ARTIFICIAL CRYPTORCHISM IN RATS

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Although cryptorchism occurs commonly in children, the changes occurring have been studied comparatively little. The pathogenesis is undoubtedly interesting: cryptorchism is usually associated with considerable functional disturbance of the undescended testis. The germinative and endocrine disturbances appear many years after a successful operation had been performed in childhood. When sexual maturity is obtained, neither spermatogenesis nor endocrine activity takes place. About 20% of such testes are hypoplastic [4].

No investigation has been made into the mechanism of these disturbances. Evidently they cannot be attributed merely to abnormal body temperature, which is thought to be the main cause of functional failure in artificial cryptorchism [11].

Artificial cryptorchism is a simplified model; however this simplification may be useful for an analysis of the pathogenesis of the condition.

EXPERIMENTAL METHODS

Artificial cryptorchism was induced in 27 young sexually mature male rats weighing 180-220 g. An incision was made in the iliac region, and through it access was gained to the testes which were gripped by the fatty tissue of the epididymis and moved into the abdominal cavity, where they were sewn by catgut to the ilio-costal muscles.

The rats were killed 2, 3, 4, 5, 10, 15, 20, 30, and 150 days after the operation (three rats at a time). The testes and accessory glands were weighed (the total weight of all the accessory glands was found), and placed in Baker's fixative. After they had remained in the fixative for 24 h, they were cut in halves, and embedded in a gelatin block. In one half a study was made of the total and neutral lipids and of the ketosteroids; the tissue of the other half was treated with chromium in order to carry out Baker's reaction and a modified Cain's reaction. Sections 15 μ thick were cut from both halves on a freezing microtome. Total lipids were determined by staining with a saturated solution of Sudan B black.

Cain's [3] Nile blue sulfate reaction was carried out according to the method prescribed, as well as in a modified manner in sections chromed after fixation by Baker's method. We were able to show that chroming progressively alters the character of the included neutral lipids.

The Seligman-Ashbel reaction [1] revealed 3-, 17-, and 20-ketosteroids. There is some doubt about the specificity of this reaction with respect to the aldehyde groups, but its originators maintain that in tissues fixed in formalin no free aldehyde groups are present. Diazotization was carried out with a solution of the salt of variamine blue B.

Baker's method [2] reveals phosphatides. A control extraction with pyridin excludes nucleo- and mucoproteins which show up only weakly in Baker's reaction.

EXPERIMENTAL RESULTS

Spermatogenesis proceeding to the stage of ripe sperms can be observed in approximately 50% of the tubules of the epididymis of a normal sexually mature rat. In the remaining tubules, the earlier stages of spermatogenesis could be made out; normally, in the parts nearest the walls the numerous small droplets stained by Sudan dye appear;

they contain phosphatides and ketosteroids, and in Cain's reaction they show the properties of neutral lipids. In the tubules containing mature sperms there was no accumulation of lipids in the other cell walls. In the testes of control rats the Leydig cells stain weakly with Sudan dye and give a moderately positive reaction for ketosteroids; they were not selectively stained by Cain's or Baker's methods.

Even two days after transplantation into the abdominal cavity, some disturbance of the testicular tissue was apparent: in part of the tubules the germinal epithelium had become disorganized, and some tubules had lost the regular arrangement of the flagellae of the sperms. However, in the cryptorchid individuals, the percentage of tubules containing mature sperms did not differ from normal. At the periphery of the tubules in which the early stages of spermatogenesis were proceeding, there was an increase in the number and size of the lipid drops. Histochemically these accumulations of lipids near the walls of the tubules resembled those found in the controls. In the interstitial tissue no morphological changes were observed.

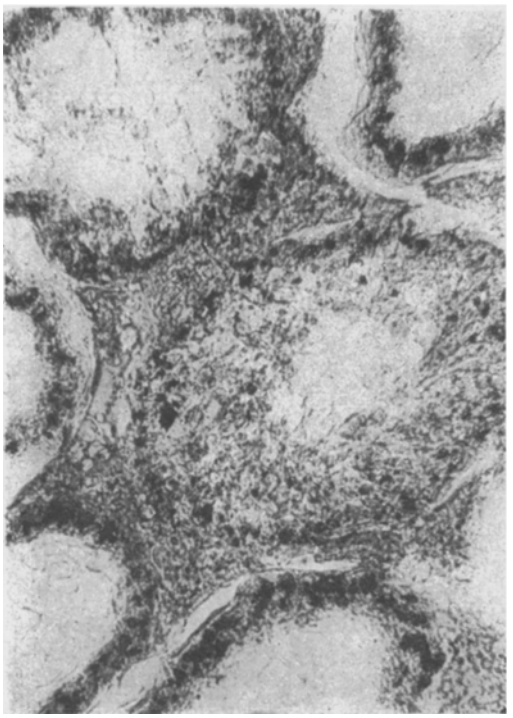

Fig. 1. Testes of a cryptorchid rat ten days after operation. Cain's reaction after chroming. The folded outlines of the tubules and a loosening and destruction of the epithelium can be seen. Around the membrane there are large neutral lipid droplets; in the center of the lumens on some of the tubules there is a fine lipid granularity. Ocular 10 x, objective 40 x.

In the cryptorchid individuals, three days after the operation there was an increased disorganization of the germinal epithelium. The mature spermatozoids were not found in more than one-third of the tubules.

In the tubules in which the earlier stages of spermatogenesis were proceeding lipid droplets distributed near the membrane were more numerous and larger. Cain's reaction showed up these lipids as large pink drops, while in the test for ketosteroids they appeared as smaller more numerous droplets.

A complete absence of spermatogenesis was observed for the first time four days after the operation. The germinal epithelium had undergone considerable disintegration and its cells had become strongly vacuolized; the diameter of the seminal tubules had been reduced, and the walls had become folded. At the same time, the first chemical changes in the properties of the lipids of the tubules became apparent: the lipid droplets lost their phosphatides. In some of the tubules these droplets were eliminated; in other tubules they were revealed in tests for ketosteroids or neutral lipids (before and after chroming). Some hypertrophy of the Leydig cells was also apparent, and their reaction for ketosteroids was less strong.

By the fifth day there were no further marked changes. In the Sertoli layer of some of the tubules, as before, a fine granularity appeared in Baker's reaction.

Cain's reaction revealed large pink droplets which were not always confined to the neighborhood of the membrane, but also occupied the center of the lumens. The Ashbel-Seligman method showed large drops rich in ketosteroids; these also spread from the periphery towards the center of the tubules.

On the 10th day, the seminiferous tubules had become still smaller in diameter, and more extensively folded (Fig. 1). The seminiferous epithelium showed only the primary stages; in certain of the tubules spermatogenesis had proceeded as far as the formation of a small number of sperms, which were distributed in a disorderly manner in the lumen. In such tubules the reaction for phosphatides was negative, and in the remainder they showed up as a dense fine granularity filling the whole of the layer of the strongly vacuolized epithelium. In the neighborhood of the membrane sometimes distinct larger droplets could be seen. With Cain's reaction, after chroming, these lipids showed up as a fine lilac granularity occupying the whole thickness of the epithelium and also as separate drops lying in the basal portion (see Fig. 1). In reactions for neutral lipids and ketosteroids the tubules appeared completely

filled with lipid droplets. The Leydig cells were noticeably hypertrophied; in them a certain amount of phosphatide could be made out, and there was an increased amount of ketosteroids.

Fifteen days after the operation the reduced degenerate seminiferous tubules contained a Sertoli layer broken up by cavities and vacuoles, and spermatogonia. In Cain's reaction the tubules appeared densely packed with pink droplets. Phosphatides appeared in the tubules in the form of small or moderately large granules occupying the whole thickness of the epithelial layer. The accumulation near the membrane of large lipid droplets could no longer be observed. They did not appear in the Ashbel-Seligman reaction. The Leydig cells were strongly hypertrophied and stained with Sudan dye, and contained ketosteroids and some small granules which gave the reaction for phosphatides.

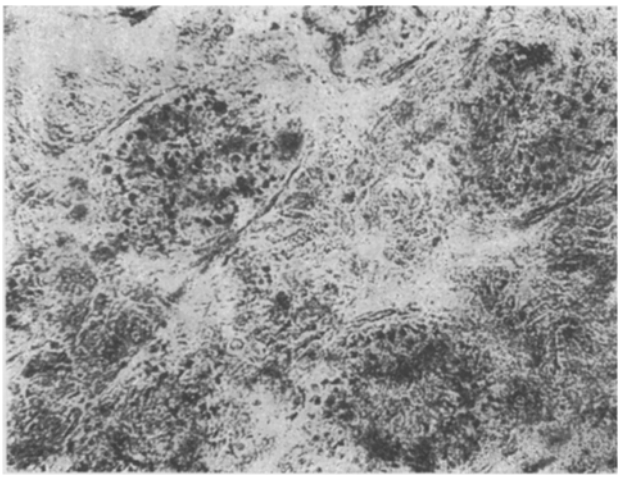

Fig. 2. Testis of a cryptorchid rat 20 days after operation. Baker's reaction for phosphatides. Droplets of small diameter separated by groups of Leydig cells. Phosphatides scattered as small and medium sized granules along the epithelium of the tubules. Some phosphatide granules are present in the Leydig cells. Ocular 10 x, objective 8 x.

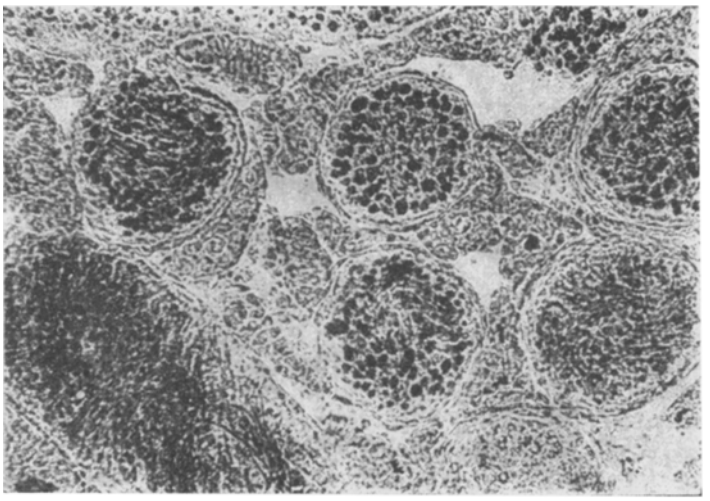

Fig. 3. Testis of cryptorchid rat 30 days after operation. Baker's reaction. The small tubules have been considerably separated by the marked hypertrophy of the masses of Leydig cells. The tubules are filled with large phosphatide droplets. Ocular 10 x, objective 8 x.

On the 20th day, the cryptorchid testes showed very little change (Fig. 2).

Marked atrophic and degenerative changes were observed after 30 days. There were no spermatogonial cells in the tubules. The Sertoli layer was thin and divided by bands; it was filled with large lipid droplets revealed by the various reactions (Fig. 3). The basement membranes of the tubules were better shown than at earlier times, but the diameter of the tubules themselves was reduced. The Leydig cells were strongly hypertrophied, and no lipids could be observed in them.

In the testes of the cryptorchid individuals five months after the operation most of the tubules were empty; there were no spermatogonial cells, and the Sertoli syncytium was loose, divided by cavities, and it usually filled the lumen of the tubules with reticulate outgrowths containing no nucleus. In it there were only occasional scattered granules of various sizes stained with Sudan dye, and these sometimes contained ketosteroids or phosphates. The tubules were greatly reduced in diameter, and their basement membrane was considerably thickened. In a very few tubules nevertheless some resemblance to spermatogenesis could be made out: large epithelial cells were formed, and sometimes a typical flagellate form which showed some tendency to form a clump in the center of the tubules could be seen. However, even in these tubules the lumen could not usually be made out. There were no scattered lipid droplets; neither Sudan dye nor the reaction of ketosteroids or phosphatides revealed any. Only in Cain's reaction were some pink droplets revealed. The Leydig cells stained with Sudan, stained positively in the Ashbel-Seligman reaction, and gave a weakly positive Baker reaction. They formed dense clumps in which could be seen numerous vessels with thickened walls which had become transparent.

Atrophy of the testes was more clearly shown during the first ten days after operation: their weight fell to less than half (see table). Subsequently, the atrophic changes increased less rapidly; by the 30th day the weight of the testes of the rats rendered cryptorchid had reached its minimum value (25-30 % of normal). This condition was stable, and by the fifth month the testes had the same weight as at the end of the first month.

The weight variation of the accessory glands was irregular. First there was a tendency for a fall in weight of 20-30% below normal during the 5th and 20th days; by the end of the experiment their weight had fallen the same amount as in the control animals.

Only a few studies have been made of testicular changes in cryptorchism. Payne [13] found that in mice spermiogenesis failed between the 3rd and 9-10th days, and that Sudanophil lipid droplets began to accumulate around the cell walls from the 2nd day onwards. Normally, in mice there are no lipids around the membranes.

Perlman [14] found that incomplete spermiogenesis from various causes always led within a few weeks to an increased concentration of lipids within the testis, and that in cryptorchism after 10 days spermiogenesis is depressed more sharply and profoundly than is the case in hypophysectomy. It is interesting that the accumulation of lipid droplets is a universal reaction: it is observed in cryptorchism, hypophysectomy, after injection of estrogen and in aging. Secretion of the follicle-stimulating hypophyseal hormone is usually reduced. It is thought that the increased lipid content of the tubules results not from their increased productivity, but from a reduction of their utilization through suppression of spermiogenesis [8, 9]. In the testes of the cryptorchid individuals it was shown that the increased content of androgens and estrogens was respectively 50 and 150% above normal.

In rats rendered artificially cryptorchid, by the 75th day, "castration cells" appeared in the hypophysis, whereas in the seminal vesicles and in the prostate gland they did not appear until the 240-400th day [12].

It is known that to prevent castrational changes in the sex ducts, it is sufficient to inject testosterone; to eliminate the changes in the hypophysis resulting from castration, the whole set of steroids is required [10].

The reason is apparently related to what we observed: that after five months there is no relationship between the condition of the testes and the weight of the accessory glands even for the very same rats.

Clegg [5, 6] studied the hormonal activity of the testes in cryptorchid rats between the 5th and 35th day after the operation. He used the amount of citric acid and fructose in the accessory glands as a criterion of the androgen concentration of the blood. He showed that by the 5th day the secretion of androgens was reduced, that it increased by the 10th day, and fell again between the 15th and 20th days after the operation; the least secretion of androgens occurred on the 21st day after transferring the testis into the abdominal cavity. At the same time there was an increase in the number of Leydig cells. Clegg thinks that the end of the third week after the operation is the end of the period of normal function of the Leydig cells in response to an increase of gonadotropins caused by a deficit of androgens in the blood. Therefore the increase in the number of Leydig cells appears to be a compensatory response demonstrating their normal sensitivity to gonadotropins, to which later the Leydig cells become less sensitive.

Comparison of our results with Clegg's suggests that the absence of lipids from the Leydig cells and the accumulation of large lipid droplets in the tubules is the morphological equivalent of a reduction of endocrine activity in the testis. This condition was observed in the first five days and between the 20th and 30th days after the operation. At the same time the presence of lipids in the Leydig cells and the fine lipid granularity in the epithelium of the tubules, observed 10-15 days after the operation in cryptorchids, appears to be the result of the active secretion of androgens.

Weight of the Sexual Organs in Cryptorchids at Various Times After the Operation

Time after operation (in days)	Mean weight when killed (in mg)		
	testes		accessory glands
	left	right	
2	1274	1346	984
3	1529	1491	1243
4	1024	1203	1124
5	1014	980	927
10	603	552	905
15	506	454	834
20	395	436	742
30	355	355	979
150	388	415	1337

SUMMARY

Changes in the lipids of the testes were studied in sexually mature rats with artificially induced bilateral cryptorchism. Observations were made from 2 days to 5 months after the operation. Failure of spermiogenesis was associated with accumulations of large perimembranous drops of lipids. These lipids were Sudanophil, neutral, and contained ketosteroids and phosphatides. By the 5th day, mature sperms had disappeared from the lumen of the tubules; simultaneously phosphatides had disappeared from the lipid drops. No lipids were present in the Leydig cells. After 10-15 days, hypertrophy of the Leydig cells and accumulations in them of minute lipid droplets could be observed. The lipids in the tubules took a different form, and were very finely dispersed.

By the 20th day, the droplet in the tubules had again enlarged, and all the testicular structures had lost their ketosteroids. After 30 days the lipids had disappeared completely from the Leydig cells; the tubules were thickly filled with large lipid drops. After 5 months the cryptorchid testes became completely empty.

LITERATURE CITED

1. R. Ashbel and A. M. Seligman, Endocrinology, Vol. 44 (1949), p. 565.
2. J. R. Baker, Quart. J. micr. Sci., Vol. 87 (1946), p. 441.
3. A. J. Cain, Quart. J. micr. Sci., Vol. 88 (1946), p. 383.
4. C. W. Charny, J. Urol., Vol. 83 (Baltimore, 1950), p. 697.
5. E. J. Clegg, J. Endocr., Vol. 20 (1960), p. 210.
6. E. J. Clegg, J. Endocr., Vol. 21 (1961), p. 433.
7. J. B. Hamilton and S. L. Leonard, Anat. Rec., Vol. 71 (1938), p. 105.
8. R. P. Howard, R. C. Sniffen, F. A. Simmons et al., J. clin. Endocr., Vol. 10 (1950), p. 121.
9. K. M. Lynch, Jr. and W. W. Scott, J. Urol. Vol., 64 (Baltimore, 1950), p. 767.
10. E. P. McCullagh and C. A. Shaffenburg, Ann. New York Acad. Sci., Vol. 55, Art. 4 (1952), p. 674.
11. C. R. Moore, in the book: Sex and Internal Secretion (Baltimore, 1934), p. 281.
12. W. O. Nelson, Proc. Soc. exp. Biol., Vol. 31 (New York, 1934), p. 1192.
13. J. M. Payne, J. Path. Bact., Vol. 71 (1956), p. 117.
14. P. L. Perlman, Endocrinology, Vol. 46 (1950), p. 347.