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Microvascular Supply of the Regenerated Rat Testis Following Cadmium Injury

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Über die Gefäßversorgung im regenerierten Rattenhoden nach Cadmiumschädigung

Zusammenfassung. 7 Monate nach Cadmiumschädigung wurde die Capillarversorgung im Rattenhoden mikroangiographisch, verbunden entweder mit histologischer oder histochemischer Methode, untersucht. Die Blutgefäße, besonders diejenigen am epididymalen Rand des Hodens, hatten z.T. durch Sprossung direkt aus der teilweise rekanalisierten Hodenarterie eine beträchtliche Regeneration erfahren. Die Architektur des „neuen" Gefäßnetzes, welches hauptsächlich auf die Oberfläche beschränkt war, unterschied sich von der normalen Gefäßarchitektur nicht unerheblich. Im Inneren der fibrotischen und geschrumpften Samenkanälchen konnten wir die Entwicklung von Capillaren nachweisen. Die Neigung zur Extravasation der Kontrastsubstanz im Inneren der Kanälchen, nicht dagegen im intertubulären Gewebe, läßt auf eine kontinuierliche Ausbildung von neuen, kleinsten Capillaren in den Samenkanälchen schließen. Die Ausbildung eines experimentellen Kryptorchismus (über 4 oder 7 Monate) hatte keine Änderung der grundsätzlichen Organisation des Blutgefäßnetzes zur Folge, ebensowenig kam durch sie eine Entwicklung von Blutcapillaren im Inneren der Hodenkanälchen zustande.

Summary. The microvascular supply of the rat testis 7 months after cadmium-induced injury was studied microangiographically, combined with either histological or histochemical examination. The regenerating intratesticular blood vessels were seen to grow through the tunica albuginea, especially from the epididymal border of the testis, partly by direct sprouting from the partially recanalized testicular artery. In architectural organization the new vasculature was completely different from the normal one and was restricted mainly to the periphery of the testis. The ingrowth of capillaries into fibrotic and shrunken tubules was demonstrated. The tendency of the contrast medium to leak into the still unorganized tubules suggested that fragile new capillaries were also growing into them. Experimental cryptorchidism for 4 or 7 months caused no change in the basic organization of the capillary bed and no growth of blood vessels into the seminiferous tubules.

The acute effects of cadmium on the testis have been studied by numerous investigators since the initial observations of Pařízek and Záhoř (1956). The primary vascular changes have been shown to lead to a rapid decrease in blood flow and an increase in capillary permeability (Gunn *et al.*, 1963; Chiquione, 1964; Niemi and Kormano, 1965; Waites and Setchell, 1966; Clegg and Carr, 1967). The late appearance of the cadmium-injured testis has been much less studied than the acute changes. The first more prolonged observation was made by Allanson and Deanesly (1962), who observed that the long-term effects of cadmium depend on the dose and that after 4 months some regeneration occurred in some of the seminiferous tubules but this did not advance within the following 4—5 months. The interstitial cells were able to recover and maintain their

endocrine function, but later Favino *et al.* (1966) observed that the secretory pattern of regenerated Leydig tissue differs from that of the normal testis. Gunn *et al.* (1965) reported a high incidence of interstitial cell tumours in the rat testis following cadmium injury.

An earlier angiographic study from this laboratory on acute cadmium-induced injury to the rat testis (Niemi and Kormano, 1965) showed that one month after cadmium administration new vasculature appears in the testis but its pattern is different from the original. It was also apparent that the process of revascularization was still going on one month after cadmium injury. As the regenerated vessels were also claimed to differ from the normal testicular blood vessels as regards cadmium sensitivity (Gunn *et al.*, 1966), it was decided to study the organization of the vascular supply to the testis at a more advanced stage of regeneration.

Materials and Methods

Male Sprague-Dawley rats, 4 months old, received a subcutaneous injection of 0.03 mM/kg cadmium chloride. Eight of these and six uninjected control animals were studied 7 months later. To produce a different type of testicular injury for correlation, 6 rats of the same age were made cryptorchid by displacement of one testis into the abdominal cavity, invagination of the processus vaginalis and closure of the deep inguinal ring. The unilaterally cryptorchid animals were studied 4 and 7 months after operation.

The vascularization of the testis was studied, using a microangiographic method described in detail in an earlier paper (Kormano, 1967a). However, owing to the tendency to extravasation of the contrast medium, an unusually low injection pressure (60—80 mm Hg) was used in some of the cadmium-treated animals. In addition to conventional microangiography, including formalin fixation and paraffin embedding for histology, the testes of two animals from both the control and cadmium-treated groups were frozen rapidly with liquid air after the blood vessels had been filled with the contrast medium. Frozen sections from these testes were microradiographed as recently described (Kormano, 1969) and thereafter used for histochemical demonstration of alkaline phosphatase activity (Gomori, 1952).

Results

1. Gross Angiography

Seven months after cadmium-induced testicular injury the intratesticular network of the blood vessels was found to be estremely fragile in every animal. The normal testes could resist an injection pressure of 120—150 mm Hg without rupture of the blood vessels (Fig. 1) but extravasation of barium sulphate suspension occurred in every testis of the cadmium group even at a pressure of 60 mm Hg. As a result of extravasation, the seminiferous tubules were seen to fill with contrast medium. This totally prevented visualization of the intratesticular arterial pattern in gross angiography (Fig. 2). In the caput epididymidis, in contrast to the testis, the blood vessels, which are known to undergo similar pathological changes due to cadmium (Gunn *et al.*, 1963), showed no signs of increased fragility. However, the epididymal network of blood vessels was dense and architecturally abnormal.

The cryptorchid testes showed no signs of increased vascular fragility at either 4 or 7 months. The general course of the blood vessels was as on the intact side, with the exception of increased tortuosity and density due to the involution of the testis (Fig. 3).

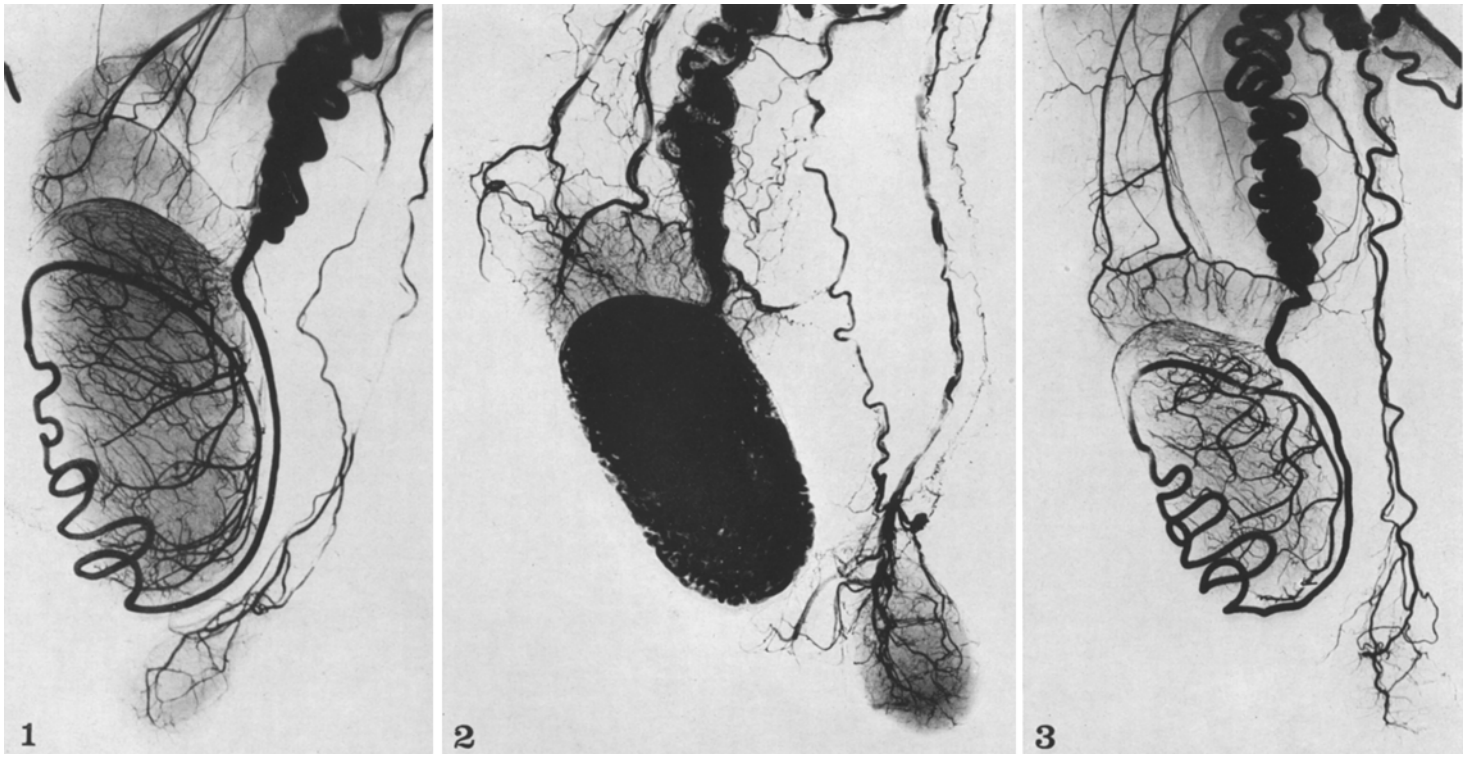

Fig. 1. Gross angiographic demonstration of the arterial supply to the normal rat testis and epididymis. ×3

Fig. 2. Gross angiography of the rat testis and epididymis 7 months after cadmium injury. The seminiferous tubules also contain barium sulphate. × 3

Fig. 3. Gross angiography of the rat testis after 7 months' cryptorchidism. × 3

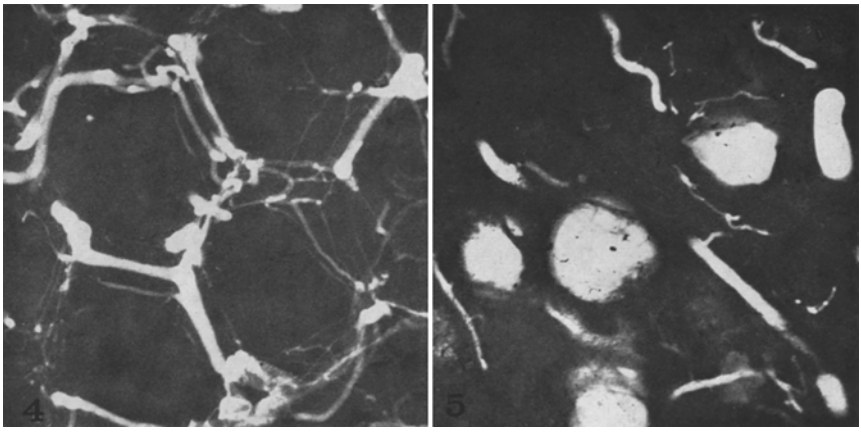

Fig. 4. Microangiographic pattern of the normal intratesticular capillary network. 2 mm section. × 110

Fig. 5. Microangiogram of cadmium-injured testis after 7 months' regeneration. Regenerated small blood vessels and some seminiferous tubules contain barium sulphate. 2 mm section. × 110

2. Microangiography

In spite of the filling of the rudimentary seminiferous tubules with contrast medium, microangiography was able to visualize the microvascular pattern between the tubules. In fact, not all the seminiferous tubules were filled with contrast medium. In contrast to the delicate pattern of capillary organization in the normal testis (Fig. 4), the small blood vessels of the cadmium-injured testes differed greatly from the normal microvascular pattern (Fig. 5). The descending part of the testicular artery was recanalized and gave numerous small branches into the testicular parenchyme directly through the tunica albuginea (Fig. 6). In some cases recanalization of even the convoluted part of the testicular artery was seen, but no normal intratesticular branching of the artery was visible. Instead, the new blood vessels had grown from the caput epididymidis and to some extent also from the cauda epididymidis through the tunica albuginea into the testis. As a result of ingrowth of the vessels the testis was supplied by newly formed and relatively straight small arteries and capillaries all over its superior and epididymal surface. The architectural pattern of blood vessels showed no features typical of this organ. The deep part of the testicular rudiment seemed to be poorly vascularized; in fact, no appreciable filling of capillaries was observed in the inner part of the organ.

The architectural pattern of the microvasculature in the cryptorchid testes was seen to remain largely normal, except for increased tortuosity of the capillary network.

3. Histology

Histologically, the cadmium-treated testes showed extensive damage of the tubules and no signs of spermatogenesis. The tubules were not all identical in condition, but could be divided into two groups, which differed in appearance. The majority of the tubules contained amorphous debris and usually also

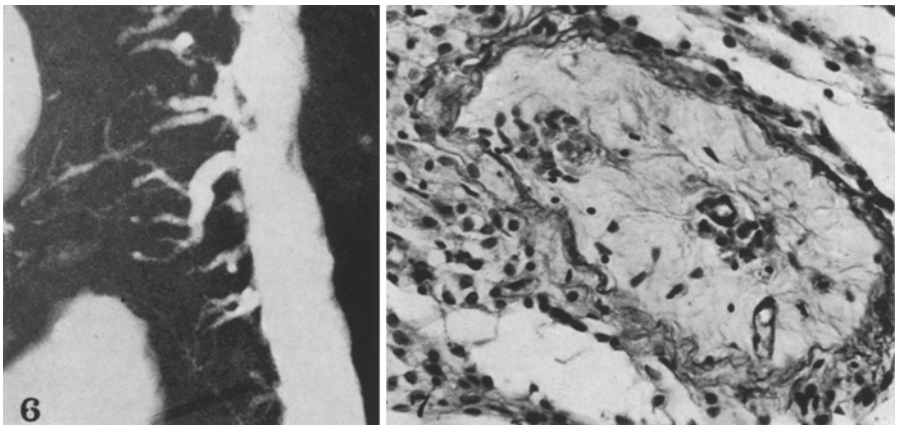

Fig. 6. Sprouting of new blood vessels from partially recanalized testicular artery 7 months after cadmium injury. Microangiographed 2 mm section. × 110

Fig. 7. Histology of a shrunken and organized seminiferous tubule, showing intratubular capillaries. × 350

contrast medium. It was impossible to discern any blood vessels that would explain the extravasation of contrast medium into these tubules but not into the intertubular space, owing to the complete disorganization of the tubular contents and the interference caused by the contrast medium. The boundary tissue was thickened in these tubules and showed no defects. Mononuclear and polymorphonuclear cells were seen both within the unorganized tubules and around them. The other variety of tubules consisted of more shrunken tubules with thick, wrinkled peritubular tissue and fibrous organization of the whole tubular lumen. The cells within these tubules had nuclei smaller than in normal Sertoli cells and abundant cytoplasm. Some tubules were seen to be in the process of organization, since they were partially filled with this loose fibrous tissue, but partially lumen and cellular debris still existed. The small organized tubules never contained leaked contrast medium, but instead, clear-cut capillary cross sections filled with barium sulphate grains were identified in some of them (Fig. 7). Regenerated interstitial tissue was only seen near the tunica albuginea.

The cryptorchid testes showed tubular damage and thickening of the peritubular tissue typical of this condition. No evidence of intratubular capillaries was seen.

4. Alkaline Phosphatase Activity

The histochemical demonstration of alkaline phosphatase activity was made in order to demonstrate the existence of blood capillaries either inside the damaged tubules or in the central part of the testis, in both of which the microangiographic method failed to demonstrate blood vessels. In agreement with the microangiographic findings, a large number of highly alkaline phosphatase-positive capillary cross sections were seen in the deep aspect of the tunica albuginea. In the centre of the testes only occasional staining suggestive of the alkaline phosphatase-positive capillary endothelium was seen. The debris-containing tubules were seen to stain heavily, owing to non-specific deposition of the

dye. This was at least partially due to the non-specific staining of the barium sulphate grains in connection with demonstration of the alkaline phosphatase activity, as observed in an earlier paper (Kormano, 1969). Therefore, no additional information could be obtained about possible vasculature in the tubules containing barium sulphate. Staining of the peritubular structures was not seen, although in normal testes there was an intense reaction.

Discussion

The present work shows that testicular revascularization after cadmium necrosis is a slow process, apparently still continuing after seven months. It also shows that the sources of anastomotic connections, the head and the tail of the epididymis, which normally contain only very small blood vessels (Kormano, 1968), are mainly used to build up a new vasculature, and that even the main trunk of the testicular artery is capable of giving off sprouts for this purpose. The pattern of the newly formed vasculature is completely different from the original, and for a long time insufficient to supply the whole degenerate organ.

In addition to the architectural difference, the newly formed vessels seem to display completely different behaviour as regards the seminiferous tubules. The normal seminiferous tubule of the testis is essentially an avascular structure. The entrance of nutrients and other necessary products into the tubules is controlled by the peritubular tissue, which functions as a blood-testis barrier (Kormano, 1967b; Setchell, 1967; Setchell *et al.*, 1969). This barrier has been shown to continue its function even in spite of disturbed spermatogenesis (Linzell and Setchell, 1969). In the present work the late vascular supply after cadmium injury was seen to be completely different. The tendency for contrast medium to enter the tubules suggests the existence of highly fragile new capillaries within the unorganized seminiferous tubules. In fact, the existence of microscopically mature capillaries was documented in the well organized tubules. Since there was no histological evidence of defects in the thickened peritubular tissue and no leakage into the interstitital tissue, it has to be assumed that the blood vessels had grown through the peritubular tissue in this particular situation, although this has never been reported to occur normally. Ingrowth of capillaries into the human seminiferous tubules occurs in granulomatous orchitis (Bünzli, 1968), a pathological condition which is also assumed to be of tubular origin. From the photomicrographs of Benson and Clare (1966) it can be assumed that capillaries may also occur in experimental spermatic granulomas of the rat. The normal testicular tissue will induce the growth of typical intratesticular vascular pattern even in a testicular transplant (Williams, 1949). Therefore this exceptional behaviour of the new blood vessels seems to be due not to a difference in the nature of the new blood vessels but to a change in the internal environment and/or cellular elements of the seminiferous tubules.

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