EXPERIMENTAL BIOLOGY

REGENERATION OF THE TESTICLES IN MAMMALS

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(Received December 24, 1955. Presented by Acting Member of the Acad. Med. Sci. USSR. N. G. Khlopin)

In work on reparative regeneration of the internal organs in mammals, that devoted to regeneration of the testicle occupies a special position. While in relation to other internal organs, investigators set about the study of conditions influencing, in one way or another, the course of regeneration, the very possibility of regeneration of the testicle in mammals has up to now been denied by the majority of authors [2, 3, 4]. In individual papers there are references to the fact that infliction of trauma not infrequently leads to complete atrophy of the testicle, although in the early stages one notes restorative processes in the spermatogenic epithelium, which quickly fade away, and do not lead to regeneration of the organ [2, 3]. In the present work, an attempt was made to create conditions contributing to manifestation of the regenerative capacity of the testicle, taking into account its special structure and function.

EXPERIMENTAL METHODS

The experiments were conducted on rats, weighing 94-130 g, guinea pigs, weighing 300-600 g, and on kittens. In the animals, the right testicle was completely removed, and half of the left one, in accordance with the findings of N. S. Artemeva [1] that on removal of one of two paired organs, more favorable conditions are created for regeneration of the other. The animals were operated on under ether narcosis, with observance of the basic aseptic rules. One testicle was fully removed after preliminary ligature of the spermal cord. Removal of part of the second testicle took place nonuniformly in the 1st and 2nd series of experiments.

In the first series, conducted on 15 guinea pigs and 20 rats, after dissection of the testicle skin and membrane, the testicle was distended by means of forceps in the incision. The caudal part of the gland was cut away with sharp scissors (about half), together with the albumin membrane in a direction perpendicular to the longitudinal axis of the testicle. Then the testicle was placed on the site, where upon the remaining seminiferous tubules often fell out of the albumin membrane. Sutures were placed on the peritoneum and skin.

The second series of experiments was conducted on 20 rats and 2 kittens. The operation for removal of part of the testicle was performed as follows. All the integuments of the testicle were dissected up to the albumin membrane, then the testicle was taken out of the incision. The albumin membrane of the caudal part of the testicle was held by forceps and cut across. Its edge was drawn out from the side and half of the entire mass of the tubules was cut off with sharp scissors in a direction parallel with the longitudinal axis of the testicle. A continuous suture was placed on the albumin membrane, and the peritoneum and skin were stitched up. This type of operation was conditioned by the following considerations. In the testicle of an adult guinea pig, or rat, almost deprived of connective tissue walls, the tubules in the elongated albumin membrane are under considerable pressure, which, in the view of many authors [3, 5], contributes to the secretion of ripe sexual products from the testicle. In the first series of experiments, by cutting out part of the albumin membrane without stitching it up, we thus created conditions, hindering secretion of sexual products, which could have led to atrophy of the organ. In the second series of experiments, we managed to remove one of the possible causes of atrophy of the testicle,

* Deceased.

and to create favorable conditions for its regeneration.

The animals were killed 3-5 months after the operation. The material was fixed by Bouin's fluid. The serial paraffin microscopic sections were stained with hematoxylin and eosin, iron-hematoxylin, and also with azan stain according to Heidenhain.

In control investigations the testicles removed during the operation were used, also testicles of animals not operated on, of weight and age uniform with the experimental animals. The control animals were killed on the day of the conclusion of the experiment.

EXPERIMENTAL RESULTS

In the first series of experiments, the restoration of the testicle only occurred in one case. In a guinea pig weighing 300 g, the right testicle was removed entirely (its weight was 380 mg), and the half of the left one (weighing 150 mg). Five months later, the pig was killed. It weighed at that time 550 g. On dissection from the left side a large testicle was found, weighing 1200 mg, oval in shape, of normal color, with a somewhat protuberant surface. Histological examination showed that a large part of the surface of the testicle was covered with a fine albumin membrane; the remaining part of the surface corresponding to the site of the former incision was marked by growth of connective tissue, in places strongly infiltrated by leucocytes. The testicle consisted of the seminiferous tubules of normal shape and size. The tubules, situated on the surface of the damaged terminal of the testicle, were full of Sertoli's cells, and almost completely deprived of cells of the spermatogenic series. Only a few tubules, also situated on the damaged terminal of the gland, contained spermatogenic cells, which, however, were abnormally distributed; they were gathered in groups, forming separate foci of spermatogenesis.

The whole lumen of the tubule was filled with nests of spermatids, surrounded on all sides by spermatocytes. The further the tubules were situated from the damaged terminal of the testicle, the more regular the distribution of cells in them. In the center of the gland, and on its undamaged terminal, pictures of normal spermatogenesis, to the point of formation of ripe spermatozoids (Fig. 1) were found in the tubules. The interstitial tissue was weakly developed, which was characteristic of the testicle of the normal guinea pig of that age.

The question arises as to whether in the given case full value regeneration of the testicle occurred, or whether, connected with the growth of the animal, there took place a normal development and growth of the remaining part of the gland. If we assume only growth of the remaining half of the gland, then its weight would have had to correspond to half the weight of the testicle in the control pig at this age. In our experiment, the weight of the testicle corresponded to the weight of the entire control testicle (Table 1), which, together with the finding of the histological examination, allows one to speak of its regeneration.

In the second series of experiments, regeneration of the testicle occurred in two kittens and in two rats. The results by weight are given in Tables 2 and 3.

As is clear from Table 2, in Kitten No. 1, three months after the operation, the weight of the testicle increased about 30 times, and was three times greater than the weight of the testicle of a normal kitten of that age. Microscopically, the testicle was oval shaped, with a slight rosy tinge, and under the membrane a thick network of vessels could be seen. On histological examination, it was discovered that the whole surface of the testicle was covered with an albumin membrane. The site of the suture was easily determined by the defect of the tissue in the albumin membrane, and infiltration of this section of the membrane by polymorphonuclear leucocytes. The tubules lying directly on the site of the defect were in no way distinguishable from the tubules lying far from it. The lumen of the tubules was full of primary and secondary spermatocytes, many of them with mitotic division. In the walls of the tubules lay Sertoli's cells and spermatogonia, in which mitoses were found. Spermatids and spermatozoids in the tubules were not observed. The interstitial tissue was normally developed, the interlobular walls were not thickened. The bulk of the testicle was made up of the seminiferous tubules.

In Kitten No. 2, three months after the operation, the weight of the testicle was less than in Kitten No. 1, but it almost reached the weight of the testicle of the normal animal (Table 2). Macroscopically, the testicle had an oval shape, yellowish hue, with no adhesion to the surrounding tissues. Microscopic examination showed that the albumin membrane, covering the whole surface of the testicle, was very thick at the site of the suture. The seminiferous tubules lying at the damaged site were no different in structure from the tubules situated far from it. They were in the main filled with Sertoli's syncytioma. At the wall of the tubules, there were single

TABLE 1

Regeneration of Testicles in Guinea Pigs

Beginning experiment					End experiment		
No. of animals	Series	Body weight in g	Weight of removed entire testicle in mg.	Weight of removed part of testicle in mg.	Body weight in g.	Weight remaining testicle in mg	Result
13 1—12 14—15	First	300 300— 600	380 400— 1 100	150 200— 500	550 600— 780	1200 30—50	Regeneration Atrophy
	Additional control (10 animals)					Weight of Testicle	
						Left	Right
					450— 525	1 200— 1 300	1 250— 1 400

TABLE 2

Regeneration of Testicles in Kittens.

Beginning experiment					End of experiment		
No. of animals	Series	Body weight in g.	Weight removed entire testicle in mg.	Weight removed part of testicle in mg.	Body weight in g.	Weight remaining testicle in mg.	Result
1	Second	1 048	85	36	2 390	1 200	Regeneration
2		870	36	22	2 210	300	Regeneration
	Additional control					Weight of testicle in mg.	
						left	right
	(1 animal)				3 100	400	350

spermatogonia , in which mitoses were noted. . In individual seminiferous tubules, giant cells containing 4-8 nuclei each, were detected. Such cells lay in groups in the lumen of the canal next to the normal spermatocytes, forming peculiar foci of spermatogenesis. The amount of interstitial tissue in comparison with normal was not increased. In the tubules of this testicle, the spermatids and spermatozoids were absent (Fig. 2). In the testicles of the control kittens of this age, spermatogenesis did not go beyond formation of secondary spermatocytes. Thus, the development of the regenerated testicles corresponded to the normal ones.

The regenerated testicles of rats (Table 3), on microscopic examination, were characterized by a moderate thickening of the albumin membrane. The interstitial tissue was poorly developed. In the tubules, all stages of spermatogenesis, including spermatozoids (Fig. 3),were observed.

In all the other animals, in both the first and second series of experiments, we observed complete atrophy of the testicle, expressed in a sharp fall in weight and size of organ (see Tables 1-3). Microscopic study revealed that the testicle consisted entirely of profuse connective tissue. Tubules were absent from the majority of such testicles. Occasional seminiferous tubules were seen, deprived of spermatogenic epithelium and Sertoli's cells.

TABLE 3

Regeneration of Testicles in Rats

Beginning experiment					End of experiment		
No. of animals	Series	Body weight in g.	Weight removed entire testicle in g.	Weight removed part of testicle in g.	Body weight in g	Weight remaining testicle in mg.	Result
4	Second	94	850	600	140	800	Regeneration
1	Second	109	900	630	230	950	"
2,3, 5—40	First + Second	95— 130	800— 1 500	400— 500	140— 250	20— 60	Atrophy "
	Additional control (20 animals)					Weight of testicle in mg. left	right
					160— 230	900— 1 350	910— 1 400

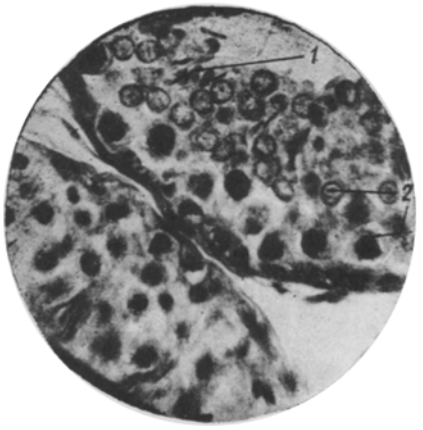

Fig. 1. Regenerated testicle of guinea pig 1) Spermatids, 2) spermatocytes; in tubules of the spermatogenic epithelial cell at all stages of development. Microphotography (x900).

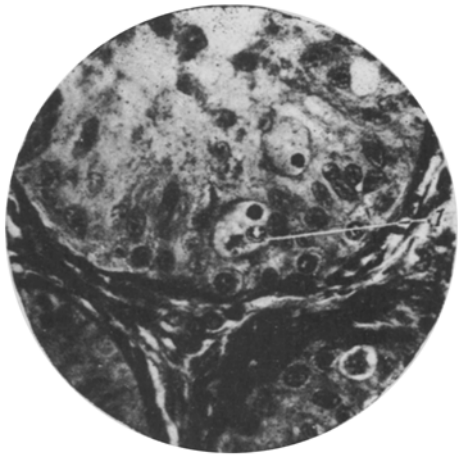

Fig. 2. Structure of the testicle tubule in cat, 3 months after operation. 1) Giant cell. Microphotography (x900).

Thus, in the first series of experiments in rats, in all cases the testicle was atrophied. The fact that in the second series of experiments regeneration of the testicle occurred in two adult rats is evidence in favor of the hypothesis concerning the significance of stitching the albumin membrane to bring about onset of the regenerative process in the testicle. But stitching of the albumin membrane is far from being the only condition guaranteeing regeneration of the testicle, as is evidenced in the absence of regeneration of the testicle in all the remaining rats in the experiments of the same series. In the literature there is abundant data on the exceptional sensitivity of the testicle to any kind of influence. The disturbance of the blood supply, toxic manifestations, compression of the spermatic cord, or of the testicle itself, may prove fatal to the functions of the testicle, and lead to complete and irreversible atrophy of the seminiferous tubules. The facile and rapidly developing atrophy of the testicle make it difficult to study its regeneration. This explains why, of the 57 animals under

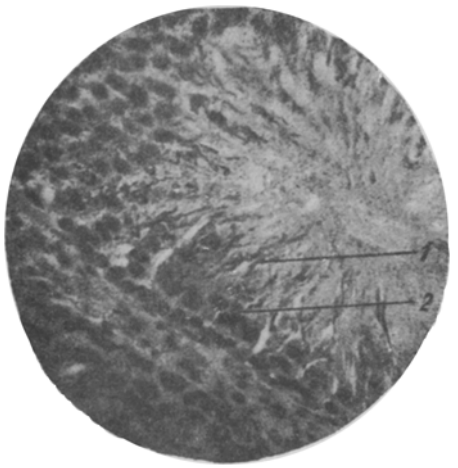

Fig. 3. Regenerated testicle of rat. 1) Spermatozoids. 2) Spermatocytes; in tubules of the spermatogenic epithelial cell at all stages of development. Microphotography (x400).

experiment, we only succeeded in obtaining regeneration of the testicle in five. Only in those cases where we managed to avoid the influence of the harmful factors arising from atrophy did complete organo-typical regeneration of the testicle occur in response to the trauma it received. Inquiries into the conditions contributing to the onset of regeneration of the testicle need to be continued.

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