Embryology. — *Sexreversal and chromosomes.* By G. KREDIET.

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Intersexes are animals formed by sexreversal, the sex of which is between male and female. They show characters belonging to both sexes. They used to be called hermaphrodites. According to the nature and structure of the genitalia they were divided into groups which were classed together in one scheme. By adding a few adjectives such a high degree of descriptive accuracy was indicated in the name that every expert knows at once with which he has to deal. Through these descriptions the appelation hermaphrodite will continue to be used.

The name intersex is of causal importance and, when preceded by male or female, indicates the true sex. This is not the case with hermaphrodites. Formerly nobody would have suspected that the male pseudo-hermaphrodite, characterized by two testes, is a female intersex. The animal belongs to the female sex and has been formed by sexreversal, as the name intersex indicates. During this process it has developed male characters, which it shows later on.

But not only the so-called hermaphrodites among the mammals belong to the intersexes. Also the free-martins and gynandromorphs are reckened among them. GOLDSCHMIDT has made the following division:

1. Time intersexes.
2. Space intersexes.
3. Hormonal intersexes.

By time intersexes he means those animals that begin their development in one sex, passed through a turning point, and finished their development in the direction of the other sex. For example, they were first female, then male, thus became intersex through the succession of sexes, that is according to time.

Space intersexes are those animals which during their development have lost one chromosome in a cleavage, for example the first, so that one blastomere has become male, the other female. If this first cleavage lay in the developing median plane of the animal, one half would become female and the other male. Then the animal would be a so-called bilateral gynandromorph. Among mammals such individuals have not yet with certainty been found, but among birds they are known. The latter are explained by assuming that a predisposed male animal having two X-chromosomes has lost one, so that all the descendants of this cell have one X-chromosome, and consequently become female cells. So there will be male and female characters lying side by side in one individual. According to this spatial division these animals are called space intersexes.

Hormonal intersexes have so far been found chiefly among cattle, but they also occur among pigs (HUGHES, HOADLEY) and goats (KELLER). A free-martin is formed in dizygote twinning of different sex, in which the two choria have been intimately fused and in which the placental circulations are closely united by a directly perceptible anastomosis, so that substances from the bull calf can circulate into the cow calf and vice versa. It is worthy of note that the bull calf almost always remains normal and the cow calf develops abnormal genitalia, in which the male characters may be more or less strongly prominent. If one calls the substances activating this process active substances or hormones the name hormonal intersexes speaks for itself.

In the time- and hormonal intersexes the sexreversal is most evident. The gynandromorphs show it only partly. So it is obvious that a comparative study can be made of free-martins and hermaphrodites. In another respect the space intersexes are more like the free-martin, because, when taking a bilateral gynandromorph as an example, one has to deal with an individual having an ovary on one side and a testis on the other,

a state of things as we find in the free-martin, which has been formed after a joint circulation of a bull calf with testes and a cow calf with ovaries. If looking at it from this point of view a third animal will have to be compared, viz. the lateral hermaphrodite, which is a time intersex and which, just as the bilateral gynandromorph has an ovary and a testis for gonads. For this reason there are some investigators who call the lateral hermaphrodite a gynandromorph. So we have to deal with three animals which have in common that during their development they were affected by an ovary and a testis, viz. free-martin, bilateral gynandromorph and lateral hermaphrodite. Curious enough they have become three very different individuals.

Comparing first the free-martins with the time intersexes there is at once a point of resemblance in the sexreversal. Also in the time intersexes this goes as a rule from female to male. On the other hand we find here one great exception. The greatest difference appears when discussing the cause of the sexreversal. To make this clear it may be useful to state shortly in what way sex is determined. In fertilization there are two chances which are about equal. The animal becomes either male or female. These equal chances are formed because there are two sorts of sperms, viz. with 1 X-chromosome and without this chromosome. The other chromosomes in the nucleus are classed under the name of autosomes; so if we write the formula of the sperms it will be $A + X$ and $A + O$. For the ripe eggcells it is always $A + X$. A female mammal has $2A + 2X$, a male $2A + X$. An $A + O$ sperm with an $A + X$ eggcell gives a $2A + X$ individual, that is a male. An $A + X$ sperm and a $A + X$ egg gives a $2A + 2X$ individual, that is a female. There are as many $A + X$ as $A + O$ sperms, therefore the chances are equal.

From this way of representation it follows that there are only two sexes, which can only be different in X. Al other differences result from this.

Seemingly at variance with this truth is the fact that every animal is predisposed bisexual. GOLDSCHMIDT has given the following explanation of it. In every animal there are male and female sexfactors, which also in fertilization have their influence. For the formation of a normal male it is not only necessary that the male factors suppress the female ones entirely, but besides they have to be much stronger. So to say, they must have superior valence. Thus there must be a certain epistasis which is to exceed a certain minimum. If this does not occur, the foundation is laid for the formation of an intersex. In case of a copulation of a "strong" male animal with a "weak" female one it may occur that the male animals are normal but that the female ones are intersexes, because in such a female animal the female factors do not get sufficient epistasis against the male ones. The influence of the male factors is not sufficiently suppressed and can assert itself. It is not explained why exactly it should go in this order that first the female are set to operate and then the male ones, so that a succession of sexes develops. This we have to accept as a fact.

The seat of the female factors F is sought in the X-chromosomes, whereas that of the male M is assumed to be spread over the autosomes. A male animal can thus be represented by MMF and a female by $MMFF$. MM must be stronger than F and FF stronger than MM if a normal sex is to be formed. In a female intersex the development begins according to the chromosomes of the fertilized egg $2A + 2X$, that is female, to be followed after the turning point by the male. The chromosome formula does not change, because we have here to do with factors which are equally balanced. That these chromosomes remain unchanged can be demonstrated by the following: A short time ago a male pseudo-hermaphrodite, of the testes of which we had made many slides, was examined again. It struck me that there were many spermatogonia which showed the chromosomes clearly apart. These spermatogonia were probably ripe for degeneration, as one can also observe in developing testes. It looks as if they are preparing for cell division, and then they are, so to say, attacked by degeneration. VON WINIWARTER and SAINMONT have also pointed out this peculiar symptom. After convincing myself that the entire nucleus was visible in the slide, I ordered the draughtsman of our institute to draw the chromosomes. For this purpose he had to project in a flat plane what he

saw in the more or less bulb-shaped nucleus. He had not been told what significance might be attached to his drawing, so that he quite objectively rendered what he saw. I myself was very anxious to know whether the female or the male number of chromosomes would appear. The male pseudo-hermaphrodite was a pig. The female has $40 = 2 \times 20$ and the male $39 = 2 \times 20 - 1$ chromosome. There appeared to be 40 (fig. 1 en 2) and as still may be seen from many chromosomes they are lying in pairs. In my

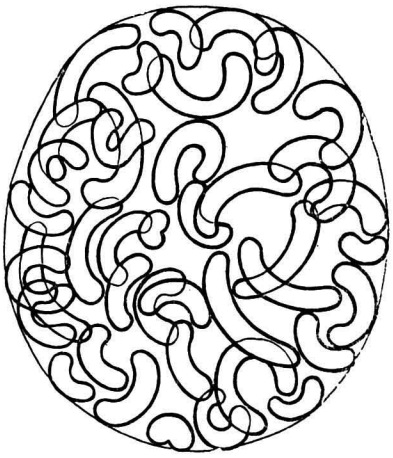

Fig. 1.

Fig. 2.

opinion this is a conclusive proof that this animal with testes was not a male but a female and consequently that a sexreversal must have taken place, for otherwise no testes could have been formed.

The question which forces itself to the front with respect to the action of the male and female chromosomal factors is what idea one must form of the way in which those factors can make their influence felt. GOLDSCHMIDT thought we had to do with an action of enzymes. These active substances are believed to be secreted by the chromosomes, first to be active in the cell and its immediate surroundings and later to pass in the circulation, so that it can perform its metabolic and morphogenic action in those places where it is needed. So one may call them chromosomal enzymes, which are secreted in all the cells of the body, for all the cells of the body possess nuclei with chromosomes. Consequently all the soma is not only sex-impregnated, but is also believed to form enzymes which permeate the whole body through the circulation.

It is difficult in one single body to furnish the proof of this hypothesis, but the placental anastomosis between two animals of different sex, as is the case with the development of the free-martin, offers a possibility to get a better insight.

In the bull calf, in which the epistasis of the male factors is greatest, the female ones are suppressed. In the cow calf the epistasis is the reverse. After the realization of the anastomosis male factors will pass into the female animal and female factors into the male. *MMF* and *MMFF* will be mixed into *MMMMFFF*. Here we have epistasis again. The *M*-factors can only then be strongest if the epistasis in the male animal is greater than in the female. KELLER and MOSZKOWICZ assumed this to be so and explain by it the sex changes which take place in the female animal. In point of fact the changes coming about in the time intersex and in the freemartin are assumed to rest on this basis. However the greatest differences appear when the animals are born. Then the twins get seperated, the free-martin is withdrawn from the action of the male epistasis, while in the time intersex things remain as they were. Is it possible that the female factors in the originally female free-martin become predominant again? Nothing of it could be observed. The animal grows into a being resembling much a castrated cow, but which

it is not. It has a somewhat different shape, so that NUMAN was already able to discriminate between them. It is still in the possession of the gonads, which often look more like testes than ovaries. Gametes have never been found in them. In RINGER's laboratory MARSMAN has made a research in order to find out whether sex hormones occur at all in the urine of the free-martin. He has not been able to find them. If this gives us a right to assume they were also non-existent in the animal itself, then it would make it clear that the gonads of these animals not only produce no gametes but that they secrete no hormones either, consequently they might just as well be absent. A research made by DE REGT to find out whether sex hormones occurred in the urine of a time intersex, a pig, has proved that they were found here in sufficient quantities, so that the gonads of these animals, although impotent as a rule, do continue their internal secretory functions. One gets a strong impression that by the sexreversal under the influence of the male chromosomal factors the free-martin has become both intersex and sexually neutral. It seems as if the chromosomal sex factors are no longer active. In castrated animals they certainly are. Geldings, castrated stallions, keep their sexual drive, although in a less degree, even if they have been castrated at an early age. Occasionally free-martins have been observed to show heatsymptoms; some are also recorded to be fertile. It is possible that these animals had become male in a slight degree or that a revival of the female factors has taken place after birth. It may also be that these animals were no free-martins, for the fact must never be accepted before we are absolutely certain that they formed in heterosexual twinning with chorionic anastomosis. So long as there is no absolute certainty in this respect it is difficult to judge about these few deviating cases.

The bilateral gynandromorphs, as have been observed a single time among birds, in which one half of the body is female and the other male, are the most puzzling beings that occur in the domain of intersexuality. Neither by means of the chromosomal factors nor by those of the sex hormones can any explanation be given. The gynandromorphs of PÉZARD may be left out of consideration because he had to deal with castrated fowl only differing in the feathers which had grown after castration. They remained animals of the female sex.

Lateral hermaphrodites are intersexes which somatically and psychically are not distinguishable from other time intersexes. The two halves of the body are equal, in contrast with gynandromorphs; only the gonads are different on both sides. Why the sexreversal showed itself in one of the gonads by the change of ovary into testis and not in the other is unknown. Perhaps we can approach it by making a series of the differences in gonads found in intersexes. It is quite common to find a testis on one side and an ovariotestis on the other. The ovarial part may be of different size. It may be larger than the testicular one. I once found a hermaphrodite in which the ovary contained such an infinitesimally small part of a testicle that it only became visible after making a series of microscopic slides. We had practically to do with an ovariotestis. If this small part is also absent, as is the case in the lateral hermaphrodites, then the series ranging from ovariotestis to ovary on one side is complete. We can only approximately conceive the fact that these animals are after all time intersexes. Therefore they must be compared with hermaphrodites in the same way as has already been done for the other time intersexes.

Up to now in my explanation of the free-martin I have left out of account the almost universally accepted theory of LILLIE, who is of opinion that the changes in the free-martin are to be ascribed to the action of the hormones, which are said to be secreted by the testes of the bull calf. The fusion of the two choria takes place when the fetus has grown to a length of 10—15 mm, the vascular development and the anastomosis taking place a little later. When the fetus has grown to a size of about 25 mm the chorionic fusion has already been established. BASCOM and VAN VLOTEN have demonstrated that in a bull fetus of 25—28 mm interstitial cells are already to be seen in the embryonic testis. Consequently the interstitial gland which is held to be the nursery of

the testicular hormones exists already a short time after the anastomosis develops. At this stage little change is to be noticed in the ovary, so that it is assumed that the internal secretion of the testis has started before there is any question of ovarial hormonal secretion. Under the influence of these male hormones the cow calf is believed to undergo the changes in the male direction, whereas nothing can happen with the bull calf.

Some objections may be raised against this theory. KELLER has directed the attention to it, that it is difficult to assume that testes of such young embryones should be capable of secreting hormones. Even in a free-martin of 32 mm BISSONETTE noticed some changes in the genitalia. It is difficult to prove that the testes in which interstitial cells are present, however young the gonad may be, secrete internally. We do know of researches by RUMPH and SMITH, which have demonstrated that a thyroid gland of a pig fetus of 7 cm secreted no hormones, but that one of 9 cm did; we also know that the hypophyses of pig fetus of 14—16 cm have no endocrine functions and those of 26—28 cm have. It would therefore be an exception if the testes of a 3 cm cow fetus already showed endocrine action.

But more strongly against LILLIE's theory militate the results of the experiments of RAYNAUD, IVY and GREENE, GREENE, BURRILL and IVY and of VERA DANTSCHAKOFF. The latter injected a male hormone testosterone proprionate into the allantoic sac of young cavia embryones, and noticed that the male parts of the bisexually predisposed genitalia of the female embryones began to develop. The gonads underwent no change. The Müllerian ducts grew into normal tubae and uterus, influenced by the female chromosomal factors. The caudal part of the genitalia proceeding from the sinus urogenitalis became purely male. After birth all the testosterinized guinea pigs resembled males, but after half a year the male part of the bisexual genitalia had been reduced to those of a castrate. Sometimes the epididymus have got lost. Ovaries, tubae and uterus continue their normal development as in a female animal. Even eggs can be fertilized, when after laparotomy sperm is put into the uterus.

Between these testosterinized cavia females and free-martins there are great differences:

1. a. *Free-martins*: gonads always have more or less developed in a male direction. Gametes have never been found.
   b. *Testosterinized cavia females*: ovaries are unchanged, ripe eggs can even be fertilized.
2. a. *Free-martins*: Müllerian ducts are strongly reduced.
   b. *Testosterinized cavia females*: Müllerian ducts are normally developed.
3. a. *Free-martins*: external genitalia almost always female.
   b. *Testosterinized cavia females*: external genitalia male.

There is some similarity in the male development of the Wolffian ducts and also partly in that of the accessory glands (seminal vesicle).

VERA DANTSCHAKOFF justly remarks that the male active substance from which the free-martin grows is unknown. The experiments of VERA DANTSCHAKOFF, RAYNAUD, IVY, GREENE and BURRILL make it clear that the testicular hormone cannot be that substance, because it acts in a different way from that which influences the cow calf in the heterosexual twinning with placental vascular anastomosis. The testosterone confines itself to the predisposed male parts of the genitalia and leaves the female untouched.

For the present it seems to me that I am fully justified in sharing KELLER's view rather than LILLIE's. In what way the sex chromosomes work is unknown, but it is probable that in some way or other they are able via the circulation to exercise their influence on the sexual development of the individual.