THE FREE-MARTIN; A STUDY OF THE ACTION OF SEX HORMONES IN THE FOETAL LIFE OF CATTLE

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TWENTY-NINE FIGURES

I. INTRODUCTION

In a preliminary paper (Lillie '16) the author has briefly discussed the theory of the sterility which is the rule, subject to a few exceptions, in the female of two-sexed twins of cattle. In such females, commonly known as free-martins, the internal organs of reproduction are usually predominantly male in character, and the external organs are usually, at least, of the female type; there are however considerable variations as will appear. The conclusion was reached that the sterile free-martin is zygotically a female, modified by the sex hormones of the male twin, which circulate in both individuals during foetal life owing to secondary fusion of the chorions and anastomosis of the foetal circulation of the two individuals. The conditions thus established in this natural experiment enable us to draw far-reaching conclusions as to the origin and the differentiation of sex-characters in mammals. A somewhat extended treatment of the data, and analysis of the facts is therefore herewith presented.

Previous studies have dealt mainly with the anatomy of the free-martin and have furnished important, though incomplete data on this part of the subject. These will be considered in their appropriate connection. A study of L. J. Cole's ('16) supplies some important statistical data referred to beyond. We shall introduce the subject by a consideration of the various theoretical points of view from which the discussion must start. In considering these it will be important to define more precisely the condition with which we are dealing. The phenomenon of sterility of the female of different sexed twins is usually consid-

ered to be peculiar to cattle;[1] it has been stated to occur in sheep rarely, but I cannot discover on what evidence. There has also been a superstitition,[2] which reappears in the press

[1] According to E. J. Davies ('13) 'hermaphrodites' are common in certain breeds of goats, for instance Toggenburgs and Anglo-Swiss. He even estimates that in 1913 "Among the total birth of kids eligible for entry in the Herd Book it is believed that at least 2 per cent belonged to the class under discussion—and the proportion is probably much greater." This statement is understood from the context to apply to the Toggenburg breed. He goes on to say that "malformed kids have recently come (a) singly, (b) as twin with a normal male, (c) as twin with a normal female, (d) as one of triplets the normal kids being male and female, (e) as one of triplets the normal kids being both males."

Assuming the malformation to mean genital abnormalities we are justified in explaining cases b, d and e on the same principle as the free-martin of cattle. As regards case a we can only say that this phenomenon is not uncommon in other mammals, whatever may be the explanation; it could be explained as due to two-sexed twin association *in utero* with early death and absorption of the male twin. Case C, an 'hermaphrodite' twinned with a normal female, is of unusual interest, for we have no evidence that this condition ever occurs in cattle; but it is to be expected theoretically in any case of blood community of male and female embryos in which the female is decidely in advance of the male in development. The male would then be subject to action of the female sex hormones, and its development would tend to be intersexual. The case cited in illustration of this association seems to be well authenticated. Unfortunately the anatomy of the malformed individual was not studied, as a kid it appeared as a female externally except for the much enlarged phallus; but it grew to male size and even developed very strongly the characteristic smell of the male goat.

[2] I do not know how wide spread this superstition may be; but that it still exists is undoubted. It has been reported to me as a common belief in certain farming communities, and it has been gravely discussed in the correspondence of the Chicago Tribune as recently as March, 1916. Formerly the idea was more wide spread, and was even countenanced by medical men as reported by Dr. James Y. Simpson of Edinburg who quotes from Burn's "Principles of Midwifery" 1843, p. 236, "It is a popular opinion, and I do not know any instance to discountenance it, that if twins be of opposite sexes the female is sterile." Simpson ('44) made an investigation of the subject which may be regarded as disposing forever of the superstitition. He investigated the family history of 123 married women born twin to males of whom 112 had families and 11 had none; he found also that this proportion of childless marriages was not greater than in the general population. Ninety-four of these cases in which he had complete histories had 409 children, an average of 4.2, which was about the same as for the general population of that time and place. He concluded that females born co-twin with males are, when married, as likely to have as many children as other females belonging to the general community. There is no basis for belief in the sterility of such females; the superstition works cruel hardship to innocent people, and it cannot be too strongly stated that there is no basis whatever for it.

from time to time, that it applies to human twins, but this is certainly not the case. In cattle, in about 87 per cent of apparently different-sexed twins, as nearly as I can ascertain, the female is sterile; about 13 per cent are normally fertile (see data beyond p. 381). We must therefore distinguish sterile and fertile free-martins, and it is important to note that the fertile individuals are not known to be in any respect inferior to other females in respect of breeding. The sterile individuals have the external organs of a female, usually, but the internal organs of reproduction are more or less of the male type. Their general bodily appearance is more or less intermediate between a male and a female—it has been compared to that of an ox or spayed heifer—so that an experienced cattle man can usually distinguish them from normal heifers. The bull twin is always normally fertile, and does not exhibit any anatomical peculiarities so far as is known.

It is essential to recognize the fact that the sterile free-martin condition is found only in association with a bull twin.[3] The

[3] Numan ('43) is, so far as I know, the only author who has questioned this; his study is by far the most extensive, and in many respects the most thorough, that has been made on the free-martin. The publication is exceedingly rare, but I have been able to study a copy from the library of the Smithsonian Institution, and as his conclusions are so often quoted from author to author, it seems worth while to give the evidence on which his dissenting statements are based. (D. Berry Hart has published an abstract of Numan's paper (Hart '12). Numan states "The anomaly occurs not only in twins of different sex, but also in female and male pairs, though more rarely." In these cases he refers first to an individual about two years old, judged by external signs alone to be a sterile free-martin; the owner stated that it was born twin to another female, which however was sold shortly after birth about two years previously. This would appear to be slender evidence upon which to base a unique exception, for it was not positively certain that the individual was a sterile free-martin, nor could Numan know unquestionably that it was born twin to another female. It is known also that cystic degeneration of the ovaries may lead to extensive assumption of male secondary sex characters in the cow (Pearl and Surface, '15); the case may belong in this category. This was the only case he had on the female side, and no others have been recorded since. In the case of male pairs he cites also a single case which is of great interest, but wrongly interpreted by him. It was a case of twins one being a normal male and the other a sexually abnormal individual. He judged the abnormal individual to be male on account of the presence of testes in the groins, and malformation of the external parts. The scrotum was absent. This is almost certainly an extreme case of modification of the

association with a male *in utero* is, therefore, in some way a necessary condition of the phenomenon. It is also an invariable rule that the male twin is normal; the reverse condition of a normal female with a defective male probably does not occur. All of my own cases were found in association with a normal bull twin; in the other possible twin combinations both individuals are normal. Of course genital abnormalities may occur apart from twinning, so that the possibility exists that in a sufficiently large collection of twins an individual might be found with genital abnormalities not due to its association with a twin. In this connection Numan ('44) notes that malformation of the sexual organs causing infertility had not yet been observed in single born heifers in his experience; but, in single born males, individuals with incomplete formation of the organs of reproduction had

female similar to one of my own cases (no. 44), and hence no exception; it is discussed *in extenso*, p. 413 of the present paper.

Numan also states, "In two-sexed twins the malformation is not confined exclusively to the heifer; but may also occur in the case of the bull, in which case the heifer is normal. However such examples appear to be very rare." This also refers to a single case viz., that of a two year old bull sent him by a veterinarian who stated that it had been born twin to a female. According to the veterinarian there was not the least abnormality discernible in the external organs of the heifer; the internal anatomy was not studied. The bull exhibited a hypospadiac condition; it possessed a split scrotum and the testes were in the abdominal cavity against the inguinal rings. Numan himself points out that such abnormalities are not rare in bulls born single. Since the actual condition of the female twin was not known, and the condition in the bull is a rather common anomaly the evidence is entirely inadequate to support the idea that the condition was due to twinning.

Numan's emphasis of these doubtful cases appears to be due in part to his fundamental objection to considering free-martins as hermaphrodites. He classified them therefore either as females or as males with defective organs of reproduction; in two-sexed twins the female was classified as male if the modification of the reproductive organs proceeded beyond a certain degree, thus establishing in his mind the occurrence of a profound genital anomaly in one individual of exceptional male pairs; this seemed to render probable a similar occurrence in the opposite case of female twins, which may seem to account for his uncritical acceptance of the case cited above, and his equally uncritical interpretation of modification of the bull as due to twinning with a female.

The entire basis for Numan's statements concerning exceptions to the rule is untrustworthy, and the exceptions cannot be accepted seeing that they are supported by no other writers.

often been found. This observation is of interest in connection with the problem stated in footnote 5, p. 389.

The theoretical interpretation of the free-martin must be based on one of two assumptions, either (1) that it and its partner, are identical, i.e., monozygotic twins, or (2) that they are fraternal, i.e., dizygotic twins. Under the first assumption, as the sex of monozygotic twins is undoubtedly identical, the free-martin would necessarily be interpreted as a modified male. Under the second assumption it would be almost equally a matter of necessity to interpret the free-martin as a female, for there is no possibility that the association of two males *in utero* should cause the transformation of one of them into a free-martin. On the first assumption the explanation of the modification must be found in the twinning process itself, i.e., in the division of the single zygote that *ex hyp*. formed the two twins. But on the second assumption no one has hitherto attempted to explain how the association of male and female *in utero* could lead to sterility of the female with a more or less pronounced male organization of the internal organs of reproduction, nor why certain females should escape the defect, nor why the phenomenon should be peculiar to cattle. It is, therefore, natural that the first assumption should have been the one followed in all previous theoretical interpretation of the free-martin, and that it formed the working hypothesis with which my own work began.

The first theoretical view that we shall consider is that of Spiegelberg ('61) who said of cattle twins: "If the twins are both female or of opposite sex, the organs of reproduction are as a rule well formed; if they are both male, it very frequently happens that one of them is an hermaphrodite." This conclusion was based on the examination of two pairs of different sexed twins in cattle; he made anatomical examination of the free-martins and found one of them a normal female, but in the other the female internal organs of reproduction were mostly absent, and were replaced by rudimentary seminal vesicles, rudimentary vasa deferentia, and a rudimentary gonad on one side which he interpreted as probably a small testis with more or less separated epididymis. The internal organs were accepted

as diagnostic of sex, and the case was interpreted as simple male
transverse hermaphroditism.

D. Berry Hart ('10) also interprets the free-martin as a male,
basing his interpretation on a comparison of the anatomical de-
scriptions of thirty cases given in the literature, and on an origi-
nal histological examination of the gonads of John Hunter's
specimens which had been in alcohol for one hundred and forty
years.

The special fact that emerges is that all the sexual glands are testes
in Hunter's cases, that adjacent structures are epididymis, and that
in none of the sexual glands are ova present.   The characteristic tes-
ticular tissue is in the form of tubuli semeniferi, and in only one are
spermatozoa present.   It seems to me, therefore, fully established that
the free-martin, when the co-twin is a potent male, is a sterile male, and
not a sterile female, i.e., they are identical male twins except in their
genital tract and secondary sexual characters.

It will be observed that Hart accepts the conditions of the
internal organs, and more especially of the gonad, as decisive
criteria of sex.   This raises a point that we shall discuss later on.
Continuing, Hart then proposes a 'Mendelian' theory as follows:
He distinguishes potent and non-potent elements in the genital
tracts of both sexes, the latter being the undeveloped rudiments
of the opposite sex; in the twinning process of a male zygote he
supposes we may either get identical male twins, or "the potent
and non-potent complex of the genital organs may be divided so
that the potent part goes to the potent bull calf, the non-potent
to the free-martin."   More specifically, "A free-martin with a
potent bull twin is the result of a division of a male zygote, so
that the somatic determinants are unequally divided, the po-
tent going to one twin, the potent bull, the non-potent genital
determinants to the free-martin."   He supposes the potent organs
to be dominant in the Mendelian sense, the non-potent recessive.

The entire argument is based on the unsupported assumption,
which it is quite possible to decide definitely by the facts, that the
free-martin is co-zygotic with its male mate.   I shall show imme-
diately that this is not the case; so that it is hardly necessary to
point out that if the gonad of the free-martin is a testis, as Hart

maintains, it can hardly be classed as a non-potent part of the genital tract, i.e., by definition undeveloped parts of the opposite sex, nor can the vasa deferentia of the free-martin be so classed; nor yet the external genital parts which are usually pure female. The theory moreover implies that as the free-martin receives the 'non-potent' genital parts, the bull twin must lack them, and must continue to propagate male individuals lacking them, for neither of which deductions is there the slightest evidence, or any attempt to produce evidence. Finally, there is not any attempt to explain why the twinning process should be attended by such extraordinary results in cattle, and involve nothing of the kind in other mammals. Hart notes that the theory implies the possibility of a similar defect in the twinning of a female zygote, and he refers to Numan's case cited before (footnote 2) as an example.

Bateson ('13, pp. 44–45) also attempts an explanation of the free-martin on the basis that it is co-zygotic with its twin,

For it is impossible to suppose that mere development in juxtaposition can produce a change of this character. It is conceivable that we should interpret it by reference to the phenomenon of gynandromorphism, seen occasionally in insects, and also in birds as a great rarity. In the gynandromorph one side of the body is male, the other female. A bullfinch for instance has been described with a sharp line of division down the breast between the red feathers of the cock on one side and the brown feathers of the hen on the other. In such cases neither side is sexually perfect. If the halves of such a gynandromorph came apart, perhaps one would be a free-martin.

The interpretations of Hart and Bateson are based on the theory that the free-martin and its twin are monozygotic, and they involve the conclusion that the free-martin is derived from a male zygote. They may be called anatomical interpretations because they are based exclusively on anatomical evidence. Cole has also come to the same conclusion on statistical grounds, which I discuss in the second part (pp. 380).

The literature on this subject is scanty, and it would serve no good purpose to continue with the few incidental citations that might still be made. Embryological evidence that would alone give a basis for correct interpretation has been entirely lacking;

my principal work has been to secure this evidence, which is herewith presented. The order of presentation is decided by the theoretical considerations, which obviously require that we should first of all determine whether we are dealing with a phenomenon of division of a single zygote, or with development of two zygotes in juxtaposition. Other considerations follow immediately from this, and the anatomical side is considered last. In conclusion the more fundamental theoretical questions come up for consideration.

II. ARE THE STERILE FREE-MARTIN AND ITS MATE MONOZYGOTIC OR DIZYGOTIC?

The first question that confronts us, therefore, is whether the free-martin and its male twin are monozygotic or dizygotic? This question can be answered decisively only by the embryological data; contributing evidence may be furnished by study of the degree of resemblance of the twins to one another, and by a statistical study of the sex ratios in twin births in cattle.

1. The embryological evidence

There are two ways in mammals of deciding whether twins are monozygotic or dizygotic: 1) If there is a single chorion for both of the twins, this would usually be regarded as evidence of monozygotic origin. But a single chorion is not decisive evidence, because, though one would expect monozygotic twins to have a single chorion, yet it is theoretically possible that two separate chorions may fuse to form one. The monochorial condition may be primary or secondary. 2) The number of ova concerned in a pregnancy may be ascertained by the number of corpora lutea, which correspond accurately. If we should find two corpora lutea for all free-martin twins, the dizygotic origin would be proved, provided that the rule holds for cattle.

I have had the opportunity of examining a large number of twin pregnancies in cattle through the courtesy of Messrs. Swift and Company of the Chicago Stockyards; and I wish to express my appreciation of their generous coöperation, without which this study could not have been made. The superintendent of

the cattle-house kept watch for uteri containing twins, and when they were not of too large size notice was telephoned to the department of Zoology of the University, and our collector, Mr. Adams, went over and brought the specimens to the laboratory. The collection has been going on for two years and a half, and 55 pairs of twins have been studied. For a long time most of the uteri were received with one or both ovaries missing, but recently special pains have been taken to secure the ovaries also attached to the uterus. A large proportion of the earlier records are therefore incomplete in this respect.

It was a great surprise to find that nearly all twins of cattle are monochorial; only two complete exceptions have appeared in 55 cases. The first case (no. 40) is of great theoretical interest, and will come up for detailed consideration later on. In a very few other cases the connection between the two halves was slight (see cases 8, 9, 10, 24, in table); generally it was broad and strong. At first I had expected to decide the question of monozygotic or dizygotic condition by the monochorial or dichorial state; so that for a considerable period not much attention was paid to the question of the corpora lutea. Later attention was directed to this question and the unexpected result was reached that in all cases in which both ovaries were present each had a corpus luteum. The exact data are: 22 cases in which both ovaries were present; in all of these there was a corpus luteum in each ovary; 7 of these were ♂ ♂, 4 ♀ ♀, 10 ♂ ♀, and one too young for sex diagnosis; 11 cases in which only one ovary was present, 9 of which had the corpus luteum present, and 2 absent; the two latter were same-sexed, one pair of males, and one of females; they may have been monozygotic, but the missing ovary may have contained two corpora lutea in each case. In 22 cases both ovaries were missing or not recorded. If we consider only those cases, 22 in number, in which both ovaries were present, there is no exception to the rule that cattle twins are dizygotic, using the corpus luteum as evidence for a separate zygote. This is a sufficiently large number to make it certain that the occurrence of monozygotic cattle twins is at least extremely rare. The free-martin condition cannot possibly be interpreted as a result of monozygotic twinning.

It may, however, be objected that we are relying on a rule which has not been proved for cattle, viz: that in a single pregnancy only one corpus luteum is present. To settle this question examination was made of 81 uteri each containing a single calf; in 45 of these both ovaries were present, and in every case only a single corpus luteum was found; in 36 cases one ovary only was present and 18 of these contained a single corpus luteum each, the other 18 lacked a corpus luteum. There can, therefore, be no doubt that the rule holds for cattle. This conclusion necessitates the inference that the monochorial condition of cattle twins is secondary. In a later section we shall consider the question of the probable time and nature of the fusion of the two chorions.

The conclusion drawn from the embryological evidence that the heterosexual cattle twins are dizygotic is supported by two other important considerations, viz: lack of close resemblance between the bull and heifer of such pairs, and by the sex ratios of all cattle twins.

2. Concerning the degree of resemblance of such twins

A careful study of this point remains to be made; but it is noteworthy that no one has recorded resemblances similar to those of identical twins in the case of the free-martin and its bull mate. My own somewhat limited observations lead me to the conclusion that the resemblances are no closer than ordinary fraternal likeness. One striking case was recently observed by me in pure bred Holstein-Friesian cattle in which the free-martin was about half black and white in patches, and the male was almost entirely black except its forehead and legs below the knees.

3. Sex-ratio of cattle twins

On this point we have observations by Cole and myself. Cole ('16) finds in a study of records of 303 multiple births in cattle that there were 43 cases male twins, 165 cases two-sexed twins (male and female), 88 cases female twins, and 7 cases of triplets. This gives a ratio of about 1 ♂ ♂ : 4 ♂ ♀ : 2 ♀ ♀ for the twins, instead of the expected ratio of 1 : 2 : 1. Cole then states:

The expectation may be brought more nearly into harmony with the facts if it is assumed that in addition to ordinary fraternal (dizygotic) twins, there are numbers of 'identical' (monozygotic) twins of both sexes, and that while in the case of females these are both normal, in the case of a dividing male zygote, to form two individuals, in one of them the sexual organs remain in the undifferentiated stage, so that the animal superficially resembles a female and ordinarily is recorded as such although it is barren. The records for monozygotic twins accordingly go to increase the homosexual female and the heterosexual classes, while the homosexual male class in which part of them really belong, does not receive any increment.

Cole thus tentatively adopts the theory, which has been worked out most elaborately by D. Berry Hart, stated also by Bateson, and implied in Spiegelberg's analysis ('61), that the sterile free-martin is really a male co-zygotic with its mate.

Cole's figures represent the only statistical evidence that we have previously had on this subject. Let us follow his suggestion and take from the two-sexed class enough cases to make the male twins equal in number to the female pairs; this will be approximately one-fourth of the class, leaving the ratio 2:3:2 instead of 1:4:2. Which one of these is the more satisfactory sex ratio I leave others to determine; I wish only to point out the fatal objection, that according to the hypothesis, the females remaining in the two-sexed class are normal; in other words, on this hypothesis the ratio of normal free-martins (females co-twin with a bull) to sterile ones is 3:1; and the ratio would not be very different on any basis of division of the two-sexed class that would help out the sex ratio. Hitherto there have been no data from which the ratio of normal to sterile free-martins could be computed, and Cole furnishes none. I have records of 24 cases statistically homogeneous, 3 of which are normal and 21 abnormal. That is, the ratio of normal to sterile free-martins is 1:7 instead of 3:1.

This ratio is not more adverse to the normals than might be anticipated, for breeders' associations will not register free-martins until they are proved capable of breeding, and some breeders hardly believe in the existence of fertile free-martins so rare are they.

My own records of 55 cases of bovine twins, all examined *in utero*, and their classification determined anatomically without the possibility of error, give 19 ♂ ♂ : 24 ♂ ♀ : 11 ♀ ♀ and 1 (no. 49) too young for determination. It will be observed that the sum of the one-sexed classes is 25 per cent greater than the two-sexed class; and the ♂ ♂ class is much larger than the ♀ ♀ class instead of being equal to it, as it should be if males and females are produced in equal numbers in cattle. The material cannot be weighted statistically because every uterus containing twins below a certain size from a certain slaughter house is sent to me for examination without being opened.[4] Cole's material shows twice as many female as male pairs, and the two-sexed class is about one-third greater than the sum of the other two classes. I strongly suspect that it is weighted statistically; the possibility of this must be admitted, for the records are assembled from a great number of breeders. But, whether this is so or not, if we add the sterile free-martin pairs of my collection to the male side in accordance with Cole's suggestion, we get the ratio 40 ♂ ♂ : 3 ♂ ♀ : 11 ♀ ♀, which is absurd. And if we take Cole's figures, divide his heterosexual class into pairs containing sterile females and pairs containing normal females according to the expectation, 7 of the former to 1 of the latter, and add the former to his male class, we get an almost equally absurd result (186 ♂ ♂ : 20 ♂ ♀ : 88 ♀ ♀). On the main question our statistical results are sufficiently alike to show that the free-martin must be interpreted as female.

Prof. Alexander Graham Bell has kindly furnished me with a catalogue of the lambs born from 1890 to 1914 in his well-known experiments on his Beinn Bhreagh Estate in Nova Scotia from which I have taken all the records of twin births, 139 in number;

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[4] The great preponderance of the ♂ ♂ over the ♀ ♀ class in foetal cattle twins of the sizes dealt with in this study appears to be real, though it must be admitted that the numbers are too small to make this quite certain. Cole's data on the other hand indicate a great preponderance of the ♀ ♀ class over the ♂ ♂ class in cattle twins after birth. It may be that abortion, which is so frequent in cattle, is even more adverse to the males in the case of twins than in single births; it is conceivable that the difference is largely a question of viability, but other explanations are possible.

of these 38 were ♂ ♂, 67 ♂ ♀ and 34 ♀ ♀, thus an exceedingly close approximation of the expected 1 : 2 : 1 ratio. In his "Problems of Genetics" Bateson cites Bernadin (La Bergerie de Rambouillet, 1890, p. 100) as to the frequency of twin combinations in Merino Sheep, viz: 87 ♂ ♂ to 187 ♂ ♀ to 83 ♀ ♀, which also approximates the expected 1 : 2 : 1 ratio. These statistics, therefore, also support the interpretation of the free-martin as female, for they show that the actual ratios of the distribution of sex among twins are as a matter of fact the expected ones in ungulates.

On the other hand in man there is a very significant and interesting departure from the expected ratio: Simpson ('44) collected statistics of 788 cases of human twins, the various sex combinations being ♂ ♂ 229, ♂ ♀ 298, ♀ ♀ 261, thus very far removed from the 1 : 2 : 1 ratio. Nichols ('07) has made a very much larger collection of statistics with the following ratios ♂ ♂ 234,497, ♂ ♀ 264,098, ♀ ♀ 219,312. It is obvious that there is a very large disturbing factor here; this is almost certainly the factor of monozygotic twinning. As the two-sexed combination must be dizygotic, we may estimate the dizygotic ♂ ♂ and ♀ ♀ combinations at one-half of the ♂ ♀ combination, on the slightly inaccurate basis of a 1 : 1 sex ratio of male and female zygotes. This would give 132,049 dizygotic ♂ ♂ and ♀ ♀ twin pairs each, and the excess viz: 102,448 ♂ ♂ and 87,263 ♀ ♀ would represent the monozygotic twin couples. This is not very far from the proportion of monozygotic twin pairs among one-sexed twin couples estimated by physical resemblance. If, then monozygotic twinning is the disturbing factor in the unexpected sex-distribution ratios of human twins, we may argue from the fact that the ratios in cattle and sheep approach expectation that monozygotic twinning either does not occur, or is very rare in them, and this is confirmed by the embryological evidence. This matter is discussed more fully in Newman's book on twins ('17) to which the reader is referred.

4. Discussion

The preceding considerations constitute an argument that the free-martin is zygotically female, which may be summarized as follows: 1) The only basis on which it could be logically interpreted as male is that it is co-zygotic with its male mate, because it is impossible to suppose that the association of two males *in utero* should cause the transformation of one of them into a free-martin in a certain definite proportion of cases. But we have seen that the free-martin and its male mate arise from separate zygotes. From this point of view the free-martin must be interpreted as zygotically female. 2) The somatic resemblances between the free-martin and its mate are not of the order of identical twins. 3) The assumption that the free-martin is male leads to an absolutely incomprehensible sex-ratio, while the interpretation that it is female. comes nearer fulfilling the expected sex-ratio. From this point of view also the free-martin is female.

The only argument that remains for its male nature rests on the anatomy of the internal organs of reproduction, which unquestionably are more or less of the male type. But, as the external genitals and the mammary gland are almost invariably of the female type, the argument from anatomy may be made to turn either way depending on what anatomical characters are recognized as diagnostic of sex. In a later section we shall go fully into the anatomical problems involved. Here it may suffice to say that the anatomical argument is necessarily inconclusive.

In what follows, therefore, we shall treat the free-martin as demonstrated to be zygotically female, and the question becomes how the association of a male and female *in utero* may so transform the female.

List of cattle twins examined in utero

NUMBER	SEX			SIZE	CHORION	MATERNAL OVARIES
	♂	♀	⚥			
1	2			35 cm. each	Single	Not observed
2	1		1	♂ 23 cm. ⚥ 21.5 cm.	Single	Not observed
3	2			25.5 cm. 26.5 cm.	Single	Not observed
4	1		1	♂ 24 cm. ♀ 22.5 cm.	Single	Not observed
5		2		17 cm. each	Single	Not observed
6	1		1	♂ 16.8 cm. ♀ 16.3 cm.	Single	Only one ovary present. Contains corpus luteum
7	1		1	About 20 cm.	? (See note 1)	Both ovaries present. Corpus luteum in each
8	1	1		♂ 26.5 cm. (wt. 2 lb., 6 oz.) ♀ 23.3 cm. (wt. 1 lb., 6.5 oz.)	Single, narrow connection	Ovaries absent
9	1	1		♂ 20 cm. ♀ 20 cm.	Single, narrow connection	Ovaries absent
10		2		23 cm. each	Nearly separated; united by narrow strand	Ovaries absent
11		2		17 cm. 16 cm.	Single	Ovaries absent
12	1		1	♂ 30 cm. (2 lb., 10.5 oz.) ⚥ 28 cm. (2 lbs., 13.5 oz.)	Single	Ovaries absent

NUMBER	SEX			SIZE	CHORION	MATERNAL OVARIES
	♂	♀	⚥			
13	1		1	♂ 20 cm. (14 oz.) ⚥ 20 cm. (14 oz.)	Single[2]	Ovaries absent
14	1		1	♂ 27 cm. ⚥ 27 cm.	Single	Ovaries absent
15		2		17.5 cm. 18 cm.	Single	Both present. Corpus luteum in each
16	2			13 cm. 12.75 cm.	Single[3]	Ovaries absent
17	1		1	♂13.75 cm. ⚥ 13.1 cm.	Single	One missing, other had 1 corpus luteum
18	2			12.5 cm. 13 cm.	Single	Ovaries absent
19	1		1	♂ 8 cm. ⚥ 7.5 cm.	Single	Ovaries absent
20	2			20.5 cm. 20 cm.	Single	One ovary absent. Other contains corpus luteum
21	1		1	♂ 27.5 cm. ⚥ 26.5 cm.	Single	Ovaries absent
22	1		1	♂ 24.5 cm. ⚥ ?	Single[4]	Both ovaries present. Corpus luteum in each
23	1		1	♂ 18 cm. ⚥ 17.5 cm.	Single	One ovary absent. Other has 1 corpus luteum
24	2			21 cm. each	Single; narrow connection	Both ovaries present. Corpus luteum in each
25	2			11.2 cm. each	? Injured	One ovary absent. Corpus luteum in other
26	1		1	♂ 12.5 cm. ⚥ 12.25 cm.	Single	One ovary absent. Other has 1 corpus luteum

NUMBER	SEX			SIZE	CHORION	MATERNAL OVARIES
	♂	♀	♀̣			
27	2			42.5 cm. each	Not examined	One ovary absent. Other has 1 corpus luteum
28	2			17 cm. each	Single	One ovary absent. Other has 1 corpus luteum
29	2			15 cm. each	Single	Both ovaries present. Corpus luteum in each
30	2			23 ᴄm. 22 cm.	Single	One ovary absent. No corpus luteum in other
31			2	12 cm. each	Single	Both ovaries present. Corpus luteum in each
32	1		1	♂ 18 cm. ♀̣ 16.75 cm.	Single	Both ovaries present. Corpus luteum in each
33	2			10.5 cm. each	Single	Both ovaries present. Corpus luteum in each
34			2	25 cm. (about)	Single	Both ovaries present. Corpus luteum in each
35	2			13 cm. each	Single	Both ovaries present. Corpus luteum in each
36	1		1	♂ 18 cm. ♀̣ 17.5 cm.	Single	Both ovaries present. Corpus luteum in each
37	1		1	♂ 16 cm. ♀̣ 15.5 cm.	Single	Both ovaries present. Corpus luteum in each
38	1		1	♂ 23.5 cm. ♀̣ 22.5 cm.	Single	Both ovaries present. Corpus luteum in each
39	2			18 cm. each	Single	Both ovaries present. Corpus luteum in each
40	1	1		♂ 10.4 cm. ♀ 10.2 cm.	Two separate chorions	Both ovaries present. Corpus luteum in each
41	1		1	♂ 22.7 cm. ♀̣ 21.8 cm.	Single	Both ovaries present. Corpus luteum in each

NUMBER	SEX			SIZE	CHORION	MATERNAL OVARIES
	♂	♀	⚥			
42—	se	e	N	ote 5		
43	2			10.75 cm.	Single	Not observed
44—	se	e	N	ote 5		
45			2	34 cm. 31.25 cm.	Single	Both ovaries present. Corpus luteum in each
46	2			23 cm. (about)	Almost separate	Both ovaries present. Corpus luteum in each
47	1		1	♂ 22.75 ⚥ 22.25 cm.	Single	Both ovaries present. Corpus luteum in each
48			2	20 cm. (about)	Single	One ovary absent. No corpus luteum in other
49	?	?		1.5 cm. each	Separate, but overlapping	Both ovaries present. Corpus luteum in each
50	1		1	♂ 21 cm. ⚥ 21 cm.	Single	Both ovaries present. Corpus luteum in each
51	2			5 cm. each	Single	Both ovaries missing
52			2	18 cm. 19.5 cm.	Single	One ovary absent. Single corpus luteum in other
53			2	19 cm.	Single	Both ovaries absent
54	2			20.5 cm. 21 cm.	Single	Both ovaries present. Corpus luteum in each
55	2			13.75 cm.	Single	Both ovaries absent
56			2	12.5 cm.	Two chorions separate	Both ovaries absent
57	1		1	♂ 19.25 cm. ⚥ 18 cm.	Single	Both ovaries absent

[1] Case 7 was received in my absence, and the entire uterus was placed in formalin; preservation of its contents was bad, and condition of chorion must be recorded as doubtful.

[2] Case 13 uterus injured by butcher; chorion cut in two.

[3] Case 16 uterus injured by butcher; chorion cut in two.

[4] Case 22 uterus injured by butcher; chorion cut in two.

[5] Cases 42 and 44 are not included because they were selected heterosexual pairs taken after birth.

III. THE TIME OF FUSION OF THE TWIN CHORIONS AND THE DE-VELOPMENT OF THE VASCULAR ANASTOMOSES BETWEEN THE TWINS

In order to form an estimate of the probable time of fusion of the twin chorions it is necessary to present a few data concerning the development of the usual single chorion. Figure 1 shows the non-pregnant uterus of the cow partly dissected. It will be noted that the horns of the uterus open by constricted apertures into the small body. The blastodermic vesicle forms in the horn of the uterus on the same side as the ovary from which the ovum was derived, as I have observed in numerous cases. It grows out into a long strand-like sac extending both distally and centrally. The embryonic area forms near the center in the sheep (Bonnet) and presumably also in the cow. The growth of the strand-like vesicle in length is extraordinarily rapid, and it soon enters the body of the uterus centrally, and penetrates into the opposite horn. By the time that the embryo is 10 mm. long the vesicle has extended completely through the body of the uterus and far into the other horn (two cases observed); the embryo is thus excentrically placed in the very long vesicle. The allantois forms later than the blastodermic vesicle; it grows from the embryo both centrally and distally, and ultimately completely fills the blastodermic vesicle and occludes its cavity. In the case of an embryo of 19 mm. length the allantois had passed from the horn of the uterus containing the embryo well into the body of the uterus. In another case of an embryo of 21 mm. length the allantois had extended through the entire horn of the uterus opposite to that containing the embryo.

I have one case of a twin pregnancy in the cow in which the embryos were only 15 mm. long (no. 49). Unfortunately the

collector did not recognize the case as a twin pregnancy until
after the uterus had been opened and one foetus removed, thus
cutting the membranes; the other foetus was also removed in
the same way, and when the uterus and specimens reached me
for examination it was necessary to reconstruct the original
condition from the parts. This was, however, successfully
done, as the central end of the one chorion was found still in
place in the body of the uterus and extending into both horns.
In this one the chorion with contained allantois had passed the
body of the uterus. In the other the end of the chorion had been
drawn out of the uterus with the foetus, but measurement showed
that it also with the allantois contained had passed the body of
the uterus. The two chorions were thus not fused at this stage,
but they overlapped and were in closest apposition in the body
of the uterus. The conditions precedent to fusion were thus
fully established at this early stage long before sexual differen-
tiation begins.

Comparison of these twins with the stages of 19 and 21 mm.
described above indicates some variation in the degree of devel-
opment of the chorion relative to the length of the embryo.
But these stages demonstrate the possibility of fusion of twin
chorions a considerable time before the stage of beginning sex-
differentiation, which I estimate at about 25 mm. Vascular
anastomosis between the twins is possible as soon as the allantoes
from the two sides meet, or even earlier, because after the allan-
tois has once fused to the chorion the blood-vessels tend to
spread out more or less in the chorion beyond the area of fusion.

Owing to the extreme difficulty of obtaining early stages of
twin pregnancies in cows the next earliest stage that we have
(no. 51) is a case of male twins 5 cm. long in which there is no
evidence of the place of fusion of the twin chorions, and there
is a perfect vascular anastomosis between the two sides. Fusion
is already perfect and any overlapping parts have entirely dis-
appeared. The next case is a two-sexed pair (no. 19) in which the
male foetus was 80 mm. and the female 75 mm. long. The twin
chorion was single with a broad connection provided with
cotyledons between the two halves; no evidence of the place of

fusion of the twin chorions remained.   The urinogenital system of the female, described in section 4, was already definitely of the sterile free-martin type.   The inference is, therefore, that fusion had taken place some time previously in order to account for the completeness of the fusion and the transformation of the reproductive system of the female.

In the case of twin pregnancies in cattle, therefore, the two vesicles starting in opposite horns of the uterus will meet in the body of the uterus before the 10 mm. stage; the allantoes of the two vesicles will not however meet until about the 15 mm. stage, and the opportunity for vascular anastomosis therefore dates from this time.

Bonnet ('89) describes a very early twin pregnancy of the sheep, which confirms in the strongest way my conclusion concerning the early time of fusion of twin chorions in ungulates. His description is of so much interest that I quote it entire, he describes a pair of sheep twins 6 mm. long, secured 18 days and 6 hours after copulation,

deren serösen Hüllen an den sich berührenden Enden auf eine Strecke von 6 cm. in einander eingestülpt und verklebt, aber noch nicht verwachsen waren. Sie liessen sich vielmehr noch leicht auseinander-lösen.   Beide Eier maassen zusammen vom freien Ende des einen bis zum freien Ende des anderen 35 cm. und waren *in maximo* 1.5 cm. weit. Die Kürze der Eier ist eine im Vergleiche zur Länge einzelner Eier in diesem Stadium auffallende; sie betrug bei einem 15, bei dem anderen 17 cm.   Wahrscheinlich behindern sich die bald einander mit den Spitzen berührenden Eier einigermaassen in der sonst normalen Längenentwicklung.

It will be noted that in this case the ova met at their apices and invaginated one another, and that the stage of such union was only 6 mm.   The sheep's uterus is of precisely the same type as the cow; fusion follows the union of the ova in the sheep as in the cow; but vascular anastomosis does not occur in the sheep, as I describe in detail later on, and for this reason the female of two-sexed twins remains unaffected in the sheep.

We have already referred frequently to the vascular anastomoses between twin foetuses of the cow, and it is now time to describe the matter fully.   The working hypothesis with which

the investigation began was that the free-martin and its twin
were monozygotic, and it was not until after 27 cases had been
examined that I was convinced that they were dizygotic.   The
real explanation of the phenomenon then for the first time be-
came evident.   No vascular injections were therefore made
during the first part of the investigation, and the evidence for
vascular anastomosis among these rests upon incidental obser-
vations, the significance of which was not realized at the time.
Relatively few of the twin ova received thereafter were in a fit
state for complete injections.   Of the 28 cases involved injections
were made only in seven cases; two of which will be described in
detail below.   But in 21 of the 28 cases vascular anastomosis
could be satisfactorily demonstrated either in the uninjected
chorions or in injections.   Some of the uninjected cases were
just as demonstrative as though they had been injected.   In
four cases of the remaining eight there was no anastomosis;
one too young (no. 49); the second was a case of normal male
twins in which the connection between the two chorions was
merely a narrow band-like connection (no. 46); the third was a
case of male twins with entirely separate chorions (no. 56);
the fourth was another case of completely separate chorions (no.
40) of the greatest theoretical interest because one foetus was
male and the other a normal female.   Finally there were three
cases with inadequate records.   Eliminating these three we
have 25 cases, in 21 of which, including the three possible twin
combinations, vascular anastomosis could be demonstrated and
4 in which it was absent (nos. 40, 46, 49, and 56).

This is not, however, the only evidence that more or less com-
plete vascular anastomosis between the pairs is the rule in cattle
twins.   I can distinctly remember the continuity of the thickened
chorionic band that carries the main arteries as the rule in the
first 27 cases, and this was recorded in certain cases in my
notes.

There cannot be the least doubt that in bovine twins fusion
of the chorions usually occurs and is followed by anastomosis of
the blood vessels of the two sides, and that intermixture of the
blood of the two foetuses results.   Nor can it be doubted that

rarely this does not occur either because the chorions fail to fuse
(cases 40 and 56) or because a slender connection is not vascu-
larized (case 46). The significance of the exceptions is very
great.

The nature and extent of the vascular connections may now be
illustrated by a detailed study of two cases. 1. Case no 33
Males, 10.5 cm. long (figs. 2 and 3). The entire arterial system of
both chorions was injected from one umbilical artery of one
partner; the mass easily passed the constriction between the
two halves of the chorion, and penetrated even into the umbili-
cal arteries of the other; every cotyledon was injected on both
sides. The venous system was also injected from one of the
umbilical veins of the same specimen; the injection mass also
passed the constriction far into the chorion of the opposite
side, but the blood present in the veins prevented as complete
an injection of the veins as of the arteries.

The two umbilical arteries of each foetus have a cross connec-
tion at the distal end of the umbilical cord, so that an injection
from one artery outwards flows both centrally and distally. The
two veins lack such an anastomosis.

The arterial anastomosis (fig. 3). The main artery from the
right of the drawing divides in three branches 1, 2, and 3 as it
approaches the center. Branch 3 need not be farther considered
as it does not anastomose with the opposite side. Branch 1 can
be followed directly through into communication with the arterial
system of the other side, branch 2 has a strong anastomosis with
the through trunk *1-1* (at *1-2*), but branches for the most part
within its own venous territory. The side branches of the
through trunk *1-1* are of considerable interest, inasmuch as some
are oriented in the direction of the blood flow from the right, and
others from the left. Thus following the trunk from the right
the first branches that we meet are directed against the blood
stream coming from this direction (*1a*); immediately after pass-
ing the anastomosis (*1-2*) we meet a branch *1b*, directed with
the blood flow from the right; the next two branches, *1c* and *1d*,
are directed similarly, but the large branch *1e* immediately
beyond has the reverse orientation. If we suppose the blood

flow to come from the left *1e* is directed with the current, *1d*, *1c*, and *1b* against it, etc. The orientation of these branches seems to indicate an alternation in direction of flow in the main trunk, as is to be expected with a beating heart at each end of it. It is significant that this is the only place in the arterial system of the membranes where reversal of orientation of branches is found in the course of a single trunk.

The venous anastomoses. The venous anastomoses are two in number (*4* and *5* in figure 3). The larger one *4* was on the opposite side from which the drawing was made, and is therefore represented as a broken line; *5* comes from the same main venous stem. There is no reversal of orientation of side branches.

The circulation. It is obvious that any arterial blood that is pumped by either foetus into the capillary system situated beyond about the line *A–B* will be taken up by the venous system to the other foetus. There must therefore be a constant interchange of blood between the two foetuses, which, considering the size of the arterial intercommunications, must be very considerable. The venous anastomoses are not significant for the intermingling of the two circulations. The direction of flow along the main arterial trunk (*1–1*) will depend on the blood pressure on the two sides. If for any reason an excess of blood is received by one of the two foetuses, this will have a tendency to raise the blood pressure on that side and thus to equalize the distribution. There is of course the possibility that the beat may alternate on the two sides, but nothing is known of this, and the effect of such an arrangement would not be easily deduced.

2. Case no. 47. ♂ 22.75 cm. and ♀ 22.25 cm. (fig. 4). Arteries injected yellow; veins blue. The injection was made first into an umbilical artery and vein of the male. The arterial injection flowed regularly into the opposite chorion and through to the free-martin; the venous injection also flowed into the opposite chorion, but not so freely. The injection was then completed from an umbilical artery and vein of the free-martin in order to fill the vessels on this side more completely.

The arterial anastomosis is a single strong vessel, the relationships of which are shown clearly in the figure and require no further description. The stage is much more advanced than the preceding case, and the cotyledons are much more developed. Most of the arterial branches are distributed directly to the cotyledons. The venous anastomosis is much less viable than the arterial; macroscopically it consists exclusively of a connection between the two veins of one cotyledon (*2*, fig. 4) one of which returns to the male side and the other to the side of the free-martin. This is the only cotyledon that appears to be connected with the umbilical veins of both sides; therefore any other venous anastomisis must be through the capillary circulation of the extra-cotyledonary chorion if it exists.

The circulation in this case must be according to the same principles as in the preceding: whenever the arterial pressure is higher on one side than the other blood must be distributed from the side of higher pressure to that of the lower pressure; it will thus reach the veins and the foetus of the opposite side; variations in pressure on the two sides must constantly occur, if there is any difference in the time of occurrence of systole and diastole of the twin hearts. The blood of the twins must therefore intermingle intimately, and internal secretions of either must reach the other.

These cases adequately illustrate the time and nature of the vascular anastomosis; we may therefore turn to the question of duration of the intermingling of the blood during foetal life.

We have seen that the vascular anastomosis probably begins at the stage of about 19–20 mm. The two cases we have considered in detail indicate a strengthening of the arterial anastomosis, and a weakening of the venous anastomosis after a certain stage as development proceeds. This is to be expected because the arterial flow is stronger and toward the center, whereas the venous flow is slower and away from the center. The circulation itself tends therefore to strengthen any primitive arterial connection, and to diminish relatively any venous connection. Moreover as development proceeds the cotyledons increase in size, and the intercotyledonary circulation in the chorion becomes

correspondingly reduced in a relative sense with the result that
the prominent arteries and veins become exclusively cotyledon-
ary with the single exception of the artery connecting the two
sides; and any intercotyledonary venous connections become
insignificant.

It is an important question whether this condition persists
throughout foetal life, even though completely sterilizing effects
on the female reproductive system are produced by the stage of
7.5 cm., as we shall see in more detail in another section. The
question therefore relates to possible influences on later stages
of the female reproductive organs, and on the somatic characters
of both twins. The latest stage that I have examined with
reference to this question was a pair of female twins, 35.3 cm.
and 31.25 cm. in length respectively. The arterial connection
was even stronger than in earlier stages proportional to the
more advanced stage of development. There is no reason to
suppose that the connection is interrupted until birth, but the
actual observations have not been made.

Thus the available records indicate a growth of the arterial
anastomosis throughout foetal life and a consequent duration of
action of the male hormones up to the time of birth. The possi-
bility exists that in certain cases the connection may be inter-
rupted at different stages of development; but so far no such
cases have appeared. In any event the decisive effects on the
reproductive system of the female are determined very early
and they are presumably irreversible in their character.

Triplets occur rarely in cattle, and cases of even more young at
a birth are on record. Unfortunately records of their breeding
history appear to be very rare. The only one that I have been
able to discover is given by Pearl ('12). In this case there were
two females and one male. The females were kept until they
were about three years old, but they never came in heat. They
were then killed, and "The man that dressed them said that they
never would have bred. Neither uterus nor tubes were recog-
nized, but the vagina apparently ended at its anterior end as a
blind sac." Both were apparently sterile free-martins. The
male was put in service and got good calves. We have here,

therefore, in all probability a case in which the circulations of the three individuals anastomosed, and in which the male sterilized both females.

IV. THE HORMONE THEORY OF THE FREE-MARTIN

We may now proceed to a consideration of the argument for the hormone theory.   In our previous considerations we have dwelt upon the separate zygotic origin of the free-martin, and the foetal vascular connections; it is obvious that these conditions suggest a hormone theory; but, before such a theory could be regarded as demonstrated an explanation of the existence of fertile free-martins would need to be offered, and the limitation of the phenomenon of sterility of the free-martin to cattle as a common occurrence would have .to be explained; the possibility of the existence of sex hormones at such an early period of the foetal life would also need to be demonstrated, and reason for limitation of the sterilizing effect to the female is needed.

We shall consider first the fertile free-martin; three cases of a normal female twin to a male have been found in my 24 cases of bovine two-sexed foetal twins.   These are readily explained *a priori* on the hormone hypothesis on the supposition that they represent cases in which anastomosis of the foetal blood-vessels did not occur.   It is important to notice that such cases are exceedingly crucial, for if we should find a case of two-sexed bovine twins in which foetal vascular anastomosis was absent, and in which the female was nevertheless a sterile free-martin, the hormone theory would have to be abandoned.

The first two cases of fertile free-martins were nos. 8 and 9 of my series (figs. 7 and 8); they were collected before the hormone theory was formed and the records are incomplete.   In my notebook I had merely recorded that the connection between the two chorions of each pair was narrow; it was probably not vascular, but this cannot be certainly known, and these cases must be left out of consideration.   Fortunately the third case, no 40, is a veritable *experimentum crucis*.   In this case organic connection of the two chorions was entirely lacking.   The central ends of the two chorions merely overlapped in the body of the uterus, and fell

apart when removed; injection of the chorion of the male showed its circulation to be entirely closed. Dissection of the female showed its reproductive system to be perfectly normal (fig. 6); sections of the gonad showed it to be an ovary (Chapin, '17); each maternal ovary had a corpus luteum in it. Even though this case stands alone, it is obvious that it fulfills all the conditions of a radical experiment; so that we can say that foetal vascular anastomosis of two-sexed twins involves the sterile condition of the female, and absence of such anastomosis its fertile condition.

The sheep and other normally uniparous ruminants should furnish another test of the theory; for though twin births are fairly common in sheep the female of two-sexed pairs is usually normal. This is a matter of common experience among breeders, and is strikingly demonstrated by Prof. Alexander Graham Bell's well-known experiments (Bell '12) on the production of a multi-nippled race of sheep; 36 per cent of the lambs born on Professor Bell's farm were twins; and in 1912, 60 per cent of the lambs born from three year old ewes were twins; the records show that the twin ewes are used commonly for breeding purpose, which would not be the case if any considerable percentage were sterile. The fact that there is no reference in this very careful series of experiments to sterility of ewes from two-sexed twins would also show that such a phenomenon must be at least very uncommon. On the other hand Bateson states that it sometimes occurs among sheep; though, on what authority, I do not know.

In response to a letter of inquiry Wm. John G. Davidson who has had charge of the breeding operations at Dr. Bell's estate for a great many years writes:

I may say that in all my experience in sheep breeding I have yet to find a case where lambs born twin to males have turned out sterile. In fact when lambs are born twin male and female if they have the desired qualifications required in the flock both lambs would be retained in the flock and I have not had the slightest trouble with either male or female being unfruitful. I know there is nothing in the free-martin theory in sheep breeding.

It was therefore very interesting to examine twin pregnancies of sheep with reference to the relations of their membranes. I

found in the four cases, that I examined, that the twins were dizygotic (in one case both corpora lutea were in one ovary) and that the membranes were fused in the body of the uterus as in cattle. But when injections were made, as was done in all four cases, it was found that the circulation of each individual was entirely closed; the injection mass could not be forced from one side to the other, either through the arteries, or through the veins. Figure 5 gives a faithful representation of one case; it will be observed that the arteries and veins of each side end in a central neutral zone that they do not cross; this zone is no doubt occupied by capillaries, and it is possible that these anastomose from the two sides, though it is uncertain. The other cases were similar, though in one of them a single centrally placed cotyledon received an artery from each side; each artery was accompanied by its own strong vein returning to the same side, which indicated that there was little, if any, intermixture of blood in the cotyledon; the starch injection masses, yellow on one side and red on the other were not forced through.

In the sheep we have, then, all the necessary conditions for the production of sterile free-martins except the actual vascular anastomosis. If the vascular anastomosis should also occur exceptionally, such a condition should be accompanied by sterility of the female in the case of heterosexual pairs. This lends probability to the assertion that this condition actually occurs occasionally in sheep.

The hormone theory thus gives a satisfactory explanation of the occurrence of occasional fertile free-martins in cattle as well as of the usual condition of sterility of the free-martin; and it fits the case of the sheep equally well. As regards other ruminants we have unfortunately almost no information. But I have been much interested to find that the famous discoverer of the circulation of the blood, William Harvey, in his "*Exercitationes de Generatione Animalium*" 1651 has some statements on the subject of twin pregnancies in ruminants: thus in Ex. 69, p. 487 (Sydenham Society edition, translated by Willis), he says of the deer, "if the conception be double, one in either horn (of the uterus), each sends its umbilical vessels to its own

horn alone; the embryo in the right horn deriving nourishment from the right part of the conception, that in the left from the left portion of the same." He made similar observations on the sheep, goat, and "other bisulcated animals" and notes that "in the dog, rabbit, hog, and other animals that produce a considerable number of young at a litter, the thing is otherwise. In these each foetus has two humors, these being severally surrounded with their proper membranes." So far as I know there are no other published observations on the foetal membranes of twins in ungulates from Harvey's time to the present with the exception of Bonnet's single case already referred to. Harvey's observations show that fusion of chorions is wide spread in twin pregnancies in ungulates; but he states definitely that in the deer the umbilical vessels of each foetus are distributed to its own side only, in which it resembles the sheep. A more careful examination of the female of two-sexed twin pairs in these animals would be of interest in order to determine the possible sporadic occurrence of sterility.

The theory requires that if the same condition of common circulation of the foetal blood were to occur in other mammals as in twins of cattle the sterile free-martin condition should occur there also. Now in multiparous mammals such conditions certainly do not occur commonly; for, if they did, the very numerous researches on their embryology would have brought them to light. In the pig one can find occasional, but rare, fusions of adjacent chorions, but I have never found any vascular connection. A number of mammalian groups could be at once excluded from consideration because the conditions of placentation are such as to prohibit chorionic fusion; in mammalian groups such as primates and many rodents in which the ovum becomes embedded in the uterine mucosa, there is of course an insuperable bar to early chorionic fusion. And in those mammalian groups in which the placenta is a highly localized organ as in the remainder of the rodents, the insectivores, carnivores, and edentates the circulation in the chorion outside of the placental area is so restricted that, even if chorionic fusion did occur, it is difficult to believe that the circulation of separate foetuses would intermingle to any great extent.

Fernandez ('15) has described fusion of dizygotic chorions in one of the armadilloes (Dasypus villosus). This form has usually two young at a birth, which may be one-sexed or two-sexed. The two chorionic vesicles are separate at first, but they gradually fuse and by the time that the embryos are 3.5 cm. long the fusion of the two vesicles is so intimate that they appear as a single one. As this is the usual condition it cannot be supposed that it is accompanied by genital abnormalities. The author does not consider the problem of vascular anastomosis; but it can hardly be supposed to occur.

Although the hormone theory invokes a cause of the utmost generality in mammals, it is obvious that the conditions leading to its intervention must be restricted to forms with a relatively diffuse placentation, i.e., ungulates for the most part, and among these to forms in which quite special conditions obtain. Such conditions are found only in normally uniparous ungulates in which the ovum grows to an extreme length very rapidly, so that the associated ova meet at an early stage which favors their organic union. Even then vascular anastomosis is not likely to occur to any considerable extent unless the development of the foetal cotyledons is relatively late, so as to be preceded by a condition of general vascularization of the chorion, before the highly specialized circulation of the cotyledons becomes dominant. Such is the condition in cattle. In sheep the development of the cotyledons appears to be more precocious; I would at least venture this suggestion, although based on relatively few observations, to explain the difference between cattle and sheep in this respect.

Although these considerations give a color of great probability to the hormone theory we have still to deal with two difficulties:— 1) it is evident that we must be dealing with specific sex hormones, for their influence is limited to sex characters so far as our present evidence goes; is there any other evidence for such early production of specific sex hormones available? 2) It is certain that the intermixture of blood of the twins must be reciprocal, but the effect is exclusively on the female; in what way can this be explained?

Both of these difficulties receive a satisfactory explanation in certain previously known facts not hitherto correlated with the phenomenona in question, and in certain new facts which are described by Miss Chapin ('17). They constitute concurrent evidence which appears to me to render the entire evidence perfectly conclusive.

The previously known facts to which I refer are (1) the early development of the interstitial tissue of the mammalian testes, from the very beginning of sex-differentiation, and (2) the fact that the differentiation of the ovary is later than that of the testis, inasmuch as in the female of mammals the first generation of ingrowths from the germinal epithelium, a complete homologue of the seminiferous tubules of the male, forms only the medulla of the ovary, and the ovarian cortex is formed from a distinct second generation of ingrowths. Thus (1) interstitial tissue of the testis is present at the time for which male hormones are postulated, and (2) the testis has a start over the ovary in this respect which results in the suppression of specific ovarian tissue from the beginning as shown by Miss Chapin's study.

On the question of the embryonic origin of interstitial cells in the testes we have the excellent studies of Whitehead ('04) and Allen ('04) on the pig; Allen also deals to a certain extent with the interstitial cells of the ovary. Allen finds that sex differentiation is strikingly shown in the structure of the testis and ovary in embryos of 2.5 cm. length. The sexes cannot be sharply distinguished at 1.8 cm. length so that the initial stages of sex-differentiation lie in between. Interstitial cells are present in both testis and ovary at the stage of 2.5 cm., but while they are very numerous in the testis at this stage they are very rare in the ovary. Whitehead finds that they appear in the testis of the pig embryo at 2.4 cm. Although these authors do not note it, it is unquestionably significant that these cells appear, and exhibit the usual evidences of active secretion at the time of the onset of sex-differentiation. It is also significant that they appear first in the testis and more abundantly than in the ovary; this is of course correlated with the fact that the cortex of the ovary in which they appear is a later formation than the seminiferous tubules or their homologue in the female, the medullary cords.

As regards the origin of the interstitial cells of the ovary we have relatively few observations. Allen notes, as we have seen, that they are very rare in the ovary of the 2.5 cm. pig as compared with the testis; they are also very short-lived, disappearing at the stage of 4 cm. "No interstitial cells are found in the rabbit ovary until the stage of 45 days after birth." They would thus appear to form a very inconspicuous and transitory feature of the embryonic ovary. It is not clear from Allen's account whether they occur in the medulla or cortex of the embryonic ovary; a point which is of some significance in connection with the following discussion.

As regards the testis Whitehead notes

Leydig's cells (the interstitial cells of the testes) pass through two phases of growth, between which a phase of atrophy intervenes. Growth is very rapid from their appearance in the embryo 2.4 cm. long until the length of 3.5 cm. is reached. This is followed by the phase of atrophy, during which the cells return almost to their first state of nearly naked nuclei (figs. 4 and 5). This process reaches its acme in the embryo 14 cm. long. Synchronous with it there is extensive growth of the seminal tubules, particularly in length, so that they are much convoluted, and the intertubular spaces are correspondingly narrowed (fig. 6).

The atrophy proceeds slowly from 3.5 to 14 cm., and is not at all marked at 5.5 cm.

In the embryo 20 cm. long the cells enter upon the second phase of growth; which attains its maximum in the pig of 28 cm., very near to term. Here the cells are enormously increased in number and size, so that they constitute the predominating feature of the microscopic picture.

It seems to me that these facts are of great significance, viz: the intensive growth of the interstitial cells of the testis at the time of most rapid sex differentiation, between 2.4 and 3.5 cm., the subsidence of their activity after embryonic sex differentiation is once attained, and the second phase of activity which probably leads on to the juvenile sex differentiation. It would thus appear that both phases of sex differentiation are covered by periods of intensive activity on the part of the interstitial cells of the testis.

These data relate for the most part to the pig, but there is no doubt that we are justified in assuming that the processes in cattle would not differ essentially; and hence that at the time of sex-differentiation in cattle there is an active secretion of male sex hormones which pass into the .blood, and thus in the case of twins by the vascular anastomosis reach the circulation of the other twin. At this time, if the other twin is a female, the cords of Pflüger have hardly begun to form; no interstitial cells can therefore be present; hence there can be no conflict of hormones. Now Miss Chapin's study of the embryonic gonad of the sterile free-martin shows that the cortex of the ovary does not develop in these animals. Hence no conflict of sex hormones arises; hence also, there can be no question of the male of two-sexed twins being influenced in its sexual development by its mate.

There is at least no escape from the conclusion that it is the circulation of the blood of the male twin in the female that accounts for the results. The probability of the presence of interstitial secretion of the testis in the blood of the male at the time of beginning sex differentiation and the limitation of the action of the male blood to the reproductive system of the female are the reasons for attributing the effect to sex hormones of the male. The possibility of course exists that the blood of the male in such foetal stages differs from that of the female in this specific respect owing to other causes than secretion of interstitial cells of the testis; but there seems to be no reason for making such an assumption.

In my preliminary paper I left open the question whether the invariable result of sterilization of the female at the expense of the male was due to more precocious development of the male hormones, or to a certain natural dominance of male over female hormones. It now appears from the results of more detailed investigation that the latter alternative probably does not arise.

The main assumptions that are involved up to the present point are (1) that cattle resemble pigs with reference to the early origin of interstitial tissue in the testis; (2) that such tissue has in foetal life properties similar to those that have been demonstrated in post foetal life by a considerable number of investi-

gators. While it will no doubt be desirable to clear up these assumptions by farther observations and experiments, it is nevertheless true that the facts that we have presented form a solid basis for both assumptions, which are in no sense unsupported or forced.

The material presents the problem whether the intermixture of other internal secretions o the twin individuals modifies any of their characters? It is obvious that modifications could be expected only in the case of differentiating characters, of which the most fundamental are those of sex; all other main features of organization are common to the sexes, and there is no evidence of individual or sexual differences in the hormones of a species except in the sexual hormones themselves. A negative answer would therefore be expected to this question in general. Individual variations of course exist, and it is an interesting question whether the blood community of foetal life tends to reduce such variations and to approximate their resemblances towards the correlation of variability of identical twins? We have seen reason to believe that this is not the case in regard to color, but the problem remains for future investigation.

According to the conceptions involved in this discussion, the deviations of the sterile free-martin from the female type are due to the action of the male sex-hormones. In order to appreciate the full extent of this action, it is necessary to understand minutely the anatomy of the sterile free-martin in comparison with normal individuals. This comparison is made in a detailed way for numerous cases in section V. The results there given will enable us to correlate the whole series of phenomena and to discuss in a very general way the phenomena of sex-differentiation in mammals.

In terminating the present section then we may repeat our main steps up to this point:—(1) We have demonstrated the separate zygotic origin of the free-martin and its male twin, (2) We have studied the foetal vascular connections in twin pregnancies of cattle. (3) We have explained the existence of occasional fertile free-martins. (4) We have explained the reason for the usual limitation of sterility of the female of two-sexed

twins to cattle.   (5) We have shown that there is a firm basis
for the hormone theory in the known data concerning the time
of origin of interstitial tissue in the testis and in the ovary.
(6) We have also shown why the effect is limited to the female.

V. THE EMBRYONIC AND ADULT ANATOMY OF THE FREE-MARTIN

We can now proceed to a discussion of the anatomy of various
stages of the free-martin with a firm basis for interpretation of
the conditions.   This will be considered under two heads, A. the
gross anatomy, B. the microscopic anatomy.   The second sec-
tion has been worked up by Miss Catherine L. Chapin from my
material, and is published as a separate paper immediately
following this.

The gross anatomy

The mammalian embryo passes through a stage of complete
sexual indifference anatomically, in which all the organs of both
sexes are represented by embryonic rudiments in each individ-
ual; the anatomical sex differentiation that follows is due to
progressive development of certain parts and degeneration of
others in different ways in the two sexes.   This is such a com-
monplace in embryology that it seems unnecessary to describe
the anatomy of the indifferent stage.

For comparison with the anatomy of the free-martins I have
introduced figures of the normal female reproductive system of
embryos 10.2 cm. long (fig. 6), 17 cm. long (fig. 9), 20 cm. long
(fig. 7) and 23.3 cm. long (fig. 8), and of the normal male repro-
ductive system of embryos 10.4 cm. long (fig. 10), 15.8 cm. long
(fig. 11), and 26 cm. long (fig. 12).   The free-martins will be
described in the order from the youngest to the oldest.   The
written descriptions are given very briefly, and the reader should
refer to the figures for details.   The exact interpretation of the
ducts is often difficult or impossible on the gross anatomical
evidence alone; Miss Chapin's descriptions should be compared.

1. Case 19.   ♂ 8 cm.; ♀ 7.5 cm. in length; figure 13, urino-
genital Septem of Female.   × 4.   (Cf. Miss Chapin's descrip-
tion of the histology of this specimen in the paper following.)

This is the youngest free-martin found; its reproductive system
is already definitely of the sterile free-martin type. The gonads
are much smaller than in the normal female (cf. description by
Miss Chapin '17, p. 455). Miss Chapin's observations de-
scribed in the following paper demonstrate a complete absence
of cortical tissue in the ovary; the medullary component is
hypertrophied as compared with the normal, and an albuginea
is present. The ducts also show a sifting in the male direction
(Chapin, p. 459), the Müllerian duct being in process of degenera-
tion. Thus already at this stage the characters of the sterile
free-martin are definitely established, and it is proved that the
primary effect of the male sex hormones is to prevent the forma-
tion of the ingrowths that normally form the cortex of the ovary.
This is a matter of great significance, which is repeated in all the
other cases.

History. Collected October 20, 1915. Maternal ovaries
absent. These twins were contained in a single chorion with
broad connection occupied by cotyledons between the two
halves.

2. The next stage (♀ 12.5 cm., case 26) was used for histo-
logical work; it is described by Miss Chapin. No study of the
gross anatomy.

3. Case 17.   ♂ 13.75 cm.; ♀ 13.1 cm. in length; figure 14,
urinogenital system of ♀.   × 4.

In this preparation the very minute size of the gonad strikes
the eye at once; (compare fig. 6, normal female 10.2 cm. long,
and 9 normal female 17 cm. long). The Wolffian body has
entirely degenerated. Phallus typically female. About mid-
way between the gonad and the genital cord the inguinal fold
arching over the umbilical arteries unites the ducts (urinogenital
fold) to the lateral body-wall; this fold is the foundation for the
round ligament in the female and is the site of formation of the
gubernaculum in the male. The ducts are interpreted as Wolf-
fian ducts, though they appear to be united in the posterior
third of the genital cord; Müllerian ducts are not visible
macroscopically.

History. Bovine twins ♂ and ♀. Collected October 9,
1915. One maternal ovary missing; the other had a single corpus

luteum in it. The chorion was single with a broad connection occupied by cotyledons between the two halves.

4. Case 37. ♂ 16 cm.; ♀ 15.5 cm., figure 15, urinogenital system of ♀.   × 2.

This figure may be compared with figure 11 (normal male of 16.8 cm.) and figure 9 (normal female of 17 cm.). The exceedingly rudimentary condition of the gonads is at once evident, about two-fifths of the length and one-third of the breadth of the normal ovary. The ducts appear to be Wolffian ducts. An extraordinary feature of the anatomy which recurs in every ♀ specimen of this or greater size is the development of a pair of gubernacula from the inguinal fold, which penetrate the musculature precisely as in the male (fig. 11) and reach the integument, extending well down to the groin. In the normal female the round ligament is definitely formed (fig. 9 A and B) in place of the gubernaculum, the horns of the uterus are definitely differentiated from the Fallopian tube, the vagina is enlarged.

History. Bovine twins ♂ and ♀. Collected January 25, 1916. Both maternal ovaries present, each with a single corpus luteum. The uterus was damaged by a cut above the cervix and the two halves of the chorion severed. Examination showed that the chorion had been single with both arterial and venous anastomosis between the two halves. Cotyledons continuous from side to side.

5. Case 6. ♂ 16.8 cm., ♀ 16.3 cm., figure 16, urinogenital system of female. (cf. Miss Chapin's description of the histology of this specimen and interpretation of the ducts.)

For comparison normal female of 17 cm. (fig. 9) and normal male of 16.8 cm., twin to this specimen (fig. 11).

The very minute size of the gonads is again apparent. Gubernacula again take the place of the round ligament. The Müllerian ducts are undeveloped. Seminal vesicles, however, have not begun to form (cf. normal male, fig. 11); the contrast between the genital cord of the ♀ and normal ♀ is very striking.

History. Bovine twins. Collected March 4, 1915. Only one of the maternal ovaries was present; corpus luteum in it. Single chorion for the twins somewhat constricted. No record of vascular conditions.

6, 7, and 8. Cases 32, 23, and 36. 16.75, 17.5, and 17.5 cm. respectively may be mentioned together (figs. 17, 18, and 19). The drawings of these three specimens show essentially the same anatomical characteristics as the preceding stage; the gonads are very small in all, but somewhat larger in 32 (fig. 17) than in the others. They all exhibit gubernacula and the ducts resemble the male ducts much more than the female; the urinogenital sinus is intermediate in length between the female (fig. 9) and the male (fig. 10).

Histories. Case 32. Figure 17. Bovine twins. Male 18 cm., female 16.75 cm. Collected January 1, 1916. Both maternal ovaries present; corpus luteum in each. The uterus was injured and the single chorion cut in two; there was an arterial connection between the halves, and cotyledons continued through.

Case 23. Figure 18. Bovine twins. Male 18 cm., female 17.5 cm. Collected October 29, 1915. Only one maternal ovary present, containing single corpus luteum. The chorion was single; broad connection between the two halves; cotyledons in connecting part.

Case 36. Figure 19. Bovine twins. Male 18 cm., female 17.5 cm. Collected January 21, 1916. Both maternal ovaries present with a single corpus luteum in each. The chorion was single with broad connection occupied by cotyledons between the two halves. Arterial anastomosis present; veins interdigitate, and may communicate.

Summary of the preceding cases. The gross anatomical findings for the stages 7.5 to 17.5 cm. thus show that the effects (either direct or indirect) of the male hormones on the reproductive system of the female are (1) to inhibit the growth of the gonad, (2) to cause the formation of typical gubernacula in place of the round ligament, (3) to favor the development of the Wolffian ducts and inhibit the development of the Müllerian ducts. The urinogenital sinus is intermediate between the male and female condition and the phallus develops similarly to that of the female; moreover the disposition of the teats is female, differing definitely from that of the male (figs. 4, 12, 25 A) and no

trace of the scrotum which is well developed in males of corresponding size is to be found. In other words the derivatives of the urinogenital ridge (gonads, Wolffian and Müllerian ducts and ligaments) tend to develop in the male direction, but other parts of the urinogenital system of such affected females are less involved.

In later stages the conditions established at the beginning undergo farther development. Drawings of various stages from 21.5 to 28 cm. are shown in figures 20 to 27, and these will hardly require detailed description after what has gone before. A rather common anomaly is the evagination of the right gubernaculum into the body cavity in place of growing into the body-wall (figs. 20, 22, 25, and 27); it is difficult to assign a cause for this; inasmuch as it rarely happens on the left side (fig. 27) it is presumably associated with the asymmetry of the urinogenital complex which is particularly striking in the case of the kidneys (see all figures of dissections). The gonad remains exceedingly small in these stages, nor is there much change in other directions. Seminal vesicles, which occur frequently at least in the sterile free-martins after birth are not yet found though they are very evident in the male from 16.8 cm. on. In the normal male the testes are drawn into the saccus vaginalis at the 26 cm. stage (fig. 11); in the sterile free-martin this may happen (fig. 25 A and B), or the gonads may remain in the peritoneal cavity (fig. 26).

History of cases:

Figures 20A and 20B. Case 2. Bovine twins. Male 23 cm., female 21.5 cm. Collected October 30, 1914. Chorion single with constricted connection occupied by cotyledons between the two sides. This being one of the earliest cases no record was made of vascular conditions in the chorion. No record of maternal ovaries.

Figure 21. Case 41. Bovine twins ♂ and ♀. Collected February 16, 1916. Male 22.7 cm., female 21.8 cm. Both maternal ovaries present, corpus luteum in each. The uterus had been cut above the cervix and membranes injured; the fusion of the two chorions was broad, and the cut ends of the arteries connecting the two were readily discovered.

Figure 22. Case 4. Bovine twins ♂ and ♀. Collected January 30, 1915. Male 24 cm., female 22.5 cm. Single chorion, constricted between the two foetuses. No other records.

Figure 23. Case 38. Bovine twins ♂ and ♀. Collected January 26, 1916. Male 23.5 cm., female 22.5 cm. Both maternal ovaries present, corpus luteum in each. The uterus was received cut off above the cervix severing the connecting part of the chorion. The large vessels run to cut ends; extensive vascular anastomosis between the two foetuses involved.

Figure 24. Case 22. Bovine twins ♂ and ♀. Collected October 26, 1915. Male 24.5 cm., female not measured. Both maternal ovaries present, corpus luteum in each. Uterus cut into destroying connection of the two halves of the chorion; the chorion was single and the maternal cotyledons occurred throughout the corpus uteri. Vascular conditions of chorion not observed.

Figure 25, A and B. Case 21. Bovine twins ♂ and ♀. Collected October 20, 1915. Male 27.5 cm., female 26.5 cm. Maternal ovaries absent. The two halves of the chorion were broadly connected, and cotyledons and blood vessels cross over from side to side.

Figure 26. Case 14. Bovine twins ♂ and ♀. Collected July 15, 1915. Male 27 cm. long; female 27 cm. long (measurements by assistant). Maternal ovaries absent; the two halves of the chorion were broadly connected with cotyledons and arteries crossing·over.

Figure 27. Case 12. Bovine twins ♂ and ♀. Collected October 2, 1915. Male 30 cm., female 28 cm. long. Maternal ovaries absent. The chorion was single, not much constricted between foetuses. Cotyledons form continuous series from side to side. No observations on vascular conditions of chorion.

The gross anatomy after birth

The anatomy of the reproductive organs of a seven weeks old free-martin (case 44) born on my farm is shown in figure 28. Incidentally it may be noted that the twins were pure bred Holsteins, and that while the male was mostly black, the female

was about half white. This is an exceedingly interesting case, as it represents the most extreme transformation of the internal organs towards the male type which I have seen. When the body cavity was opened gonads appeared to be entirely absent; however the ducts were found to perforate the body wall; and farther dissection revealed the gonads lying in peritoneal sacs situated in the groin on each side between the skin and abdominal muscles. There is no difficulty in interpreting the parts (fig. 28): the vulva is typical; it leads into a short urinogenital sinus beyond which there is no trace of vagina, uterus, or tubes. Opening into the dorsal wall of the urinogenital sinus at its anterior end are two Wolffian ducts (vasa deferentia); laterally to them seminal vesicles. The Wolffian duct of the left side is much larger than that of the right. They run in a broad membranous septum corresponding to the broad ligament of the uterus and enter peritoneal evaginations, open to the body cavity, which perforate the abdominal muscles in the manner already described. When the peritoneal sacs are opened, as shown in the insert figures, figure 28, B and C, they are found to contain testis-like gonads, with an . epididymys-like organ associated. It is an interesting fact that the smaller right gonad is associated with a smaller Wolffian duct and a smaller seminal vesicle; this is the side on which disturbances in relation of parts is found in foetal stages. Sections of the testis show a superficial dense albuginea and entire absence of cortical ovarian tissue. The interior is occupied by exceedingly wide branching tubes lined by a one-layered epithelium. Connective tissue is abundant between the tubes.

History. Figure 28. Case 44. Holstein-Friesian twins ♂ and ♀ born March 12, 1916. The female (free-martin) was killed and dissected April 29, 1916.

In the foetal stages of the free-martin, which we have considered, the gonad presents a rudimentary aspect and seminal vesicles are not formed, although they appear in the males at much earlier stages (fig. 1). On the other hand, the gonads may attain a very considerable size after birth, and seminal vesicles appear usually to be present (cf. literature). It therefore fol-

lows that there must be a belated growth of the gonads and formation of seminal vesicles in the later foetal stages which I have not had an opportunity to examine, in some cases at least.

The literature contains descriptions more or less complete of the anatomy of the reproductive system of about 30 free-martins. These have been tabulated by D. Berry Hart ('09–'10). All agree in one fundamental respect, viz: that the gonads never show the least evidence of possessing the structure of an ovary; Graafian follicles have never been described. In some cases the gonad is represented merely by clumps of fat, or is absent; in others it is rudimentary and situated in the body cavity, or may be of considerable size in the same situation, or finally may be found in the groin. Whenever present it presents more or less superficial resemblance to a testis, sometimes an exceedingly close resemblance. Histologically also it resembles a testis. However it is probable that spermatozoa are never formed, and even that the earliest stages of spermatozoa are lacking in the tubules. All agree likewise in the very great reduction or complete absence of vagina, uterus, and tubes, and the presence of Wolffian ducts in a greater or lesser degree of development. However, it would be merely tedious to review the variations in detail.

The very interesting question presents itself how far the modification of the female reproductive organs towards the male side may be carried. I have already described the most extreme case that I have met (no. 44, fig. 28); but one of Numan's cases indicates that the transformation may proceed considerably farther. This case seems to be well authenticated in all respects, and on account of the rarity of Numan's publication I include a full description of it: a pair of calf twins was delivered by Caesarian section in 1832 at Maarssen in Holland, one was male and the other was supposed to be a free-martin. Numan bought the latter the following year; on examination a small opening was found in the perineum a short distance below the anus through which urine dropped, the urethra passed over the pubic symphysis and opened externally about two hands-breaths below the anus through an opening surrounded by an

apparent female vulva, within which appeared an imperforate glans penis; the vulva was provided ventrally with a tuft of hair as normally. The animal was slaughtered in 1835, and the anatomy of the reproductive system studied. Numan's figure is here reproduced (fig. 29). It will be seen that the internal anatomy is quite similar to my case 44 (fig. 28), but the external parts are also modified in this case to a very considerable extent in the male direction.

If this case is well authenticated, as it appears to be, we would have to conclude, contrary to the evidence from my cases that the external organs of reproduction of the female are also susceptible of modification in the male direction by the male hormones; it may be that this occurs in cases of exceptionally early action of the male hormones. The embryological history of the external male and female organs shows that the sexual type is fixed very early.

It is of course by no means certain that the extreme possible modification of the female reproductive system by male hormones will be found in free-martins, indeed when we consider the various exigencies of the admission of the male hormones to the female circulation under the conditions of production of the free-martin, it seems improbable that the optimum conditions for modification of the female reproductive system are ever realized in such cases: the onset of the action can not be much before morphological sex differentiation has begun in any case, and its intensity must certainly be of a low order quantitatively at first not only on account of the minuteness of the first vascular inter-connections, but also on account of the rudimentary character of the interstitial gland of the testis at first, if we suppose that the effective hormone is derived entirely from this source. Nevertheless search should certainly be made for more pronounced cases of transformation of the free-martin towards the male condition. But it is obvious that definitely controlled experimental investigation will be the only means of deciding where the real limit of action lies, whether or not the complete transformation of a female zygote of mammals into a male individual by hormone action is possible.

VI. GENERAL DISCUSSION

The precision and definiteness of the transformation of the specific organ system, and the undoubted character of the primary cause of the transformation appears to offer a more definite basis than we have hitherto had for the analysis of the origin and differentiation of some fundamental sex characteristics of mammals. At the present time there is a general recognition of the primary zygotic determination of sex in mammals; we have some scattering data on sex limited inheritance, a great deal of miscellaneous information on the effects of castration, and a little on the effects of cross transplantation of gonads. There is also a large literature on hermaphroditism and pseudohermaphroditism of mammals. But these data are by no means capable of arrangement in any general scheme. They are on the whole exceedingly confusing in spite of the recent advances concerning the zygotic character of the initial sex impulse, and the analyses of sex hormone action in post-foetal life.

The free-martin gives us additional evidence of considerable value concerning the problem of sex-determination and sex-differentiation in mammals, especially in its suggestion that the course of embryonic sex-differentiation is largely determined by sex-hormones circulating in the blood. The evidential value of this case is, however, limited, in the first place by the fact that only the female is affected,—we have no information on the reverse situation—and in the second place by the fact that we cannot study separately the effect of early embryonic castration of the female, but only as it is modified by the simultaneous presence of male hormones, On the male side there is complete absence of information as to the effects of early embryonic castration and the possible effect of the presence of female hormones[5]

[5] A curious problem however presents itself in this connection viz: how it happens that the sex-hormones of the mother do not affect the reproductive system of the unborn sons. Steinach's results on feminization of infantile male rats by castration and implantation of ovaries demonstrates the far reaching effects of female sex hormones in the male system; and we can hardly doubt that even greater effects would result in embryonic life. It would appear probable, therefore, that the embryo is in some way protected from the sex hormones circulating in the mother's blood. Either there is cessation of production of sex

in the absence of male hormones. These facts must be kept
clearly in mind as definitely fixing the very provisional character
of such speculations as we may make.

The present standpoint for the analysis of sex-characters of
mammals would have to include (1) primary zygotic determina-
tion of the male and female sex and (2) secondary differentiation
of the sex characters, in which internal secretions play a very
specific and fundamental rôle. The production of intermediate
zygotic conditions is theoretically possible, since Goldschmidt has
demonstrated all grades of intersexuality in the gypsy moth de-
pending on variable conditions of the gametes. But it is obvious
that such conditions are not involved in the present case and
that we have to consider only the secondary factors.

It follows from the data that the female zygote must contain
factors for both sexes; the primary determination of the female sex
must therefore be due to dominance of the female factors over
the male. If we think of this as a simple quantitative relation,
as Goldschmidt ('16) has done, we can explain the intersexual
condition of the free-martin as due to an acceleration or inten-
sification of the male factors of the female zygote by the male
hormones. The degree of the effect which is quite variable, as
we have seen, would of course be subject to all quantitative
variations of the hormone. Thus the case of the free-martin
could come under the same general point of view as that of
the intersexes of Lymantria according to Goldschmidt with the
one exception that the quantitative differences between the male
and female factors of the female zygote necessary for the differ-
entiation of female characters, are reduced in the free-martin by
internal secretions instead of by variations of potency of the

hormones during foetal life, or they are neutralized in some way, or the placenta is
impervious to them. The first possibility does not seem consistent with our
knowledge of the physiology of the mammalian ovary, or with the cytology of the
organ during pregnancy; the second one offers no point of attack at present; the
third seems the most probable *a priori*, and is no doubt susceptible of experi-
mental analysis. But whatever explanation may prove to be correct it seems
probable that disturbance of the equilibrium that protects the male from the
sex hormones of the mother would result in malformations of the male sex char-
acters to a degree commensurate with the extent of the disturbances. There is,
therefore, here a possible explanation of the greater mortality among male
foetuses, and of certain types of intersexes (pseudo-hermaphroditism).

male factors in different varieties as in the intersexual hybrids of Lymantria.

The case of the free-martin shows that a gonad with a primary female determination may form a structure which is morphologically a testis (Chapin '17), through suppression of the cortex and overdevelopment of the medullary cords and urinogenital union under the influence of male sex-hormones. Lesser degrees of transformation are of course possible, so that it is certain that the gonad of a mammalian female zygote is capable of most, at least, of the series of transformations that may exist between an ovary and a testis. Whether the transformation in the male direction may proceed under such conditions to the production of true spermatocytes and spermatozoa is at least doubtful. Such elements have not hitherto been described for free-martins, if we except D. Berry Hart's statement concerning the gonads of Hunter free-martins, that "in only one are spermatozoa present." More than six words seem necessary to establish so important an exception.

Pick's investigation of 'true hermaphroditism' in man and mammals shows that in all bilateral cases the gonad is part ovary and part testis; but that whereas normal germ cells may arise in the ovarian part, there is no trace of spermatocytes or spermatozoa in the testicular part. Four such cases are described for the hog, and it would seem that the theoretical possibility exists of explaining them as due to embryonic foetal anastomosis of blood-vessels between opposite sexes, by making the farther assumption that such anastomosis was only temporary and ceased after the transformation of part of the genital ridge. The interpretation of 'hermaphroditism'[6] in mammals is in any

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[6] The term 'hermaphroditism' has so many and various connotations that it seems better to drop it from our vocabulary, so far as mammals are concerned, and to describe the conditions hitherto gathered under this head simply as intersexual. Some conception of the confusion that results from present methods of classification of these cases may be felt by stating that our case 44 (fig. 28), would be classified as Hermaphroditismus spurius masculinus externus (Neugebauer '08): 'spurius' because both kinds of gonads are not present; 'masculinus' because the gonads are apparently testes; 'externus' because the external organs are female. But the animal is female, not male, and it represents merely a certain stage in a series. The animal is not a sex-mosaic, e.g., male in front of a certain transverse level and female behind ('transverse hermaphroditism'); it is a step in a series of sex intergrades.

event a very difficult problem, and it seems worth while to indicate the hitherto unconsidered possibility of action of heterologic sex hormones, however brought about, in this connection.

Regarding other parts of the internal reproductive system we have seen that the free-martins exhibit a graded series of inhibition of the female ducts, and of development of the male ducts which may obviously correspond to variable time of onset, intensity, and perhaps duration of action, of the male sex-hormones. The series extends nearly to the normal male limit in exceptional cases (no. 44, fig. 28). There is indicated a rough parallelism at least between the grade of transformation of the gonad and that of the remainder of the internal reproductive system. The external organs of reproduction are the least liable to modification, but they do not escape in all cases, and may even exhibit considerable transformation in the male direction if we can accept Numan's case described on p. 413 and illustrated in figure 29.

The fundamental determining factor in these events is undoubtedly the male sex hormones as has been argued previously (p. 396); but the entire causal nexus is by no means clear. We do not know what the results of embryonic castration of the female might be in itself, and hence we are unable to assert definitely in just what positive ways the male hormones act on the female zygote, because the earliest determinable result of such action is the suppression of the ovarian cortex, which must be regarded as practically equivalent to castration. This action at least is due to the male hormones; how much of the subsequent events is due to mere absence of ovarian tissue, and how much to positive action of male sex-hormones is more or less problematical. It is well-known that spayed females of certain birds and mammals tend to develop male characters; heifers with cystic degeneration of ovary also develop certain male characteristics (Pearl and Surface, '15), so that we must admit in principle the possibility that much of the male development in the free-martin is due to the lack of inhibitions normally furnished by the ovary.

It is also probable that the various parts of the reproductive system have other means of correlation, and act and react on one another in various ways. Certain indications of this are

seen in lateral variations, as for instance in figure 28 where a large gonad on one side is associated with a large Wolffian duct, and seminal vesicle, and a much smaller one on the other side with a correspondingly smaller duct and vesicle.

When, therefore, we attribute the free-martin condition to the male hormones we only. mean to assert that they are the primary cause, and not that they are the decisive factors in each member of the series of events.

It follows from this discussion that sex in mammals cannot be diagnosed by the character of the gonads alone because a testis-bearing individual may develop from a female zygote. The unexpected result is reached that the external genitalia and the mammary gland are more reliable criteria of the female sex than the internal parts.

We have no comparable data on the male side, and we can only speculate as to the transformation that would be produced in the male reproductive system by the action of female hormones beginning before sexual differentiation. Theoretically we would have to assume that the male zygote contains female as well as male factors, but the male zygote may not be capable of such extensive transformation as the female, owing to the embryological fact that the male gonad never forms normally any homologue of the cords of Pflüger in the female, i.e. of the ovarian cortex, whereas the female does form the homologue of the seminiferous tubules before the cords of Pflüger begin to arise. In the case of the free-martin we do not find that male hormones cause the development of any structure which is not represented embryologically in the normal female; the hormones act in this case merely by inhibition or stimulation of normal embryonic rudiments. If this should hold as a principle also on the male side we could not expect that the transformation of a testis into an ovary should ever occur, although suppression of complete testicular development would probably happen. But apart from this admittedly uncertain principle there is no reason for assuming that the possibility of the male zygote for acquisition of female characters may be any less than the reverse case.

For their proper evaluation the results concerning the free-martin should be associated with the other studies indicating that

zygotic determination of sex is not irreversible predestination, but a quantitative overbalance in the direction of one sex on the other. I refer more especially to the studies of Richard Hertwig ('06), Kuschakewitsch ('14), of Whitman-Riddle on pigeons (Riddle '16), and especially the very convincing demonstration of reversibility of sex in the gypsy moth by Goldschmidt ('16). These studies demonstrate that sex differentiation is controlable within variable limits in certain groups, to which mammals must be added as a result of the present study. To determine the means, limits and subsequent results of such control is now one of the most important tasks of biology.

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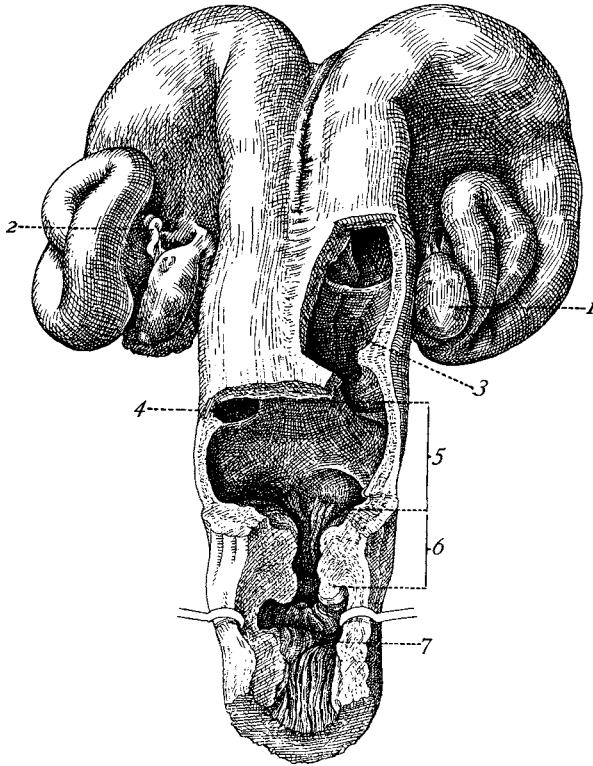
Fig. 1[7] Cow's uterus strongly distended with formalin; ventral view; parts cut out. $\times \frac{2}{5}$. *1*, left ovary; *2*, right fallopian tube; *3*, left horn of uterus cut open; *4*, opening from right horn into body of the uterus; *5*, body of the uterus; *6*, cervix; *7*, vagina.

[7] Drawings made by Kenji Toda.
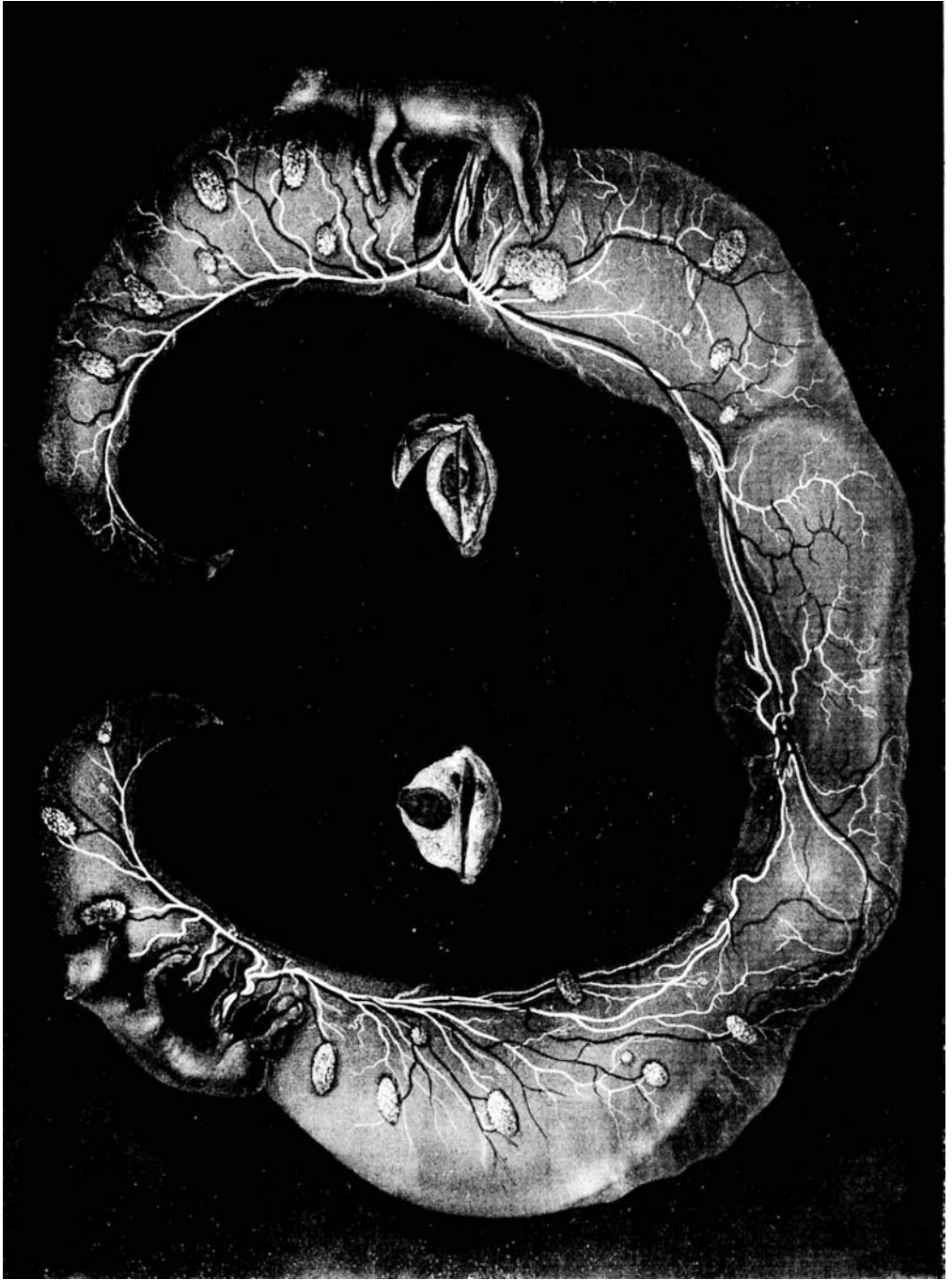
Fig. 2 Twin chorionic vesicle of cow; double injection; case no. 33 ♂♂, 10.5 cm. each. × ⅓. The figure also shows the two ovaries of this pair of twins cut so as to exhibit the corpus luteum in each. Amnions entirely removed. The arteries are represented in white, the veins in black. (See text for further description.)
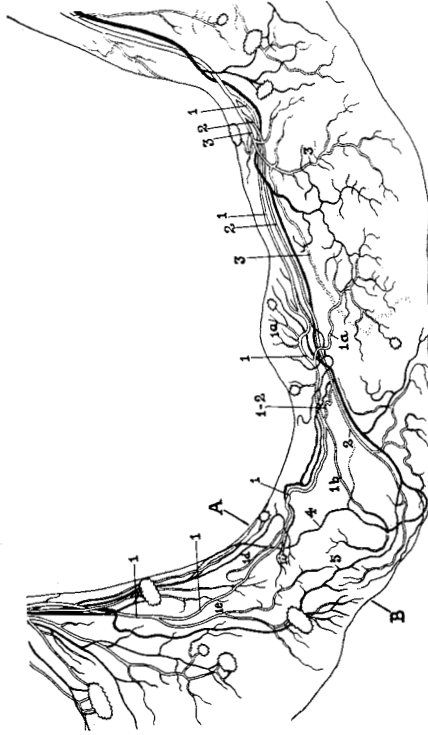
Fig. 3 The central part of the twin chorionic vesicle shown in figure 2 slightly schematized. See text p. 393 for description.

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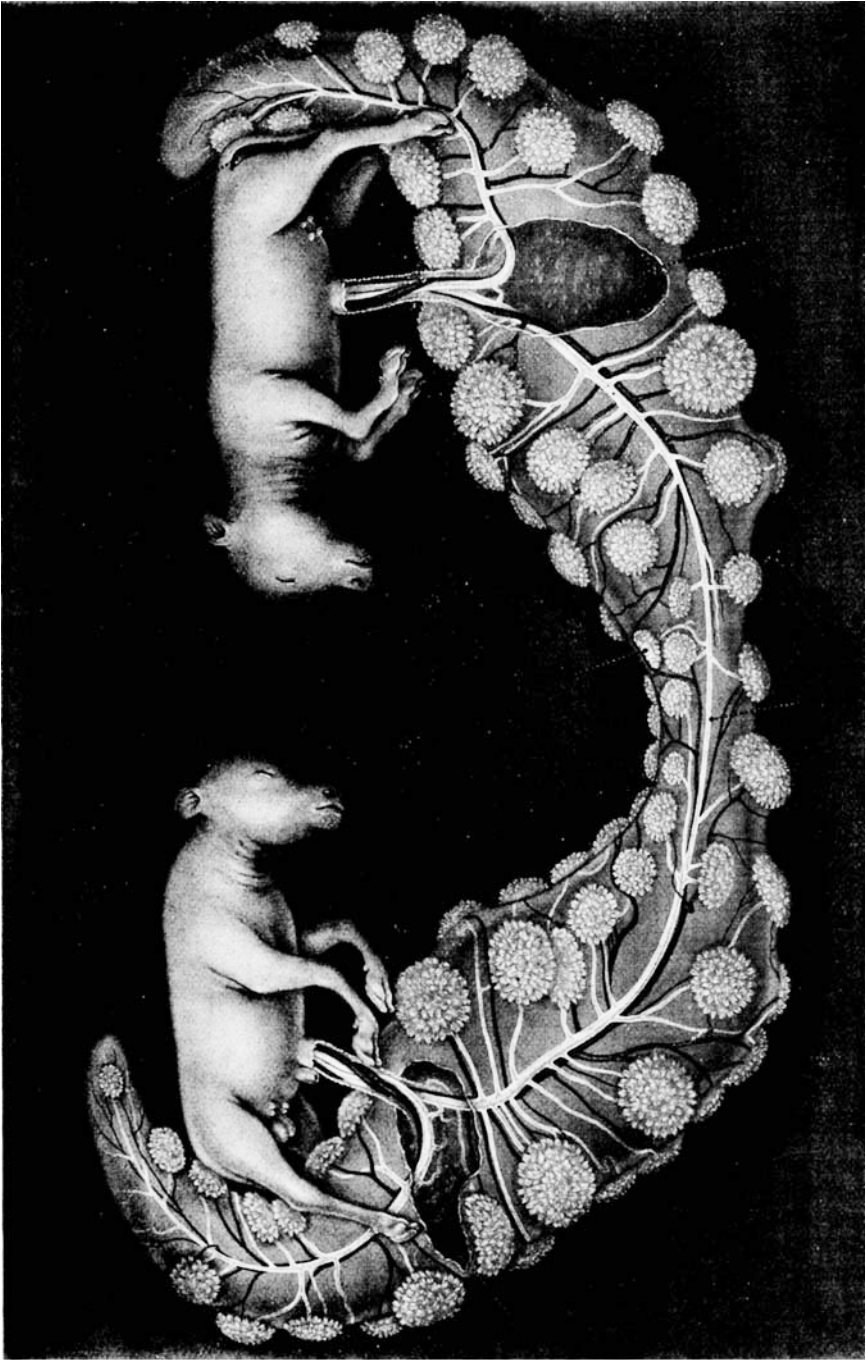
Fig. 4 Twin chorionic vesicle of cow; double injection; case no. 47. ♂ 22.75 cm. ♀ 22.25 cm. ×¼. 1, arterial through trunk; 2, cotyledon with venous connection with both sides; 3, amniotic sacs opened; 4, clitoris of free-martin; note female arrangement of teats; cf. with male. (See text p. 394 for further description.)
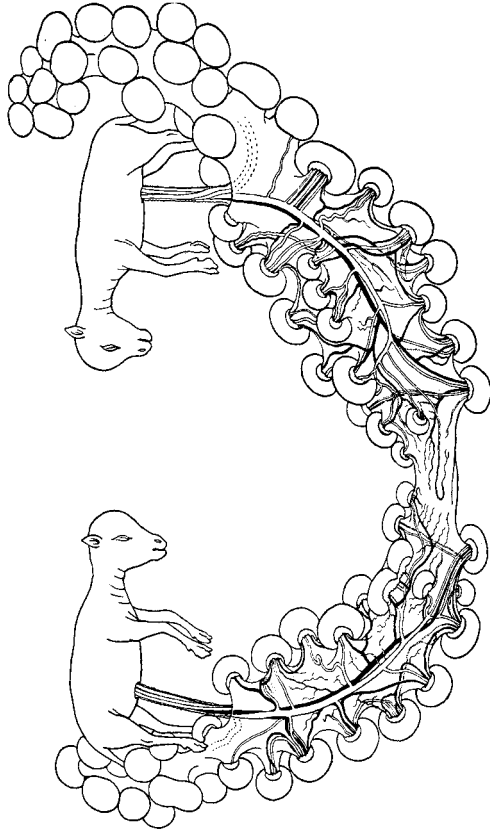
Fig. 5 Twin chorionic vesicle of sheep. ×⅖. Double injection toward the center. ♀ ♀ 13 cm. each. The maternal ovaries had a corpus luteum in each. The chorionic vesicles have fused, but there is no anastomosis of blood-vessels from the two sides.

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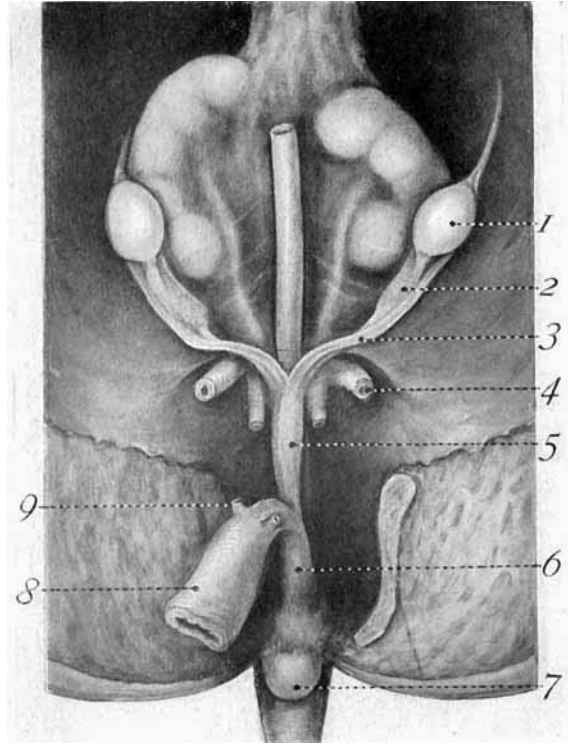
Fig. 6 Case 40. Reproductive organs of fertile free-martin; 10.2 cm. long. × ⅘. In this case there was no fusion between the two chorions, and the reproductive system is normal. See figure 10 for male twin. *1*, ovary; *2*, remains of Wolffian body; *3*, Müllerian duct; *4*, umbilical artery; *5*, vagina; *6*, urino-genital sinus; *7*, clitoris; *8*, neck of allantois; *9*, ureter.
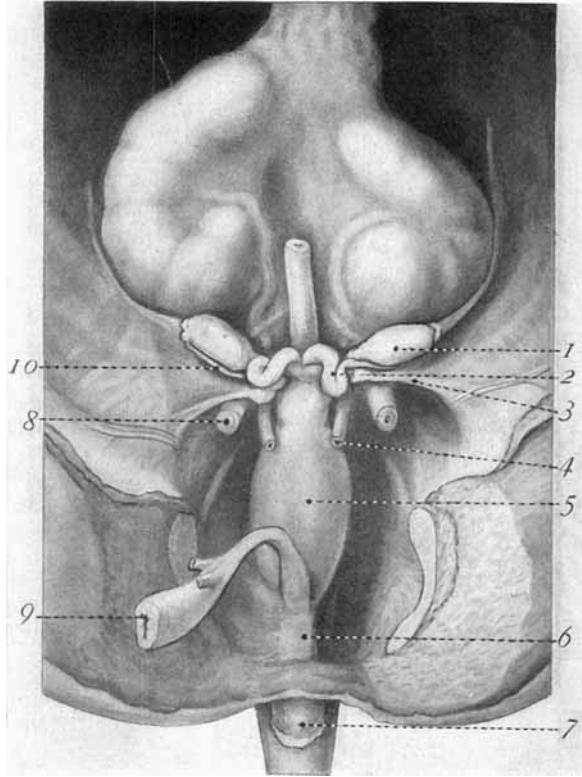
Fig. 7 Case 9. Reproductive organs of fertile free-martin 20 cm. long. × ⅔. The chorion of the twins was single with a narrow connecting part between the two halves devoid of cotyledons for a space of about three inches. No record of vascular connections, which were presumably lacking. *1*, ovary; *2*, left horn of uterus; *3*, round ligament; *4*, ureter; *5*, vagina; *6*, urinogenital sinus; *7*, clitoris; *8*, umbilical artery; *9*, neck of allantois; *10*, fallopian tube.
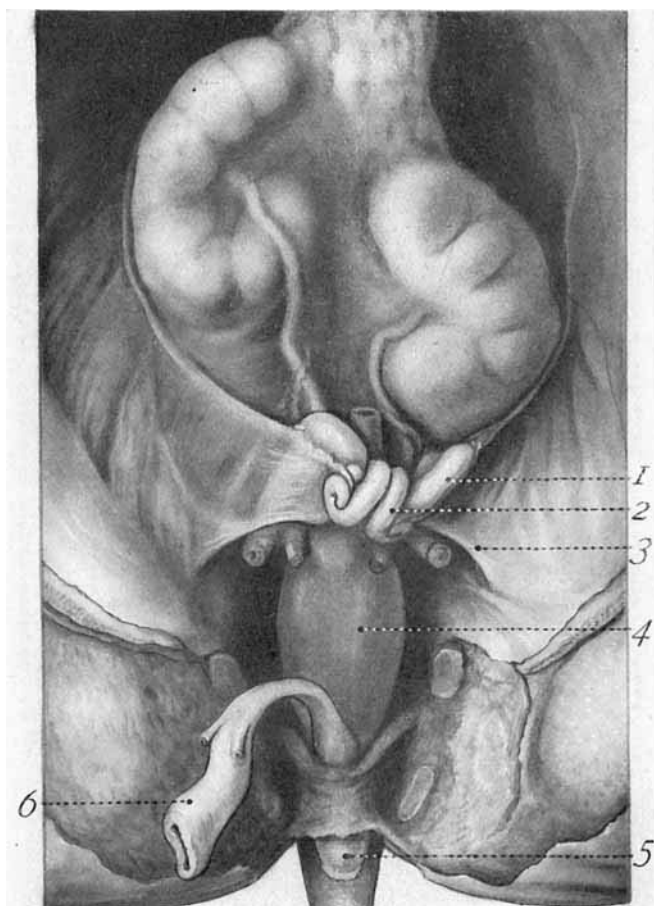
Fig. 8 Case 8. Reproductive organs of fertile free-martin 23.3 cm. long.
$\times \frac{4}{3}$. The chorion of the twins was single with a narrow connection between the
two halves. Presumably no vascular anastomosis. The male was 26.5 cm. long,
nearly 80 per cent heavier than the female, and its skin was unpigmented, whereas
the female was darkly pigmented. *1*, ovary; *2*, left horn of uterus; *3*, round liga-
ment; *4*, vagina; *5*, clitoris; 6, neck of allantois.
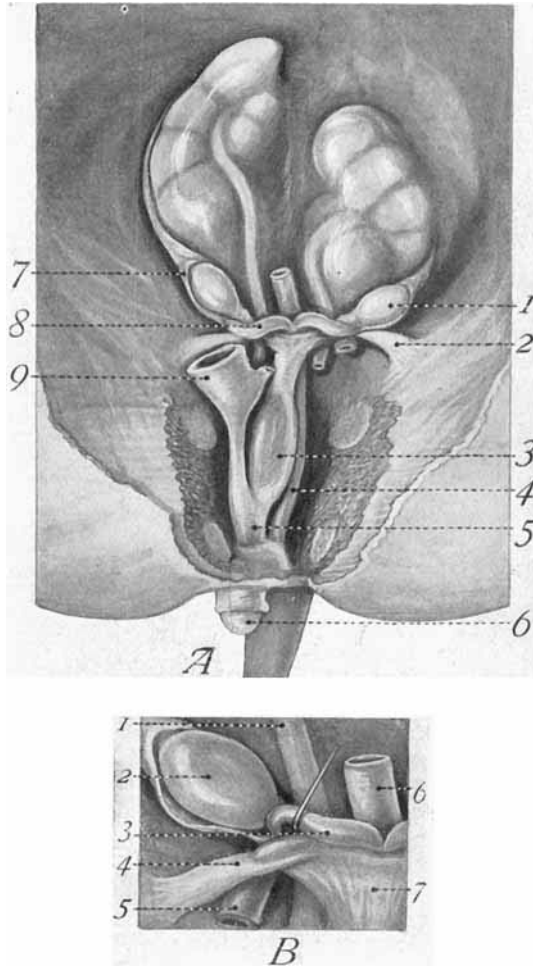
Fig. 9   Urinogenital system of normal female from twin females, pair 5.
17 cm. long.   Collected February 11, 1915.   Chorion was single constricted be-
tween the two foetuses.   Figure B shows part of the right side × ⅔ with the
horn of the uterus lifted to show the insertion of the round ligament.   Contrast
the round ligament of the female with the gubernaculum of the male (fig. 11)
to which it corresponds exactly in position.   The horns of the uterus begin to
show the spiral coil; body of the uterus small; vagina distended; the urinogenital
sinus much shorter than in male (fig. 11).

A.   × ⁴⁄₃.   *1*, ovary; *2*, round ligament of uterus; *3*, vagina; *4*, rectum; *5*,
urinogenital sinus; *6*, clitoris; *7*, fallopian tube; *8*, right horn of uterus; *9*, allantois.

B.   × ⅔.   Part of same specimen with horn of uterus raised to show inser-
tion of the round ligament.   *1*, ureter; *2*, ovary; *3*, right horn of uterus; *4*, round
ligament; *5*, umbilical artery; *6*, rectum; *7*, body of uterus.
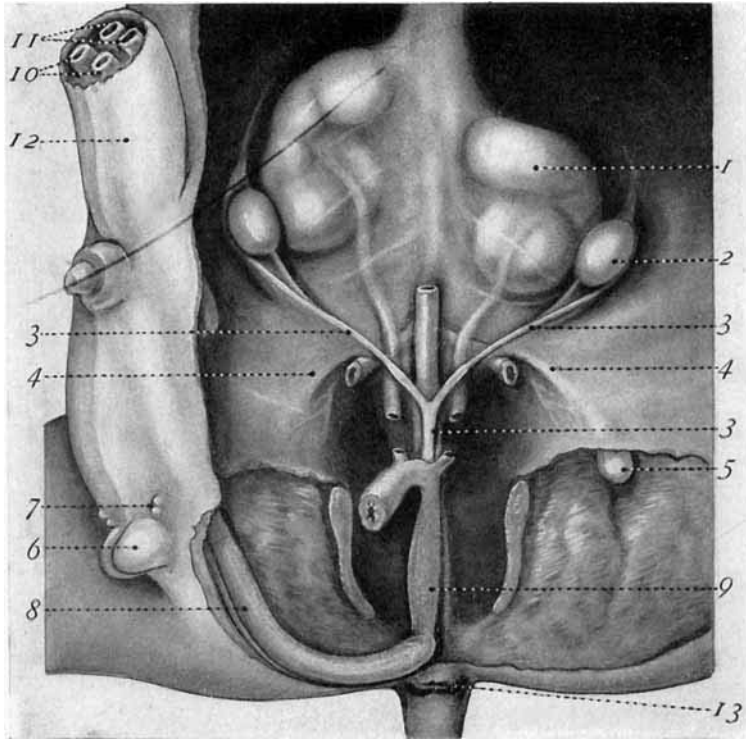
Fig. 10   Normal male 10.4 cm. long, from two-sexed pair 40. $\times \frac{8}{3}$.   Note that the gubernaculum is well formed on the left side, but not yet on the right.   *1*, kidney; *2*, testis; *3*, Wolffian ducts; *4*, inguinal fold; *5*, left gubernaculum; *6*, scrotal sacs; *7*, teats; *8*, penial tube; *9*, urinogenital sinus; *10*, umbilical arteries; *11*, umbilical veins; *12*, umbilical cord; *13*, anus.
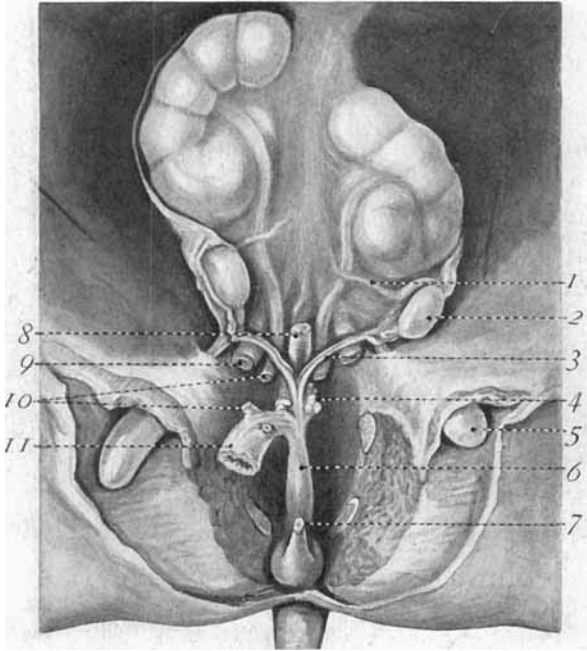
Fig. 11   Normal male 15.8 cm. long. $\times \frac{4}{3}$.   From two-sexed pair 6 collected March 4, 1915.   Chorion was single, constricted between the two foetuses.   The long gubernacula have grown into the groin but have not yet entered the scrotal sacs.   The testes are still in the body cavity, though close to the entrance to the saccus vaginalis.   The left testis, posterior in position to the right, corresponding to the more posterior location of the left kidney.   The seminal vesicles are well formed with distal buds.   The urinogenital system of the free-martin twin is shown in figure 16.   *1*, spermatic artery; *2*, testis; *3*, Wolffian duct (vas deferens); *4*, vesiculae seminales; *5*, gubernaculum; *6*, urinogenital sinus; *7*, root of penis; *8*, rectum; *9*, umbilical artery; *10*, ureter; *11*, allantois.
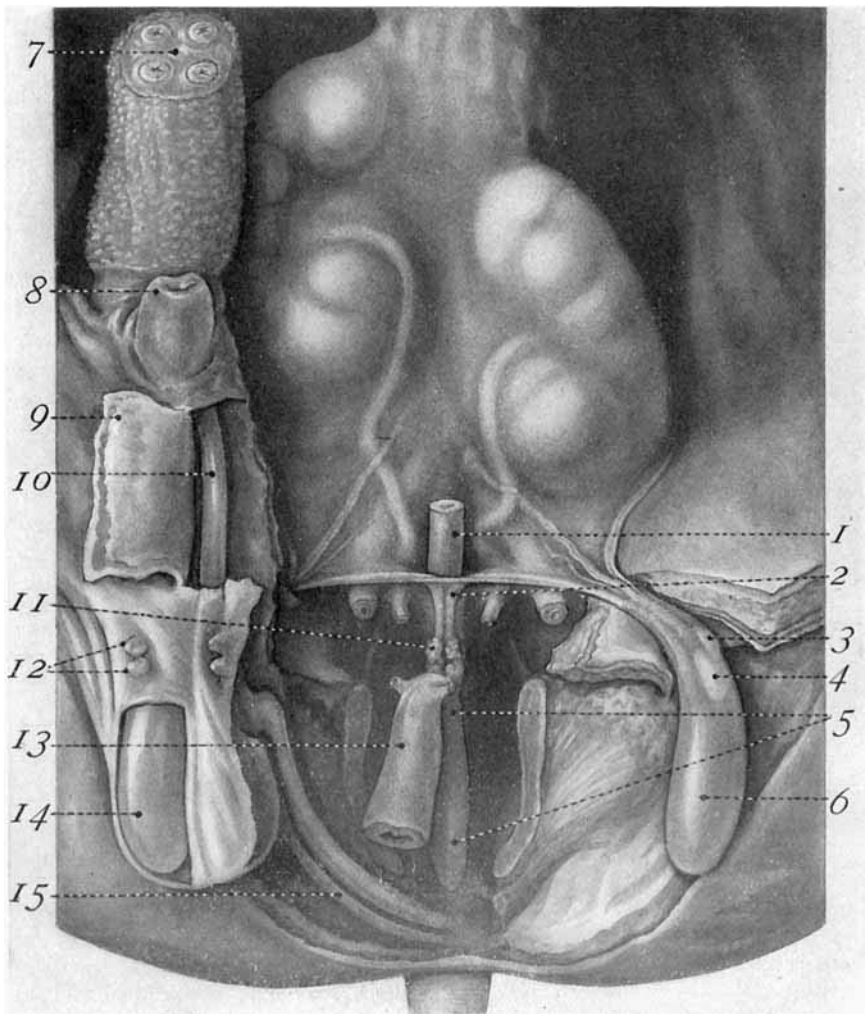
Fig. 12   Normal male 26 cm. long; from twin one-sexed male pair 3; collected
January 28, 1915.   × ⅓.   Single chorion, constricted between the two foetuses.
This figure shows the entire male urinogenital system; the gubernacula have en-
tered the scrotal sacs.   The testes are drawn into the vaginal sacs.   The disposi-
tion of the teats for the normal male should be noted.   *1*, rectum; *2*, vasa deferentia;
3, epididymis; *4*, testis; *5*, urinogenital sinus; *6*. gubernaculum withdrawn from
scrotal sac; *7*, cut end of umbilical cord; *8*, prepuce; *9*, wall of penial sheath;
10, penis; *11*, vesiculae seminales; *12*, teats; *13*, allantois; *14*, gubernaculum in
scrotal sac; *15*, retractor muscle of penis.
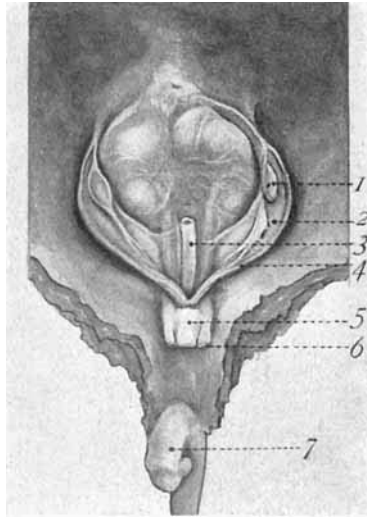
Fig. 13 Urinogenital system of sterile free-martin 7.5 cm. long. × $\frac{8}{3}$; case 19. Specimen fixed in Flemming's fluid; not so fully dissected as the following cases, as it was preserved for microscopical study. (Other drawings from the same specimen are figures 4 and 5, Chapin '17.) *1*, gonad; *2*, Wolffian body; *3*, rectum; *4*, genital duct; *5*, allantois; *6*, umbilical artery; *7*, clitoris.
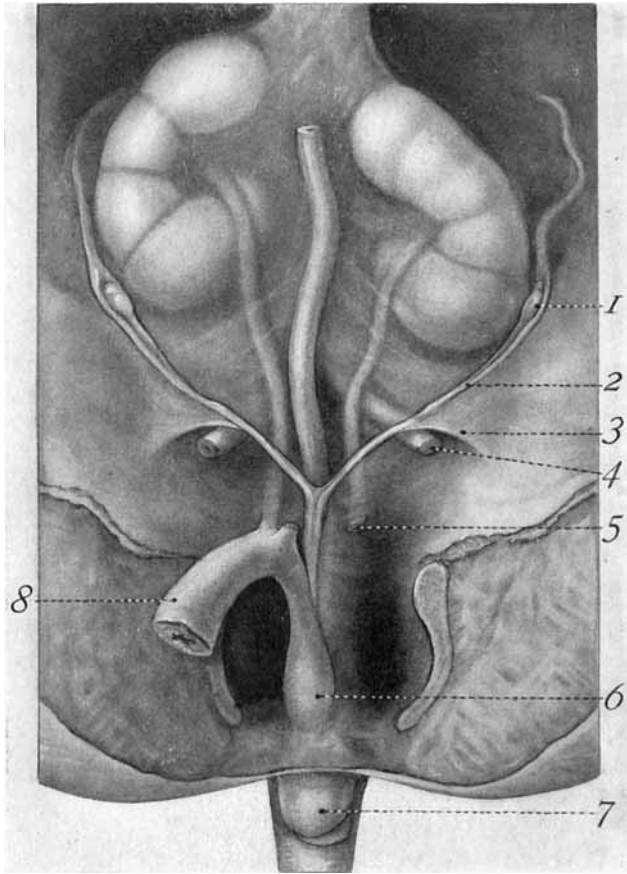
Fig. 14 Sterile free-martin 13.1 cm. long. $\times \frac{5}{3}$. From two-sexed pair 17. Collected October 9, 1915. Gubernacula are not developed; cf. male of earlier stage (fig. 10). *1*, gonad; *2*, Wolffian duct; *3*, inguinal fold; *4*, umbilical artery; *5*, ureter; *6*, urinogenital sinus; *7*, clitoris; *8*, allantois.
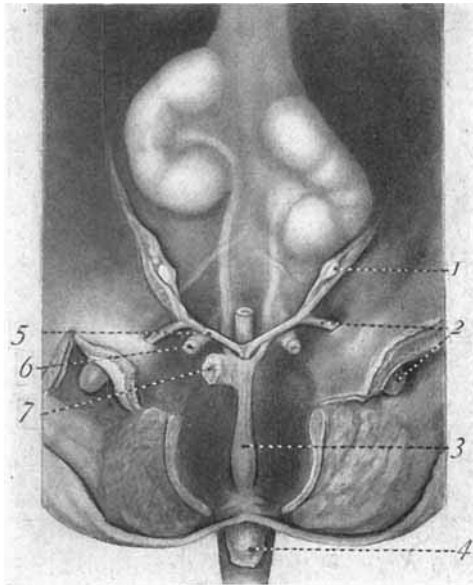
Fig. 15 Sterile free-martin 15.5 cm. long. × ⅓. From two-sexed pair 37. Collected January 25, 1916. Gubernacula are developed as typically as in a male (figs. 10 and 11). Gonads small. Ducts also appear as in male. *1*, gonad; *2*, gubernaculum; *3*, urinogenital sinus; *4*, clitoris; *5*, Wolffian ducts; *6*, umbilical artery; *7*, allantois.
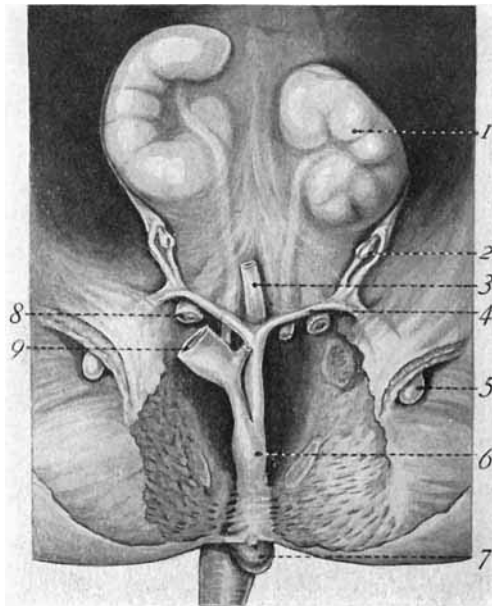
Fig. 16 Sterile free-martin 6, 16.3 cm. long. $\times \frac{1}{3}$. (Cf. fig. 11 for male twin.) Gubernacula are somewhat smaller than in the male (fig. 11). Small gonads (cf. figs. 9 for normal size, and fig. 7 of Chapin for histological structure). The urinogenital sinus is intermediate in length between male and female (fig. 9); no seminal vesicles. *1*, kidney; *2*, gonad; *3*, rectum; *4*, Wolffian duct; *5*, gubernaculum; *6*, urinogenital sinus; *7*, clitoris; *8*, umbilical artery; *9*, allantois.
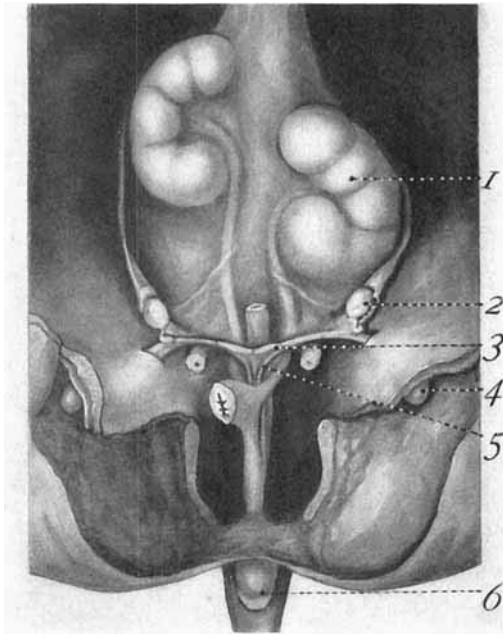
Fig. 17 Sterile free-martin 16.75 cm. long. $\times \frac{4}{3}$. From two-sexed pair 32. Similar in all essential respects to figure 16 except that gonads are slightly larger, and possible rudiments of Müllerian ducts occur in the broad ligament. *1*, kidney; *2*, gonad; *3*, rudiments of horns of uterus; *4*, gubernaculum; *5*, Wolffian ducts; *6*, clitoris.
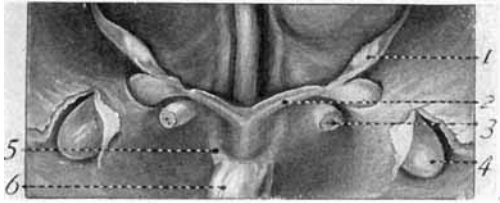
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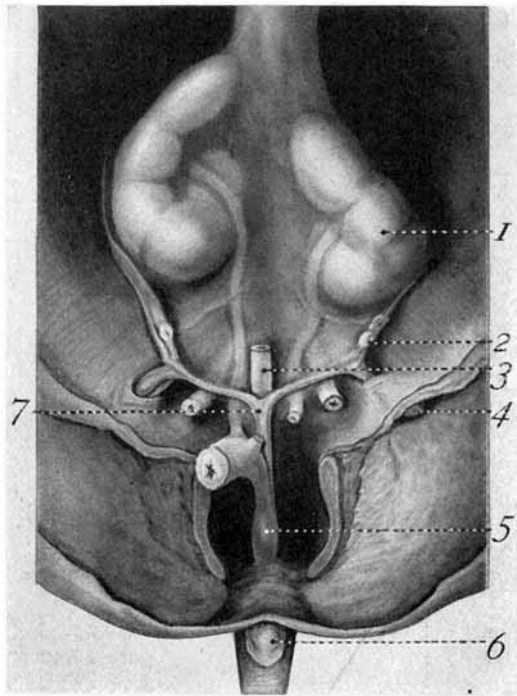
19

Fig. 18 Part of urinogenital system of sterile free-martin 17.5 cm. long. × ⅘. From two-sexed pair 23. The gonads are unusually rudimentary. *1*, gonad; *2*, urinogenital fold with ducts; difficult to interpret; *3*, umbilical artery; *4*, gubernaculum; *5*, ureter; *6*, allantois turned back.

Fig. 19 Urinogenital system of sterile free-martin 17.5 cm. long; × ⅘, From two-sexed twin pair 36. The gonads are exceedingly rudimentary in this specimen; the gubernacula are present, but not as well developed as in figure 18. *1*, kidney; *2*, gonad; *3*, rectum; *4*, gubernaculum; *5*, urinogenital sinus; *6*, clitoris; *7*, Wolffian ducts.
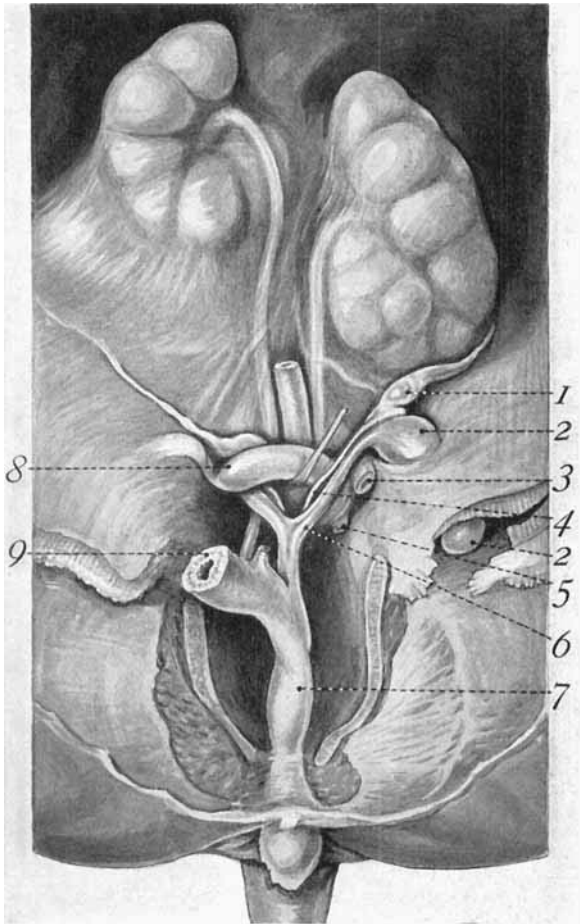
20A
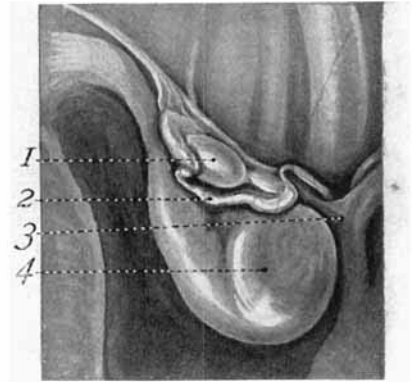
20B

Figs. 20A and 20B   Urinogenital system of sterile free-martin 21.5 cm. long. From two-sexed pair 2.

20A.   × ⁴⁄₃. On the right side the gubernaculum, instead of growing into the body wall has evaginated into the body cavity and lies partly in the utero-rectal recess.   Both Wolffian and Müllerian ducts appear to be present; latter very rudimentary.   Gonads very small.   *1*, gonad; *2*, gubernaculum, left side; *3*, umbilical artery; *4*, Wolffian duct; *5*, ureter; *6*, Müllerian duct; *7*, urinogenital sinus; *8*, gubernaculum of right side evaginated into body cavity; *9*, allantois.

20B.   × ⁸⁄₃. Part of 20A with the right gubernaculum withdrawn from the utero-rectal recess and turned over to expose the gonad and Wolffian duct. *1*, gonad; *2*, Wolffian duct; *3*, Müllerian duct; *4*, gubernaculum.
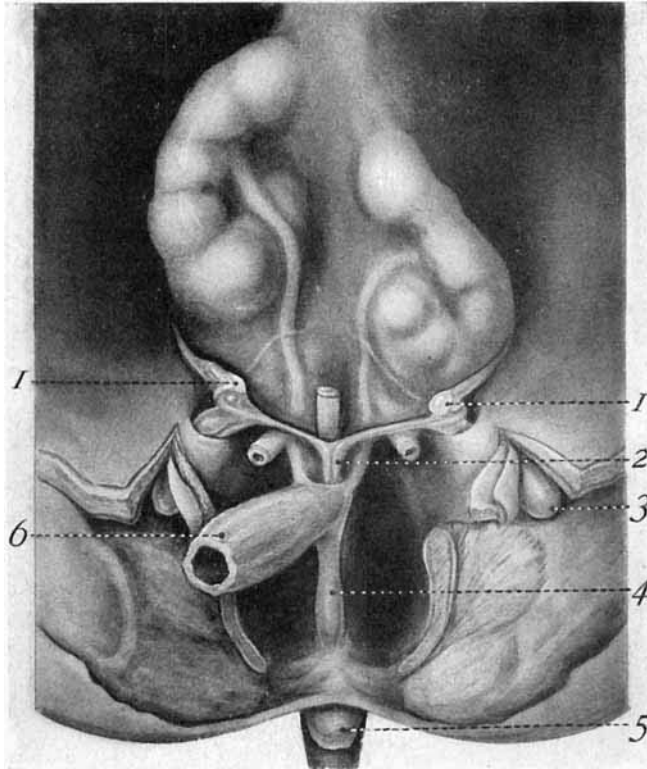
Fig. 21 Urinogenital system of sterile free-martin 21.8 cm. long. $\times \frac{4}{3}$. From two-sexed pair 41. Exceedingly small gonads drawn close to entrance of the saccus vaginalis. *1*, gonad; *2*, Wolffian duct; *3*, gubernaculum; *4*, urinogenital sinus; *5*, clitoris; *6*, allantois.

Fig. 22A Urinogenital system of sterile free-martin 22.5 cm. long. $\times \frac{4}{3}$. From two-sexed pair 4. The right gubernaculum is evaginated into the body cavity.

22B. Part of 22A. $\times \frac{8}{3}$ with the right gubernaculum turned over to show the gonad and Wolffian duct. *1*, kidney; *2*, gonad; *3*, Wolffian ducts; *4*, gubernaculum; *5*, allantois; *6*, clitoris; *7*, umbilical artery; *8*, ureter.

22A

22B
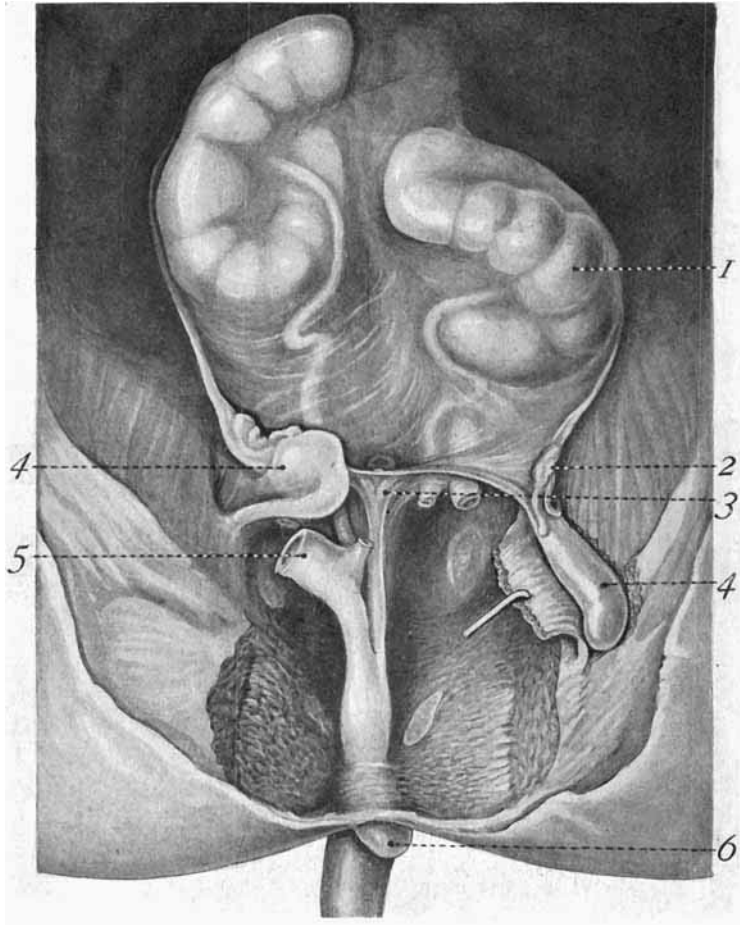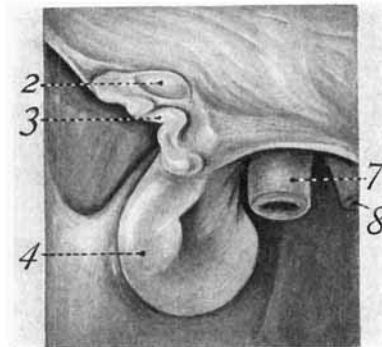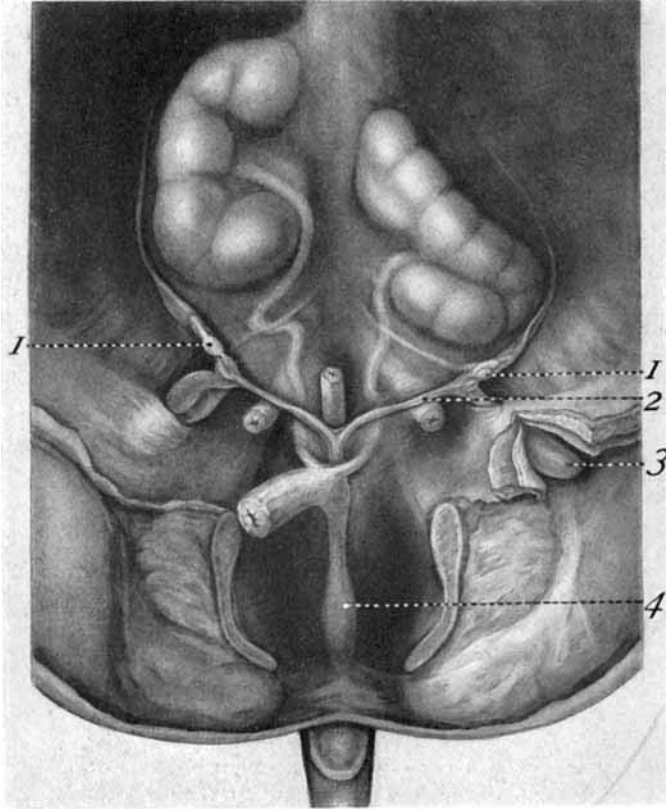
Fig. 23   Urinogenital system of sterile free-martin 22.5 cm. long. $\times \frac{4}{3}$.   **From two**-sexed pair 38.   The typical features recur here; the gubernaculum of **the right** side has grown only partly into the body wall; compare the left side.   *1*, gonad; *2*, Wolffian duct; *3*, gubernaculum; *4*, urinogenital sinus.
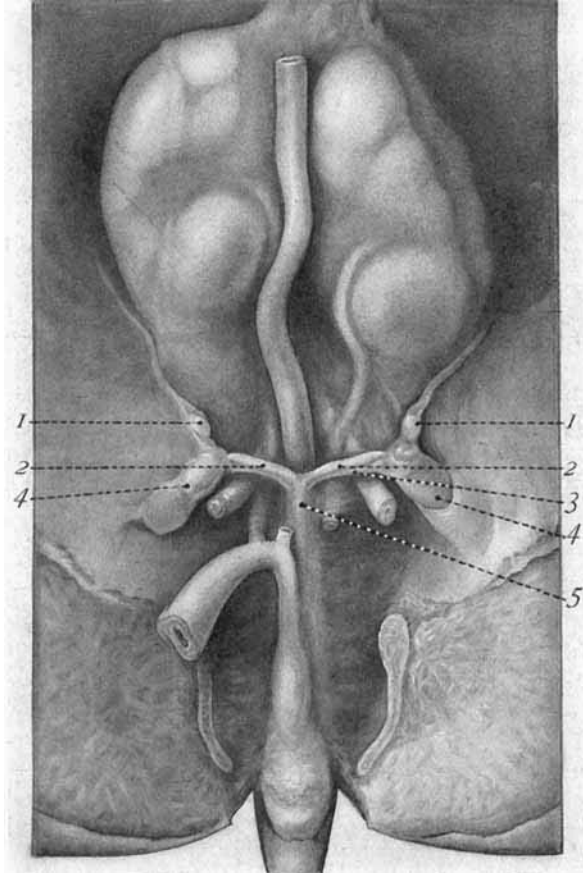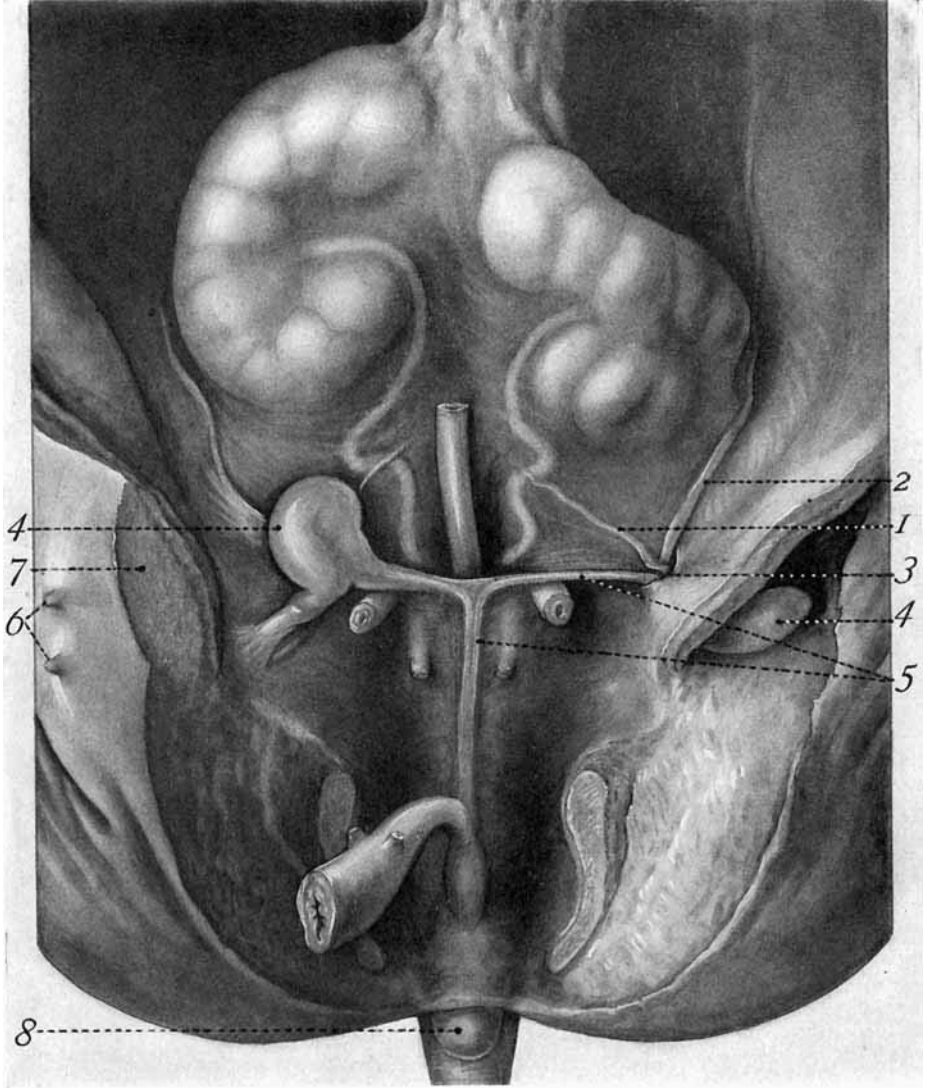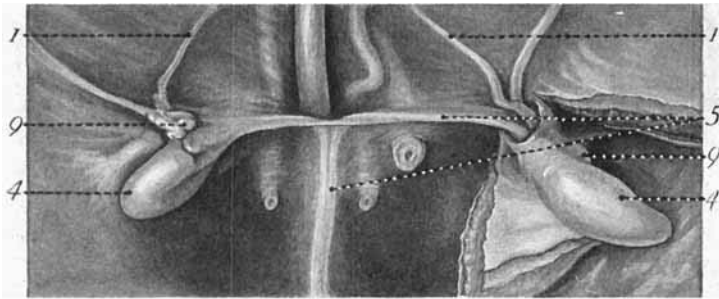
Fig. 24   Urinogenital system of sterile free-martin about 24 cm. long.   × ⅓.
From two-sexed pair 22.   The typical features recur here again; the right guber-
naculum lies in the body-cavity and is relatively undeveloped; compare the
left side.   Parts of the cornua uteri seem to be developed in this case.   *1*, gonad;
2, Müllerian ducts (cornua uteri); *3*, Wolffian duct; *4*, gubernaculum; *5*, rudi-
ment of corpus uteri, cervix and vagina.

25A

25B

Fig. 25A   Urinogenital system of sterile free-martin 26.5 cm. long. $\times \frac{4}{3}$.
From two-sexed pair 21.   In this case the gonad of the left side has entered the
saccus vaginalis; compare figure 12 for normal condition of the male.   The right
gubernaculum has grown into the body-cavity.   Observe the female disposition
of the teats and development of the glandular tissue of the mammary gland,
and compare male (fig. 12).

Fig. 25B   Part of 25A further dissected to show the left saccus vaginalis
containing the gonad on the left side; on the right side the gubernaculum is
turned over.   Designations for 25A and 25B.   1, ovarian artery; 2, remains of
urinogenital ridge; 3, entrance to saccus vaginalis; 4, gubernaculum; 5, Wolffian
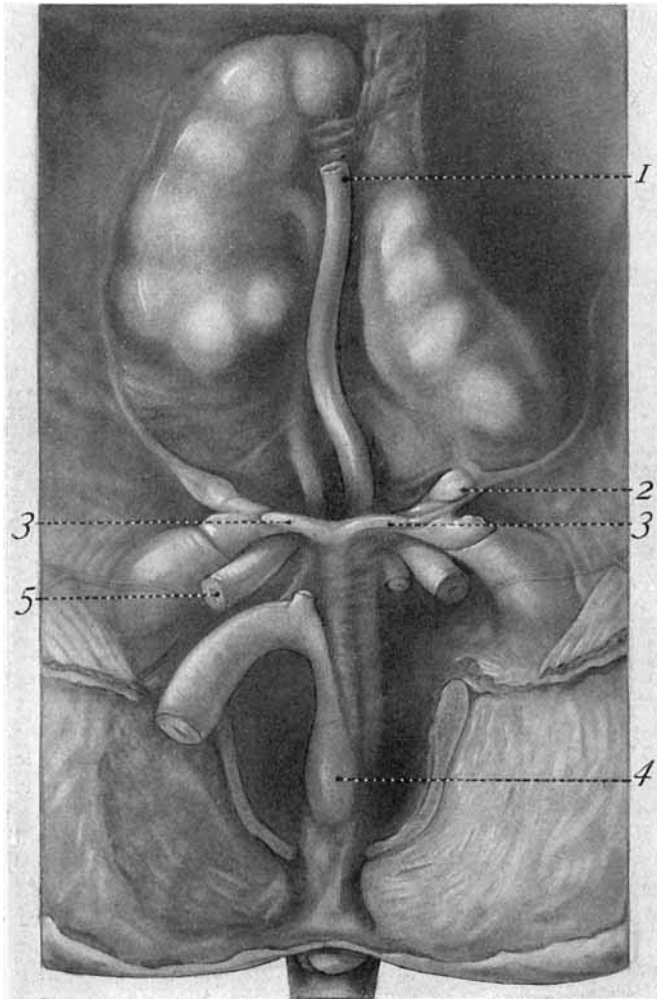duct; 6, teats; 7, mammary gland tissue; 8, clitoris; 9, gonad.

Fig. 26 Urinogenital system of sterile free-martin 27 cm. long. × ⅓. From two-sexed pair 14. Although this case is beyond the stage in which the testes of the male normally enter the saccus vaginalis, the rudimentary ovaries are here in the body cavity. In this case there appear to be rudiments of the cornua uteri. *1*, rectum; *2*, gonad; *3*, cornua uteri; *4*, urinogenital sinus; *5*, umbilical artery.

Fig. 27 Urinogenital system of sterile free-martin 28 cm. long. × ⅓. From two-sexed pair 12. In this case both gubernacula have grown into the body cavity; the right gubernaculum lies in the utero-rectal recess of the body-cavity. In figure B the gubernacula are rearranged, the right one being drawn out of the recess, and the genital cord is cut across and turned forward. Rudiments of both Müllerian and Wolffian ducts present. *1*, gonads; *2*, gubernaculum; *3*, sex-ducts; *4*, cornua uteri; *5*, corpus uteri, cervix and vagina; *6*, Wolffian ducts.
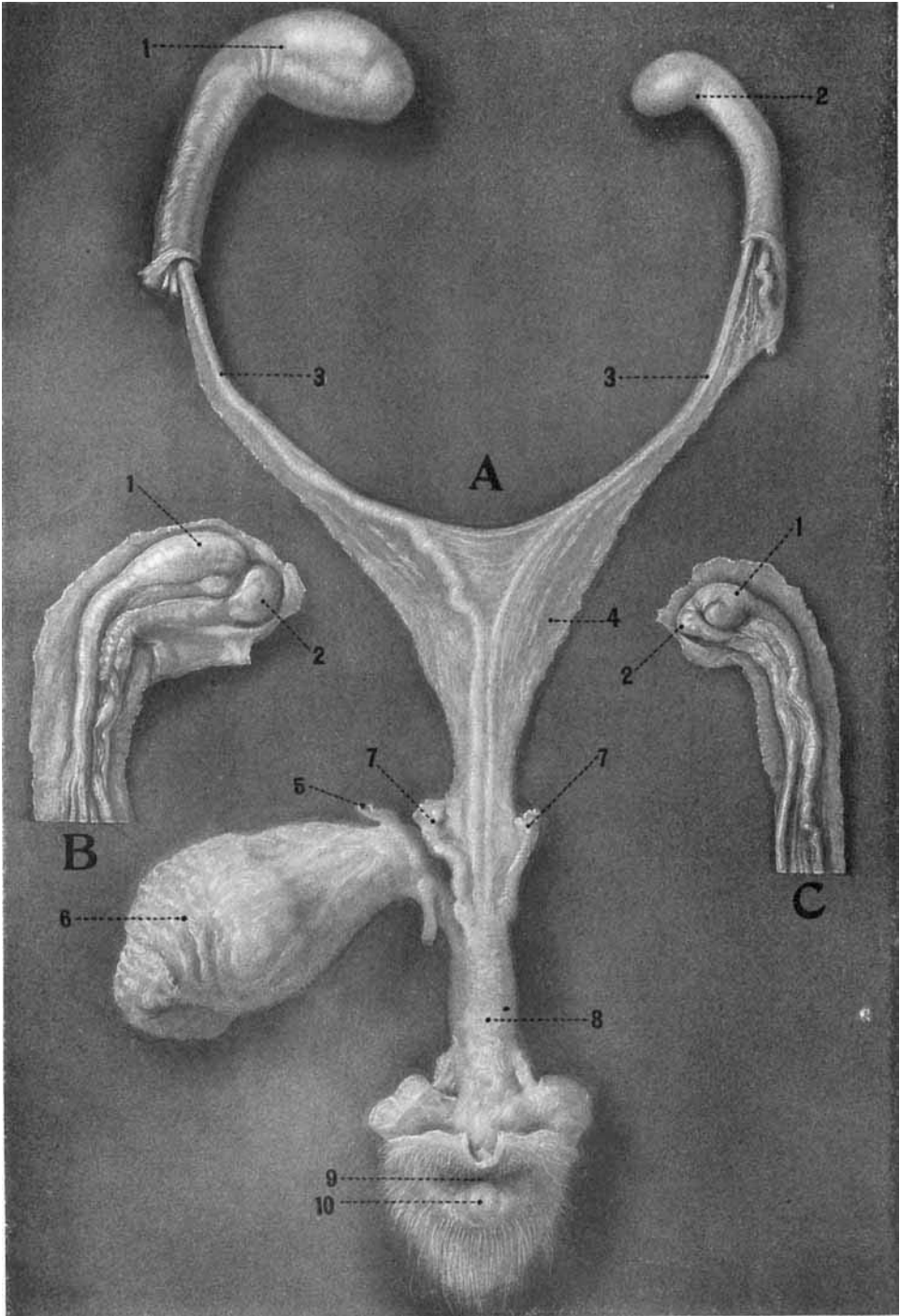
448
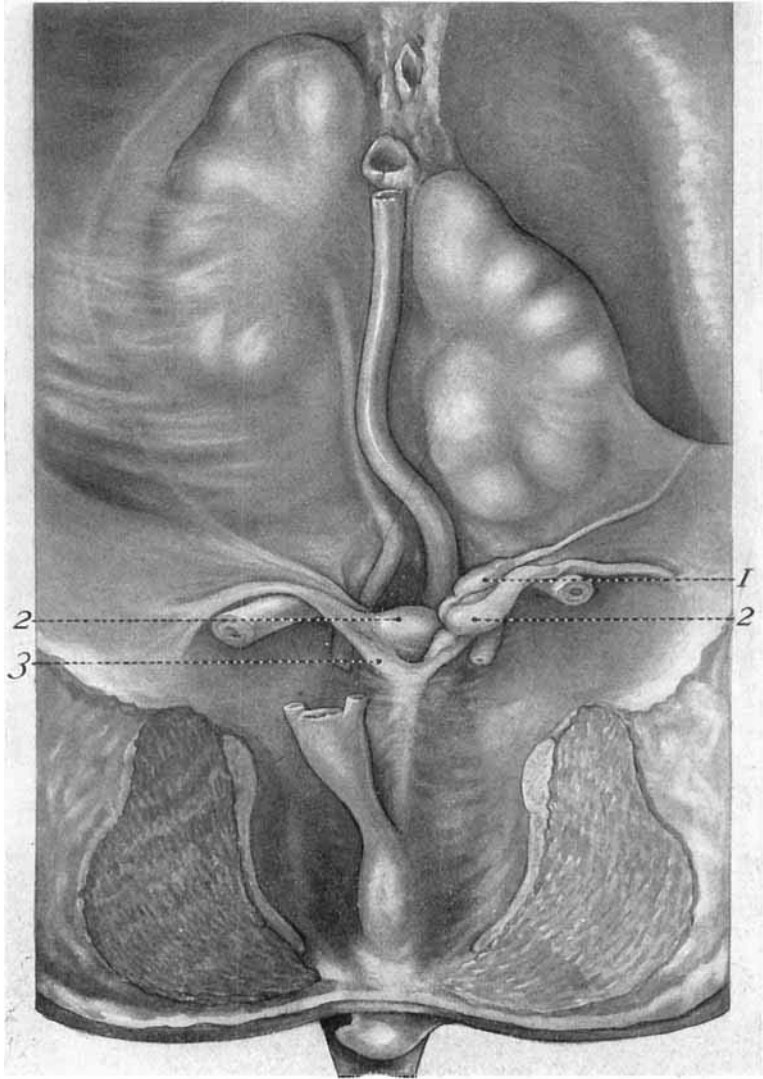
27A
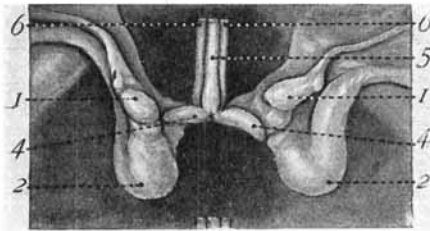
27B

Fig. 28   Reproductive organs of a seven weeks old free-martin. $\times \frac{5}{13}$.   Born twin to a male, case 44.   The dissection shows a dorsal view.   Description in text.

Fig. A   *1*, left saccus vaginalis containing gonad; *2*, right saccus vaginalis containing gonad; *3*, Vas deferens (Wolffian duct); *4*, broad ligament; *5*, ureter; *6*, bladder; *7*, seminal vesicles; *8*, urogenital sinus; *9*, vulva; *10*, clitoris.

Figs. B and C   Left and right sacci vaginales opened.   *1*, testis; *2*, epididymis.

Fig. 29   Reproductive organs of a free-martin described by Numan ('43) from his plate XI.   Description in the text (p. 413).   The description of this plate was missing.   The explanation of the letters is therefore my own interpretation.   *a*, epididymis with testis above; *b*, Saccus vaginalis; *c.c*, cut wall of saccus vaginalis; *d.e.f*, spermatic artery, vein and nerve (?); *g*, Vasa deferentia (Wolffian ducts); *h*, bladder; *i*, broad ligament; *k.l*, ligaments of bladder; (?); *m*, ureters; *n*, seminal vesicles; *o*, entrance of vasa deferentia into the urinogenital sinus; *p*, urinogenital sinus; *q*, prostate; *r*, penis; *s*, retractor muscles of penis; *t*, ?; *u*, external opening of urinogenital sinus (urethra) beneath the glans penis; *v.w*, accessory openings in the urethra; *x*, vulva; *7*, anus; *8*, perineum.