On the Descended Testes of Sex-Intergrade Pigs.

By

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With 8 Text-figures.

Introduction.

In a previous paper (2) I described the anatomy of a number of sex-intergrade pigs, and discussed the inheritance of a tendency to the abnormality as well as the physiology of its development. In the present paper I describe in some detail the minute structure of the testes of four sex-intergrade pigs, the anatomy of three of which was described in my earlier paper.

In all the four pigs described in this paper the testes were extra-abdominal. Except in one case they did not project as much as do those of a normal boar, but they were readily palpable. I have chosen these four pigs for description of the minute structure of the testes because cryptorchid testes are always abnormal. Had I chosen to describe the testes of
cryptorchid sex-intergrades, it would have been difficult to decide which abnormalities were due to cryptorchism and which, if any, to the sex-abnormality.

The problems which I have attempted to solve are these:

1. To what extent is the sex-abnormality associated with aberrations from the normal development of spermatogonia to spermatozoa?
2. What is the sex-chromosome equipment of sex-intergrade pigs?
3. Do the interstitial cells of sex-intergrades secrete actively or not? In other words, is there evidence that the interstitial hormone is produced in these animals? If it were not, the absence of hormone might be the cause of the abnormality.

Problem (1) calls for no comment at this stage. In connexion with problem (2) it is essential for the reader to remember that Wodsedalek (7) has found that the boar has two sex-chromosomes, while the sow has four. Wodsedalek regards the two nucleoli which are always present in the interkinetic spermatogonium as the sex-chromosomes, though, in the pig, he has not actually proved that this is the correct interpretation. (In the bull he has established the identity of the spermatogonial nucleolus with the sex-chromosome.) I give in fig. 1 an outline drawing of two of Wodsedalek’s figures, representing the early (left) and full-sized (right) spermatogonial nucleus of the boar. The small nucleoli are inconstant in number and of

**Text-fig. 1.**

Outline drawing of Wodsedalek’s figure (7) to show the nucleoli of the young (left) and of the full-sized (right) spermatogonium. The two large nucleoli in each nucleus are supposed to be the sex-chromosomes. nl, nucleoli.
doubtful significance; the large nucleoli are the supposed sex-chromosomes.

With regard to problem (3) it is necessary to review the literature at some length. It is by no means the case that all authors agree as to the structure of the functioning interstitial cell of the boar. All authors admit that routine histological methods give a central denser region of cytoplasm and a peripheral less dense vacuolated region, and further that there are small particles of fat which are never abundant and which do not occur in every cell. But with regard to cytoplasmic inclusions other than fat there is divergence of opinion.

Ancel and Bouin, in their classical researches on the interstitial cells (1), describe the following cytoplasmic inclusions in the interstitial cells of the adult boar:

(a) 1 Granules of very variable size—some very small—which stain with iron haematoxylin or with acid fuchsin after fixation by potassium bichromate.

(b) Inclusions varying in size from small granules to large lobed structures lying in the periphery of the cell and occupying the position of the vacuoles seen in ordinary preparations. They are rendered evident by fixation in bichromate acetic followed by Weigert's copper haematoxylin. This technique involves the embedding of the tissue in celloidin and its impregnation with the mordant (copper acetate) while so embedded. The sections are stained in haematoxylin, differentiated in a mixture of borax and potassium ferrocyanide, and mounted in gum-syrup.

Whitehead (5 and 6) also describes two sorts of inclusion other than fat particles:

(a) Numerous mitochondria, much smaller than the granules next to be described.

(b) Granules of 1–2μ diameter lying mostly in the peripheral parts of the cell. Each granule lies in a distinct vacuole. They are destroyed by fixatives containing much acetic acid or potassium bichromate, though they are occasionally seen

1 The letters (a) and (b) do not correspond with the notation used by the authors.
in Flemming material. They are best fixed by absolute alcohol or '10 per cent. formalin'. They are well stained by iron haematoxylin or by the basic radical of neutral gentian-orange, as used by Bensley. On the other hand, they take the eosin of Mann's methyl-blue eosin. They are stained by Altmann's acid fuchsin and by Sudan III. Whitehead thinks it possible that they are derived from mitochondria by the incorporation of fat.

Hanes (4) again describes two types of granule:

(a) Granules staining with Altmann's acid fuchsin, lying chiefly in the central, denser portion of the cell.
(b) Granules lying in the peripheral portion of the cell, staining a brighter red with Altmann's acid fuchsin than do the first-named granules. They can also be stained by iron haematoxylin and by Weigert's method.

My interpretation of these three different accounts is as follows. Some of the mitochondria of the interstitial cell undergo a progressive enlargement by the incorporation of other substances, one of which is probably fat. The larger they are the more peripherally they tend to lie. During their enlargement their resistance to acetic acid and to alcohol increases, while potassium bichromate becomes less essential to their fixation. The result is that in the end they may be fixed with fluids containing much acetic acid or with absolute alcohol. Fixation by the latter fluid shows how little of their mitochondrial nature remains. When fully developed they are best fixed by acetic bichromate and stained by Weigert's copper haematoxylin. Other fixatives tend to shrink them and make them appear to lie in vacuoles.

The mitochondria sensu stricto are Whitehead's granules (a) and the smallest of Ancel and Bouin's granules (a) and of Hanes's granules (a). The earlier stages of the enlargement and alteration of these mitochondria are represented by the larger of Ancel and Bouin's granules (a) and of Hanes's granules (a), and also by the smaller of Ancel and Bouin's granules (b). The final stages of their enlargement and alteration are represented by Whitehead's granules (b) by Hanes's
granules (b), and by the larger of Ancel and Bouin's granules (b).

I incline to the view that Whitehead is mistaken in his statement that the gentianophil granules (i.e. his granules (b)) are destroyed by potassium bichromate.

**TECHNIQUE.**

The following fixatives were used:
- 0.9 per cent. sodium chloride in 4 per cent. formaldehyde.
- Bouin's fluid.
- Flemming's fluid.
- Regaud's fluid.

For nuclear characters formalin and Bouin material was stained in Heidenhain's, Delafield's, and Ehrlich's haematoxylin, and Flemming material in safranin.

For fat Flemming material or post-osmified formalin material was mounted unstained or stained only with safranin.

For cytoplasmic inclusions other than fat Regaud material was post-chromed and treated with a mixture of pyroligneous acid and chromic acid and then stained (as in the Champy-Kull technique) with acid fuchsin, toluidin blue, and aurantia. In other cases material which had been fixed in formalin and had lain in it for some months was treated in just the same way as the Regaud material, with favourable results. Formalin material was also stained with neutral gentian-orange, dehydrated in acetone, and differentiated in oil of cloves.

**RESULTS.**

Sex-intergrade No. 3.\(^1\) Age 7 months. In this pig the right testis was visible externally, but was not in a properly formed scrotum, while the left testis was abdominal. The opening of the vulva was rendered small and crescentic by the

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\(^1\) The numbering of the pigs corresponds with the numbers used in my earlier paper (2), which should be referred to if fuller anatomical details
enlarged clitoris. The uterus was quite fairly well developed, especially the cornua; the vagina was thin-walled.

The following description refers to the right descended testis.

The seminiferous tubules are without lumen and quite degenerate, there being only a single peripheral row of nuclei which are probably Sertolian. Fat-globules are abundant within the much vacuolated protoplasm.

The interstitial cells are present in about the normal amount. Bouin's fluid, haematoxylin, and eosin give the characteristic picture (fig. 2 A). With post-chromatization and treatment in bulk with the pyroligneous-chromic mixture followed by staining with acid fuchsin, toluidin blue, and aurantia, the numerous centrally placed mitochondria and the peripherally placed larger granules are rendered evident (fig. 2B). No valid distinction can be drawn between granules staining darkly and those staining brightly with the acid fuchsin, there being intermediate gradations. In formalin material Whitehead's granules (b) are sometimes stained by the gentian radical of Bensley's neutral gentian-orange (fig. 2C), though I have not succeeded in staining them by this method in more than a few cells in a section, and then not brightly. I attribute this failure to the fact that the material had lain for some months in formalin. As mentioned in the introduction, I am inclined to regard the gentianophil granules...
as the last term in the modification of the mitochondria, and therefore as the same as the larger of the granules stained by the acid fuchsin in fig. 2 b.

I take it that the interstitial cells of this testis are in a state of normal functional activity.

Sex-intergrade No. 9.—Age 2½ months. The testes

Part of seminiferous tubule with adjacent interstitial cells of sex-intergrade no. 9. Bouin; haematoxylin. f.sh, fibrous sheath of seminiferous tubule; i, interstitial cell; n, nucleus in seminiferous tubule; v, vacuole.

projected as do those of a normal boar of this age. The only female characters were the externals—namely, the presence of a vulva and the absence of a penis.

Here again the seminiferous tubules are degenerate. In a pig of this age one expects to find a row of ‘germinative’ nuclei peripherally with here and there a larger nucleus of a spermatogonium. This latter type of nucleus is absent (fig. 3), and further the tubules are loaded with particles of fat (fig. 4).
The interstitial cells are about normal in number. As far as can be judged from Bouin material, they are normal also in structure (fig. 3).

Sex-intergrade not described in my earlier paper.—This pig was castrated at the age of about 2½ or 3 months in my presence.¹ The testes were palpable but not visible externally, and there was a typical female vulva. This pig is still alive, so that the internal organs are not known.

The seminiferous tubules are not degenerate, but are those characteristic of a boar of this age. Both 'germinative' and spermatogonial nuclei are present, each of the latter having

¹ Thanks to the kindness of Mr. E. B. Adams.
the characteristic group of mitochondria associated with it. There is no fat within the tubules. The interstitial cells are present in the normal quantity. They are smaller than those of no. 8, but they are in a state of great functional activity, being loaded with granules similar to those drawn in fig. 2 b, but much more numerous.

Sex-intergrade No. 8.—This specimen I obtained in the New Hebrides (Pacific Ocean), where sex-intergrade pigs are common. I guess the age at 8 months. The testes just showed externally, but did not project as much as those of a normal boar. The vulva was that of a normal female with a very slightly enlarged clitoris. There was no uterus.

The left testis differs from those of all my other sex-intergrades in that it shows spermatogenesis in progress. Nearly every tubule shows spermatocytes in synizesis or in the pachytene stage (fig. 5), and a few tubules show apparently abnormal spermatids and spermatozoa (fig. 6). A few tubules show degenerate nuclei of a characteristic appearance (fig. 7); the chromatin has become plastered on to the nuclear membrane in deeply staining patches.

A study was made of the nucleoli of the spermatogonia to ascertain the sex-chromosome equipment in accordance with Wodsedalek's statement that the large spermatogonial nucleoli are probably the sex-chromosomes. Several authors have traced the identity of the spermatogonial nucleolus with the sex-chromosome in various mammals. It was found that the nuclei of the spermatogonia of this sex-intergrade have sometimes two (fig. 8, a and b), sometimes three (fig. 8, c and d), and sometimes four large nucleoli (fig. 8, e and f). The facts suggest that the abnormality of sex may be due to irregularity of sex-chromosome distribution, either in the mitoses of the developing sex-intergrade itself, or in one of its parents at or before the meiotic division. The latter alternative would account for the fact that certain pigs are known to

1 Thanks partly to the generosity of the Trustees of the Percy Sladen Memorial Fund.
Text-FIG. 5.

Part of seminiferous tubule with adjacent interstitial cells of sex-intergrade no. 8. Bouin; iron haematoxylin. Note spermatocytes in synizesis and in the pachytene stage.

Text-Figs. 6, 7.

Fig. 6.—Spermatid nuclei and spermatozoa of sex-intergrade no. 8. Bouin; iron haematoxylin.

Fig. 7.—Degenerate nucleus in seminiferous tubule of sex-intergrade no. 8. Bouin; iron haematoxylin.
produce one or more sex-intergrades in nearly every farrow of which they are parents.

It must be remarked, however, that the nuclei with three

TEXT-FIG. 8.

Spermatogonial nuclei of sex-intergrade no. 8. Bouin; iron haematoxylin. A and B have two large nucleoli. C and D have three large nucleoli. E and F have four large nucleoli. nl, nucleolus (supposed sex-chromosome).

and with four nucleoli may really be degenerate nuclei in which the nucleoli have become broken up. In a few spermatogonia more than four nucleoli—perhaps rather smaller than the ordinary large nucleoli—are found. These are probably degenerate nuclei.

For the most part Sertoli cells are not abnormally numerous;
but here and there they are found to have multiplied considerably, as is often the case in degenerate testes.

It would be very interesting to know whether many of the sex-intergrade pigs of the New Hebrides have testes in which spermatogenesis is in progress; whether, in fact, the pigs of the New Hebrides exhibit a different type of intersexuality from those of Great Britain.

The right testis is small and entirely abnormal, consisting for the most part of connective tissue. The other cell elements are difficult to interpret, and certainly indicate extreme degeneration.

**DISCUSSION.**

It emerges from this study that the seminiferous tubules of sex-intergrade pigs tend to be degenerate even when the testes have descended. An exception to this rule is no. 9, but this specimen was so young that one could not pretend to say that subsequent development would result in normal spermatogenesis.

What hinders normal spermatogenesis is unknown, but perhaps the metabolism, and particularly the fat metabolism, tends to be of female type. It can be imagined that this might interfere with the usual course of development of the male germ-cells.

With regard to the interstitial cells, these are usually present in normal or greater than normal amount in sex-intergrade animals, and my work tends to show that they are actively functional. At first sight this appears to be opposed to the view, strongly grounded on experimental facts, that they are the cells which produce the male-determining hormone. But if we assume, with Crew (3), that the hormone must be produced at some definite time early in development if it is to have its full normal effect, the difficulty disappears. My own hypothesis to account for mammalian intersexuality (2) is that in the early stages of the development of every sex-intergrade both testicular and ovarian tissue was present. On this hypothesis
also active secretion by the interstitial cells is not irreconcilable with the belief that their secretion is the male hormone.

I wish to record my thanks to Professor E. S. Goodrich and to Professor J. S. Huxley for their valuable criticism of the typescript of this paper.

**Summary.**

The descended testes of four sex-intergrade pigs were studied with the following results:

1. The seminiferous tubules are usually quite degenerate but may contain a few abnormal (?) spermatozoa.
2. Perhaps the sex-chromosome equipment of sex-intergrades is abnormal.
3. The interstitial cells are normal and functional.

**References.**