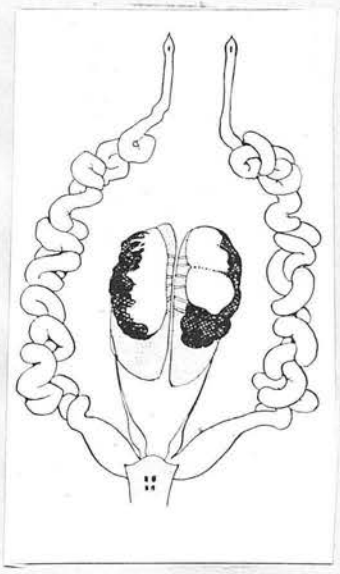
Left gonad. An ovo-testis with a larger six-lobed ovary-portion, upon the inner border of which, at about its mid-point, was situated a small spherical lobule of testicular substance.

Vasa efferentia entered into functional relationship with the spermatic and ovarian tissues of both gonads.

Seminal vesicles small and fusiform.

Müllerian ducts well-developed.

On/



Crew (2)

On section, the ovarian patch upon the right gonad contained but a single ovum, and the rest of this gonad was purely testicular. The testicular patch upon the left gonad had the structure of normal testis but at one point it contained a well-developed ovum.

10. CREW. (2). R. temporaria. Adult. Killed June. Secondary sexual characters typically male.

Right gonad. An ovo-testis having the appearance of a malformed testis, irregular in outline and with its surfaces scored with deep grooves; which bore along its outer border a broad band of dense black pigment, which in some places was flatly applied to the surface of the testis and in others was piled up into nodular prominences resembling ova.

Left gonad. An ovo-testis consisting of three lobes, of which the anterior and middle were testicular, but bore a band of dense black pigment similar to that of the opposite gonad along the outer border, while the posterior lobule was ovarian, the pigment/

pigment of which was continuous with the band upon the outer border of the other two.

Vasa efferentia present on both sides.

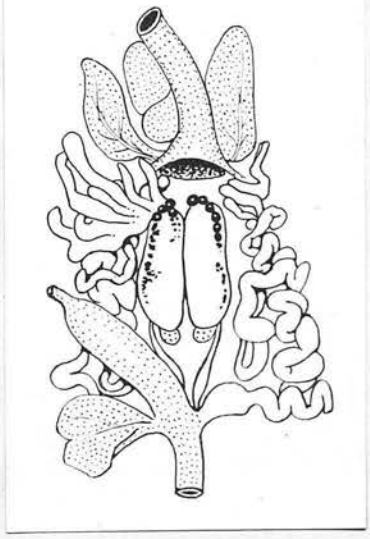
Seminal vesicles, pigmented and of moderate size were present.

Müllerian ducts were well-developed.

On section, the right gonad consisted of normal spermatic tissue in all parts, save those involving the pigment upon its outer border. The band of pigment was composed of dense masses of polygonal pigment granules within a fibrous tissue matrix and included many ova, all of which showed signs of degenerative changes. Ovary- and testis-portions were quite discrete but the line of demarcation was very irregular and the pigment appeared to ramify along the lines of the intertubular connective-tissue of the testis-portion. The anterior and middle lobes of the left gonad had a structure similar to the above, whereas the posterior lobe was entirely ovarian but pathological.

There was ciliated epithelium upon the peritoneum.

11./



Marshall (B)

11. MARSHALL. (B). R. temporaria. Adult. Killed during the winter. Secondary sexual characters typically male.

Right gonad. An ovo-testis having the appearance of an elongated and irregularly shaped testis bearing an irregular band of black pigmented material along the anterior 2/3rds of the anterior border, and at the anterior pole, a clump of spherical bodies strongly pigmented and closely resembling ova in appearance. At the extreme anterior end there was a pigmented lobe separated from the main body of the gonad by a deep constriction.

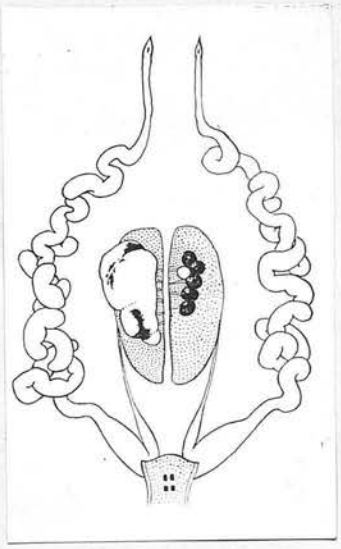
Left gonad. An ovo-testis similar in appearance to the right save that there was no pigmented lobe at the anterior pole.

Vasa efferentia on both sides as those of the normal male.

Seminal vesicles small and spindle-shaped.

Müllerian ducts well-developed.

On section, the greater part of each gonad had the structure of normal testis, but ova were found in considerable numbers lying/



Crew (3)

lying between the seminal tubules. The great majority of these ova were immature and showed signs of degenerative changes. The pigmented bands were composed of densely packed granules of pigment and hyperplastic connective-tissue. The anterior lobe of the right gonad consisted partly of normal testicular tissue and partly of ova in a state of extreme degeneration. The ovarian tissues were not discrete from the spermatic and were pathological.

12. CREW. (3). R.esculenta. Adult. Killed November. Secondary sexual characters typically male.

Right gonad. An ovo-testis having the appearance of an irregularly shaped testis with bands of dense black pigmented material running in deep grooves along its twisted outer border.

Left gonad. An ovo-testis, consisting of six lobes, five of which were ovarian, and the other, testicular in appearance. The testicular lobule was placed centrally and medially in relation to the others.

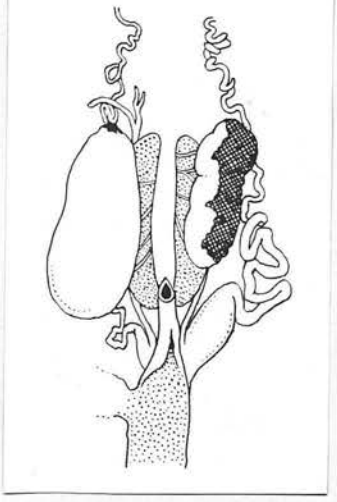
Vasa efferentia, - of the ordinary male/

male pattern on the right: on the left four in number and connected with both ovarian and spermatic lobes of the gonad.

Seminal vesicles present but small and spindle-shaped.

Müllerian ducts well-developed.

On section, all those parts of the right gonad not including pigment had the structure of normal testis. The pigmented nodules consisted of densely packed polygonal masses of pigment granules and hyperplastic connective-tissue. No ova were found amid this pigment, but near to this two ova were seen lying between the seminal tubules of the spermatic tissue. The pigment had the appearance of following the lines of the intertubular connective-tissue. The left gonad consisted of two distinct portions. The five pigmented lobes were composed of pathological ovarian tissue in which degenerate ova were included among dense polygonal masses of pigment and hyperplastic connective tissue. The spermatic lobe, discrete from the rest, was entirely testicular in structure. The renal vessels of both sides lay/



Ridewood.

lay in deep pigmented channels upon the surface of the kidneys and the pigment was identical microscopically with that of the ovary-portions of the ovo-testes.

13. RIDEWOOD. R. temporaria. In first year. Killed November. Secondary sexual characters typically male.

Right gonad. An ovo-testis having the appearance of an exceptionally large testis bearing a small pigmented excrescence antero-externally.

Left gonad. An ovo-testis with a 3-lobed ovary-portion situated externally.

Vasa efferentia as those of the normal male.

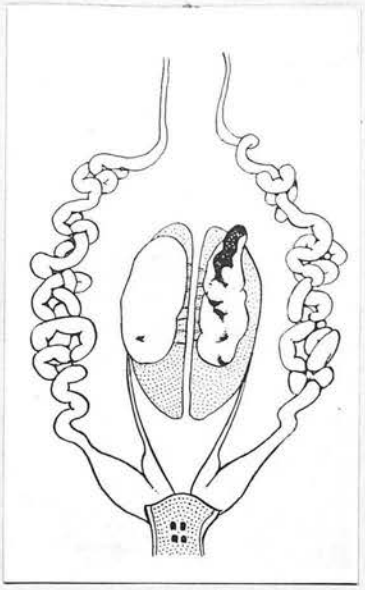
Seminal vesicles present.

Müllerian ducts well-developed, more especially the left.

No sections were made.

14. CREW. (4). R. temporaria. Adult. Killed May. Secondary sexual characters typically male.

Right gonad. An ovo-testis which save for a small pigmented pit upon the outer border/



Crew (4)

border had the appearance of a testis.

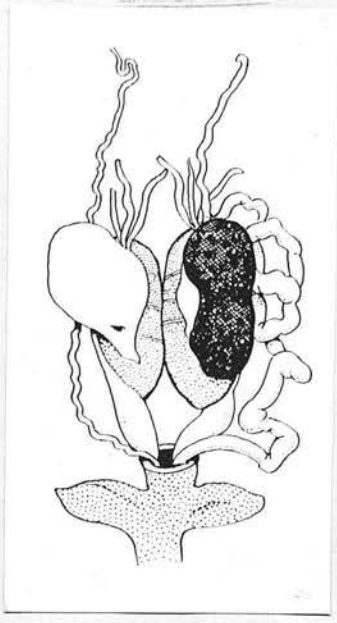
Left gonad. An ovo-testis having the appearance of an irregularly shaped testis which bore along its outer border a prominent pigmented crest, jet-black in colour and consisting of four spherical nodules in its anterior part.

Vasa efferentia on both sides as those of the normal male.

Seminal vesicles well-defined.

Müllerian ducts moderately well-developed.

On section, the greater part of the right gonad had the structure of normal testis. Sections involving the pigmented pit showed the presence therein of polygonal masses of pigment and broken down granular material. The left gonad was mainly spermatic in structure but the pigmented parts consisted of masses of pigment, hyperplastic connective-tissue, and a few very degenerate ova. There was ciliated epithelium upon the peritoneum.



Ognew

GROUP C.

THOSE CASES in which, on inspection ONE GONAD was an OVO-TESTIS and the other A TESTIS.

SUB-GROUP 1. The testis was abnormal in appearance.

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15. OGNEW. R. temporaria. Adult. Secondary sexual characters typically male.

Right gonad. An exceptionally large testis.

Left gonad. An ovo-testis with a small ovarian portion placed laterally.

Seminal vesicles were present.

Müllerian ducts. Right one moderately, left one well developed.

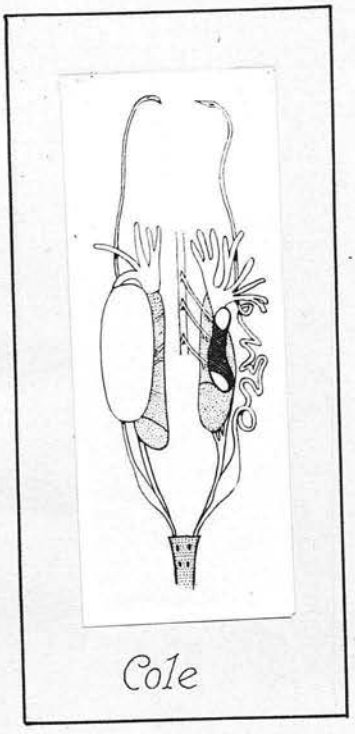
No sections were made.

16. COLE. R. temporaria. Young and apparently immature. Killed during the summer. Secondary sexual characters typically male.

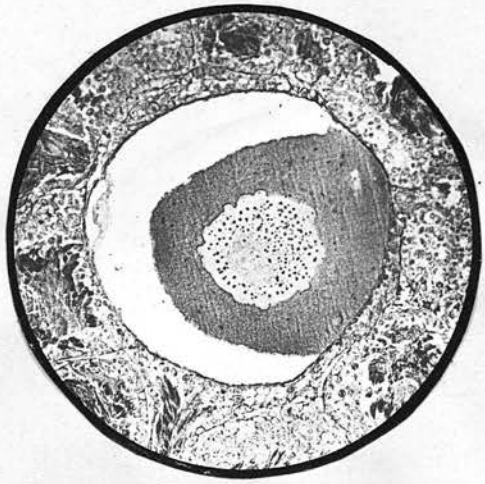
Right gonad. A testis somewhat enlarged and non-pigmented.

Left gonad. An ovo-testis with a larger densely pigmented ovary-portion and

a/



Cole



amaller non-pigmented papilliform testis-portions at its poles.

Vasa efferentia typically male on the right: on the left, only two but"the divisions of the renal artery to the ovo-testis might well have been mistaken for efferent canals".

Seminal vesicles not present.

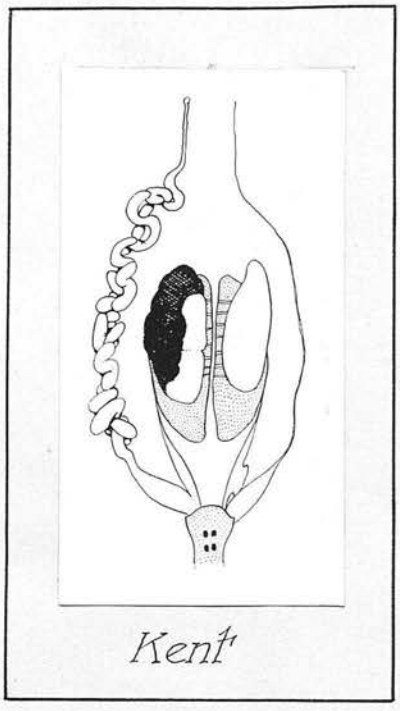
Müllerian ducts. That of the right side was rudimentary and not convoluted, but had a distinct and densely pigmented uterine segment. That of the left side was pigmented, convoluted, and moderately well-developed, although its uterine segment was non-pigmented.

On section, the right gonad was a normal testis and with the exception of one small spherical patch and two or three problematical granular bodies, similar to those found in the opposite ovo-testis, pigment was entirely absent. One ovum at "a stage of development corresponding to that found in female frogs of between one and two summers" was found near the surface close to the junction of the anterior and middle thirds of the gonad, and the neighbouring/

neighbouring portions of the testis were less conspicuous than the remainder of the gland and contained the most immature spermatozoa.

The left gonad was "for the most part made up of the polygonal pigment cells which occur normally in the frog's ovary, with, however, patches of normal testis at its poles. The anterior pole consists of testis with a thin zone of the pigment cells invading one side, whilst the posterior pole is somewhat over half testis. The remainder of the gland is occupied by the pigment cells alone. Further a horizontal section through the middle of the gland of this side, exposed five circular spaces in the pigment mass, containing, surrounded by fibrous capsules, spherical masses of lightly pigmented granular matter. Three out of the five completely filled their respective spaces - the other two did not, one of the latter also containing a large vacuole. The significance of these bodies, which may be free or lie indiscriminately among the pigment cells, and of the abnormally developed pigment mass, is, of course,

on/



Kent

on the available data, impossible to determine. The former may plausibly be looked upon as disintegrated ova (undergoing resorption) - the latter as preceding the development of ova and the formation of a true ovo-testis".

17. KENT. R. temporaria. Adult. Killed January. Secondary sexual characters typically male.

Right gonad. An ovo-testis consisting of an inner testis-portion divided by a well-defined transverse constriction into anterior and posterior halves, and bearing along the anterior part of its outer border the strongly pigmented ovary-portion.

Left gonad. A testis of unusual shape.

Vasa efferentia of the male pattern present on both sides.

Seminal vesicles were present but were of unequal development, the left one being the larger.

Müllerian ducts. The right one was well-developed and much convoluted, but its uterine segment was small and non-pigmented. The left one was rudimentary and straight, but/

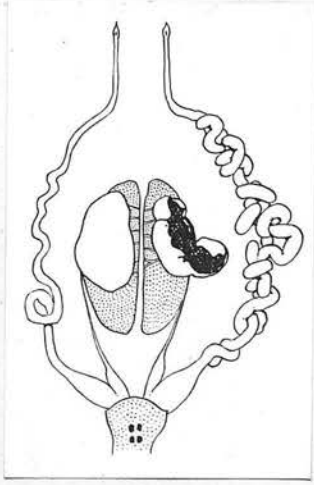
but possessed a well-defined though small non-pigmented uterine segment.

On section the right gonad was seen to be composed of two discrete portions. The testis'portion consisted everywhere of normal spermatic tissue, but the ovarian was pathological. The left gonad was spermatic in structure, but throughout the gland ova were found some within and others between the seminal tubules. The presence of an ovum within a tubule was associated with deformity of the spermatozoa, which were obviously compressed. The presence of an ovum between seminal tubules was associated with fibrous overgrowth in the intertubular connective-tissue, and this produced deformity of the tubules in the neighbourhood. There was ciliated epithelium upon the peritoneum.

18. HUXLEY. (2). R.temporaria. Adult. Killed October. Secondary sexual characters typically male.

Right gonad. An irregularly shaped testis with uneven outlines and a scarred surface.

Left gonad. An ovo-testis. A three-lobed testis-portion twisted upon itself, bore/



Huxley (2)

bore a prominent crest of pathological ovarian tissue upon its outer border.

Vasa efferentia of the usual male pattern on both sides.

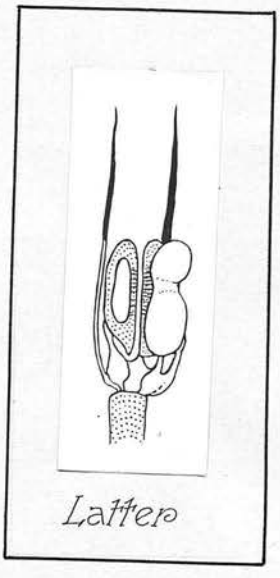
Seminal vesicles were present but were small and fusiform.

Müllerian ducts. Right one weakly developed but convoluted and with a well-defined and pigmented uterine segment. The left one was well-developed.

On section, the right gonad had the structure of ordinary spermatic tissue save that six ova were found, two actually within and the rest between seminal tubules. Some of these ova had a normal appearance, others were degenerate. Accumulations of pigment, ovarian in origin, were found also between seminal tubules.

The left gonad consisted of an inner normal testis-portion and an outer pathological ovary-portion. The two were quite discrete and the ovary portion contained but few ova and all of these were degenerate. The greater part of the ovary-portion was composed of polygonal masses of pigment

and/



Latter

and hyperplastic connective-tissue. The cavities of the lobulated ovary were obliterated by this pigment but the divisions of the lobules were still distinct on section. There were ciliated cells upon the peritoneum.

SUB-GROUP 2. The testis was normal in appearance.

Nil.

GROUP D.

THOSE CASES in which, on inspection, BOTH GONADS WERE TESTES.

SUB-GROUP I. The TESTES were abnormal in appearance.

19. LATTER. R. temporaria. Adult. Killed September. Secondary sexual characters typically male.

Right gonad. A faintly pigmented testis smaller than usual.

Left gonad. A faintly pigmented testis larger than usual and irregular in shape.

Seminal vesicles present.

Müllerian/

Müllerian ducts only slightly developed, not convoluted, and solid in their anterior portions, but expanded in their posterior portions.

On section, ova were found in both testes, some within and others between the seminal tubules. Besides these ova degenerate cell-masses were found in similar situations.

20. MITROPHANOW, R. esculenta. Young. Secondary sexual characters male.

Right gonad. A testis much smaller than usual.

Left gonad. A testis smaller than usual.

Seminal vesicles present and well-developed.

Müllerian ducts well-developed.

On section, the right gonad contained one true ovum and many doubtful ones. A lobe at the anterior pole of the gonad was regarded as a rudimentary Bidder's organ. The true ovum lay within a seminal tubule. The left gonad was also a testis but contained doubtful ova only.

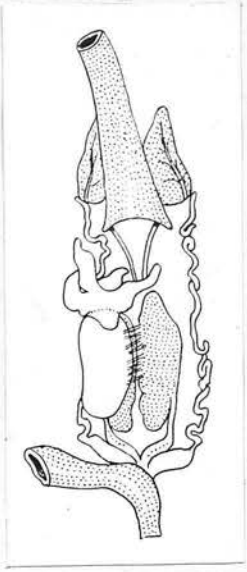
21. PEDASCHENKO. R. temporaria. Adult. Secondary sexual characters typically male.
Both gonads missing. Müllerian ducts well developed.

22. MARSHALL (E). R. temporaria. Adult.
Secondary sexual characters typically male.
Both gonads missing.
Seminal vesicles present.
Müllerian ducts well developed.

23. HOOKER. (A). R.fusca. Adult. Secondary sexual characters typically male. Killed April.
Left testis missing.
Seminal vesicles small.
Müllerian ducts weakly developed with narrow uterine segments.

24. MARSHALL. (C). R. temporaria. Adult.
Secondary sexual characters typically male.
Right testis replaced by fat.
Vasa efferentia normal on the left; absent on the right.
Seminal vesicles present.
Müllerian ducts well-developed.

SUB-GROUP 2. The TESTES were normal on inspection.



Marshall (A)

25. MARSHALL. (A). R. temporaria. Adult. Secondary sexual characters typically male. Seminal vesicles were small. Müllerian ducts well-developed.

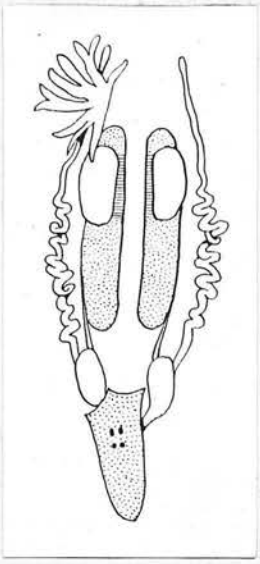
26. GERHARTZ. R. esculenta. Adult. Secondary sexual characters typically male. In addition to the usual male accessory sexual apparatus, well developed Müllerian ducts were present.

27. TARNANI. R.esculenta. Adult. Secondary sexual characters typically male. Seminal vesicles were not present . Müllerian ducts were only moderately developed.

28. TICHOMIROW. R. esculenta. In an otherwise normal male moderately well-developed Müllerian ducts were present.

29. SUTTON. R. temporaria. In an otherwise normal male, slightly developed Müllerian ducts were present.

30. KORTSCHAGIN. R. esculenta. In an otherwise normal male, slightly and imperfectly developed Müllerian ducts were still present. The right one had separate anterior and posterior/



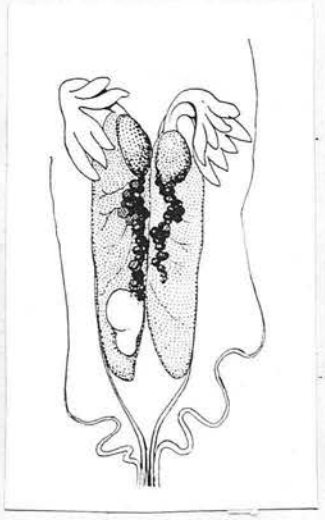
Sumner

posterior portions, it had no anterior opening and was solid.

31. SUMNER. (I). R.virescens. Adult. In an otherwise normal male, Müllerian ducts slightly developed and with no anterior openings were present.

32. SUMNER. (2). R. virescens. Adult. In an otherwise normal male, very slightly developed Müllerian ducts with no posterior openings were present.

DIVISION/



Cerruti (A)

B . B U F O .

DIVISION II. SECONDARY SEXUAL CHARACTERS TYPICALLY MALE.

GROUP A. ONE GONAD an OVARY, the other an OVOTESTIS.

SUB-GROUP 2, the ovary abnormal in appearance.

33. CERRUTI. (A) Bufo vulgaris. Adult. Killed January. Secondary sexual characters typically male. (When captured was actually in copulation with a large female and appeared strongly excited.)

Right gonad. From behind forwards this consisted of (1) a pyriform testis-portion; (2) an ovary-portion; and (3) a Bidder's organ.

Left gonad. Posteriorly, an anomalous ovary, and in front of this, a Bidder's organ.

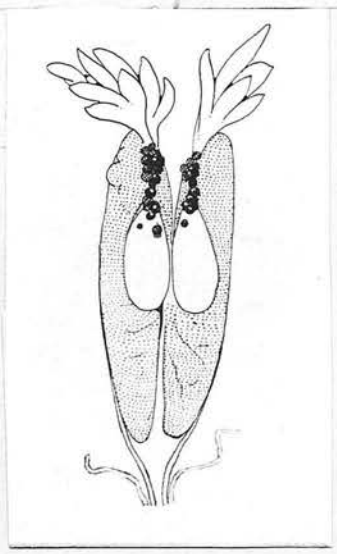
Vasa efferentia. "Serial sections revealed the lack of a rete testis".

Müllerian ducts. Right one relatively long/

long and thin, terminating blindly; left one relatively shorter and also terminating blindly. On section the testis-portion of the right gonad showed the presence of sperms of normal appearance mingled with other cellular elements having variable form and size, many of which are to be considered as spermatids or spermatocytes in more or less advanced stages of degeneration". The intercanalicular connective-tissue was abundant around the tubules towards the centre of the testis-portion and between the tubules were numerous cells pigmented yellowish-brown.

The ovarian tissue of both gonads presented common characters. The smaller oocytes presented a normal structure but the larger ovules exhibited signs of degeneration and contained numerous granules of yellow-brown pigment.

Since the Müllerian ducts ended blindly the ova, even if produced, could not be emitted.



Cerruti (B)

GROUP B. BOTH GONADS OVOTESTES.

34. CERRUTI. (B.). Bufo vulgaris. Adult.

Secondary sexual characters not described.

Right Gonad. Posteriorly, a pyriform testis-portion; anteriorly, an ovary-portion. From the anterior part of the testis-portion "one sees projecting from the testicular stroma some ovules of notable size and browned with darkish pigment". Bidder's organ missing.

Left gonad. Similar to the right.

Vasa efferentia. "Relation to the kidneys normal".

Müllerian ducts had been cut away before the examination was made. On section the testis-portions had the normal structure. The ovules seen projecting from the testicular stroma were in an advanced stage of degeneration, being full of a granular substance - "in which are present numerous granules/

granules of a yellowish-brown pigment and a rare nucleus belonging to immigrant cells (perhaps leucocytes)." The ovary-portions contained ova of all stages of development. The young and medium were mostly normal though "in some the cytoplasm does not appear homogeneous but in sections presents striae which stain deeply with acid dyes. The nucleoli appear homogeneous or vacuolated and the chromatic threads stain with great difficulty". "The larger ova 850-900 μ. seem like normal oocytes of equal size, but sections of them show easily that they are in a very advanced stage of degeneration, being invaded by immigrant elements, probably leucocytes, and containing numerous granules of yellow-brown pigment".

(In one figure is shown a dark mass due to an accumulation of granules of normal yellow-brown pigment, which Cerruti suggests represents an egg in a more advanced stage of degeneration.)

35. SPENGEL. Bufo cinereus (vulgaris.) Adult. Secondary sexual characters typically male. Each/

Each gonad consisted from behind forewards of a testis-portion, an ovary-portion of several ovarian chambers and containing ova as large as those seen in a female of 2 or 3 years.

36. KNAPPE. Bufo vulgaris. Adult. Secondary sexual characters typically.

Each gonad consisted from behind forwards of a testis-portion, a Bidder's organ, an ovary-portion, another Bidder's organ.

37. KING. Bufo lentiginosus. Adult. Killed March. Secondary sexual characters male.

Right gonad. An ovo-testis with testis-portion posteriorly. No Bidder's organ.

Left gonad. An ovo-testis, similar to that of the right side. The ovary was placed where normally the Bidder's organ would have been.

Vasa efferentia present.

Müllerian ducts still present but without terminal dilatations.

On section, the testis-portions had the structure of normal spermatic tissue; while all the larger ova within the ovary-portions showed degenerative changes.

GROUP D2. BOTH GONADS TESTES NORMAL IN APPEARANCE.

38. SPENGEL. Bombinator igneus. Adult. In an otherwise normal male, the right Müllerian duct was as well-developed as the oviduct of a female and the left, though smaller, was abnormally large.

(C) PELOBATES.

DIVISION II. Secondary sexual characters typically male.
GROUP C2. ONE GONAD an OVOTESTIS, the other a TESTIS of NORMAL APPEARANCE.

39. SPENGEL. Pelobates fuscus. Adult. Secondary sexual characters typically male.

Right gonad. A normal testis.

Left gonad. An ovo-testis of which the posterior part consisted of a 2-lobed ovary, containing pigmented ova of the size of normal mature ova.

Accessory sexual apparatus as that of a normal male.

Müllerian ducts extremely rudimentary.

(D)/

(D.) HYLA.

DIVISION II. Secondary SEXUAL CHARACTERS typically male.

GROUP D.2. BOTH GONADS TESTES, NORMAL in APPEARANCE.

40. SWEET. Hyla aurea. Adult. Secondary sexual characters typically male.

Right and left gonads both testes normal in appearance.

In addition to the usual male accessory sexual apparatus, Müllerian ducts were present, being well-developed, but having no posterior openings.

On section, ova were found within both testes.

THE SPECIES & the EXTENT of the ABNORMALITY.

The species which contributed the abnormalities and the nature of the abnormality are shown in the following Table:-

TABLE/

TABLE I.

DIVISION.I. The secondary sexual characters were not typically male.

GROUP.A. One ovary; one ovo-testis.
SUBGROUP.1 The ovary was normal in appearance.
SUBGROUP.2 The ovary was abnormal in appearance.

GROUP.B. Two ovo-testes.

GROUP.C One ovo-testis; one testis.
SUBGROUP.1. The testis was abnormal in appearance.
SUBGROUP.2 The testis was normal in appearance.

GROUP.D. Two testes.
SUBGROUP.1 The testes were abnormal in appearance.
SUBGROUP.2 The testes were normal in appearance.

DIVISION.II. The secondary sexual characters were typically male.

GROUP A. 1, 2
GROUP.B.
GROUP.C. 1, 2
GROUP.D. 1, 2
as above.

	RANA TEMPORARIA.	RANA ESCULENTA.	RANA FUSCA.	RANA VIRESCENS.	BUFO VULGARIS.	BUFO LENTIGINOSUS.	PELOBATES FUSCUS.	BOMBINATOR IGNEUS.	HYLA AUREA.	
GROUP A. 1	2									2
GROUP A. 2	3									3
GROUP.B.	1		1							2
GROUP.C. 1										0
GROUP.C. 2										0
GROUP.D. 1										0
GROUP.D. 2										0
										7
GROUP A. 1					1					1
GROUP A. 2	5	1	1		3	1				11
GROUP.B.	4						1			4
GROUP.C. 1										1
GROUP.C. 2										6
GROUP.D. 1	4	1	1					1	1	10
GROUP.D. 2		4		2						33
	21	6	3	2	4	1	1	1	1	
		32			5	1	1	1	1	40

GROUP A 6
" B 13
" C 5
" D 16

Of the 40 Cases described, 21 were furnished by R.temporaria. This does not mean, necessarily, that this species is more prone to abnormality than any other, however, and the explanation of these figures possibly lies in the fact that the Common Grass Frog is used for laboratory purposes more commonly than others. Only 40 Cases have been thought worthy of detailed description, but there is little doubt that similar cases have been encountered in every Zoological Laboratory yet have not been recorded. Nevertheless, such abnormality must be regarded as very rare, though certainly occurring in all species of the Anura.

THE PRIMARY SEX-GLANDS of these ABNORMAL INDIVIDUALS.

Spermatic and ovarian tissues were present, as noted in the tabulated List of Cases, separately and in combination, in the following forms.

SPERMATIC TISSUES.

1. Normal healthy spermatic tissue. (Let the formula Sp.1. represent such.)
2. Healthy spermatic tissue with ova and pigment included within its structure. (Sp.2.)

OVARIAN/

OVARIAN TISSUES.

1. Normal healthy ovarian tissue. (Ov.1.)

2. Ovarian tissue in which a considerable proportion of the ova were degenerate and in which there was overgrowth of the connective tissue and an increase of the pigment. (Ov.2.)

3. Ovarian tissue in which the degenerative changes were more pronounced, the ova being all degenerate and the hyperplasia of the connective tissue and the density of the pigment being more marked. (Ov.3.)

4. Ovarian tissue in the extreme stages of degeneration. No ova remained and all that was left was a nodular mass of pigment and fibrous tissue. (Ov.4.)

Using these formulae, it is possible to analyse the Cases.

Sp. Spermatic tissue;

Ov. Ovarian tissue:

? Details not available:

♂ As in the male:

♀ As in the female:

\- Less than:

-- Much less than:

--- Very much less than

Abs. Absent:

Rt. Right:

Lt. Left.

	CASE	RIGHT GONAD · SP.	OV.	LEFT GONAD · SP.	OV.	VASA EFFERENTIA	SEMINAL VESICLES	MULLERIAN DUCTS
A. IA	1	–	1	1	1	♀	♀	♀
	2	1	2	–	2	♀♂	♀♂	♀♀
A2	3	–	2	2	2	Rt.♀:Lt–♂	–♂	♀♀
	4	–	3	1	3	–♀♂	–♂	♀♀♀
	5	1	3	–	3	Rt.♂:Lt–♂	♀♂	♀♀♀
B	6	1	2	1	2	♂	–♂	♀♀♀
	7	?	?	?	?	?	♀♂	Rt–♀:Lt.♀
IIB	8	1	1	1	1	♂	♀♂	♀♀
	9	1	3	2	?	♂	–♂	♀♀
	10	1	3	1	3	♂	♂♂	♀♀
	11	2	3	2	4	♂	–♂	♀♀
	12	2	4	1	3	Rt.♂:Lt–♂	–♂	♀♀
	13	?	?	?	?	♂	♂♂	♀♀
	14	1	4	1	3	♂	♂♂	–♀
C1	15	?	–	?	?	♂	♂♂	Rt–♀:Lt.♀
	16	2	–	1	3	♂	–♂	Rt––♀:Lt–♀
	17	1	3	2	–	♂	♂♂	Rt–♀:Lt––♀
	18	2	–	1	3	♂	–♂	Rt–♀:Lt.♀
D1	19	2	–	2	–	♂	♂♂	––♀
	20	2	–	2	–	♂	♂♂	♀♀
	21	–Abs.	–	–Abs.	–	Abs.	♂♂	♀♀
	22	–Abs.	–	–Abs.	–	Abs.	♂♂	♀♀
	23	1	–	–Abs.	–	Rt.♂:Lt.Abs	–♂	––♀
	24	–Abs.	–	1	–	Rt.Abs:Lt.♂	♂♂	♀♀
D2	25	1	–	1	–	♂	–♂	♀♀
	26	1	–	1	–	♂	♂♂	♀♀
	27	1	–	1	–	♂	♂♂	–♀
	28	1	–	1	–	♂	♂♂	–♀
	29	1	–	1	–	♂	♂♂	––♀
	30	1	–	1	–	♂	♂♂	––♀
	31	1?	–	1?	–	♂	♂♂	––♀
	32	1?	–	1?	–	♂	♂	––♀
B.								
IIA2	33	2	2	2	2	♀♂	♂♂	––♀
IIB	34	1	2	1	2	♀♂	♂♂	––?
	35	?	?	?	?	♂	♂♂	?
	36	?	?	?	?	♂	♂♂	?
	37	1	2	1	2	♂	♂	––♀
IID2	38	1	–	1	–	♂	♂	Rt.♀:Lt–♀
C.								
IIC2.	39	?	–	?	?	♂	♂	♂
D.								
IID2	40	2	–	2	–	♂	♂	–♀

Reviewing only the cases of abnormality in Rana it is seen that the various forms of ovarian and spermatic tissues furnish gonads as follows;-

	CASE & GONAD	TOTAL
Ov. 1	1 rt.	1
Ov. 2	3 rt. 2 lt.	2
Ov. 3	4 rt. 5 lt.	2
Ov. 4	nil	0 = 5
Sp. 1	23 rt. 24 lt. 25-32 both	18
Sp. 2	16 rt. 17 lt. 18 rt. 19 & 20both	7 = 25
Sp. 1	Ov. 1 1 lt. 8 both	3
Sp. 1	Ov. 2 2 rt. 6 both	3
Sp. 1	Ov. 3 5&9 rt:4 lt.10 both 12 lt. 14 lt. 16 lt. 17 rt. 18 lt.	10
Sp. 1	Ov. 4 14 rt.	1 = 17
Sp. 2	Ov. 1 nil	0
Sp. 2	Ov. 2 3 lt.	1
Sp. 2	Ov. 3 11 rt.	1
Sp. 2	Ov. 4 11 lt. 12 rt.	2 = 4
	In the case of 13 gonads sections could not be or were not made.	13 = 13
		64

ONE GONAD	THE OTHER	CASE	
Ov. 1	Sp. 1 Ov. 1	1	1
Ov. 2	Sp. 1 Ov. 2	2	1
Ov. 2	Sp. 2 Ov. 2	3	1
Ov. 3	Sp. 1 Ov. 3	5 & 6	2
Sp. 2	Sp. 1 Ov. 3	16,17,18	3

When the abnormalities of the reproductive system are thus arranged in a graduated series, they appear to be merely phases of one process at the beginning of which the gonad has the appearance of a normal typical ovary, and at the end of which the same gonad, is, in every way a typical testis. At the beginning, the gonad is an ovary of considerable size, and is capable of functioning as such and of producing mature ova (CASE 1. Right), it contains many ova in various stages of development, and a considerable amount of undifferentiated germ-tissue, much of which is situated upon the inner border of the gonad. Then upon this inner border, one or several patches of spermatic tissue make their appearance. (CASES 3 & 4 left). These have developed from the hitherto undifferentiated germinal tissue of this region. Very soon after this, the ovarian tissue of both gonads begins to show signs of degenerative changes, such as an increase in the relative amount of pigment, a diminution in the size of the nuclei of the ova, and a shrinking of the cytoplasm with vacuolation, (CASE 2.) The spermatic tissue progressively increases in amount, and the ovarian undergoes progressive degeneration, becoming less and less in amount, and more and more solid in consistence, while the/

the pigmentation becomes more and more intense. Fewer and fewer ova are found within its shrinking bulk (CASES 4 lt. 5 rt.), until at length the gonad assumes the form of a testis irregular in shape, and with its surfaces scarred with deep grooves, which bears upon its outer border a sub-peritoneal crest of jet-black nodules of pigment cells and fibrous tissue (CASE 14 rt.). This pigment, now a harmful foreign body, is slowly removed by the blood-stream, and can be identified within the renal veins, and the testis, becoming healed, ultimately assumes the form and appearance of the normal gonad of the typical male (CASES 25-32).

It seems clear that at the beginning one gonad - usually the right - is the seat of this transformation, for when both gonads are affected, they exhibit two different phases of the process, that on one being more advanced than that in the other, and it seems that the occurrence of this phenomenon in one gonad favours its onset in the other, since the degeneration of the ovarian tissue is often equally pronounced in the ovary of the opposite side, it is reasonable to assume that in every case in the Lists, both gonads have been, are, or would have been affected.

Ova/

Ova are often found amid the spermatic tissues of the testis portion of an ovo-testis and of an otherwise normal testis. Normally ova are extruded from the surface of an ovary. In an ovo-testis they are extruded from the ovarian portion into the testis-portion (or the actively growing spermatic tissues envelope ova and pigment along the line of junction of ovary- and testis-portions), where they remain more or less healthy for a considerable time, resisting destruction by virtue of their maturity, and being nourished and protected by the surrounding tissues, while the other ovarian structures are destroyed and removed. The presence of such ova is sufficient indication that the testis which includes them has been an ovo-testis. These ova are doomed and ultimately become resorbed, leaving the testis perfectly normal.

The question as to whether these ova are to be found actually within the seminal tubules is of some importance, since if they are found in this situation, it is not unreasonable to suggest, that such cells may be the results of the division of spermatogonia. There is, however, no reason why ova produced by the ovary-portion of an ovo-testis, should not be found within a seminal tubule of the testis-portion of the gonad, and if these cells are also found between the tubules, then force is given to the contention that they are ova, and that they are the products/

products of ovarian and not of spermatic tissue.

When such an ovum is found within a seminal tubule, the spermatozoa also present therein are greatly compressed. The ovum thus may have the normal structure, while the spermatozoa may be deformed and degenerate. But this degeneration is the result of local pressure, and is not an indication that the ovarian tissue generally, is healthy, and spermatic degenerate. When the ovum is situated between the seminal tubules, these, in the neighbourhood, are contorted and misshapen. This is the result of local hyperplasia of the intertubular connective tissue, which follows the irritation set up by the presence of the foreign body, - the ovum and its pigment.

It is seen that Ov.4. type ovarian tissue, does not occur alone, but only in association with spermatic tissue. Spl.1 tissue is such as is found in the very earliest stages, and in the closing stages of this process; in the first it is associated with type Ov.1. ovarian tissue. But in the closing stages, ova which have been extruded from the ovarian tissues, so that these are such as have been designated by the formula Sp.2., and all that remains of the other ovarian tissues, is the pigment which

is/

is massed along the outer border of the gonad.

There is no gonad with the constitution Sp. 2: Ov.1. This is because in the case of type Ov.1. ovarian tissue, there is, as yet, no appreciable degeneration and no considerable degree of growth on the part of the spermatic tissue. It is by the active enveloping growth of the spermatic tissue that ovarian ova and pigment become included within them.

In those cases in which one gonad was a testis and the other an ovo-testis, it is seen that the tissues of the testis are of the formula Sp.2. This definitely proves that the testis, which contains ovarian pigment and ova, is a gonad in which the transformation from ovary to testis is more advanced than in the gonad of the opposite side.

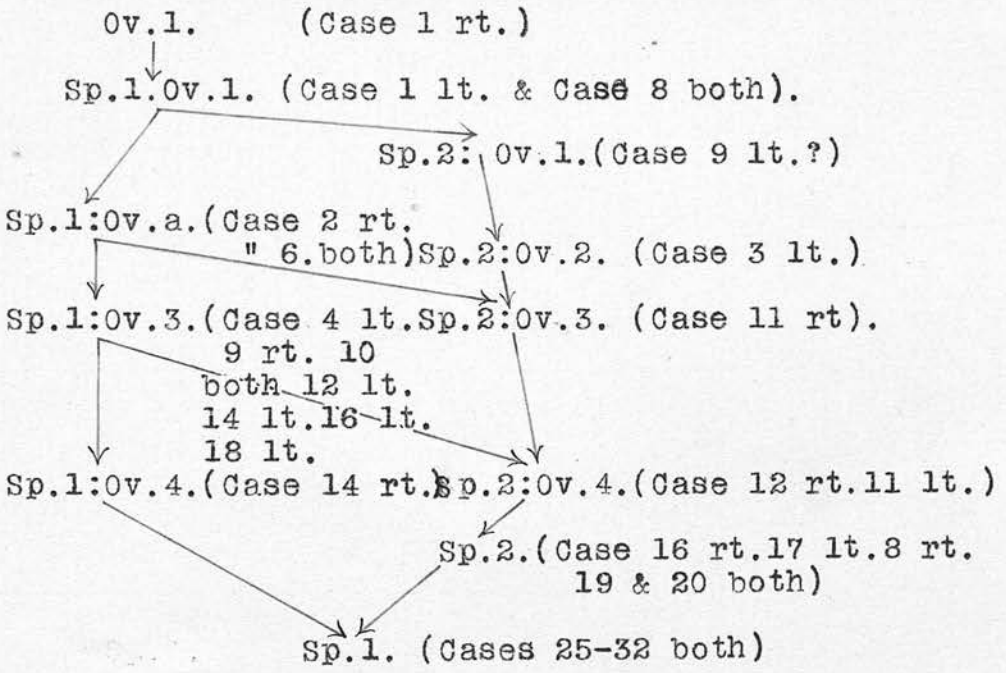
Although the process may begin in either gonad apparently, yet in the 18 cases in which anything approaching a true estimate can be made, the right gonad was first affected in 13 cases: the left one in 5 only.

It is the rule that the testis-portion of an ovo-testis is situated upon the inner border of the gland, a position in every way convenient for its association with vasa efferentia. The ovary-portion is always placed externally to the testis-portion, - and this fact has to be remembered when the question/

question as to whether a crest of pigment containing no ovum is ovarian tissue or not, is raised.

It would seem then, that if spermatic tissue becomes expressed in a gonad which previously has possessed the characters of an ovary, it makes its appearance in a definite situation. This points to a localisation in the germinal tissue.

The process thus outlined can be depicted graphically by the use of the formulae employed above. (Ov.1., it will be remembered, is ovarian tissue which on microscopical examination, has the normal structure.)



The degenerative changes in the ovarian tissue/

tissue of both gonads quickly follows the expression of spermatic tissue in one and the degree of this degeneration is more or less equal on the two sides. There is no case in which the ovarian tissue was thoroughly healthy in one gonad, whilst degenerate in the other. If the presence of the spermatic tissue in one gonad is responsible for the ovarian degeneration, then the agent which produces this must be blood borne, after the manner of a hormone.

THE ACCESSORY SEXUAL APPARATUS.

VASA EFFERENTIA.

It is seen that every gonad described as a testis, whether it was normal or abnormal, was equipped with these efferent ducts. The testis-portion of every ovo-testis also, was linked up with its kidney, but in these cases the vasa efferentia were commonly reduced in number, when compared with the same structures of the typical male. In those cases in which one gonad was an ovary, the findings of different investigators have differed, but in some cases efferent ducts were found connecting up gonad and kidney. Similarly, in the case of an ovo-testis, vasa efferentia were found, not only in connection with the testis,-but with the ovary-portion of the gonad too.

Spermatic/

Spermatic tissue in a gonad, therefore, is invariably equipped with vasa efferentia, even though its amount is relatively small, and ovarian tissue of an ovo-testis and of an ovary, is frequently found to be supplied with these efferent ducts. The less the relative amount of spermatic and the greater the amount of ovarian tissue in the gonads, the less likelihood there is that vasa efferentia of the male pattern will be found.

It is very exceptional to find any suggestion of efferent ducts in a typical female. During the examination of many hundreds of individuals, no trace of such has been found. Therefore, it is reasonable to assume that whilst the gonads have the constitution represented by the formula Ov.l., no efferent ducts will be present, and that they make their appearance at the time of the early growth of the testis-portion of the ovo-testis (Sp.l: Ov.l.), and thereafter keep pace with the development of the testis.

THE SEMINAL VESICLES.

These were absent in 5 Cases (1, 2, 5, 7, & 8), and less developed than in the typical male in 10 others (3, 4, 6, 9, 11, 12, 16, 18, 23 & 25). Most of these occur towards the beginning of the Table/

Table, so that the nearer the individual approximates with respect to the nature of the gonads, and to the nature of the secondary sexual characters, to these characters in the typical female, the more likely are the seminal vesicles to be either smaller than those of the typical male or even absent altogether. Conversely, as has been pointed out previously, in those cases in which the gonads and the secondary sexual characters generally approximated those of the typical male, the seminal vesicles were also, almost without exception, as those of the typical male.

These facts suggest that there is some definite relation between the nature of the gonads and the development of the accessory reproductory apparatus. COLE'S conclusion that "a good diagnostic feature as regards sex is the seminal vesicle, which has only been observed in hermaphrodites predominantly male" is justified, for it is seen that the vesicles are commonly poorly developed or absent altogether in those cases in the Lists which were more female than male as regards their secondary sexual characters and the nature of their gonads, in which a considerable amount of ovarian tissue was present. In several cases the vesicles were small or absent although there was no ovarian tissue present in the gonads which were composed/

composed entirely of spermatic tissue. In most of these cases unfortunately, the age of the individual and the season of the year are not stated, and consequently a true estimate of the importance of the condition of the vesicles cannot be made. Reviewing the whole of the cases, however, it is seen that there is some connection between the development of the seminal vesicles, and of the vasa efferentia also, and the presence of spermatic tissue in the gonads.

In this connection, it is noteworthy that NUSSBAUM in 1905 found, that the size of the seminal vesicles was controlled by the internal secretion of the testis.

It will be noticed that the degree of development of the seminal vesicles and that of the vasa efferentia are connected, for in Cases 1, 2, 3, 4, 5, 7, 12, both of these structures differ in degree of development from those of the typical male, in that both were poorly developed. Whatever affects the development of the one, therefore, also affects that of the other.

MÜLLERIAN DUCTS.

The degree of the development of these typically female ducts in the Cases tabulated, varies from almost/

almost the rudimentary condition normally found in the typical male, - fine straight strands of tissue as described by MÜLLER, BURRO, and RATHKE, - to that of the fully developed oviduct of the typical adult female. In 18 Cases these ducts were found to be well-developed, moderately well developed in 2, slightly or weakly developed in 6; & in 5 other Cases the development of the ducts of the two sides was unequal.

It will be seen that it is the rare exception for these ducts to be anything but well developed in those Cases in the first half of the Table, that is, in those Cases in which a considerable amount of ovarian tissue still was present, and further that towards the end of the Table, the condition of those ducts is more and more frequently described as moderately, slightly or weakly developed. In the last 14 Cases, the ducts were well developed in 6, moderately developed in 2, and slightly developed in 6, and in all these only spermatic tissue was present in the gonads.

So that in an otherwise normal male, Müllerian ducts in form and size equal to the oviducts of the adult female are found in the presence of actively functioning spermatic tissue. HARMS and MEISEN-

MEISENHEIMER claim to have demonstrated that the internal secretion of the male gonad can encourage the development of the accessory reproductive apparatus of the opposite sex, and if this is so, then in such cases the unusual development of the Müllerian ducts may have been a response to the action of the internal secretion of the testes. Experiments conducted to test the findings of HARMS & MEISENHEIMER have failed to confirm their results, and there is reason to believe that the occurrence of Müllerian ducts in form and size equal to the oviducts of a mature female, in an otherwise typical male, is an indication that functional ovarian tissue of considerable amount has been present in the gonads of the individual.

It is of interest to note, that in 1885, SUTTON made the following generalisation.

> "When a male frog develops a Bidder's organ or ovary in conjunction with a testis, the Müllerian duct or oviduct then assumes some considerable size",

and from the results of the examination of more than 250 specimens of Rana temporaria, he concluded -

> "that as a rule the amount of development of these ducts is in direct proportion to the size of the Bidder's organ".

It/

It is gathered from the Tables that the presence of spermatic tissue in the gonads is associated with a fuller development of vasa efferentia and seminal vesicles, and that the Müllerian ducts attain their fullest development only in the presence of ovarian tissue. It is suggested, therefore, that the presence of well-developed oviducts is, in itself, an indication that ovarian tissue either is or has been present in the gonads of the individual possessing them. When once they have attained a considerable size, as they do in association with the development of ovarian tissue, the oviducts persist, even though the ovarian tissue is removed. This can be demonstrated by extirpation of the ovaries, after which the oviducts retain their form and size certainly for several months. They do not attain a fuller development during the breeding-season in cases thus operated upon, but they certainly do not atrophy. In the Cases in the Lists, it is seen that only in those in which there still remained some ovarian tissue capable of producing ova, though they were abnormal and degenerate, did the oviducts exhibit a seasonal increase in size and activity. In the majority their glandular portions still retained the power of manufacturing their particular secretion, for in water

the ducts became much swollen, and this is a further proof that the ducts do not undergo any considerable degree of atrophy.

There is no evidence that the seasonal activity of the testis in these cases produces a corresponding increase in the size and activity of the Müllerian ducts, as would be expected from the results of the experiments of HARMS & MEISENHEIMER. It is seen, as has been stated above, that this increase is only shown in those Cases in which ovarian as well as spermatic tissue was present in the gonads.

It seems probable, therefore, that when the gonad has the constitution represented by the formula Ov.1., oviducts become developed up to the female standard, that when the gonad's composition becomes that represented by Sp.1: Ov.1. vasa efferentia and seminal vesicles make their appearance, and that coincidently with the fuller development of the testis-portion of the ovo-testis, the male accessory reproductory apparatus attains the form and size of that of the typical male, while the oviducts, having reached a considerable size already, retain their form throughout the process, which is converting a young female into a male.

Granting that the hormones of the gonad are responsible for the stimulus which calls forth the development/

development of the accessory reproductive apparatus, it is seen that the exhibition of the specific male hormones, does not cause the development of any structure which is not represented embryologically in the normal female, and that hormones act merely by inhibition or stimulation of normal embryonic rudiments.

The Müllerian ducts were of unequal development in a few cases, and in these the better developed duct was on the side of the body on which more ovarian tissue was still present. (cf.Aves).

CASE.	Rt.GONAD		M.D.	Lt.GONAD		M.D.
7	Sp. ?	Ov. ?	-♀	Sp. ?	Ov. ?	♀
15.	?	-	-♀	?	?	♀
16.	2	-	--♀	1	3	-♀
17.	1	3	-♀	2	-	--♀
18.	2	-	-♀	1	3	♀

There is, therefore, some evidence that the degree of development of the Müllerian duct is controlled to some extent by the activity of the ovary of the same side. It would seem that the Müllerian ducts are developed under the direction of the ovaries, but that the degree of their development in these/

these Cases is determined by the time during general development at which the spermatic tissues first become expressed. If this is expressed relatively early, then these ducts will not have attained their full development, and their conditions will be as in Cases 14, 27, and 28. If spermatic tissue becomes expressed even earlier still, then the ducts will be as in Cases 20, 23, 29, 30, 31 and 32. If, on the other hand, this expression is relatively later, then the ducts will have already attained their full development and will retain their characters.

In the cases in which these ducts were of unequal development, it would seem that the process of transformation of ovary into testis began, as is usual, in one gonad, and later extended to the other, and that the interval between the beginning of the process in the two gonads was prolonged, so that the duct of the side on which spermatic tissue had yet to become expressed in the gonad, would be permitted to develop a little further, as the ovarian tissue of this side would be affected to less extent than that of the other.

A point of interest arises in CASES 30, 31 and 32. In CASE 32 these ducts had no posterior openings, while in the others they were solid in their anterior portions.

The/

THE SECONDARY SEXUAL CHARACTERS.

Of the 30 frogs in the tables of which sufficient details were given as to their secondary sexual characters, 25 - (83·3%) - were definitely and typically male. Four others were definitely but imperfectly male. (13·3%), and in the remaining Case the secondary sexual characters were female. (3·3%).

In every case functioning spermatic tissue was present, and in those Cases in which ovarian tissue was also present, and in which sufficient description of its histological structure was given, it has been shown that this tissue was pathological and undergoing removal.

The presence of imperfectly developed male secondary sexual characters, is not associated with the development of female characters, as is seen in Cases 2 & 4, and the secondary sexual characters can remain fully and typically male in the entire absence of the gonads, as is illustrated by Cases 21 & 22.

These characters do not become at all obvious while the individual is still young, and the female characters are, for the most part, negative in nature. Assuming that there is some intimate relation between the primary and secondary sexual characters, a review of the Cases suggests that the superimposition/

super-imposition of male upon female secondary sexual characters, is a simple matter in the Anura. The presence of patches of ciliated epithelium upon the peritoneum round about the suspensory ligament of the gonad, does not interfere with the assumption of the male characters which are developed in quite different situations. The wartiness of the skin is a seasonal phenomenon and only occurs in the presence of functioning ovarian tissue.

It has been shown that the development of spermatic tissue upon the inner border of a gonad, which previously had every appearance of an ovary, occurs at an early stage in the life-history of the individual, and that coincidently with its appearance the ovarian tissue begins to degenerate. If then, the female secondary sexual characters are not developed to any extent, until the normal female is well matured, the development of the spermatic tissue in these abnormal individuals, and the consequent destruction of the ovarian tissue, will prevent the assumption of the female characters.

The degree of development of these characters bears no relation to the amount of germinal tissue present, but this tissue must be healthy and functioning. A small amount of healthy spermatic tissue/

tissue is associated with well-defined male secondary sexual characters, although in the individual, there is a greater amount of ovarian tissue which is pathological. It is seen that the exhibition of the male sex-hormones is not attended by the development of any structure which is not represented embryologically in the normal female, and that as in the case of the accessory reproductive apparatus, the hormones act by inhibition and stimulation of normal embryonic rudiments.

The cases of abnormality in Bufo, Bombinator, Pelobates & Hyla, are too few to permit of a similar treatment to that adopted in the case of Rana. They are, however, exactly similar in their nature and there can be no doubt that the conditions found are, with minor differences, identical with those described in Rana.

The relative position of ovarian and spermatic tissues in an ovo-testis is somewhat different, in that the ovarian tissue is placed anteriorly and the spermatic posteriorly. Further, it would seem that the comparatively smaller amount of spermatic tissue is associated with the assumption of male secondary sexual characters in the case of Bufo than in Rana.

The gonad is Bufo is subject to considerable variation/

variation. CERRUTI figures a Bufo vulgaris in which he observed, in front of each testis, a Bidder's organ, another piece of testis, another Bidder's organ and lastly the fat-body; and in another case, quoted in the Lists, in which in place of Bidder's organ, there was found in front of each testis, an ovary. KNAPPE describes a case, quoted also in the Lists, in which he observed in front of each testis, a Bidder's organ, an ovary, another Bidder's organ and lastly the fat-body. KING has dealt with such abnormalities at length, and she is of the opinion that the component cells of an embryonic testis or ovary, in response to the stimulus of altered blood supply, can assume the characteristics of a Bidder's organ. It certainly does appear that cells which normally should develop into typical testis or ovary or Bidder's organ, can, on occasion, develop into another of these tissues.

In Cases 34 & 37 the position of the ovarian portion coupled with the fact that the Bidder's organ was missing, strongly suggests that these had been derived, in part at least, from those cells which normally would have given rise to Bidder's organ. Further, as KING points out, as the testis-portion in these Cases was shorter than the normal testis of the typical male, it may be assumed that the primordial germ/

germ-cells in the anterior part of the germinal ridge, which normally would have developed into spermatic tissue, had also taken part in the formation of the ovary. She suggests that the causal agent, whatever it may have been, must have acted at a very early period in the life-history of the individual, since in the normal toad, the cells which develop into Bidder's organ become differentiated when the tadpole is about two weeks old.

In Cases 33, 35 & 36, an ovary was found between the Bidder's organ anteriorly and the testis posteriorly. This can be interpreted as the condition in which the most anteriorly situated cells of the germinal ridge have developed, as is usual, into a Bidder's organ, those situated posteriorly into a testis, while the cells of the more anterior part of the middle region of the ridge have developed, for some reason or other, into an ovary. In Case 39 one gonad was an ovo-testis with the ovary-portion posteriorly situated. There is in this a strong suggestion that either the forerunners of testis, ovary, and Bidder's organ are normally present in the apparently undifferentiated primordial germ-tissue, or perhaps, that the foundation primordial germ-cells, in response to the appropriate stimulus, can become any one or all of the three types of adult germinal tissue.

It is seen then, that an individual, to all appearance a female, becomes transformed into a functioning male. The question naturally arises as to whether such an individual actually is a female which ultimately assumes the male organisation.

Before the answer can be given to this question, it is necessary to review what is known of the development of the gonads, and to examine the work that has been done in connection with the sex-ratio in frogs.

III./

III.

THE DEVELOPMENT of the GONAD.

In the Anura the gonad first becomes apparent as the genital ridge - a longitudinal prominence, which runs along the dorsal wall of the splanchnocoele on either side of the middle line and projects into the splanchnocoele cavity. Only a part - the gonal part - of the ridge is destined to take part in the formation of the sex-gland, while the rest remains sterile and in the anterior portion forms the fat-body. As development proceeds, the ridge deepens to become a fold, consisting of a thickened area of peritoneum - the germinal epithelium - covering a supporting and vascular core of mesenchymatous connective-tissue. Differentiation of the component cells of this germinal epithelium in certain areas, then occurs, and the cells show recognisable indications of nuclear and cytoplasmic features,

which/

which are characteristic of the gonad later on. These cells are the gonocytes and the localisation of these gives to the embryonic gonad a suggestion of metameric segmentation.

Many authorities maintain that cells, arising quite independently of the coelomic lining, migrate into the germinal area and ultimately become functional gametes. NUSSBAUM observed this migration in Rana, and concluded that the cells came from the vitellus, while BOUIN found them at the root of the mesentery. Whatever may be their exact origin, the gonocytes become aggregated along the free margin of the genital fold, and it is this portion, by a multiplication of the cells, following a preliminary reduction of their number, which becomes the functional gonad, while the attached portion is suspensory in its function. The actively proliferating gonocytes spread into the interior of the developing gonad and constitute the ancestral oogenia or spermatogonia, as the case may be, while immigrant mesenchyme cells pass into the gland along its attached border to form the vascular and connective tissues.

It is not possible to distinguish between the gonad which is to become a testis from one which is to become an ovary until about the time of the metamorphosis, for in all young larvae, the gonads are/

are very similar in histological structure, all having the characters as described and pictured by WITSCHI. Each consists of a strand of tissue which contains the germ cells and in the interior of which a cavity - the primary genital cavity with its walls lined with germinal epithelium - is formed. Into this cavity, there grow from the base of the genital fold, the segmental cell-cords - the sex-cords.

In Rana at this time, in the gonad which is to develop into an ovary, the gonocytes which later will become functional ova, increase rapidly in size and indifferent cells of the germinal epithelium become converted into follicular or 'nurse' cells which minister to the metabolic needs of the growing ova. Characteristic egg-nests are thus formed and in the cells of these one finds synaptic phenomena. Then the growing oocytes soon become isolated and the formation of yolk begins. The sex-cords in the female have no special significance and soon become absorbed. The further processes of development do not present any special feature. This activity is only local, for the greater part of the germinal epithelium retains its primitive character, and it is this which in successive years provides the successive crops of ova.

The histogenesis of the spermatozoa, though/

though fundamentally equivalent to that of the ova, differs in detail. It is slower and the great increase in size of the gonocytes is not seen. Further there is an additional phase in their development. The development of a testis from what may be regarded from the histological point of view, at any rate, as an indifferent gonad, has features distinct from those described above. The germ cells leave the germinal epithelium and lay themselves on the sex-cords, according to GOLDSCHMIDT, and close to them the seminiferous tubules become differentiated, first being vesicular and later tubular in form: so that ovary and testis can be further distinguished at this time by obvious differences in the plan of development of the urino-genital network of tubuliferous tissue, for in the testis, connection between the sex-cords and the pronephros is established.

SWINGLE has shewn that the germ cells first appear as a median ridge of entodermal-like cells, just dorsal to roof of archenteron in 7 mm. embryos. The ridge is cut off from the underlying entoderm by:

(1) closing of lateral plates and formation of mesentery:

(2) active migration of the germ cells.

The germ ridge splits longitudinally, and the halves separate to form the paired gonadial ridges of the larva/

larva. In larva of the first season, (four to six months of age) the gonads are mere hollow sacs with walls made up of a single or double layer of sex cells. Despite undifferentiated character of the gonads and immaturity of the larvae, the germ cells undergo a very precocious and abortive sexual cycle, culminating in degeneration and resorption of the cells. Maturation phenomenon is normal up to the first maturation division, when fragmentation of the centrosome occurs with consequent formation of poly-asters and destruction of the chromosomes. A few giant spermatids are formed by growth of an axial fibre from the centrosome of undivided first spermato-cytes. The cells and chromosomes are considerably more like those of Urodeles than like cells and chromosomes of adult Anura. A few spermatogonia, lineal descendants of the primordial germ cells, per-sist unchanged through the abortive sexual cycle and give rise to a second generation of germ cells in two-year-old larva. Many of these cells undergo a second and developmental cycle and give rise to sper-matozoa in the tadpole. Thus there occurs in the larval bullfrog two sexual cycles: The first is very precocious and abortive, the second is normal. This phenomenon is interpreted as a recapitulation in the germ-cell cycle to past phylogenetic conditions of the Anura.

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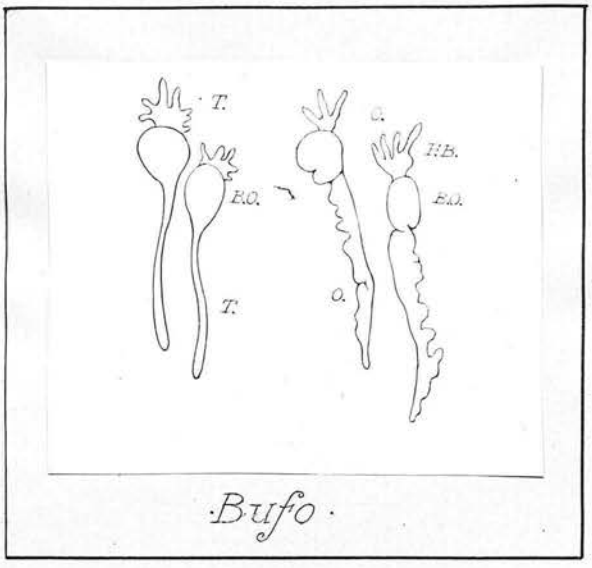

Bufo

In Bufo at the time of the metamorphosis, a testis can be distinguished from an ovary by the following features. A testis is a long cylindrical body, with a smooth outline, whereas an ovary is relatively broader and has an irregular outline. At the anterior end of the gonad, ovary and testis, there is a relatively large rounded body called Bidder's organ, which, in all young toads of the same age, is more or less of the same size and histological structure. But whereas it persists throughout the whole lifetime of the male, it disappears in the case of the female towards the end of the second year.

The distinction between ovary and testis in Bufo, is readily made, but in Rana it is made difficult by the occurrence of a third type of gonad which, in naked-eye and microscopic appearances, appears to be intermediate between the two.

IV.

THE INTERSEXUAL GONAD of the FROG.

The history of this third type of gonad of the frog begins with BORN, who found that of 1443 young frogs (R.temporaria), which he had raised under laboratory conditions, 1371 turned out to be females while but 72 were males. He sought to explain this remarkable sex-ratio by assuming that the environmental conditions under which these frogs had been reared, must have been responsible for the death of so many males in the earliest stages of development. PFLÜGER, also arguing that the true explanation was simply that there had been a higher death-rate among the males, repeated the experiment on a larger scale and more critically. He reared frogs from parents obtained from Bonn, Utrecht, and Königsberg, and found on examination that 35% were males and 65% females in the Bonn stock, that of the Utrecht lot 13% were males and 87% were females, and that of those from Königsberg, 48·5% were males and 51·5 were females. As controls he had consignments of young frogs/

frogs from the three localities which had developed under natural conditions, and found among these that the percentages of males and females were practically identical with those of the frogs reared under artificial conditions. Among the controls from Utrecht, 13·2% were males, among those from Königsberg - two lots of over 500-47·2% were males, and this notwithstanding the fact, that as a result of a high temperature, half of one of these consignments from Königsberg had succumbed upon the journey. He himself collected 228 young frogs which had grown under natural conditions near Bonn, and found among these 35% males.

Then he made investigations as to the relative numbers of males and females among adult frogs in the three localities, and found that in all three males and females were ordinarily present in equal numbers.

Bonn	49·4 & 51·% ♀
Utrecht	48·8%
Königsberg	50%

PFLÜGER was convinced that a differential mortality could not explain the sex-ratio among the artificially raised young frogs and seeking a solution, he ultimately came to the conclusion that there/

there must be three forms of young frogs, males, females, and a third form which might become either males or females, and which by becoming males produced an equality in the numbers of the two sexes among adults. Many of the individuals which had been regarded as females were actually hermaphrodites which ultimately became males. These have been designated since this time as" PFLÜGER'S hermaphrodites."

GRIESHEIM also repeated the experiments of BORN and reached conclusions substantially the same as those of PFLÜGER, HERTWIG was the next to investigate this question, and he also found that in addition to those young frogs, which undoubtedly were males or females according to the constitution of their gonads, there were others the sex of which could not be determined exactly. He described the microscopical anatomy of these 'indifferent' individuals, and maintained that it was impossible to foretell whether they were destined to develop into testes or ovaries. In certain of his experiments these 'indifferent individuals' seemed to take the place of females, one female for example, produced 52 males and 50 females with spermatozoa from one male, and 54 males and 69 'indifferent individuals' with that from another. He concluded that the indifferent gonad/

gonad was a rudimentary ovary, with its female characters so diminished that it might develop into a testis.

SPENGEL in discussing PFLÜGER'S results, quotes F. MEYER, who states that a testis with ovarian inclusion and ovaries with testicular inclusions are not uncommon.

SCHMITT-MARCEL, a pupil of HERTWIG'S, next carried out most extensive investigations into the origin and ultimate fate of these individuals, with very great care and thoroughness, and his results seem to leave no doubt that there is an intermediate form of gonad, which, by transformation into a testis, produces an equality of the sexes among adult frogs. He objects to the term 'hermaphrodite' in any description of the gonads of the young frogs of indefinite sex, pointing out that PFLÜGER'S investigations were inexact and incomplete, and that the description of the histological structure of the gonads given by PFLÜGER and confirmed by HERTWIG does not warrant the use of the term, as in no case was there to be seen the normal gonal tissues of both sexes existing side by side in one and the same individual.

He suggests that the sex-glands in these cases should be designated 'intermediate gonads', in as much as they possess a structure and an external appearance which are intermediate between those of a testis and those of an ovary.

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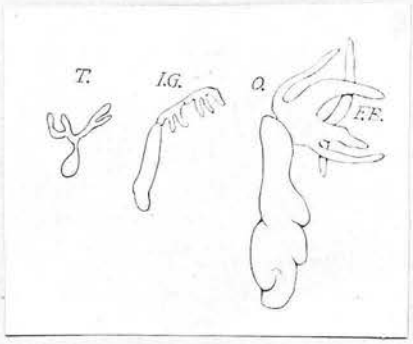

·Rana·

The condition itself he describes as Pseudo-hermaphroditism. His results can be summarised as follows:-

Until two months after the metamorphosis, only two forms of gonad are encountered in young frogs, - immature testes and immature ovaries, but after this time a third form appears. A testis, the smallest of the three, averages 1-1½mm. in length, is about as long as it is broad, and has a perfectly unbroken outline. An ovary, the largest of the three, averages 4-5 mm. in length, is about twice as long as it is broad, and has a notched outline. The third form of gonad is about twice as long as the testis, but only of the same width, while its outline is undulating, though never notched.

On section, the testis shows the structure of normal immature spermatic tissue throughout; the ovary presents an overwhelming preponderance of immature ova; while the third form, more ovarian than testicular, contains fewer ova, more undifferentiated germinal tissue, and a relative preponderance of interstitial tissue, than the ovary.

He was convinced that the intermediate gonads become testes, and all the intermediate forms ultimately became males, and that this transformation was achieved by one of two processes.

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In the first of these, the change is heralded by the separation of the ova by masses of interstitial tissue, and by the disintegration of the ova, the cytoplasm of which becomes like 'glass' and the nucleus broken down. This degeneration of the ova makes room for an increase of the undifferentiated germinal tissue, which gradually assumes the characters of normal spermatic tissue, so that, at length, the gonad presents all the features of a typical testis. The second method of transformation consists of a preliminary disintegration of the ova, and an increase in the amount of undifferentiated germinal tissue, but in addition, there is a process of cavity formation. These cavities are lined with epithelium and their origin remains a matter of speculation. They may arise by an extension of the coelomic cavity into the developing gonad, or it may be, that they are the result of extensive disintegration of the ova, but whatever may be their exact origin, they ultimately serve as efferent ducts. The process is completed by an ever-increasing undifferentiated germinal tissue, gradually assuming the characters of normal spermatic tissue.

SCHMITT-MARCEL argues that before the second month after the metamorphosis, intermediate gonad cannot be distinguished from ovary, and that this/

this is the reason why, if the relative numbers of males and females are estimated by an examination of the gonads during this period, there is such an overwhelming preponderance of females. He finds that up to the tenth month after the metamorphosis, there is a progressive increase in the percentage of intermediate forms associated with a corresponding decrease in the percentage of the females, while the number of the males remains stationary. After the tenth month, there is a progressive decrease in the percentage of the intermediate forms associated with a corresponding increase in the percentage of the males, while the percentage of females remains more or less what it was at the tenth month. He submits that -

> "even the comparison of these statistical numbers leads to the supposition that these intermediate forms, by their change into typical males, are intended to balance in the later stages the great disparity in the numbers of the two sexes in the young stages".

This indirect development of a testis has been even more fully described, more recently, by WITSCHI, another disciple of R. HERTWIG. The process is undoubtedly one by which a gonad, to all appearances an ovary, becomes a testis. The essential feature is, that the sex-cells of the peripheral parts of/

of the ovary tissue become detached & migrate into the sex-cords. Then from these sex-cords, normal testis tissue develops in the central parts of the gonad. At this stage, the gonad is centrally a testis, peripherally an ovary. Gradually the female parts of the gland are destroyed and only in rare cases do eggs, which may reach maturity, remain in the completed gonad.

GOLDSCHMIDT regards these PFLÜGER'S hermaphrodites as temporary intersexuals, the condition of the gonads varying according to time at which the transformation into males begins. In the sexually normal races, the sex differentiation takes place relatively early in the life history of the individual, whereas in these indirectly produced males, the decision as to sex is retarded. He submits that the experimental results in the case of the frog, can be brought into line readily with those in LYMANTRIA & BONELLIA.

SPENGEL had previously given as his opinion that in the case of a hermaphrodite which later on becomes a male, if the ovarian tissue was strongly developed, the gonad would assume the characters, - macro- and microscopically, of an ovary, but that nevertheless the animal, later on, would become a male.

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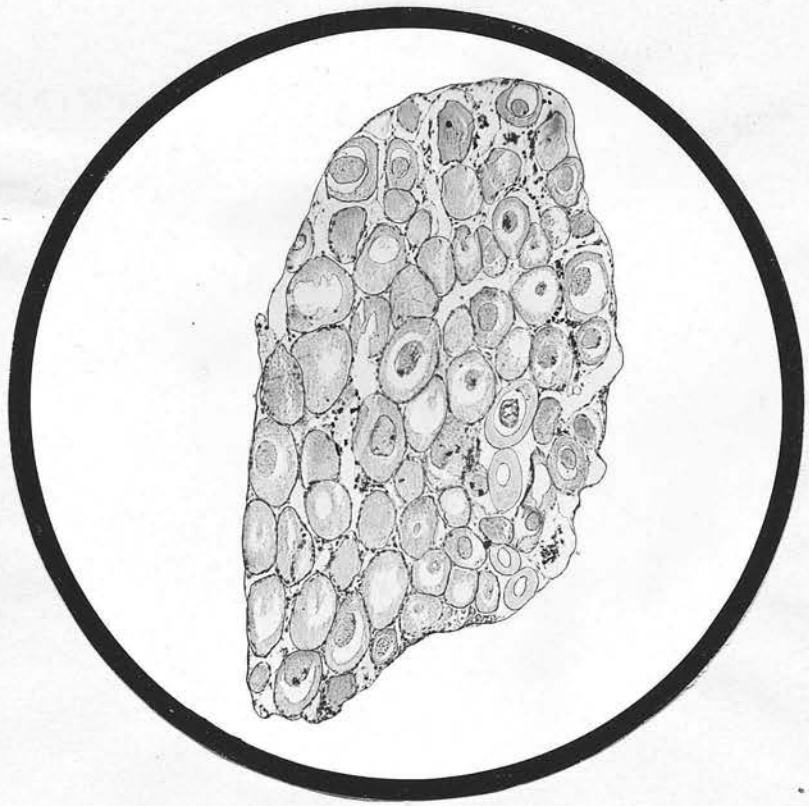


Bidder's organ

In Bufo there is no intermediate gonad and a testis can always be distinguished from an ovary, for the latter has a central cavity which is lacking in the former. Nevertheless, in the testis of the young toad, there are cells which possess a very similar appearance to the ova in the intermediate gonad of the frog. Such cells constitute the Bidder's organ, the significance of which is not yet clear. BIDDER himself regarded it as an accessory male gland which served to carry on the earlier stages of the development of the spermatozoa, but most authorities have decided that they are rudimentary ovaries.

JACOBSON came to this conclusion as a result of his work on Bufo cinereus, and von WITTICH, strongly upholding this view, argues that the condition affords trustworthy evidence of the primitively hermaphroditic nature of the generative glands of Amphibia.

MARSHALL subscribes also to this opinion, but SPENGEL submits that as Bidder's organ is found in males and females both, it is extremely unlikely that it is a rudimentary ovary.

HOFFMAN, who considered Bidder's organ as a hermaphrodite gland, refers to the normal presence in the interior of this organ in Bufo cinereus (vulgaris) of/

of rudimentary testis tubes in which, however, there have not developed spermatozoa.

CERRUTI, comparing HOFFMAN'S figures with his own preparations, is convinced that these rudimentary seminal tubules are really ovules in degeneration and invaded by immigrant cellular elements (perhaps leucocytes).

KUSCHAKEWITSCH inclines to the opinion, that this organ of the toad has the same morphological significance as the intermediate gonad of the frog, and that it represents an archaic gonad, in the first or female phase of its development.

KING is satisfied, that it is undoubtedly a rudimentary ovary, the component cells of which have marked characteristics which sharply distinguish them from ovarian ova. She finds that the cells which are destined to form Bidder's organ, are directly continuous with the primordial germ-cells which later, form the true gonad, and that they are exactly similar to these in their structure and development up to the synezesis stage, and concludes from these facts, that the cells which constitute Bidder's organ are degenerate germ-cells.

GOLDSCHMIDT, entirely accepting Bidder's organ as a rudimentary ovary, cites its occurrence as the classical example of the condition

which/

which he designates "Accessory Hermaphroditism" a variety of "Non-functional Hermaphroditism". He points out that it undergoes changes which are parallel to the sexual cycle and that in the case of the male, there is increased activity and regeneration of its constituent cells, co-incident with the formation of spermatozoa.

HARMS maintains that Bidder's organ is an organ of internal secretion and has conducted experiments which show that the development of the typically male finger-pads, is controlled in some part by this organ, for, while extirpation of the testis or of Bidder's organ has no influence upon the development of the pads, yet if, after extirpation of both testes and Bidder's organs, a Bidder's organ is grafted in the dorsal lymphsaes, the typical seasonal development of the pigmented pads follows in due course.

GOLDSCHMIDT submits, therefore, that Bidder's organ is a rudimentary ovary which has assumed a particular function in Amphibia.

CERRUTI, who has recorded the presence of sperms in Bidder's organ, from his extensive investigations, also is satisfied that Bidder's organ undoubtedly is a rudimentary ovary.

KING is of the opinion that primordial

germ/

germ cells which fail to undergo the normal processes of development assume the characteristics of rudimentary ova, regardless of the sex of the individual in which they occur.

In addition to the cells of Bidder's organ, others closely resembling rudimentary ova are frequently found, singly or in groups, throughout the testes of young toads which have but recently completed their metamorphosis. Such have been described in Bufo vulgaris by - SPENGEL, HOFFMANN, KNAPPE, and FRIEDMANN, in Bufo lentiginosus by KING, and in both Bufo and Rana by BALBIANI.

Similar cells are also found in the ovary of the female as described by KING. Since there is no communication between testis and Bidder's organ, it is very improbable that the cells develop in the latter and later migrate into the spermatic tissues. KING is of the decided opinion that these cells are really primordial germ-cells in which for some unknown reason the course of development has become changed, so that they increase in size rapidly, and assume the characters of rudimentary ova, and that they must, therefore, be regarded as degenerating cells. With very few exceptions, all cells of this sort become absorbed early in the life-history of the individual.

Histologically/

Histologically the structure of such a testis of a young toad is very similar to that of the intermediate gonad of the young frog. The two are compared by KING who states that in both cases, large cells having the general appearance of rudimentary ova are found among primordial germ-cells which are in an apparently indifferent state. These cells develop to a certain size and then undergo processes of degeneration and absorption, leaving the gland wholly male or female as the case may be. The chief differences between such anomalies in Rana and those in Bufo consist in the fact that the large cells which appear in the sex-glands of young toads are, as a rule, segregated into one or several masses which can be seen under a low magnification before the gland is sectioned, and have very distinct features that distinguish them from normal ova, while in Rana according to SCHMITT-MARCEL, these cells are scattered throughout the sex-gland and appear in every respect like normal ova.

"Rudimentary ova" have been described as occurring in the testes of adult frogs in the following cases. PFLUGER, R. fusca (3 cases). KING, Rana pipiens; HOFFMANN, Rana fusca, in its first/

first year, rudimentary ova in both testes between the seminal tubules) EISMOND, Rana fusca: and FRIEDMANN, Rana viridis, (killed August; ten normal and three degenerate ova within the seminal tubules of one testis and five normal in the other.)

CERRUTI points out that in the figures of BALBIANI, LATTER & FRIEDMANN, which show well-developed ova in the interior of seminal tubules, yolk-plates are not drawn.

Such a cell closely resembling rudimentary ovum may be one of three things, an ovum; a second form of spermatozoon; or the result of aberrant cell-division of a normal spermatogonium or spermatocyte. The gonad, therefore, may be an intermediate gland: a testis of normal but unusual structure: or a testis in which the processes of spermatogenesis are abnormal.

Some authorities have not hesitated to describe these cells as ova, while others have referred to them as large cells resembling ova. Some make no attempt at compromise. BEARD, for example, confidently maintains that they are spermatogonia which, did they attain their full development, would furnish a second form of spermatozoon, & regards these cells in the testis as abortive gametes in as much as in the Anura they seldom develop further than the spermatocyte stage./

stage, and thus bear a superficial resemblance to ova. He points out that ripe ova or ova anywhere near ripening have never been seen in the testes of the frog or of the toad, whereas two forms of spermatozoa have been observed.

Von la VALETTE ST. GEORGE found large spermatozoa of double the usual size occasionally in Bufo calamita and states that they are abundant in Rana esculenta but rare in Hyla. BROMAN has described a second form of spermatozoon in Bombinator igneus. This is the evidence which supports the conception that these cells are the doomed forerunners of a second form of spermatozoon and it is clear that the appearance of these cells can be interpretated equally well by the assumption that they are the results of abnormal cell-division on the part of the ordinary constituent spermatogonia of a testis. And abnormal cell-division has been observed and described frequently.

Vom la VALETTE ST. GEORGE remarked upon the frequency of amitotic divisions among the archispermatocytes, and KING, though stating that it does not occur in the spermatogenesis of Bufo lentiginosus describes it in the development of the cells of Bidder's/

Bidder's organ. CHAMPY maintains that polymorph nuclei are found only in the archispermatocytes and distinguishes two kinds, one, an easily stainable and but slightly crenated, and the other showing a greater degree of polymorphism and not readily stainable. FLEMING observed these giant-cells in Salamandra and BROMAN in Bombinator and describe pluripolar mitosis as occurring in the testes of these associated with a multiplication of the centrosome apparatus.

LEVY finds that all mitosis in spermatogenesis may be pluripolar and describes archispermatogonia some with one round nucleus, some with two, some with kidney-shaped nuclei, some with several, and some with many nuclei, and finds suggestions of correlation between the size of the mass of nuclear material and the size of the cell. He points out that one form of these is seductively like the cells described by FRIEDMANN as rudimentary ova and has exactly the appearance described by CHAMPY as "degenerescence oviforme". The outstanding feature of all these cells was that in no case was there any sign of even commencing division of the cytoplasm. Similar conditions were found by LEVY in all the phases/

phases of spermatogenesis, - cells with one round nucleus formed the majority but in a few cases, especially those examined between August and October, cells were seen with two or more free or coalescing muclei and extra centrosomes.

While disagreeing with many of BROMAN'S conclusions, he confirms the latter's statment that abnormal mitosis is responsible for the developement of these atypical sperms.

MAXIMOW explains the appearance of these giant spermatids by assuming either an amalgamation of normal gametes has occurred or that the nucleus of a normal spermatid, becoming hypertrophied and hyperchromatic, divides amitotically.

There is strong evidence, therefore, that the cells which have been accepted by certain authorities as a second and distinct form of spermatozoon are nothing more than the result of abnormal cell-division of the ordinary spermatogonia and spermatocytes, by which the nuclear material increases in bulk, whilst the cytoplasm remains undivided. The graduated series of conditions found by LEVY can explain the origin of the two-headed giant spermatozoa described by von la VALETTE ST. GEORGE and BROMAN.

Similar/

Similar cells have been described as occurring in other tissues by several writers. For example SCHMIDT, quoted by LEVY, describes giant epithelial cells, either with a single large nucleus or multinuclear, in the skin of the Green-frog, which bears a striking resemblance to ova and which in their origin, LEVY believes, are exactly similar to the giant cells of the testis. Moreover, similar cells were found by KING in the ovary of the female toad.

MAXIMOW found that after injury regeneration produces giant-cells which he thought arose by fusion or overgrowth, while the results of the experiments of MEYNS indicate that the intermediate gonad is a developmental stage rather than a reversionary rudiment. He found that a transplanted testis of the frog is brought back to a very primitive type of structure before regeneration occurs in it, and that both spermatozoa and ova are formed before the latter degenerate and the spermatozoa progressively develop. Moreover in the earlier experiments of LOEB and BANCROFT in which frogs' eggs were made to develop without fertilisation, the only larvae which grew sufficiently to permit an examination of the sexual organs being made had gonads which possessed the characteristics/

characteristics of the intermediate gonad as described by SCHMITT-MARCEL. In the experiments of 1918 LOEB however raised such larvae through the metamorphosis to almost or quite adult frogs and out of nine reared to an age at which the sex could be distinguished with certainty, seven were male and two were females.

KUSCHAKEWITSCH accepted these cells as ova and sought to explain their presence by suggesting that it is possible that there are specific male and female areas in the germinal epithelium, but FRIEDMANN contests this view maintaining that it is unlikely since the cells are found scattered throughout the gonad quite irregularly.

It is to be noted that KNAPPE and HOFFMANN describe them as being scattered here and there between the seminal tubules while in FRIEDMANN'S case these degenerating ova were within the tubules.

So far then, the facts marshalled are as follows. In the early stages of the development of a testis, or at any rate of a gonad which ultimately becomes a testis, and also occasionally in the functioning testis of an adult, large cells with ovarian characters are sometimes found. These cells are somewhat/

somewhat similar to those which constitute Bidder's organ of the toad, and are also occasionally found in the ovary. They undergo processes of degeneration and are ultimately removed, being not uncommon in young gonads, but extremely rare in those of an adult individual. As to their exact significance, there has been much controversy and three interpretations have been suggested. One is that such a cell is the forerunner of a second form of spermatozoon which in the Anura never attains functional and complete development, but which degenerates in the spermatocyte stage and is removed. If this is so, then according to the observations of SCHMITT-MARCEL this form appears in considerable numbers, before the form which does attain maturity makes its appearance. Another view is, that such a cell is the result of aberrant cell-division on the part of a normal constituent cell of the gonad. This view is supported by the evidence of BRÖMAN, who observed abnormal mitosis of such, and by that of LEVY, who has described the process. Further, SCHMIDT has observed the presence of cells exactly resembling those in the testis in the skin of the frog, while KING has found them in the ovary of the toad. These cells appear during the regeneration of an injured testis/

testis, and are also found in a transplanted testis, which is brought back to a primitive type of structure before regeneration occurs. LOEB found that the gonads of his parthenogenetic frogs in their early stages contained these cells, whereas those of such frogs which lived to attain maturity did not.

Others have not hesitated to describe these cells as ova and in consequence have held that the primordial germ cells are sexually indifferent. The fact that "in young toads, and possibly also in young frogs, primordial germ-cells that fail to undergo normal processes of development assume the characteristics of a rudimentary ova, regardless of the sex of the individual in which they occur", can be explained, as pointed out by KING, on the assumption that HAECKEL'S view that the hermaphroditic condition is the primitive one, for then it is not very remarkable that -

"in the male Amphibian at the present time many of the the primordial germ-cells still have the power, under certain conditions, of developing into ova, which, since they cannot leave the sex-gland or come to maturity, are destined to degeneration and absorption. Except in very rare cases the primordial germ-cells in the female are no longer able to develop into spermatogonia".

It/

It is sufficient to state that the above doctrine is not generally accepted, and that GEGENBAUR'S dictum that 'the hermaphrodite stage is the lower and the condition of distinct sexes has been derived from it', is opposed by that of PILSENEER, who maintains that 'Unisexuality is primitive, the hermaphrodite state is secondary'. According to the latter "the study of Molluscs, Myzostomidae, Crustacea, and Pisces, shows that in these groups the separation of the sexes preceded hermaphroditism; various cases in other groups tend to show that this is true universally; and the same conclusion applies to plants. In Mollusca, Crustacea, and Pisces at least, hermaphroditism is grafted upon the female sex".

BEARD maintains that - "the idea of the original or primary nature of hermaphroditism was never founded at all in facts, and with the lapse of time and the accumulation of the results of research, it has not emerged unscathed. In those instances, where the relations of the hermaphrodite and dioecious states to each other have been looked into, it has, as a rule, turned out, that the former had been derived from the latter, and that in all cases it was upon the females, that the hermaphroditism had been superimposed./

imposed. This has now with greater or less certainty been established in Cirrepedia, certain Teleosti (BROCK), Sacculina (YVES DELAGE) Molluscs (PAUL PILSENEER), Myzostoma (BEARD), Myxine, (J.T.CUNNINGHAM) and, lastly, various Nematoda (E. MAUPAS)".

It is of interest to note that BEARD and DELAGE quite independently in 1884, first took up the stand that 'Hermaphroditism, probably all hermaphroditism, had its origin in a unisexual condition' and this doctrine was substantiated by the investigations of Pilseneer in 1895.

Unless this conception that there is a sexually-indifferent phase in the life-history of certain individuals is true, then much of the experimental work of YUNG, BORN, MAUPAS, and others, based as it was, upon the conception that the animals used were either of no predetermined sex, or else were hermaphrodite, proves nothing more, as BEARD maintains, than what percentage of either sex will survive under given, usually utterly abnormal conditions. On the other hand, if it can be shown that sex-differentiation is controllable within limits, then it is clear that the determination of sex is not irreversible predestination. And if a gonad can produce ova/

ova or sperms or both synchronously then it is probable that a variety of circumstances may incline the individual to the male or the female side. In examining SCHMITT-MARCEL'S results it is almost impossible to avoid the impression that there is a type of young frog which starting life with a gonad exactly similar to the ovary of the typical female, that is to all appearances actually a female, and which becomes an intermediate form and ultimately a male possessed of typical testes. The following table shows the number of frogs examined mostly at intervals of a month, together with the percentages of females, males and intermediate forms.

FROGS	FEMALES	INTERMEDIATE FORMS	MALES
225	85%	0	15%
310	85	0	15
390	75	8	17
332	70	12	18
215	70	12	18
200	64	18	18
220	55	24	21
210	54	24	22
200	54	20	26
200	54	15	31
180	53	12	35
200	53	7	40
180	52	4	44
200	52	0	48

These/

These figures seem to prove with certainty that the percentage of the females among young frogs is reduced to about 50 by a corresponding increase in the percentage of the intermediate forms. The intermediate forms arise from the females or from individuals indistinguishable from such and are therefore individuals whose gonads present a structure which is intermediate between an ovary and a testis. As the frogs approach maturity the percentage of the males rises to about 50 and this is associated with a progressive diminution in the percentage of the intermediate forms until none of these remain. The males then are produced from the intermediate forms by a process which transforms the gonads of these last into typical testes, and therefore the males have arisen from individuals which had been regarded previously as females.

The standard by which the sex of the individual was judged in this experiment was one in which the female had gonads which included a preponderance of ova, the male had gonads with the structure of typical testis, and the intermediate, gonads more ovarian than testicular in structure but suggestive of both. Now if the constituent cells of the intermediate/

intermediate gonad which were ovarian in character are regarded as the forerunners of a second form of spermatozoon or as the results of abnormal cell-division of the ordinary spermatogonia, the clean dove-tailing of the percentages as shown in the table cannot be explained. "Even the comparison of these statistical numbers leads to the supposition that these intermediate forms, by their change into typical males, are intended to balance in the later stages the great disparity of the numbers of the two sexes in the young stages". On the evidence of SCHMITT-MARCEL'S work and the more recent work of WITSCHI there is an intersexual individual, and this temporary intersexual individual normally and with very rare exceptions becomes in every way a typical male.

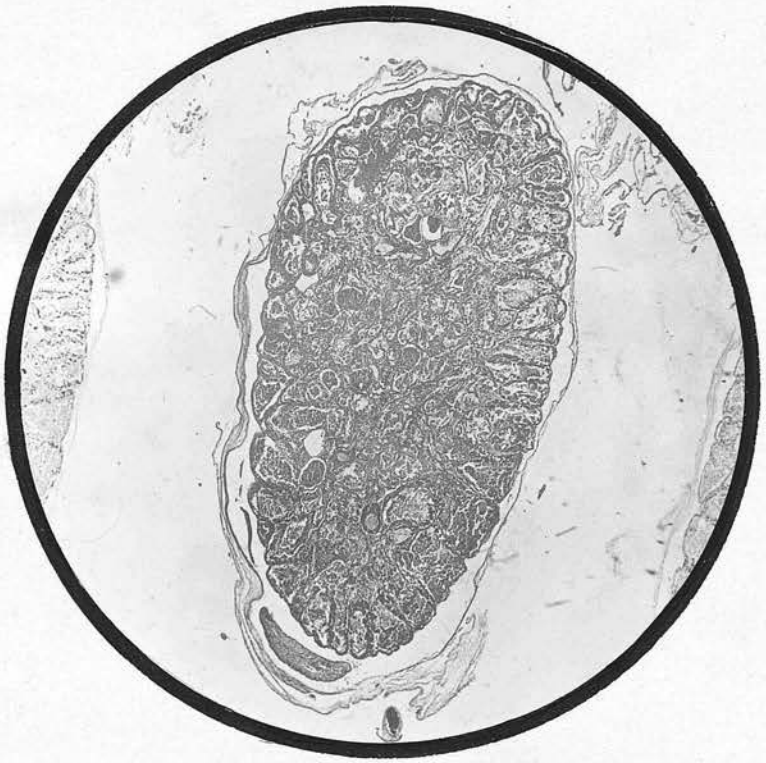
Cytology alone obviously cannot decide the question as to whether these cells are spermatic or ovarian in origin and nature, and the personal factor must enter into any interpretation of cytological appearance.

BRAUN, ROUGET, NUSSBAUM, HOFFMANN, EIGENMANN and ALLEN among others, have all emphasised the remarkably close resemblance which exists between ovum/

R. temporaria
(dislodged testis)

ovum and spermatozoon in their earlier stages of development. The evidence of other methods of investigation must be sought. The experimental work of MAXIMOW and of MEYNS has already been mentioned. In support of their conclusions that a testis which includes these rudimentary ova-like cells is one which presents the characteristics of a normal testis at a certain stage of its development, the following case may be cited.

A frog, Rana temporaria, was killed in April 1916. It was an adult, measuring 5.2 cm. from from the tip of its snout to the symphysis of the pubis, while its accessory sexual apparatus and its secondary sexual characters were entirely and typically male. During the dissection it was noticed that while the left gonad was a testis, normal in every respect, the right gonad was missing. But there was a tumour 10 mm. long and 6 mm. broad, encased in a fibrous tissue capsule and firmly attached to the muscle-sheaths of the m. rectus abdominis and m. triceps femoris and lying upon and distal to the right groin. Naturally it was assumed that this tumour was the missing testis and search was made for vasa efferentia but none could be found for the tumour/



Section of the dislodged Testis.

tumour was not connected with any part of the genital system. Further dissection showed that there was a hernia the sac of which was provided by the abdominal peritoneum and which was covered by the stretched fibres of the m. obliquus externus. The neck of the sac was almost completely obliterated by fibrous overgrowth and was adherent to the adjacent surfaces of the m. rectus abdominis and m. triceps femoris at the site of their origins, while the dorsal surface of the tumour was firmly adherent to the ventral surface of the latter muscle. It is of interest to note that the location of this tumour is very nearly that of a testis in the scrotum, it lay close to where the external ring of the inguinal canal would be.

On section, the left gonad had the structure of normal testis. The tumour proved to be the missing testis but its structure was peculiar in that while the tissues near the periphery had the normal structure of a testis, the central parts of the gonad consisted of a loose matrix of indefinite tissue amongst which many large cells closely resembling ova were found. The gonad had the structure of the intermediate gonad as defined by SCHMITT-MARCEL and of the testis of indirect development as described by WITSCHI/

WITSCHI. These cells which had the appearance of ova, were either ova or spermatogonia of an abnormal design. The opposite testis was perfectly normal and it is reasonable to assume that the conditions found in this dislodged testis, were the result of the dislocation.

The testis was encased in a thick capsule of fibrous tissue and muscle fibres and branches of the femoral artery provided a blood-supply by which the gonad was nourished.

No vasa efferentia were present and the spermatozoa produced by the functioning peripheral parts of the gland were doomed to resorption. The central parts of the testis - the parts furthest away from the blood-supply during its re-establishment - exhibited the signs of abnormal cell-division, or else the structure of a primitive gonad which characteristically, according to MEYNS, forms both ova and spermatozoa.

Of the two suggestions, the first is the more reasonable, for such abnormal cell-division would appear to be a natural consequence upon severance of a delicate organ from its trophic supply.

On the other hand, if these cells are ova then it would appear that a gonad which previously had been a normal testis can, on occasion, produce both/

both ova and spermatozoa.

Before coming to a final decision as to the exact nature of these cells, it was necessary to compare them with cells of similar appearance found amid the spermatic tissues of individuals in which undoubt- ovarian and spermatic tissues were both present. These cells are, certainly, very much like those found amid the spermatic tissues of such an individual; they have every appearance of rudimentary ova. But the structure of the whole gonad is very different in the two cases, so different that they have very little in common. Further in this case the accessory reproductive apparatus of the other gonad were typically male in structure and design, whereas these organs were also abnormal in the cases cited in the table.

In this case, therefore, it is reasonable to interpret these cells as the result of abnormal development on the part of the ordinary component cells of a previously normal testis, and not as the products of previously existing ovarian tissue or as ova produced by the spermatogonia.

The/

The bulk of the evidence goes to prove that in an otherwise normal male, cells closely resembling ova found among the spermatic tissues, result from abnormal division of the ordinary constituent cells of a testis. The only evidence actually supporting the contention that such cells are ova, is that of the percentages of SCHMITT-MARCEL.

It can be assumed that in the frog sex-determination is effected by means of a sex-chromosome mechanism, one sex, the homo = gametic, possessing two sex-chromosomes; the other, the heterogametic, one and a dummy. In certain groups, typified by Drosophila, the chromosome-constitution is symbolised as ♂xy, ♀xx, the male being heterogametic and producing two classes of spermatozoa, whilst the female being homogamatic produces but one sort of egg. In other groups, typified by Abraxas, the constitution is ♂zz, ♀zw, so that there are two classes of eggs but only one of sperm.

In the frog, the sex-chromosomes have not yet been demonstrated but since in Necturus it is of the ♂xy, ♀xx type, it is reasonable to assume that the frog will be found to possess a chromosome-constitution similar to this.

V./

V. THE SEX RATIO.

(1). In Nature the sex-ratio in frogs and toads, is taken to be ♂ 1. ♀ 1., but there is considerable evidence that the sex-ratio varies greatly among the different species.

CUÉNOT, who collected the statistics which had been published, found that in different cases the number of females ranged from 49 to 86·8% in the different species of Rana.

PFLÜGER and GRIESHEIM found 63·7% among Rana fusca;

YUNG 53 and 57% in R. esculenta.

FISCHER-STEWART found an excess of females in Hyla arborea during the breeding season. During the summer term of 1920, 428 adult Rana temporaria were used for dissection. They had been procured from several localities, and among them were 343 males, 80%: 84 females, 19·62%; and one, ·23% - an intersexual. It is of interest to note that whereas in April and May, the breeding months, there were many more males than females, in June and July, their numbers became more or less equalised.

(1)/

(1) APRIL.	MALES	FEMALES.		
	57	3		
	55	8		
	24	4	& 1 intersexual.	
	54	5		
	38	15		
	27	17		
	29	27		
	343	84	1	Total = 428.

During the winter term the numbers were as follows:-

(2) NOVEMBER	MALES	FEMALES	
	51	32	
	24	19	
	53	22	
	25	19	
	37	42	
	26	34	
	33	18	
	46	31	
	31	19	
	22	16	
	53	22	
	28	22	
FEBRUARY	23	17	
	448	313	Total = 765
	59%	40.9%	

Most/

Most observers, however, have recorded that ordinarily there is a greater number of females among both young and adult frogs, the excess of females ranging from 1.05 to 73%. In Bufo however, KING has recorded an excess of males among adult B. lentiginosus particularly during the breeding-season and BOULENGER found a similar excess in B. vulgaris and B. clamata. DUNCAN MATTHEWS states that of 276 toads used for dissection in the Natural History Department of the University of Edinburgh in the summer of 1884, 230 were males (83.33%). He continues their description by stating that at the anterior end of each testis a small ovary was attached and that the Müllerian ducts, though in the majority of cases nothing more than distinct straight lines, were still present. The ovaries were Bidder's organs, of course, and there was nothing remarkable in the condition of the Müllerian ducts.

(2) The sex ratio varies also among frogs from different localities as is clearly shown by the classical example of PFLÜGER'S specimens from Bonn, Utrecht and Königsberg. But for locality one may read lineage since physical isolation would produce distinct/

distinct inbred races.

Bonn	♂	35%	65	♀.%
Utrecht		13	87	
Königsberg		5	51.5.	

(3) The sex-ratio also varies among frogs or toads from the same locality in different years. This is clearly shown in KING'S records of the young toads used as controls in her experiments. The normal variation is seen to range from 32.21 to 56.83% in the case of the females and from 29.76 to 48.61% in the case of the males. These controls were the offspring of many and indiscriminate matings and were gathered without any attempt at selection as to ancestry. The table also shows that the rate of development also varies with the seasons as is shown by the fact that the percentage of the individuals whose sex could not be ascertained varied from 0 to 38.7%.

Table/

YEAR	TOTAL NO.	% of FEMALES	% of individuals of undetermined sex.	% of MALES
1904	500	51.80	0	48.20
1907	600	56.83	0	43.17
	651	55.14	0	43.86
1908	140	54.28	0	45.72
1909	323	51.39	0	48.61
1910	210	32.85	36.19	30.95
	1250	32.21	38.72	29.76
	201	37.61	30.38	32.33
	200	53.00	0	47.00
1911	350	43.71	8.00	48.28
	350	44.57	4.57	50.85

(4). The sex-ratio also varies very considerably among the offspring of different parents. HERTWIG found that different males gave conspicuously different sex-ratios among their offspring. For example, eggs of one female fertilised by the sperm of one male yielded 52 males and 50 females, while other eggs of the same female fertilised by another male produced 64 females and only three males. In another experiment the eggs of one female fertilised by one male yielded 140 males and 142 females, whereas other eggs of the same female fertilised by another male produced 109 indifferent offspring. In other cases various percentages of males, females and indifferent individuals/

individuals were produced, and HERTWIG showed that the eggs of females belonging to a strain in which the indifferent condition was usual, when fertilised by the sperm of a male of a race in which this condition was not usual, could yield indifferent offspring. In another experiment he found that eggs which when fertilised by a male of a race which produced only males and females, yielded these two forms only, when fertilised by a male of an indifferent-producing race, yielded indifferent offspring, while eggs of a female of an indifferent-producing race fertilised by this male produced females.

In one of KING'S experiments eggs were taken from the right uterine segment of a female toad, divided into four lots and fertilised with the sperm from three different males with the following results.

MALE	SPERMATOZOA	INDIVIDUALS	SEX ASCERTAINED IN	MALES	FEMALES
1st.	Lt. testis	255	126	52.39%	47.61%
	Rt. do.	403	224	48.22	51.78
2nd.	Lt. do.	380	301	47.51	52.64
3rd.	Rt. do.	221	124	43.36	55.64

HERTWIG/

HERTWIG describes a series of experiments in which the eggs of two females were divided into six lots and fertilised with spermatozoa from six different males. The mortality in several lots was very high and the results showed great variation in the sex-ratio, some lots yielding over 90% of females while others gave an excess of males. But in spite of this mortality and lack of uniformity HERTWIG considers that his results indicate that the male has some influence in determining sex in Rana.

MORGAN suggests that most of these cases can be explained on the assumption that there are two forms of spermatozoa and that more of one kind are injured or that internal processes may lead to the production of more functional spermatozoa of one kind than of the other. If then there is a male-producing sperm and a female-producing sperm and conditions altered the usual proportion of these two forms altered sex-ratios would result.

KING points out in connection with this suggestion that the numerous investigations that have been made of the spermatogenesis of the various species of Amphibians have, so far, failed to demonstrate the slightest evidence of the existence of two forms/

forms of functional spermatozoa. In any case the experiments of KING clearly demonstrate that even if there are two forms of spermatozoa, they are produced in approximately equal numbers in both testes so that if the male alone is responsible for sex-determination then practically equal proportions of the two sexes should be produced in any large lot of individuals whether the eggs are fertilised with sperms from one or several males.

KING has similarly shown that if there are male and female producing ova then the two kinds must be produced in approximately equal numbers in each ovary. She took the contents of the two uterine segments of a female toad and fertilised each lot separately with spermatozoa from one male. She points out that there is no guarantee that one uterine segment contains only the ova produced by the ovary of the same side but that it may be assumed that, for the most part, the ova from one ovary do enter the oviduct of the same side. Her results were as follows;-

OVA	SIDE	MALES	FEMALES	%
300	Right	131	169	56.33
300	Left	128	172	57.33

Further/

Further to test the theory put forward by RUMLEY DAWSON which suggests that the eggs producing one sex are from the ovary of one side and those producing the other sex are from the ovary of the other side, KING took the contents of the right uterus of a female toad. These ova, mostly at any rate from the right ovary, when fertilised with spermatozoa from a single male yielded 45.8% males and 54.2% females, a not unusual ratio.

It is seen then that the sex-ratio among the Anura varies considerably under natural conditions as well as under those of the laboratory. It is also seen that the individual exerts a considerable influence in the production of a sex-ratio in its offspring, and that the male and the female parent both play their part in the establishment of the ratio.

HERTWIG in his earlier papers suggested that the sexual character of an egg changes during the course of its development in the ovary, tending to produce a male in the earlier stages of its ripening, a female in the middle stages and a male when over-ripe, these changes being related to the ratio of the nucleus to the cytoplasm at different periods of/

of the maturation of the ovum. Later however, this conception was modified by the suggestion that the spermatozoon exerts a definite influence in sex-determination.

(5) There is considerable evidence that the relative condition of the two gametes at the time of fertilisation exerts an influence upon the sex-ratio and that this can be disturbed experimentally. The following table gives the results of certain experments of HERTWIG and of KUSCHAKEWITSCH which cannot be explained by a selective mortality. In many of these experiments more than one male was employed and this introduces a complication. The percentages are those of male off-spring.

HOURS	0	6	18	24	36	42	54	64	89
R. Hertwig	58%	54%		55%			87%		
	49%				58%		59%		
	48.5%		37%			50%		88%	
Kuschakewitsch	53%								100%

In this table it is shown that if a portion of the ova were fertilised naturally the percentage of males in the individuals arising from them ranges from 48.5 to 58%. If the remaining ova are kept within the female/

female, and this is accomplished by removing the male before all the ova has been extruded by the female, for a period which varied from 8 to 89 hours when the male is placed once more with the female, the percentage of males rises with the length of time between the fertilisation of the two portions of the ova. After an interval of 89 hours the second batch of ova yielded all males.

MORGAN endeavours to explain these results by suggesting that they may be due to "late fertilised"ova, being in reality not fertilised at all but partheno- or arrheno-genetic. But, as is emphasised by HUXLEY, there is as yet no direct evidence in favour of this view.

(6) KING finds that by treating the eggs of Bufo in various ways which reduce their water-content the proportion of females is raised very considerably, and that on the other hand by treating them with very dilute acid, and thereby causing them to absorb water, the proportion of males is correspondingly increased. She is of the opinion that sex in Bufo is determined at or near the time of fertilisation, and that external factors, acting during this time, may influence the sex-determining mechanism in such a way as to cause/

cause it to produce one sex or the other. In discussing her results she submits that "unless by chance, therefore in picking out the individuals to be reared, I selected in each case tadpoles that would give a great majority of females when developed, I can see no alternative but to assume that sex in Bufo can be altered by changing the water content of the eggs at the time of fertilisation".

MORGAN submits that such results as these can be readily explained on the assumption that there are male- and female-producing ova and that one kind is more deleteriously affected by experimental processes and urges that it is essential in such experiments to obtain accurate data as to the proportion of the eggs which are fertilised.

DONCASTER suggests that KING'S results can be brought into line with those of HERTWIG, who explains his by the assumption that over-ripe ova tend more or less constantly to extrude the female-determining factor whereas, in the ordinary course of events, it is a matter of chance whether it remains within the nucleus of the egg or passes into that of the polar body, by assuming that an increase in water-content tends to cause the female-determining factor, an/

factor-an X-chromosome or its equivalent - to be extruded with the polar nucleus, while decrease of water-content causes it to be retained in the nucleus of the ovum.

RIDDLE has shown that in pigeons a large excess of females can be produced by various depressing agents, and that this disturbance of the sex-ratio is not due to any differential mortality.

STRONG has proved, by breeding experiments involving a sex-limited character, that these individuals have a chromosome constitution normal to their sex. The true explanation would seem to be that depressing agents cause the W-chromosome to remain in the egg.

HERTWIG further found that the age of the spermatozoa had no effect upon the sex-ratio. He fertilized ova with spermatozoa from males at very different stages of the breeding-season, but in no case could any effect be noted.

(7) YUNG, BORN, MAUPAS, BALBIANI, HENNEGUY, all conducted experiments based upon the assumption that all tadpoles pass through a period of transitional hermaphroditism, or sex-neutrality, and were convinced that they had disturbed the sex-ratio by manipulation of the diet.

BORN noted that out of 1272 young Rana fusca which/

which had been well nourished during the larval period, 1209 were females, (95%), while in 160 young frogs taken from their natural environment, only 52% were females. But no account was taken of the many hundreds of tadpoles which had died during the course of the experiment, and the gonads were not sectioned, the larger ones being regarded as ovaries, and the smaller as testes.

In one set of experiments YUNG fed tadpoles of Rana esculenta upon different kinds of food, and found that the sex-ratio of the different lots varied considerably.

LOT. FED ON	NO. of YOUNG FROGS	MALES.		DOUBTFUL		FEMALES		LOST.
A. on Fish	24	4	17·39%	2	8·69%	17	73·91%	1
B.Fish & Beef	33	6	18·18	2	6·06	22	75·75	-
C.White of egg	10	3	30·00	0	-	7	70·00	-
D.Yolk of egg	7	0	00·00	2	28·57	5	71·42	-

In another set of experiments, in three sets of controls, the proportion of females to males was as follows:-

54 : 46,
61 : 39
56 : 44. Average 57% females.

Feeding/

Feeding one set of the first lot upon beef, was associated with a rise in the percentage of females from 54 to 78; a set of second lot when fed upon fish shewed an increase in the percentage of females from 61 to 81; and some of the third lot fed upon frog-flesh yielded 92% females.

CUÉNOT fed tadpoles of Rana temporaria upon a vegetable diet and kept them in a confined space. He found that their development was retarded and that their gonads were small, and that of the 26 which survived the metamorphosis, every one was a female. A second lot kept under similar conditions, produced 3 females and 4 males. A third lot kept in an aquarium with cool running water, underwent metamorphosis two and a half months later, and yielded 3 females, 28 males, and one hermaphrodite. A fourth lot having fed upon the albuminous envelopes of the eggs for some days were then kept on the diet as shown below, [illegible] with the following results:-

GROUP FED ON	MALES		DOUBTFUL		FEMALES.	
1. Vegetable food	57	49·13%	8	6·89%	51	43·96%
2. Animal "	22	61·11	0	-	14	38·88
3. Mud & their own dead.	12	34·28	0	-	23	65·71.

CUÉNOT/

CUÉNOT concludes that the results of the feeding experiments on Rana are contradictory and that therefore it is evident that nutrition is not an absolutely dominating factor in sex-determination. Having repeated the experiments he was able to come to no more definite conclusion than that there was a strong possibility that sex is determined already in the egg at the time of fertilisation. BEARD quotes CUÉNOT'S experiments as demonstrating that no external conditions such as hunger, rich food or poor, age, and other factors can influence the determination of sex. But the experiments of CUÉNOT were uncritical and the numbers used too limited to warrant any such generalisation.

The experiments, however, have been repeated by KING. In one set of experiments she examined the following contentions.

1. That an abundant nutrition leads to an excess of females;
2. That a scarcity results in an excess of males. (SCHENK'S theory);
3. That a diet rich in protein yields an excess of females, (YUNG);
4. That, on the contrary, a nitrogenous diet plays no part in sex-determination,(SCHULTZE)

Her/

Her results are summarised in the following table;-

1. From the fertilised eggs of one female.

LOT	DIET	TOTAL NO. of INDIVI-DUALS	MALES %	DOUBTFUL %	FEMALES %
A.	Meat	300	48.66	5.66	45.66
B.	Wheat	300	39.66	12.66	47.66
C.	Natural	300	34.33	36.00	29.66
D.	Lecithin	200	27.50	24.50	48.00
		1100	38.45	19.27	42.27

2. From the fertilised eggs of another female.

A.	Meat	200	36.00	9.50	54.50
B.	Wheat	200	38.00	8.00	54.00
C.	Natural	200	43.00	21.50	35.50
D.	Lecithin	200	28.00	37.00	35.00
		800	36.25	19.00	44.75

3. The two series combined.

A.	Meat	500	43.60	7.20	49.20
B.	Wheat	500	39.00	10.80	50.20
C.	Natural	500	37.80	30.20	32.00
D.	Lecithin	400	27.75	30.75	41.50
		1900	37.52	19.15	43.31
			56.68%		62.47%

Control/

Control. In a lot of 500 young toads collected from their natural habitat there were 51.8% females and 48.2% males.

During the course of this experiment every tadpole which died was examined, and the results of this examination incorporated in the above tables. KING found that tadpoles of one sex did not die in greater numbers than those of the other.

It is seen that in every case the figures are well within the limits of possible normal variation and that therefore it is not established that sex-determination is influenced by the quality or by the quantity of the food. This is in agreement with the conclusions of CUÉNOT and SCHULTZE.

The tadpoles fed on meat (Lot A.) were noticeably larger than the others; they developed hind-limbs very quickly; and they were much more heavily pigmented. "This result does not support YUNG'S contention that an excess of nitrogenous food leads to the development of a greater proportion of females, and it seems to indicate that food of this character has no influence in determining sex in Bufo. The tadpoles fed on cereal (Lot B.) were smaller but more uniform in size".

According/

"According to YUNG, a purely vegetable diet is insufficient to transform a frog-tadpole into a frog. Such a diet does not seem to be equally injurious to toad-tadpoles."

The tadpoles fed on a mixed diet such as they would obtain under natural conditions were the smallest of all; showed most variation in size, developed most tardily and had the highest death-rate. In both lots (C) there was an excess of males.

In lots (D), yolk of egg which contains 8.43 - 10.72% of Lecithin, according to GAUTIER, was given to test DANILEWSKY'S statement that this has a marked influence upon development. The tadpoles showed the most rapid development but there was nothing remarkable in the sex-ratio.

It is not proven then that food, either by reason of its quantity or quality, can disturb the sex-ratio. The results of these investigators which would seem to show that the sex-ratio can be influenced by dieting are mostly uncritical since no account was taken of the great numbers of tadpoles which died, and, further, the decision as to the sex of an individual was made on a most superficial and certainly untrustworthy examination of the gonads.

HERTWIG/

(8) HERTWIG and KING have both examined the effects of temperature upon the sex-ratio, HERTWIG divided the fertilised eggs of one female into two lots. The first lot was kept at a temperature, first of 15°C. and then of 16°-18° C.: while the second lot was kept at 30°C. The second lot metamorphosed in about a month and yielded 344 males and 319 females, whereas the first lot metamorphosed between six and ten months later and gave 260 males and only 85 females. The mortality in the first lot was very heavy, but HERTWIG was satisfied that this did not account for the difference.

In another experiment from one lot of fertilised eggs, kept at 25°C., 200 tadpoles were produced of which but 67 passed the metamorphosis. Four were males and 63 females. A second lot kept at 13°C developed so tardily, that their sex could not be ascertained.

In a further experiment one lot of tadpoles were reared in a temperature of 22°-30°C. and a second lot at 13°-21°C. The first yielded 245 males and 127 females (34·13%), while the second lot gave 282 males and 54 females. (16·07%).

In another experiment of HERTWIG & WITSCHI, when the tadpoles of a certain strain of frogs which normally/

normally yielded the usual sex-ratio, were reared in a temperature of 20°C., only females were produced, whereas other tadpoles of the same parentage reared in a temperature of 27°C., produced males only and all these were indirectly produced by the transformation of females.

From these and other similar experiments, HERTWIG was convinced that cold favours the development of males.

KING repeated these experiments and came to the conclusion that:- "it is conceivable that a low temperature might act more injuriously on the female-producing spermatozoa than on those that are male-producing, if it be that there is a dimorphism in the spermatozoa of Bufo and that the male determines sex. This would, of course, greatly increase the chances that an egg laid in cold water would be fertilised by a male-producing spermatozoon. If, on the other hand, sex is determined in the egg, it is possible that the sex-determining mechanism is so very evenly adjusted that the temperature under certain conditions may turn the scale in one direction or the other".

Her results with Bufo lentiginosus were as follows:-

TEMPERATURE.	FERTILISED EGGS of	OUT of 250 SEX ascertained in	FEMALES	MALES.
22°-30°C.	Frog A.	63	44 (69·84%)	19
	Frog B.	39	9 (23·07%)	30
14°-18°C.	A.	169	101 (61·58%)	68
	B.	117	44 (37·60%)	73

It is seen that the eggs of Frog A. in both warmth and cold produced an unusually large proportion of females, while those of Frog B. yielded an unusually small proportion of females. This points to individual variation rather than to any suggestion that temperature plays a part in sex-determination.

(9) The sex-ratio is markedly disturbed as a result of inter-generic, - specific, and - varietal crosses. GOLDSCHMIDT and HARRISON with moths, and RIDDLE with pigeons have shown that such crosses give a large excess of males (in these groups the homogamatic sex). Data concerning the results of such matings in other groups are not available in any quantity, but there is strong reason to assume that the same rule holds good. It would seem that strange sperm has the effect of causing the W-chromosomes - in the case of the/

the pigeon, for example - to be thrown out in the polar body.

It is seen then, that the sex-ratio is subject to considerable variation in Nature and that it can be profoundly disturbed experimentally by careful selection of the individuals and by inter-generic, -specific, and -varietal matings, and by altering the relative physiological condition of the gametes at the time of amphionixis. An altered ratio can be explained by the following hypotheses.

(1). That there has been differential maturation. In the frog, granting that the chromosome-constitution is of the ♀xx type, both egg and polar body would contain an X-chromosome so that differential maturation cannot explain an unusual ratio.

(2). That there has been differential fertilisation. It has already been stated that in the case of the frog, there is no direct evidence in support of this suggestion.

(3). That there has been a differential mortality of gametes or of zygotes in the earlier stages of their development. Many experiments resulting in an unusual ratio have been carried out, in which the possibility of/

of this factor has been most critically considered and found to be negligible. There is no direct evidence to support the view that a modified sex-ratio results from a differential mortality of gametes.

(4). That an X-chromosome becomes converted into a Y-, (or Z into W-) or vice versa. There is no evidence of any sort to support the contention that this could happen. This hypothesis is highly improbable and can be dismissed.

(5). That a female assumes the organisation of the male or vice versa. This postulates that an individual possesses the rudiments of the equipment of both sexes, but the chromosome constitution of but one, and that the chromosome-constitution may become over-ridden as a result of the action of external agencies, so that an individual possessing the chromosome-constitution of one sex may come to possess the organisation of the other.

This hypothesis was greatly elaborated by GOLDSCHMIDT, as a result of his experiences with experimental breeding work on Lymantria.

BRAKE, GOLDSCHMIDT, and others have produced large numbers of intersexual individuals by crossing Lymantria dispar Linn. with Lymantria dispar var. Japonica Motsch, and have found that these intersexual forms were absent in the offspring of some cross-matings and present in definite proportions in others, and that by making certain assumptions these numbers followed the scheme of Mendelian inheritance.

GOLDSCHMIDT submits that both sexes contain the anlagen for either sex: that in both sexes, irrespective of zygotic composition, both anlagen may become patent; and that which is to appear depends entirely upon the quantitative relation of both. He assumes that the male and the female factorial sets act independently, and with a definite quantitative strength and finds that in different races of the gipsy-moth the valencies of the female and of the male factorial sets are specifically different, one having, for example, a comparatively low potency of the female set, another a very high potency of the male set, and so on, so that by crossing appropriate/

appropriate races he is able to produce any degree of the intersexual condition and to show that the intersexual forms occur in definite proportions in these various crosses. The intersexual individuals thus produced furnish a complete series from an almost female as regards external characters and internal structure to a very nearly complete male beyond which "the next step would be the complete transformation of the would-be females into males. And this can be obtained too".

GOLDSCHMIDT concludes that the male-determining factor of varying potency is borne by a sex-chromosome and is opposed to a female-determining factor borne by the egg-cytoplasm. He finds that the male-determining factor behaves as a Mendelian character while the potency of the female factorial set is transmitted only by the female and remains constant in all the descendants in the female line. From this he concludes that the female factor is a cytoplasmic factor borne by the egg and not by the spermatozoon.

But though there are many similarities in the conditions found in the intervarietal hybrids of Lymantria and in the Anura yet the parallel is not very/

very close. In order to explain the progressive transformation of an individual with female characters into one with male, it can be assumed that the relative valency of the male factorial set became enhanced by some means with the result that the female factorial set becomes swamped, and if to this hypothesis is added that elaborated by LILLIE in his work on the "Free-martin", then the intersexual individual in the Anura may be explained.

LILLIE has shown that in the free-martin, the intersexual condition is due to an acceleration or intensification of the male factors of the female zygote, the female being heterozygous for the sex-factors, by the male hormones, and points out that the degree of the effect, which is quite variable, would be subject to all quantitative variation of the hormone, the quantitative differences between the male and female factors of the female zygote necessary for the differentiation of female characters being reduced in the free-martin by internal secretions instead of by simple variation in the potency of the male factors.

He found that twins of cattle are derived exclusively from separate zygotes so far as the evidence from sixty-one cases goes. The embryonic membrane/

membrane of such twins, however, fuse in an early stage (embryos of about 30 mm.) and the blood-vessels of the two individuals anastomose. If one is male and the other female the reproductive system of the latter fails to develop its usual characters, and characters of the male appear instead to a variable extent which appears to depend upon variations in time and degree of the vascular anastomosis. Such individuals have long been known as free-martins. The gonad is testis-like in form and structure owing to complete suppression of the ovarian cortex and hypertrophy of the homologue of the seminiferous tubules. The Müllerian ducts usually degenerate, and the Wolffian ducts may develop into quite typical vasa deferentia; gubernacula arise as in the male; but, save in very exceptional cases, the external organs of reproduction and the mammary gland conform to the female type. In rare cases (about one in eight cases of two-sexed twins) the vascular anastomosis fails to develop, and in such cases the female is normal. No abnormalities of the reproductive system of the male arise in two-sexed twins.

Sex-determination in the zygotic sense is thus seen not to be the exclusive determiner of sex-differentiation in mammals, even in respect to the most/

most fundamental sex-characteristics. The possibility of complete sexual inversion, by means of hormones of the opposite sex, and of control of sex-determination in this sense, is thus postulated.

CHAPIN who made a microscopic study of the reproductive system of foetal free-martins demonstrated that there is a fusion of the embryonic membranes and a subsequent anastomosis of blood vessels of the cattle twins. If one twin be male and one be female, the latter is commonly sterile. This is the result of the introduction of the interstitial secretion of the male into the blood of the female. It is manifested by the development toward the male condition, of those organs in the free-martin which are present in the indifferent stage (rete, first set of sex cords, primary albuginea), and the absence of those organs which develop in the normal female at sex differentiation or later (cords of PFLÜGER, definitive albuginea, union of Müllerian ducts to form uterus).

There is much variation in the reproductive organs of the free-martins. This is due to two variable factors: (1) The time at which the interstitial secretion of the male embryo may first enter the circulation/

circulation of the female embryo, and (2) the amount of secretion which may be introduced.

WILLIER who has more recently investigated the structure and homologies of the gonads of the free-martin shows that in the free-martin (a sterile female co-twin to a normal male in cattle) an indifferent gonad with a primary female determination, under the influence of sex hormones from the male twin, may develop variable degrees of transformation in the male direction. So far as the structural evidence from sixteen cases (seven foetal and nine postnatal) goes, the reproductive glands of free-martins may be placed into three distinct groups, which may be characterized as low, medium and high degrees of transformation in the male direction, and constitute, therefore, a chain of connected links between an embryonic ovary and a testis. Every organ of these modified free-martin gonads is affected. The sexual cords exhibit a series of gradations between medullary cords and seminiferous tubules (complete except that male sex cells are lacking). The rete ovarii transforms into a rete testis chiefly by developing connections (tubuli recti) between the rete tubules and the epididymal tubules. In the least transformed gonads the epididymis is/

is absent; in gonads exhibiting a moderate degree of transformation the head of the epididymis alone is present, and in the most completely transformed gonads a complete epididymis is developed. The distribution of the blood-vessels ranges from a typical ovarian to a typical male arrangement. It is thus seen that the most fundamental sex organ may be rather completely inverted by means of hormones of the opposite sex. Sex differentiation, then, is not exclusively determined by the union of the gametes.

If in a young frog, the relative valencies of the male and female factorial sets are such that the epistatic minimum is not present, both anlagen will become patent. This being so both spermatic and ovarian tissues will be laid down. If that portion of the spermatic tissues which is concerned with the production of the internal secretion of the testis, is developed before that part of the ovarian tissues which is responsible for the production of the internal secretion of the ovary, then the latter hormone will be suppressed since the ovarian tissues undergo no further development and such as exist undergo degeneration and ultimate removal.

It is an established fact that in certain animals the part of the spermatic tissues which is concerned/

concerned with the production of the sex-hormone of the male is the interstitial tissue of the testis. In the intermediate gonad described by SCHMITT-MARCEL attention is called to the relative preponderance of stroma and to the abundance of undifferentiated germinal tissue. Much of this may be regarded as spermatic and hence the tissue which is responsible for the production of the internal secretion of the testis is present at a very early stage in the development of the gonad. If spermatic and ovarian tissues can exist side by side they must be such as are found before the exhibition of their hormones. No question of any dominance of one hormone over the other arises, since the hormone of the testicular portions of the intersexual gonad is produced and exhibited before that of the ovarian portions of the gland, the result being that further development of the ovarian tissues is prevented and that the ovarian elements already present in the gland become degenerate and are removed. Slowly but surely the gonad becomes transformed into a typical testis and the individual into a typical male, and associated with this ovarian destruction and suppression and with the active development of the testicular tissues, the accessory sexual apparatus and the secondary sexual characters assume the/

the form characteristic of the normal male and the Müllerian ducts are not developed beyond the male standard.

GOLDSCHMIDT suggests that the non-participation of the secondary sexual characters is explained by the fact that they are only formed after the completion of the sex differentiation. It is certain that these secondary characters are not developed until the individual has attained a considerable size and equally certain that in the majority of cases the temporary intersexual has completed its indirect development into a male during the first eighteen months of its life.

But as is illustrated in the cases of abnormality already described, the spermatic tissues may not become expressed until the ovarian have attained functional maturity and it has been shown that the appearance of a small amount of spermatic tissue upon the inner border of a gonad which previously has had every character of a normal ovary, is associated with the immediate degeneration of the ovarian tissues and a gradual but inevitable assumption on the part of the individual of the male organisation.

In these circumstances, if the gonal tissues exert their influence by means of internal secretions/

secretions, then that of the testis must be more powerful by far than that of the ovary for the former wipes out the ovarian tissues and directs the imposition of male characters.

There is, in the frog, an antagonism of sex hormones and the ovarian is powerless in the presence of the spermatic. Quantity, apparently, is not a factor, for a very small nodule of testicular substance can still provide sufficient of the male hormone to wipe out the great mass of ovarian tissue and its abundant internal secretion.

A difficulty naturally arises. If the above be true, then there is no reason to question the accepted statement that Bidder's organ is a rudimentary ovary. This organ persists in the male but disappears in the female, which suggests that it is spermatic rather than ovarian in nature, but so deficient in interstitial tissue, that the hormone of the ovary becoming exhibited in the absence of the hormone of the spermatic tissues, produces its ultimate destruction. In this connection, it will be remembered that CERRUTI found spermatozoa actually within a Bidder's organ and that HOFFMANN described rudimentary seminal Tubules therein, whilst the experimental work of HARMS supports the idea that Bidder's organ is male in nature.

GOLDSCHMIDT/

GOLDSCHMIDT himself, in his latest book, "Mechanismus und Physiologie der Geschlechtsbestimmung", has enlarged his previous theories of sex-determination, and now includes the action of the sex-hormones among the determiners of sex. He submits that "each individual of a bisexual species contains the materials, the action of which may evoke one or the other sex; that these materials are distributed in relatively different quantities among the individuals; that there is a special mechanism which controls their distribution, as is revealed by Mendelian experiments and made visible in the sex-chromosomes. The action of these materials, like enzymes, is proportional to their concentration and the quantitive difference between the female and male factorial sets permits automatically the female or the male enzyme first of all to complete the decisive reaction. The reaction is the production of the specific hormones of sexual differentiation. The formative action of these hormones is not quite clear. It is probable, however, that they produce a differential state of metabolism which conditions the formative processes".

He calls attention to the biochemical nature

of/

of development - the chemical causes at work, urging a complex of cells to grow into a particular form, to become symmetrical or asymmetrical, in short, to show all the differentiations which in their totality represent the multiplicity of organisation, and argues that all the results of experimental embryology indicate that two processes have to be distinguished in the differentiation of organisms in development.

(1) The development of inherited anlagen and their proper distribution.

(2) The completion of the process of growth leading to the specific end-form of the organism, under the direction of substances designated as hormones.

He further postulates that the sexfactors themselves are enzymes or substances of similar physico-chemical character and that each of these enzymes of differentiation - male and female - is necessary for the completion (acceleration) of the reaction/

reaction, the products of which are the specific hormones of sexual differentiation. In forms in which the female is the heterozygous sex - the female enzyme is inherited purely maternally, so that each egg is identical with respect to the factor of femaleness. The male enzyme, on the other hand, is carried along with the X-chromosome to half the zygotes only. The absolute as well as the relative quantity of both enzymes is characterisation of the race.

But as a result of his most recent work, GOLDSCHMIDT has shifted his ground somewhat and now agrees that his results can be equally well explained on the hypothesis that zygotes with the chromosome-constitution of one sex, actually may become transformed into functioning individuals of the opposite sex. He has bred from ZW males, and ZZ females - from individuals, that is, which possessed the chromosome constitution of one sex but the organisation of the opposite. Sufficient data are not yet available, but theoretically it is obvious that such a male (ZW) mated to a normal female (ZW) must produce a generation consisting entirely of females (ZW), and that such a female (ZZ) mated with a normal male (ZZ) must yield a generation of males (ZZ). It is obvious that the activities of a feminised male or of a masculinised female could thoroughly disturb the sex-ratio among the offspring of that generation.

In/

In the experiments of HERTWIG and KUSCHAKEWITSCH, in which a generation consisting solely of males was produced, the results can be explained by the hypothesis that the effect of abnormal conditions resulted in the overriding of the chromosome-constitution and that one half of these males were masculinised females or, to use SHULL'S term, only "somatic" males of constitution XX.

There are several experiments which go to prove that chromosome-constitution can be overridden. SHULL'S investigations into the phenomena of hermaphroditism in Lychnis dioica demonstrate that the individuals were males genetically, and that they were only 'somatic' hermaphrodites, their chromosome-constitution having been overridden by external causes.

Moreover, STRASBURGER & DONCASTER found that female plants of Lychnis dioica, when attacked by Ustilago violacea were transformed towards hermaphroditism, an observation which supports the conclusions of SHULL.

LILLIE'S work on the free-martin can be interpreted as further proof that in female (presumably XX) embryos in cattle, co-twins of males, the whole organisation can be so altered that even the gonad itself takes on the characters of testis and the accessory sexual apparatus becomes more male than female The primary sexual characters can be modified in the zygote/

zygote, therefore, in spite of the presence of a definite chromosome-constitution. DONCASTER in one of his latest papers, suggested that the sterile tortoiseshell tomcat is possibly a female chromosomally which becomes transformed into an individual with an almost complete male organisation by a similar mechanism, - the circulation in a communicating bloodstream of substances emanating from its male co-twin.

If, then, a generation which shows a preponderance of one sex succeeds a generation in which the opposite sex was the more numerous, that is, if there is a swing-back, it is reasonable to suspect that in the first there were individuals which possessed the chromosome-constitution of one sex but the organisation of the other.

It so happened that the individual referred to in the Lists as No.14, when received was actually in copulation, and was reserved with others for breeding experiments. This frog was marked and ultimately was killed and dissected in May. The abnormalities were then noticed for the first time and in consequence every attention was paid to the examination of his mate and of his offspring. The female proved to be normal in every way. It was intended that she should be bred from again, but unfortunately, she died in November.

The eggs and tadpoles resulting from this union/

union were raised according to the directions given by H.D.KING and every effort made to rear as many individuals as possible. The fertilised eggs from seven other couples were kept under exactly similar conditions to furnish controls.

The eggs nearer the centre of the egg-mass gave rise to few tadpoles and every egg that was fertilised did not hatch for numbers of such were arrested in their development by death. The question of selective mortality naturally enters into a consideration of the sex-ratio of the individuals which did survive therefore. But there is no evidence to show that the sex-ratio would have been different if every egg had hatched, & every tadpole survived.

As the tadpoles passed the metamorphosis, they were removed to outdoor pens under conditions as natural as possible, the different lots being kept separate.

During twelve months, the frogs were examined in batches at intervals of two months. Every one that died was examined and included in the records. The controls showed no great variation in the relative numbers of the sexes and towards the end of the experiment only two lots were maintained.

The gonads were sectioned and compared to the/

the standards given by WITSCHI. No case of the indirect method of development of a testis was encountered, the gonads were either ovary or testis or else the tissues were too undifferentiated to allow of a decision being made. In all 2356 tadpoles and frogs were examined.

But of the frogs which resulted from the union of the male with the abnormal reproductive systems and a normal female every one of the 774 examined and found with gonads sufficiently developed, was a female normal in every respect. Those of the control lots were typically male or female and no case of abnormality was met with among them. The relative numbers of males and females varied in the different lots but only to a slight degree, the average being 46% males, 54% females. The average of all the lots was 23% males and 77% females, which is distinctly different from the figures of the parental generation (80% males and 19% females), constituting, in fact, a very complete swing-back.

By pure chance, therefore, it became possible to breed from a frog which, if the foregoing inferences are sound, was a female in chromosome constitution which had come to possess the organisation of the male. The results, as shown in the sex-ratio of the offspring, furnish proof - as conclusive as proof can be - that this/

this frog was indeed a female chromosomally, but that it had become possessed of the male sex-equipment. Therefore, probably by the action of the sex-hormones, complete sex-reversal actually does occur in the frog.

VI./

VI.

THE RELATION of the PRIMARY and SECONDARY SEXUAL CHARACTERS.

It has been shown that the assumption of the secondary sexual characters is evoked by the activities of the primary sex-glands and that the degree of their development is correlated to the strength of this action, and varies according to the stage during growth at which it operated.

There is some doubt as to what particular gonal tissue is responsible for the stimulus which evokes the assumption of the secondary sexual characters, and the recent work of CHAMPY on Triton alpestris shows that the conclusions of ANCEL and BOUIN regarding the role of the interstitial gland may not be applied to all Vertebrates, for he has demonstrated that while there is no definite correlation between the development of the interstitial tissue of the testis and the development of the secondary sexual characters in the Amphibians, there is a constant correlation between these characters and the phases of spermatogenesis, while the interstitial tissue always is least active when spermatogenesis is at its height.

In the Rat, MOORE recently transplanted a portion of a sex gland to a rat of the opposite sex, twenty/

twenty to fifty days after birth, and found that the transplanted gonads grew in the animal of opposite sex into which they were grafted and persisted for a period of over eight months. Histological sections of the grafts after recovery showed that a transplanted ovary grows and persists in a functional condition in a male animal possessing one testicle, and growth and differentiation of graafian follicles proceed in a normal manner up to the period of maturation, showing that the ovary remains functional. After the period of maturation, the follicles undergo atresia instead of ovulation and the follicular masses are converted into interstitial cells.

Portions of a testis transplanted into a female persisted for a period of eight months in the presence of an ovary, but did not produce spermatozoa, and, as in all cases of transplantation in mammals without exception, is followed by destruction of the germinal epithelium. In the white rat there is no apparent sex-gland antagonism.

MINOURA has demonstrated in the chick, by experiments in which he grafted a piece of testis or ovary on the chorio-allantoic membrane of developing embryos, that specific materials - (sex hormones) secreted by testes and ovary stimulate development/

development and differentation of the homologous sex organs and inhibit those of the opposite sex. His results show that sex-differentation can be controlled experimentally to some extent and that reversibility of primary sex characters can be experimentally accomplished.

In the following experiments the frogs were anaesthetised with Ethyl Chloride as this was found to give the best results. It was sprayed on to a mat of cotton-wool lying at the bottom of a square glass jar which had a closely fitting glass cover. Within two minutes anaesthesia was complete in the majority of cases; if it was not, a little more Ethyl Chloride was sprayed into the jar. When the frog could be rolled about by tilting the jar, making no effort to maintain its normal position, it was taken out and secured upon its back by means of pins bent over its extended and abducted limbs. A small incision was then made slightly to one side of and parallel to the anterior abdominal vein and the gonads being withdrawn through it one after the other, the mesorchium (or mesovarium) was severed. In a few cases an incision was made just lateral to the vertebral column on either side and the gonad withdrawn/

drawn by this route, but no appreciable advantage was gained and this method was relinquished in favour of the single ventral incision, save in the case of certain males in which the testes could not be found readily. In such cases the ventral incision was sutured and the dorsal ones then made.

The anaesthesia lasted for about ten minutes and so gave ample time, and a douche under the cold water tap produced a quick recovery. All the wounds healed by first intention and no death was due to the actual operation.

In all cases measurements of the right arm just above the elbow were made; an impression of the right hand was taken in dental composition and a plaster cast made; and while the frog was under the anaesthetic, the right hand was blistered and the raised skin cut away from the region of the finger-pad. The gonads were sectioned.

After the operation the frogs were kept in small wooden boxes lined with damp moss and grass, and fed frequently. The blistered hand healed quickly leaving no scar.

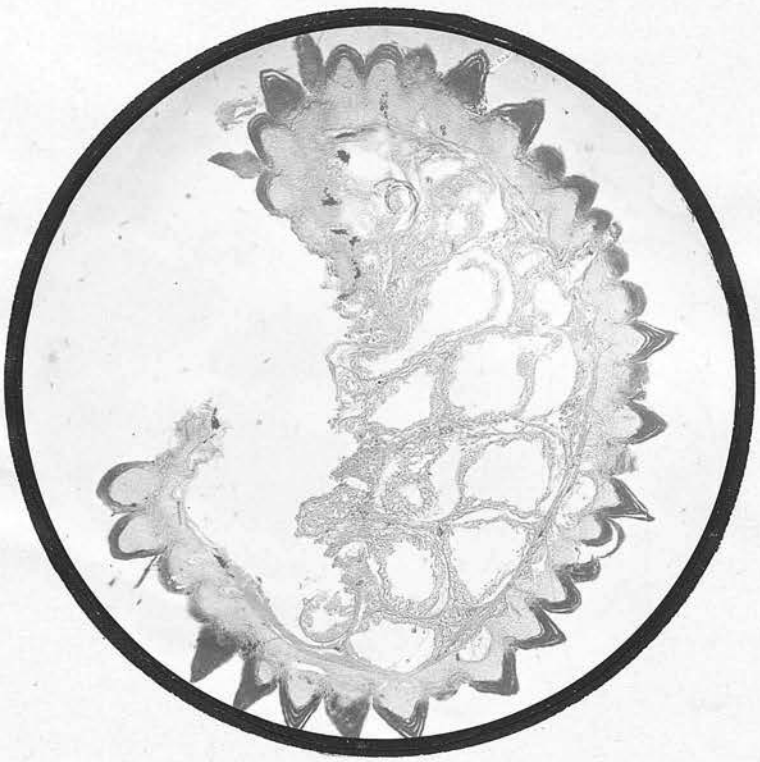
It was hoped by these experiments to define the exact relation between the primary sex-glands on the/

the one hand and the accessory sexual apparatus and the secondary sexual characters on the other.

It has already been recorded that the testes are smallest after the breeding season in Spring and largest from September onwards to the time of the breeding-season of the following year. They gradually increase in size from March to September and after this undergo no change in size until after the next breeding season. The sudden diminution in size is associated with the evacuation of the spermatozoa during copulation and the gradual increase in size coincides with successive phases of spermatogenesis, for during the summer months the spermatogonia are proliferating to form the spermatocytes and these during August and September divide to produce the spermatozoa. But when these have been produced the testicular tissues remain inactive and in a state of quiescence until the time of the breeding season of the following year.

Equivalent cyclical changes occur in the case of the ovary, while the oviducts of the female and seminal vesicles of the male exhibit phases of increased activity at the time of the breeding season. At this time too, the skin of the back and flanks of the female becomes roughened and warty and the gen-

eral/



R. temporaria
(Section of Finger Pad during breeding season.)

general colouration of both sexes becomes more pronounced.

The rough, intensely-pigmented, finger-pad of the male loses its more superficial layers immediately after the breeding-season to become much less rough and of a colour similar to that of the rest of the palm. It gradually becomes quite smooth and this is due, as was pointed out by SMITH and SCHUSTER, to a proliferation of the epidermal tissues and not to a further desquamation of the colourless papillae that remain after the pigmented layers are cast off. From August onward, that is, after the testis has completed its seasonal increase in size and the spermatozoa have been produced, the pads gradually become more and more pronounced until they have again attained the characters of the breeding-season. The development of the pad is not coincident with the formation of the spermatozoa but follows on their production. Moreover the pad is not entirely smooth although non-pigmented after the desquamation of the pigmented layers.

GONADECTOMY /

GONADECTOMY.

I. IN THE MALE. (Rana temporaria.)

CASE I.

Both testes were removed in March from a well-developed rutting male, whose secondary sexual characters were well-defined and typical. The testes on section had the normal structure and contained functional spermatozoa in great numbers. The seminal vesicles on inspection were well-defined and pigmented. The pigmented finger pads were desquamated 19 days after the operation and were never again replaced. The frog was killed in February, 11 months after the operation and the seminal vesicles were seen to be smaller than they had been previously; no spermatic tissue could be found; the finger-pads on inspection and on section were smooth and unpigmented; the rut-muscle was well-developed; and the measurement of the arm was identical with that taken a year before. The bones of the arms were as those of a typical, well developed male.

CASE II.

As CASE I. Killed in February, eleven months after the operation.

CASE/

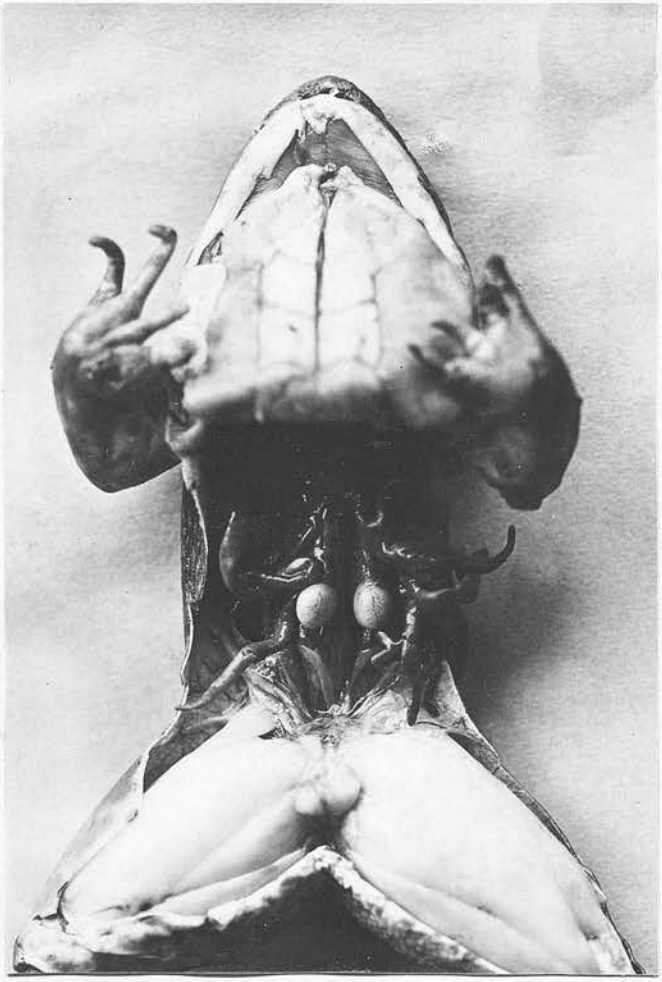
CASE III. As CASE I. Died in March 12 months after the operation. Both testes removed. Three other frogs were similarly operated upon, but died at intervals before the following breeding-season.

CASE IV.

Both testes removed from a male in October. The finger-pads were well-defined, slightly pigmented and rough. The testes were normal in structure but were inactive. The seminal vesicles were of moderate size. No desquamation of the finger-pads followed the operation. The pads remained unchanged throughout the following breeding-season. Killed April.

Two other frogs were operated on in November but these died before the end of the year.

The results of these experiments demonstrated that castration of the male performed during the time of the breeding-season was quickly followed by a shedding of the pigmented superficial horny layers of the finger-pads, and that during the following breeding-season the characters typical of this time did not appear. Further, it was found that if castration was performed during the winter months, no change in the characters of the pads followed the operation, but that the characters typical of the breeding/



R. temporaria
(regeneration of Testes after Gonadectomy.)

breeding-season were not exhibited when this season arrived.

It was found also that in the absence of testicular tissue the seminal vesicles did not undergo the characteristic enlargement at the time of the breeding-season, and that following castration the muscular and skeletal male characters remained unchanged for at least a year.

CASE V.

Operation as in CASE IV., but the frog assumed the typical male secondary sexual characters in the following February. On examination both testes were found to have regenerated, small rounded nodules about a third of the size of an ordinary testis occupying the normal position. Some small amount of testis-tissue must have been left behind at the time of the operation.

NO. II. IN the FEMALE.

CASE VI.

The ovaries were removed from a female in March, when she was obviously ready for egg-laying. Ovariotomy is a much simpler operation than castration, for both ovaries readily protrude/

protrude through a very small incision and can be gently stroked out and the mesovarium cut. The skin of the flanks and back was warty and the oviducts very well-developed. The skin gradually lost its wartiness and then the frog remained unchanged throughout the rest of its life and when it was killed twelve months later, no ovarian tissue was found, but the oviducts were still well-developed and became much swollen when placed in water.

CASE VII.

As CASE VI.

CASE VIII.

The ovaries were removed in October. The results were similar to those in CASE VI. save that the skin was not warty at the time of the operation. (Three other females were operated upon, 1 in March, and 2 in October, but died within 2 months of the operation.)

AUTO-TRANSPLANTATION.

I. IN the MALE.

CASE IX.

The testes of a well-developed male with well-defined pigmented finger-pads, were severed from/

from their natural position and pushed among the abdominal viscera, in the month of March. The seminal vesicles were seen to be well-defined and pigmented. Seventeen days afterwards, the pigmented layers of the finger-pads were desquamated. During the breeding season of the following year the pads assumed their pigmented horny characters. The frog was killed in March after he had mated with a normal female. The eggs from this mating were divided into a larger and a smaller lot and the smaller lot were mixed with the spermatozoa of another male. The smaller lot produced tadpoles, the larger did not yield a single one.

On examination of the sterile frog, one testis of average size was found to have secured firm attachment to the internal aspect of the peritoneal operation scar.

On section in the peripheral portions of the testis the seminal tubules contained spermatogonia and spermatozoa, but the latter were undergoing processes of disintegration. The more central portions of the gland were occupied by a loose matrix of fibrous tissue, amongst which were many cells closely resembling rudimentary ova/

ova which were most probably the results of aberrant division of the normal constituent cells of the testis. The gland was well supplied with blood-vessels. No sign of the other testis could be found. The seminal vesicles, though empty, were well-developed, as was also the rut muscle. The arms were massive and the bones of the male pattern. There was no means of egress for the spermatozoa.

CASE X.

As in CASE IX.

CASE XI.

As in CASE IX. save that the testes were placed within the lymphsacs.

CASE XII.

In this case the breeding-season characters of the pads were not exhibited during the following spring and on examination no trace of either testis could be seen, and the conditions were as those which follow double orchidectomy.

In another Case which died before the following breeding-season, the testes had secured new vascular attachments, but were undergoing degenerative changes. Spermatogonia could still be/

be found and the spermatozoa were all disintegrating.

It was found, therefore, that the testes are left within the abdominal cavity, they usually readily secure new attachments and continue to produce spermatozoa. But since there can be no egress for these, they inevitably degenerate as they approach maturity, and become absorbed.

Further, it was found that the testis does not retain its normal structure, for while the spermatogonia of the more peripherally situated tubules survive, yet the more central parts showed signs of abnormal cell-division, with the production of large cells lying amid a matrix of fibrous tissue and somewhat resembling rudimentary ova in appearance, but sufficiently graded to indicate their true origin and nature. Following the severance of the testes from their natural connections, the pigmented finger-pads if present, are desquamated, but if the testes secure new vascular attachments, these are renewed in the following breeding-season.

II./

II. IN the FEMALE.

CASE XIII.

The ovaries were severed from a female in October and allowed to remain within the abdominal cavity. No change followed the operation. In the following breeding-season, there were no signs of egg-production, and the skin of the back did not become warty. When the frog was killed and examined, two small masses of pigmented fibrous tissue adherent to the peritoneum were all that remained of the ovaries. The oviducts were well-developed and became swollen when placed in water.

CASES XIV., XV., & XVI.

As in CASE XIII.

Apparently the ovary does not so readily secure new attachments as does the testis, and, therefore, atrophies. The oviducts, when once well-developed, do not atrophy, even though the ovarian tissue disappears.

ALLO/

ALLO-TRANSPLANTATION.

I. IN the MALE.

CASE XVII.

The testes were removed from a well-developed rutting male, and those of another male placed in its abdominal cavity, their surfaces being roughened and stitched against the peritoneal wound. The sequelae were as those in CASE I., and when the frog was examined in the following March, all that remained of the testes were two small masses of fibrous tissue.

CASES XVIII. & XIX.

As in CASE XVII, save that the testes were not completely replaced by the fibrous tissue which invades the degenerating gonad and the spermatozoa were degenerating and undergoing removal by phagocytosis.

CASES XX. & XXI.

The operation was performed in October. The results were the same as in CASE IV.

CASES XXII., XXIII., XXIV & XXV.

Ovaries instead of testes were stitched within the abdominal cavity of a male in October. In CASE XXV., only one testis was removed and in its place an ovary was grafted. The results/

results were as those in CASE XVII. All that remained of the ovaries in the following April were small pigmented masses of fibrous tissue. The pigment was typically ovarian.

Three other males were castrated and ovaries then transplanted. The frogs died within 5 weeks following the operation.

II. IN the FEMALE.

CASES XXVI., XXVII., & XXVIII.

In March, the ovaries of a female which had produced offspring were removed and those of another stitched in their place. The results were as those in CASE XIII.

CASES XXIX. & XXX.

Testes instead of ovaries were stitched within the abdominal cavity of an ovariotomised female in October. In CASE XXX. the operation was unilateral. Nothing remarkable ensued and in the following April, it was found that the transplanted gonads had become replaced by fibrous tissue. (In 3 other CASES which died at various intervals during the six months following the operation, the testes were found to show various stages of degeneration).

It was found that allo-transplantation was invariably unsuccessful as the transplanted gonads without/

without exception atrophied and were replaced by fibrous tissue. Their presence did not affect their host in the least, and the conditions found were the result of the gonadectomy and not of the transplantation.

These findings are in absolute agreement with the results of GERHARTZ & of SMITH & SCHUSTER but fail entirely to confirm those of NUSSBAUM and MEISENHEIMER.

NUSSBAUM castrated two male frogs, one in May and the other in June, and it is stated that at this time the finger-pads were smooth. During September and October both frogs were injected with testis-extract, or had pieces of testis transplanted into them, and it was found that the roughness on the pad developed increasingly. In the cases of castrated frogs to which nothing further was done, the pad became reduced in size. In frogs castrated out of the breeding-season, the pads failed to develop and there was no increase in the size of the characteristically male muscles. In the transplantation cases the beneficial effects were transitory for the pieces of testis were gradually absorbed. NUSSBAUM also states, that if the nerves supplying the first digit were severed, the pad did not develop. Also, if/

if the nerves of the m.pectoralis, flexor carpi radialis, and abductor indicis longus are severed, these muscles did not develop. From this, he concludes that testis forms an internal secretion which is concerned with the production of the typical male secondary sexual characters, and that this secretion exerts a specific action upon certain local groups of ganglion cells, which are connected with the nerves of the arm.

PFLÜGER, on the contrary, argues, that the effect of section of these nerves, is a loss of sensation of the parts supplied, and that as a result of this, the tissues are not guarded from injury.

Further, he points out that the effect of one-sided gonadectomy is general rather than local, and that such an operation has little or no influence in destroying the symmetry of the secondary sexual characters.

RIBBERT states, that after a one-sided gonadectomy, the remaining testis undergoes a compensating hypertrophy, and MARSHALL (F.H.A.) concludes, that if this is so, it affords an additional indication that the testis is an organ of internal secretion.

MEISSENHEIMER claims to have confirmed NUSSBAUM'S/

NUSSBAUM'S results, and to have extended them, for, he states that he has induced the development of the finger-pad, not only by the implantation of pieces of testis, but also by the implantation of pieces of ovary.

SMITH and SCHUSTER repeated these experiments in 1910, and came to this conclusion:-

> "Now without wishing to throw the least doubt on the good faith of these observations, we hold them to be insufficient, because there is no real evidence produced, apart from the mere inspection of the thumb at different times during the experiment, to show that the small papillae of the size shown in NUSSBAUM'S figures, depicting the condition at the end of the experiment, were not present at the beginning of the experiment as well".

Similar objection is applied to the experiments of MEISSENHEIMER. They find that the cycle of changes in the thumb of the male frog is conditioned by the presence of testicular tissue in a normal functional condition, and prove satisfactorily that ovariotomy, with or without subsequent transplantation of testes or injection of testis-extract, has no effect in making the thumb of the female assume any male characters,/

characters, and that castration in the male, except during the breeding-season, has no marked effect in reducing the papillation of the finger-pad; if performed during the breeding season, a rapid desquamation of the outer layers of the skin over the pad follows. They show that the implantation of testis, or the injection of extracts, into a castrated male, has no effect upon the secondary sexual characters.

GERHARTZ maintains that the extirpation of one testis has no effect upon the secondary sexual characters; that if complete castration is performed when the sexual characters are well developed, these will atrophy; and that if the operation is done whilst they are poorly developed, they will remain stationary. Further, if the mature testes of newly caught frogs are implanted into the lymph-sacs of the castrate, the typical secondary sexual characters will appear again, even although a year has elapsed since the operation, but as these transplanted testes are not supplied with either nerves or blood-vessels, as they lie in the lymph-sacs, their influence is of short duration as they soon degenerate.

BRESCA found that after castration the crest of the male Triton did not develop.

These experiments show, however, that the presence/

presence of functional gonal tissue is necessary for the normal cycle of changes in the secondary sexual characters, and in the accessory sexual apparatus.

They do not prove beyond question that the stimulus which evokes the assumption of the breeding-season characters, is of the nature of a hormone, for, as SMITH & SCHUSTER point out:-

> "The fact that the developmental cycle of the thumb depends for its normal course on the presence of normal living testicular tissue, can be equally well explained on the theory that the testicular cells enter into a chain of metabolic processes in the body, which do not pursue their normal course in the absence of the testicular cells".
>
> "The gonad may equally well exert its action by taking up some substance or substances from the blood, thus altering the composition of the blood, and perhaps stimulating the continued production of these substances in some other organ of the body".

The frog is, obviously, not a suitable material for an investigation as to which of these two theories is the correct one. SMITH & SCHUSTER have shown/

shown conclusively that injection of gonal extracts does not affect the cyclic changes in the secondary sexual characters, and the results obtained by NUSSBAUM are open to grave criticism. The present experiments cannot decide this point, but they do support the opinion that in the frog both healthily functional ovary and testis cannot exist in one and the same individual. A gonad transplanted into an adult individual of the opposite sex may secure fresh trophic connections, but, nevertheless, it perishes. It does not follow that an internal secretion of the remaining gonad is responsible for the destruction of the grafted gonad, for allo-transplanted testes are destroyed in a male just as are ovaries.

In the frog since allo-transplantation is invariably unsuccessful, the conditions found in the cases of abnormality cannot be reproduced experimentally and a most critical method of examination of the exact nature of the abnormalities is not available.

VII. S U M M A R Y .

(1) It is shown that the recorded abnormalities of the reproductive system obviously suggest that there is a process by which an individual which at one time in its life history possessed only the complete sex-equipment of a female, comes to exhibit the organisation of the male, the only abnormality remaining being an over-development of the Müllerian ducts.

(2) A review of what is known of the development of the reproductive system supplies considerable proof that sex-determination is effected by a very delicate mechanism and that there is cytological and embryological support for the conception that the rudiments of both sexes are present within the embryonic sex-glands.

(3) A study of the sex-ratio provides conclusive proof that an individual possessing the chromosome-constitution of a female may come/

come to possess the organisation of the male. Experimentally it has been shown that certain factors such as raw crosses in mating, as between different genera, species, varieties, or strains, and others which affect the relative physiological conditions of the gametes at the time of amphimixis, such as temperature and altered water-content, can override the chromosome-constitution of a zygote and cause a definite reversal of the sex of the individual.

(4) Such a masculinised female or 'somatic' male, functioning as a male, was allowed to mate with a normal female. Every one of the offspring of this mating which attained a sufficient size to permit an examination to be made, was a female. These offspring incorporated in their generation disturbed the sex-ratio producing a swing-back, as in this there was a preponderance of females, whilst in the preceding generation there was a preponderance of males. This swing-back confirms the conclusion that in the previous generation there were individuals of female chromosome-/

chromosome-constitution, the chromosome-constitution of which had been overridden by external agencies.

(5) It is demonstrated that the conditions found in the cases of abnormality cannot be reproduced experimentally by allo-transplantation but that the assumption of the secondary sexual characters is conditioned by the presence within the individual's body of the appropriate functioning gonal tissue.

VIII.

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IX. INDEX of AUTHORS.

INDEX of AUTHORS (Contd.)

INDEX of AUTHORS (Contd.)

INDEX of AUTHORS (Contd.)

INDEX of SUBJECTS.

INDEX of SUBJECTS (Contd.)

INDEX of SUBJECTS (Contd.)

INDEX of SUBJECTS (Contd.).

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