

**EXPERIMENTS ON THE INTERNAL SECRETION OF
THE SEXUAL GLANDS, ESPECIALLY ON EXPERI-
MENTAL HERMAPHRODITISM. BY KNUD SAND.**

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THE point of view with regard to the internal secretions of the sexual glands has changed considerably in the last decade. The great progress made is, in the first place, due to Steinach. Later Athias, Brandes, Goodale, Pézard and I have experimented on the subject. I propose in this Paper to give a brief account of some experiments which are part of a larger work published in 1918 as a monograph in Danish(1).

In the years 1911-13 Steinach(2) published his ingenious experiments on the transformation of the sexual characters in mammals by exchanging the sexual glands. In the latter part of 1916(3) he described new experiments, in which he succeeded in producing a somatic and psychical hermaphrodite animal by subcutaneous ingrafting of both kinds of sexual glands at the same time and on the same infantile castrated male animal. Provided both the organs had become fixed, the experimental animal showed a development of the homological as well as of the heterological characters, both somatic and psychical; the psychic character being periodically either male or female.

Among the researches into different sexual problems which I began in the summer of 1914 there were experiments on masculinisation and feminisation. By simultaneous grafting of male as well as of female sexual glands on the same organism I also took up the question of the eventual immunity of the organism from the heterological sexual glands and the question of the antagonism between the sexual glands, which had been presumed by Herbst(4), and observed by Steinach. Finally it became my special object to throw light upon the modern conception of hermaphroditism and abnormal sexual conditions by experimental production of hermaphrodites.

My experimental animals were guinea-pigs and rats, mostly operated within the first month of their lives. Apart from a series of experiments

on ligature of vas deferens and experimental cryptorchism which I will not mention here, my experiments were based upon transplantation.

I. *Experiments on transplantation.*

A series of experiments were made on the following modes of grafting sexual glands:

A. Autotransplantations.

B. Isotransplantations (Homoiotransplantations)

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| } | (a) Homological: between individuals of the same sex. |
| } | (b) Heterological: between individuals of different sex, so-called "experiments on transformation." |

In the transplantations of the testis I found a special technique very useful, namely *punctura testis*, in that before the grafting I made a series of punctures in the albuginea to make the vascularisation easier. Also "transplantation in two tempi" with primary moving up of the testis on the peritoneum, and after a fortnight, cutting the vessels and vas deferens, a method which Steinach also has used, gave me excellent results in testis-autotransplantation. All of the testes-transplantations showed the importance of the Leydig-cells for the internal secretions.

A great number of ovary-transplantations were made. At the conclusion of the experiments the transplanted organs were examined in serial sections, and the effect of their hormones was estimated in relation to their total mass, and to their contents of the different ovary-elements. As to the question of the hormone-supplying tissue in the ovarium, the results gave considerable support to the opinion which in recent writings (Biedl⁽⁵⁾ and others) obtains more and more prevalence, namely, that the three elements interested, the follicles, the theca-lutein tissue and the corpora lutea, morphologically and physiologically, may be looked at from the same point of view, and are able, under different circumstances, to supply the place of one another, in their influence on the female "accidental" (secondary) sexual characters. These results were obtained by experiments that showed widely different combinations of the follicles, theca-lutein tissue and corpora lutea in connection with the effect of the hormones on the transplanted organs concerned. This point of view has been particularly clearly worked out on theoretic grounds by Bucura⁽⁶⁾, with whom on the whole I agree. Like Bucura I take the follicles, and, especially their granulosa-cells, to be the

original mother-substance for the hormone-production, "the primary hormone-cells," which before puberty (*i.e.* until the atresy of the follicles with the growth of the theca-lutein-cells, and the formation of corpora lutea has reached a higher development) are able to produce the number of hormones necessary, even if their chief object is, as I believe, the nourishment of the ovule.

When the follicle perishes (atresy), its hormone-forming function is taken over by the theca interna cells and these hypertrophy. When ovulation occurs, the function is taken over by the granulosa cells which hypertrophy and form the corpus luteum with intensified hormone-development. In this stage, the theca-lutein tissue and the corpus luteum may perhaps be described as "secondary hormone-cells."

Accordingly, I agree with Bucura that the theca-lutein tissue and corpus luteum are elements, which through hormone-depositing and an intensified hormone-production, caused by hypertrophy of the hormone-cells (no doubt together with the follicles constantly present), produce the varying number of hormones necessary for the constantly changing sexual processes of the mature female organism (development of puberty, menstruation, rutting, gravidity, lactation).

As to the heterological isotransplantations, the "experiments on transformation," I was able to confirm the discoveries of Steinach, as well with regard to masculinization as to feminization of the animals. With reference to the masculinization I was from the outset observant of the possibility of producing a somatic reaction in animals, corresponding to the mamma hypertrophy of the feminized animals. It was, I think, an obvious conclusion to presume a hypertrophy of the clitoris. In a series of cases in my experiments (on rats) I succeeded in producing an undoubted hypertrophy of the clitoris ordinarily quite rudimental. The hypertrophy was so great that the organ spontaneously protruded as a 4 or 5 mm. long, turgescient "peniculus" in the transformed animals which also psychically were decided males. Here I had a reliable, somatic reaction noticeable at an early stage, of an undoubted masculinization of female animals, corresponding to the mamma-hypertrophy of feminized male animals. The microscopical examination of the ingrafted animals showed strongly augmented Leydig-cells among atrophic tubuli seminiferi.

When I began these experiments in 1914 I wondered why Steinach in his treatises had mentioned nothing about the conditions of the clitoris in his masculinized animals. Not until 1917, a long time after the conclusion of my experiments, did I find a communication by Lipschütz (7),

according to which he had made the same observation in a guinea-pig, masculinized by Steinach, as I had made in a series of rats. Lipschütz found in this animal a considerable hypertrophy of the clitoris with two corpora cavernosa and also development of the quill-shaped growths found on male animals. Corpus cavernosum urethræ was altogether lacking, the penis-like organ represented a totally hypospadical penis.

II. *Experiments on experimental hermaphroditism.*

Steinach (1912) called attention to the fact that castration of the experimental animal is a condition *sine qua non* for the ingrafting and efficiency of the heterological gonads, the transplanted organ perishing if castration be omitted. On the whole I can verify this observation.

Steinach assumes this condition to be an *antagonism* between the sexual hormones. I am of opinion that perhaps it would be more plausible to assume an *immunity* of the non-castrated organism from the heterological sexual glands, without anticipating the nature of this immunity.

In order to examine the point in question I made different experiments.

A. With the object of making conditions equal for both gonads from the outset, I made a *simultaneous transplantation of a testis and an ovarium on the same castrated infantile animal*. By doing this it was my intention to avoid the "preponderance" with regard to its efficiency, which the homological gonad might be in possession of by heterological transplantation on a non-castrated animal, and which would perhaps influence a transplanted organ of this kind.

A single positive result may be briefly reported.

Guinea-pig (*a*). Castrated on the 17th August, 1916, at the age of three weeks, whereupon one of its testes and a consanguinal ovarium were transplanted in subperitoneal "pockets." After two months the penis was partially developed, and there was a marked hypertrophy of the mamma, out of the turgid, strongly pigmented papillæ of which normal milk could be pressed.

After three months: penis 0.6 cm. (normally about 1 cm.), breadth of the base of the vesiculæ seminales 0.4 cm. (normal) swelled with secretions.

The mamma, as above described, decidedly hypertrophied, the papilla measuring 0.6 cm. (normally 0.1 cm.). Natural milk (microscopy) can be pressed out and plenty of glandular tissue is found.

This somatic hermaphroditism was combined with a decided bisexualism of the psycho-sexual character, in that the animal showed, even during the course of a single hour, momentary change from female to decided male character, according to the animals (males, newly-born young ones, females) with whom it was brought into contact.

Conditions found in the well-grafted organs: testes: enormous development of Leydig-cells among atrophied tubules. Ovarium: numerous mature follicles and some interstitial, glandular tissue. Mamma (microscopy): plenty of glandular tissue almost of the same type as in a puerperal mamma.

The animal was accordingly a decided example of experimental hermaphroditism both somatically and psychically. Thus, independent of Steinach, I obtained the result that it is possible to produce an artificial hermaphrodite through simultaneous transplantation of male and female sexual glands on an infantile mammal.

B. *Experimental hermaphroditism through formation of artificial ovario-testes.* Steinach states that he has succeeded in making the transplanted organs grow together to a whole, by placing them directly against each other. In 1914 I made use of a direct formation of ovario-testes by special technique, placing the ovaries in the middle of the testes and leaving the latter in their natural position, also in short: *Intratesticular ovary transplantations.* In experiments on rats I succeeded in producing artificial ovario-testes by this method. After about four months' observations I found, as well in adult as in infantile animals, the ovarium containing the follicles, interstitial glands and corpora lutea lying in the middle of testis tissue with perfect spermatogenesis and normal Leydig-cells. The results showed:

I. That the ovarium grows and, what is more, grows comparatively easily into the testis tissue itself, develops further here and is able to form corpora lutea.

II. That the testis, although the heterological gonad lies in the middle of it, and is nourished through its substance, is still able to develop until it reaches perfect spermatogenesis, and

III. That it is, accordingly, possible artificially to produce hermaphroditic sexual glands, ovario-testes, and both the gonads of different sex easily thrive in intimate union without injuring each other, apart from the traumatic injury.

On account of the lack of predisposition for mammæ by the male rats, these experiments did not with certainty illustrate the effect of the artificial ovario-testes on the accidental sexual characters, even if the animals psycho-sexually showed a somewhat changed appearance. Unfortunately the technique of the experiments on guinea-pigs is exceedingly difficult, the testes of these infantile animals being much smaller than those of the rats, but as the ingrafting of the ovarium on these latter had succeeded so well, and hoping that a positive ingrafting on guinea-pigs would give distinctly observable double reactions,

I also made some experiments on guinea-pigs. These gave a positive result. The following example may be given here:

Guinea-pig (*b*). On the one-month old animal intra-testicular ovario-transplantations were made on both sides. After one month the penis, as well as the mamma papillæ, began to increase in size, the latter however most. The section three months after showed the penis 0.6 cm. long (*i.e.* not as large as that of the normal control animal), there were rather well-developed vesiculæ seminales, containing secretions and with a breadth at the base of 0.3 cm., but yet a little smaller than those of the normal control animal (breadth of base 0.4 cm.), at the same time the papillæ showed an enormous hypertrophy, were large and filled with secretions, intensely pigmented with a broad areola, normal milk could be pressed out.

Psycho-sexually the animal had without doubt shown decided bisexuality.

The section showed both of the testes lying adherent to each other in the abdomen, *i.e.* a condition of cryptorchism had at the same time been produced.

The microscopical examination (serial sections) showed that both of the organs had been transformed into ovario-testes, with a well-ingrafted ovarium lying in the middle containing several mature follicles and plenty of interstitial glandular tissue in intimate contact with the testis tissue, the latter of which showed infantile character (cryptorchism):

The mamma showed the same character as in the case with guinea-pig (*a*), thus being of puerperal type.

This experiment, then, gives a new proof that the ovarium finds good conditions for existence in the middle of the testis, and that the gonads do not directly influence each other deleteriously, but, at the same time, it also proves that the formation of ovario-testes makes it possible, by intra-testicular ovary-transplantations, to produce a somatic and psychical hermaphrodite animal.

I will not here enter further into all the considerations to which the above-mentioned phenomena might give rise, not least as a biological basis for critical examination of hermaphrodite and other abnormal sexual conditions. On the whole I can agree with the views Steinach has put forth, and Hirschfeld⁽⁸⁾ has supported. I have only to add that through numerous other experiments, especially ovary-transplantations on rats, I came to the result that auto- and homological isotransplantations (homoiotransplantations) have succeeded better than transplantations on castrated male animals, in these the ovarium showed a lesser growth and more tendency to atresy, a fact that Steinach has called attention to. I must, however, especially emphasize that a corpus luteum development was very rare in these animals. Neither did I ever succeed in a real ingrafting of the heterological gonad on non-castrated animals, whereas there was, as mentioned before, a positive result if the gonads had immediately been brought under somewhat the same conditions by simultaneous transplantation on the same animal, or by the intimate union formed by the production of ovario-testes.

These phenomena do not, I think, point so much to a real antagonism, *i.e.* a reciprocal contrary influence, as to a kind of immunity of the normal organism from the heterological gland. This *immunity* can perhaps be conceived as an "atrepical immunity," which may perhaps be explained as follows: in every organism are found certain substances which are necessary for the sexual glands, and these substances the latter try to absorb to the greatest possible extent. The normally situated non-transplanted gonads have the best chances of being able to absorb these substances, for which reason heterological (perhaps also homological) gonads, transplanted on normal organisms, cannot get enough of these substances and therefore perish. Homological and heterological gonads, which have been transplanted at the same time to the same organism, are both able to grow in, having both about the same opportunity of absorbing the substances.

Further the phenomenon that ovaries ingrafted in the testes find good conditions for developing there, can probably be explained by a similar theory, in that the substances of the normal male organism, necessary for the gonads, are perhaps stored up in the testes, both kinds of gonads thus being able to make use of them.

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