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Vascular Lesions in Testes Associated with Male Infertility in Cameroon

Possible Relationship to Parasitic Disease

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Summary. Testicular biopsies in 40 of 41 infertile males with severe oligospermia in Cameroon presented massive subendothelial "fibrinoid" deposits in the small and medium sized vessels. Fibrinogen, complement and IgM were demonstrated in these deposits by immunofluorescence. Evidence strongly suggestive of parasitic testicular involvement was also observed in 2 cases.

It is postulated that the "fibrinoid" deposits are the result of repeated formation and deposition of circulating immune complexes by reaction of antibodies with antigens. These antigens could be of various origins and in the cases described here they could be derived from living or dying parasites in the region. The accumulation and incorporation of the "fibrinoid" deposits may lead to vascular stenosis resulting in chronic ischaemia, tubular atrophy and fibrosis, and finally oligospermia.

Key words: Testis – Oligospermia – Sub-endothelial deposits – Immune complexes – Parasite.

Introduction

Fragmentary evidence suggests that infertility is a major health problem in Cameroon[1] (Nasah and Drouin, 1976). Clinical studies in approximately 300 infertile couples have shown that 40% of these could be considered to be due to primary infertility and the remaining 60% to secondary changes. Among females, tubal pathology was the primary cause of infertility in 22% of the former and 32% of the latter group. 5% of the women had proven tuberculosis (WHO Technical report Series No. 582, 1976). The male component has never been investigated in any detail but of nearly 90 males of unions characterized as exhibiting primary infertility, sperm analysis showed oligospermia in 50%,

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Table 1

Sperm count/cu.ml	No. of cases	Percentage	Rating
11–20 million	9	21.95	Mild
6–10 million	7	17.07	Moderate
1.5 million	20	48.78	Severe
< 1 million	5	12.20	Aspermia
Total	41	100.0	

with up to 40% oligospermia in those characterised as secondary (Nasah and Drouin, 1976; WHO Technical Report Series No. 582, 1976).

We report here vascular lesions in the testes of 40 males with secondary infertility who showed evidence of severe oligospermia and discuss the role of these lesions in producing testicular abnormalities and scarring.

Patients and Methods

Seventy-one males, 25–50 years, were studied because of infertility, their wives having been shown to be normal in this respect. Forty-one (57.7%) showed indications of various degrees of oligospermia, and these were selected for testicular biopsies.

Forty of the 41 patients studied were normal on physical examination. One patient had sequelae of poliomyelitis. A past history of mumps and other causes of orchitis was sought, and details of past medication recorded. Laboratory examinations included blood, urine, stool and skin snips in search for parasites. Serological test for syphilis were carried out in the majority of cases as well as a search for active gonorrhea. The haemoglobin genetype was also studied.

A subjective estimation of testicular size and consistency was made. The semen samples were produced after 3–4 days of continence and were examined for volume, sperm concentration, motility and morphology. The specimens were all examined within 1–2 h of production. All patients had oligospermia of varying degrees of severity (Table 1) with about 50% of the cases presenting severe oligospermia. Considered qualitatively (i.e. motility 60% after 2 h), 25 cases (61%) showed no motility, while in 10 cases the motility was 50% or less.

Current medications (especially hormones) were stopped at least two months prior to biopsy in all patients. In all cases, except No. 3, one biopsy was taken from one testis. One third of the specimen was fixed in formalin, and the remaining two thirds in Carnoy. All specimens were paraffin embedded and cut at 5 μ. Sections were stained with haematoxylin-eosin, van Giesen-Verhoff, Masson's trichrome, PAS, Gomori, PTAH and, in some instances, with Congo red. In 19 cases, portions of the biopsies were immediately frozen at −60° C for immunofluorescent studies.

Clinical Findings

The ages of the patients varied between 25–50 years, average 33.6 years. No cardiovascular abnormality was clinically evident. One patient was hypertensive; no patient had diabetes. A small hydrocele was noted in 7 patients during testicular biopsy, one of these showed a calcified plaque within the tunica albuginea. One other individual had been operated on for a hydrocele 18 years previously. In another patient in whom the testis was small and firm the tunica vaginalis was noted to be thickened and firmly adherent to the tunica albuginea. Unilateral atrophy of the testis in one patient was presumed to have been a complication of mumps which had occured 8 years previously. On one other patient, there was bilateral, asymmetrical testicular atrophy.

Urinary schistosomiasis (Sch. Haemato.) was confirmed in one case. While stool examinations (stool concentration) were negative for this parasite, they showed evidence of ascaris in 3 cases and trichocephale in 5 cases. In 7 out of 10 randomly selected patients, rectal scrapings revealed the presence of schistosoma ova with lateral or terminal spines. Of the 22 cases in which blood and skin snips were examined for microfilaria, 2 (each of which had a hydrocele at biopsy) were positive for loa-loa and onchocerca.

Results

Pathological Findings

Forty of the 41 specimens (97.56%) showed two major abnormalities. These were:

1. Intramural arterial deposits.
2. Testicular scarring.

Intramural Arterial Deposits

These consisted of irregular, massive and often eccentric eosinophilic subendothelial deposits in the small-sized arteries and arterioles in 37 cases (90.25%) (Fig. 1a–c). This substance, "fibrinoid" in appearance, stained yellow with van Giesen, red with Masson's trichrome, was sometimes slightly positive with PTAH, strongly PAS-positive and did not stain for amyloid. It was frequently segmental, partially or completely obliterating the vascular lumen. In only one instance was a fibrin thrombus observed. The older lesions appeared as eccentric hyalinized or sclerosed areas of the vessel wall. In addition to these changes, some of the smaller vessels (9 cases) showed concentric fibrotic thickening of their wall, with narrowing of the lumen. There was no vascular necrosis nor perivascular inflammatory cellular reaction. It is to be noted that the veins, especially those immediately next to the often thickened tunica albuginea, were of the most part hyalinized and thickened but patent.

Testicular Scarring

Scars were variable in size and consisted of large fibrous bands containing numerous partially and/or completely atrophied, hyalinized seminiferous tubules (Fig. 2a, b). Some of the vessels within and/or in the neighbourhood of the scars presented intramural subendothelial "fibrinoid" deposits in different stages of evolution and in some cases their lumena were partially obliterated.

Other Histological Observations

A typical *Sertoli-cell only* appearance was observed in only 9 cases (22.5%) (Fig. 3a). This was sometimes focal or patchy with some tubules showing germinal cell arrest and others showing vacuolization of the epithelium (Fig. 3b).

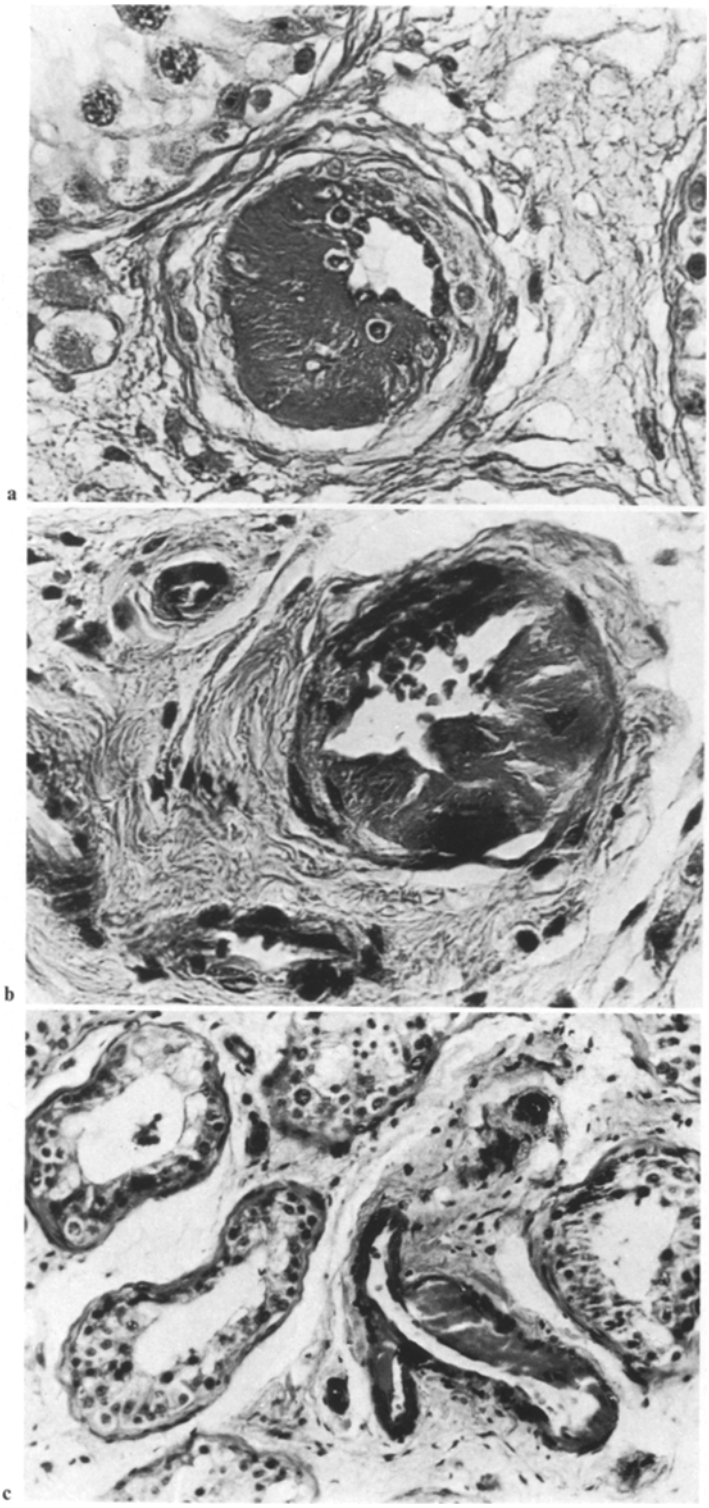

Fig. 1. **a** Fibrinoid sub-intimal deposits. PAS positive, negative with Congo red. (HE, ×300). **b** Hyalinization and incorporation of the deposit in the wall partially obliterating the lumen. (HE, ×300). **c** Polysegmental nature of deposits. (HE, ×180)

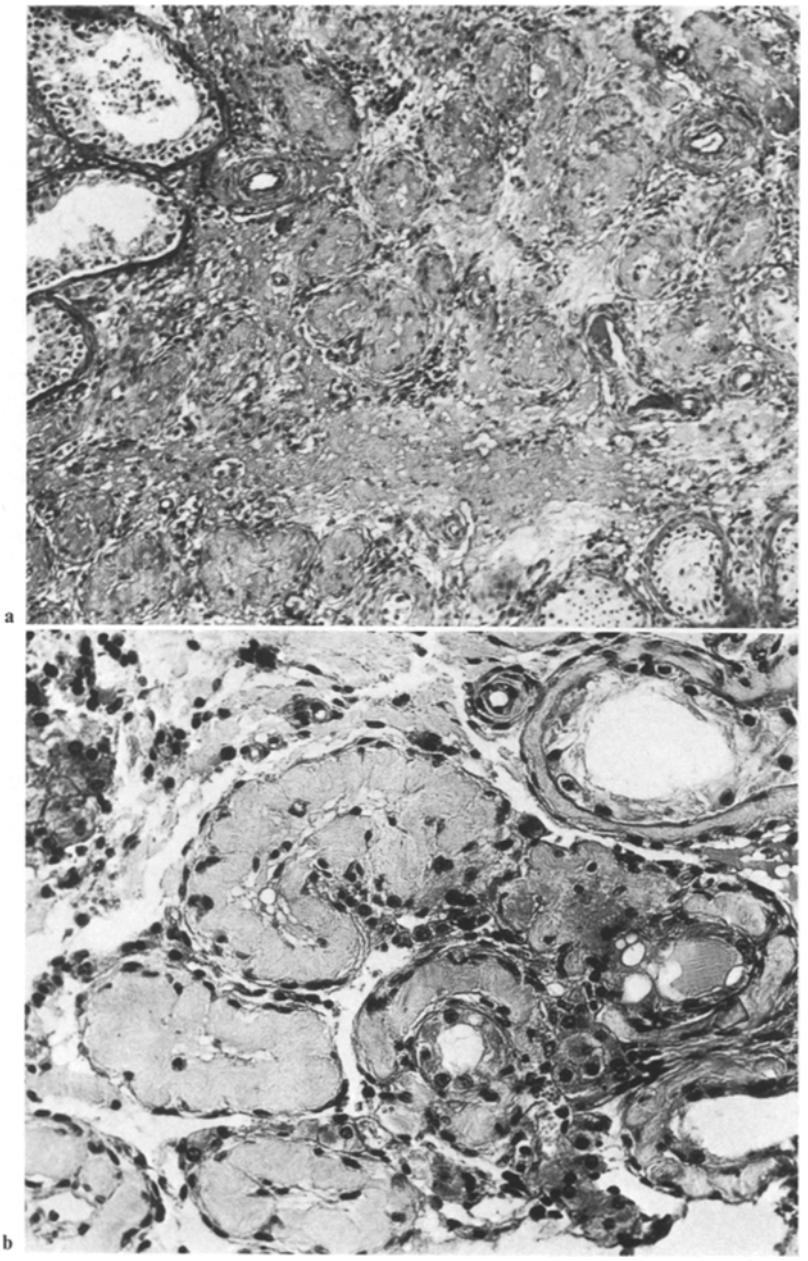

Fig. 2. a Scarring with tubular hyalinization and atrophy. Note deposits partially obliterating vessel lumen on the lower right hand corner. (HE, ×60). **b** Sclerosis of tubular basal membrane and hyalinosis. Absence of spermatogenesis. (HE, ×180)

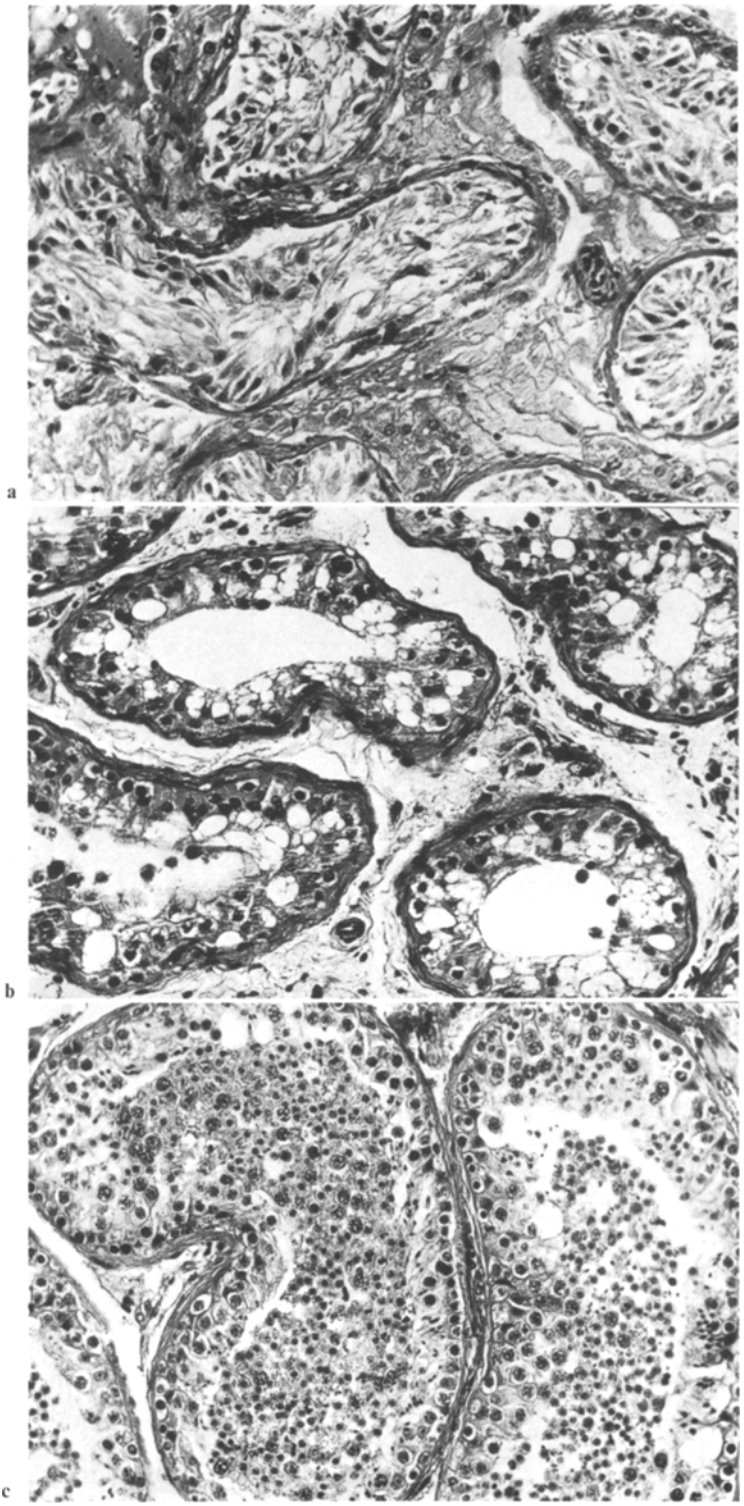

Fig. 3. **a** Sertoli-cell only appearance (HE, ×240). **b** Germinal cell arrest and vacuolization of the epithelium (HE, ×240). **c** Sloughing of germinal epithelium. (HE, ×240)

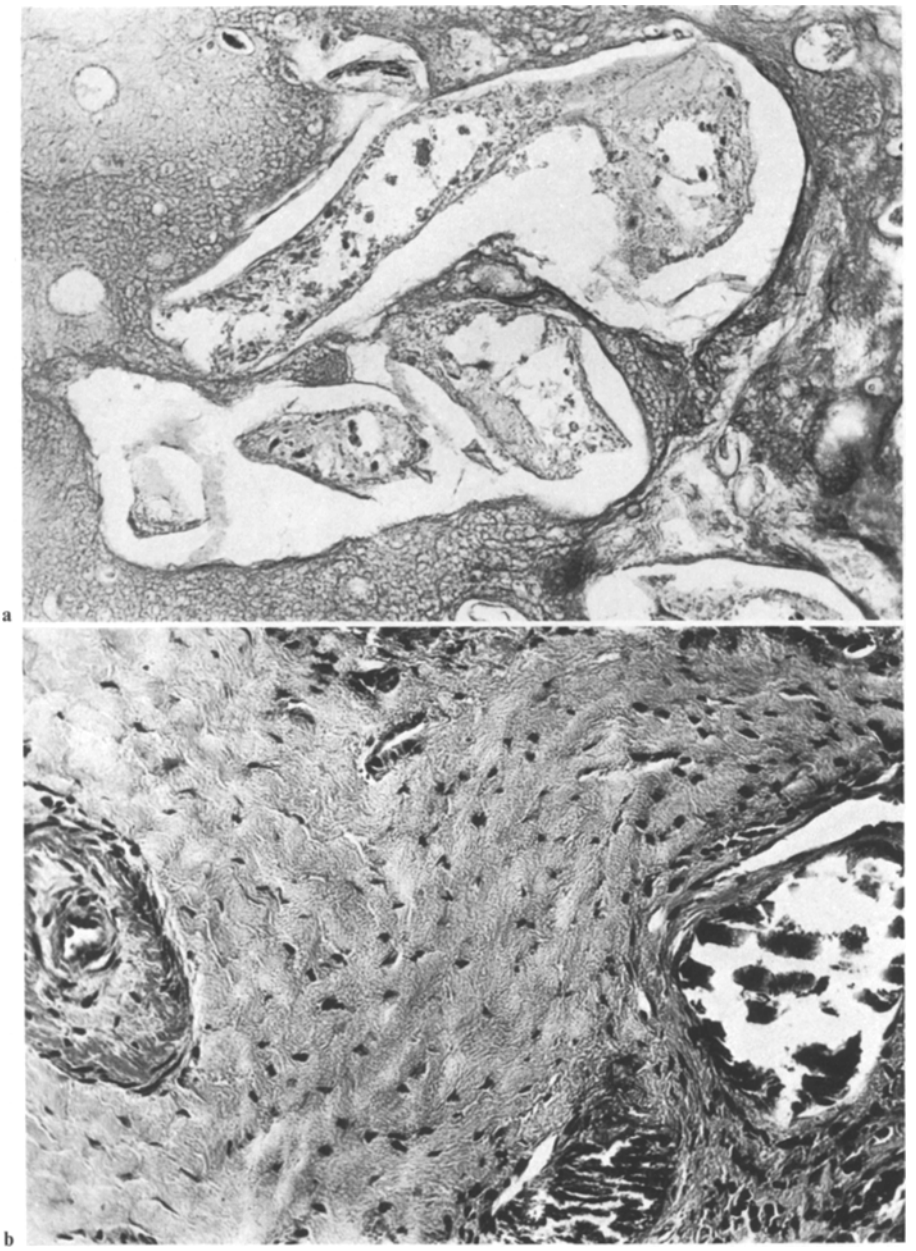

Fig. 4. a Parasitic elements in calcified nodule consistent with filariasis (HE, ×180). **b** Fibrosis of testis with calcified element on the right and partially oblitered, hyalinized artery on the left. (HE, ×180)

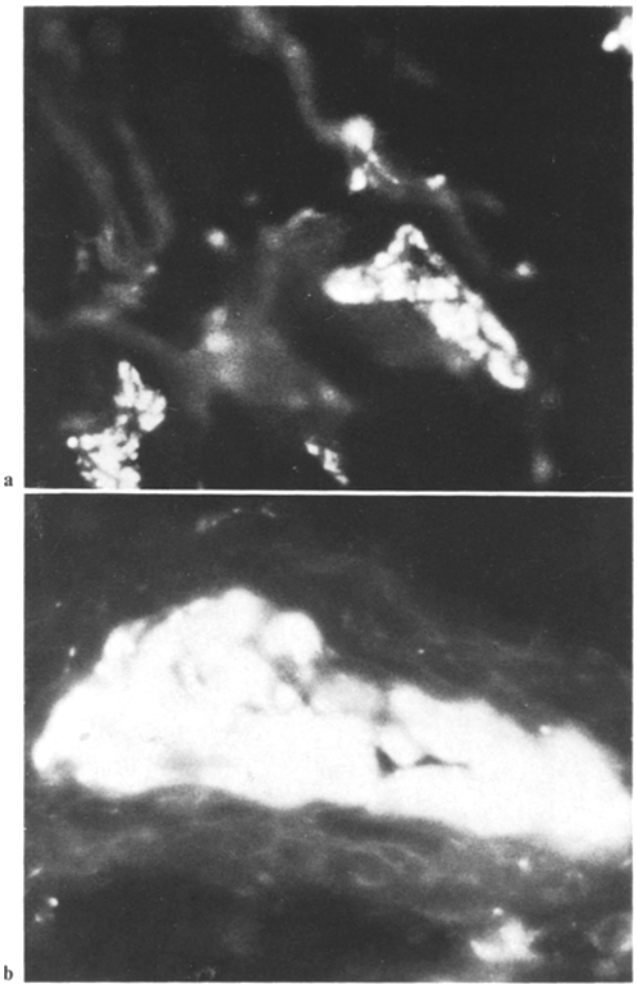

Fig. 5. Immunofluorescent staining showing bright fluorescence in the vascular deposits. **a** Staining for fibrinogen. **b** Staining for IgM

Germinal cell arrest was noted in 12 cases (30%) and *sloughing of germinal epithelium* (Fig. 3c) in 21 cases (52.5%).

Edema was a prominent feature, occurring in 36 cases. This could have been partly due to technical reasons. The Leydig cells were often seen to be undergoing hydropic or granular degenerative changes. In 11 instances there was some degree of Leydig cell hyperplasia, either focal or adenomatous (2 cases).

The whitish plaque which, in one patient, fixed the tunica vaginalis to the albuginea, was partly calcified and contained fragments of parasites. These resembled ghost elements consistent with filarial parasites (Fig. 4a). The corresponding testis showed the vascular changes and scarring described in the other biopsies.

The remaining testicular specimen was completely fibrosed, no testicular structure was apparent. Numerous calcified nodules were found with a major foreign body giant cell reaction but no inflammatory cells. In two areas, there were structures which strongly suggested partially calcified parasitic elements. The vessel walls were thickened, sclerosed and sometimes showed "fibrinoid" deposits (Fig. 4b). This specimen correlated well with the small, firm testicle observed clinically.

Immunofluorescent Studies

Cryostat sections of the frozen material from 19 patients, cut at 8–10 μ, were studied by immunofluorescence for immunoglobulins (Ig total, IgA, IgG, IgM), complement and fibrinogen. In each of 8 cases, 2 different specimens were examined, and in 3 cases 3 specimens. Two testicles, one from a newborn who died with the respiratory distress syndrome and one from a 19-year old male who died as the result of an automobile accident, were used as controls.

Bright fluorescence for IgM was demonstrated in the arterial wall in 9 cases, while positive fluorescence for complement and fibrinogen was present in 11 cases. These were often patchy, segmental deposits or diffuse granular green-yellow masses within the wall of the arteries (Fig. 5a, b).

Discussion

The arteriolar changes described here are similar in many respects to previous reports (Buergi and Hedinger, 1959; Hatakeyama et al., 1966; Rubli, 1977; Suoranta, 1971). In the present series, the vascular changes were the most persistent and striking features. The abundant subendothelial, eosinophilic, strongly PAS-positive deposits might be the cause of marked narrowing of the vascular lumen with resultant reduction in blood flow within the organ. These strongly PAS-positive deposits contained, in some instances, fibrin as demonstrated by the Masson and PTAH stains. Specific immunofluorescent stains revealed that they also contained IgM, fibrinogen and complement in a significant number of cases. These reactions suggest an immunological origin.

The severe tubular damage, appearing as sloughing, tubular atrophy and, more often, thickening of the basal membrane in the form of wide hyaline bands, together with degenerative changes in the germ cells, might represent manifestations of chronic ischaemia. This would result from repeated deposition and incorporation of sub-endothelial deposits resulting in multisegmental stenosis of the vessel lumen. Similar lesions have been described in experimental animals after partial or complete ligation of the testicular artery (Kaya and Harrison, 1975; Oettle and Harrison, 1952; Steinberger and Tjide, 1969; Tjide and Steinberger, 1970). Oettle and Harrison (1952) were among the first to show the high sensitivity of rat testes to ischaemia. Since then, several experimental models have been described in which temporary or permanent ischaemia of the testes has been studied in detail (Kaya and Harrison, 1975; Steinberger

and Tjide, 1969; Tjide and Steinberger, 1970). The seminiferous tubular changes and scarring described in those experiments are comparable in most instances to those lesions observed in the testes of the patients studied here, and are similar to those described by Buergi and Hedinger (1959), Rubli (1977) and Suoranta (1971).

The combination of the various histological changes could explain, at least in part, the pathogenesis of the oligospermia in most of these cases. These lesions could lead to complete aspermia as a result of total fibrosis of the testes, as was observed in 2 cases. It has been shown by Steinberger and Tjide (1969) that the result of permanent long-term ischaemia of the testes leads to fibrosis of the organ similar to that produced by other injurious stimuli (e.g. heat, X-rays, cytotoxic agents).

Histological findings in the testes in male infertility are well documented (Alexandre, 1971; Amelaz, 1966; Charny, 1963; Girgis et al., 1969; Girgis et al., 1971; Le Lorier et al., 1970, Scott et al., 1976; Tillinger, 1957), and numerous aetiological factors have been incriminated (Alexandre, 1976; Charny, 1963; Dubin and Amelaz, 1971; Garduno and Mehan, 1970; Meinhard et al., 1973; Morita, 1971). Among those often listed are varicocele (Alexandre, 1976; Comhaire and Vermeulen, 1974; Dubin and Hotchkiss, 1969; Engle, 1947; Etriby et al., 1967; London, 1972), tubular insufficiency (Dubin and Amelaz, 1971; Li, 1974; London, 1972; Ragab et al., 1961; Steward and Montie, 1973), cryptorchidy (Etriby et al., 1967; Friberg et Kjessler, 1975; Lipschultz, 1976) and chromosomic defect (Halin et al., 1973; Kretser et al., 1972; Li, 1974; Scott et al., 1976).

Oligospermia is not uncommon in the African male but there are few published reports on the subject (Adadevoh, 1974). There are even fewer documents dealing with the possible aetiology of this condition in the African continent (Adadevoh, 1974; Nasah and Drouin, 1976; WHO Technical Report Series No. 582, 1976). Van Zyl (1975) studied male infertility in South Africa but did not mention whether there were African patients among his subjects.

The seminiferous tubular changes described here are similar in many respects to other previous reports on testicular biopsies in cases of male infertility in various parts of the world (Charny, 1940; Girgis et al., 1969; Girgis et al., 1971; London, 1972; Morita, 1971; Ragab et al., 1961; Scott et al., 1976; Tillinger, 1957). Surprisingly in only one of these reports, to the best of our knowledge, was there mention of blood vessel changes which the author considered as a possible pathogenic factor in oligospermia (Morita, 1971).

The pathogenesis of the subendothelial deposits remains obscure. Hatakeyama et al. (1966) have shown that they were composed partly of granular glycoliprotein from the plasmatic fluids. Our immunofluorescent studies have shown that the early lesions contain IgM, complement and fibrinogen. It is therefore possible that the modification of the vessel wall observed in 40 of the 41 patients (97.6%) could be the result of repeated formation and deposition of circulating immune complexes by reaction of antibodies with antigens. The latter could be released from living or dying parasites, or many other potential sources. Similar vascular deposits are known to occur in other continents (Buergi and Hedinger, 1959; Hatakeyama et al., 1966; Suoranta, 1971). These antigen-

antibody complexes, once deposited within the vessel wall, would finally become incorporated and appear as hyaline sclerosed thickening. It is thus quite possible that similar deposits could be deposited in the vessel wall of other organs and/or systems. Evidence for the participation of immune complexes in some aspects of parasitic diseases is well documented (Brito et al., 1969; Lambert and Houba, 1974; Quizoz et al., 1973; Silva et al., 1970).

In filarial endemic zones, it is not uncommon to find microfilaria in hydrocele fluids (Vassilakos and Cox, 1974). Furthermore, testicular involvement in filarial infection, especially that of the lymphatic system in this anatomical region, has been documented in previous reports (Marcel-Rojas, 1971; Pugh, 1976). It is possible that the parasitic elements observed in two cases of the present series are remains of dead filaria.

Whatever the aetiology, vascular lesions should be considered among the factors responsible for oligospermia. Clearly further observations should be made, particularly where parasitic diseases are endemic and male infertility is a problem.

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