serum dilution. The amount of AFP in serum specimens was estimated by multiplying the reciprocal of the highest specimen dilution that prevented particle agglutination by 0.2. An additional control of test specificity using purified mouse albumin (Miles Laboratories, Elkhart, Ind.) at 0.1 to 100 µg/ml showed no inhibition of the AFP reference agglutination.

The antiserum and C-AFP reagents stored at 5 °C for up to 22 months gave the same results as the initial titration. In 10 consecutive tests of the standard AFP preparation over the same 22-month period, the minimum inhibitory concentration was 0.2 µg/ml.

Undiluted sera from 19 of the 56 normal adult mice were inhibitory; 1 of these 19 was also positive at a 1:2 (but not a 1:4) dilution. Thus, the serum AFP concentrations of the Nya:NYLAR and the C57L/J tumor-free mice were 0.4 µg/ml for one mouse and ≤ 0.2 µg/ml for the rest. Pihko and Ruoslahti[3] determined by radioimmunoassay that the normal AFP levels for 4 strains of adult mice ranged from 0.03 to 0.35 µg/ml. Thus the charcoal particle test sensitivity is within the upper normal range for the adult mouse population.

The serum AFP concentration of the 13 pregnant mice ranged from 8 to 16 µg/ml, with a mean of 12.3±1.2 SEM.

For the 57 tumor-bearing mice the mean serum AFP concentration 3 days after transplant was 3 µg/ml, about 10 times the maximum normal level (figure 2). It then rose exponentially to 2662 (±1020 SEM) µg/ml on day 21 postimplantation and apparently changed little thereafter through day 28. A parallel increase in serum AFP levels was obtained by RID: day 10, 408 (±142); day 14, 1003 (±247); day 21, 3543 (±480), and day 28, 4374 (±771) µg/ml.

The charcoal particle test procedure offers a number of advantages. It requires little technical skill; no special instrumentation, materials or radiochemicals; and less than 2 h to complete. It is about 50 times more sensitive than double-diffusion in agar[8]. The C-AFP indicator and reference antiserum are stable for at least 22 months. The use of purified AFP rather than antibody on the charcoal indicator particle avoids nonspecific complexing with serum macroglobulins and interference due to antigen excess[9]. The results with the mouse model also suggest the possibility of a similar test for human AFP to aid in clinical studies of neoplasia.

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Immunological and morphological consequences of vasectomy in the rabbit[1]

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Summary. Cell-mediated immunity to spermatozoa was detected in vitro 6–18 months after vasectomy in the rabbit. The autoimmunity was accompanied by aspermatogenic orchitis in the testes and epididymides.

Vasectomy has rapidly become the most popular sterilization technique in the human male because it is simple and rapid. As a result of ligation and sectioning of the vas deferens in a healthy individual, spermatozoa are confined to the epididymis and vas deferens. The spermatozoa degenerate and antigens reach the circulation. In the rabbit, a few reports[2,3] have documented the development of humoral antibodies after vasectomy accompanied by observations in the testis resembling those found in male rabbits after injection of spermatozoal antigens in Complete Freunds Adjuvant (CFA). The animals develop specific autoimmune aspermatogenic orchitis characterized by intertubular mononuclear infiltration and desquamation of the seminiferous tubules. The development and role of cell-mediated immunity (CMI) to spermatozoa after vasectomy in the rabbit has been little investigated. This report presents preliminary evidence that cell-mediated immunity is present in the rabbit 6–18 months after vasectomy.

Materials and methods. 3 sexually mature white rabbits were used. In one the vas deferens was located, ligated at 2 points and a piece removed. In another, the vas deferens was resected without ligation to deliberately extravasate spermatozoa and enhance the development of antisperm autoimmunity. The remaining rabbit was sham-vasectomized. CMI to sperm was determined by an in vitro correlate of CMI: the capillary tube leucocyte migration inhibition technique modified from Brannen and others[4]. The animals were tested 3, 6, 12 and 18 months after surgery and peripheral blood leucocytes were used as indicator cells (4×10^7/ml) and 4 times washed ejaculated sperm as antigen source (1×10^7/ml).

After 18 months the animals were killed, reproductive tracts removed and inspected grossly for distension, presence of cysts and granulomata and weighed. The tissues were fixed in Bouins fluid, dehydrated in alcohol, cleared in xylene, embedded in paraffin wax, sectioned at 6 µm,

Migration inhibition indices at various intervals after surgery

Time after surgery (months)	Method of vasectomy		
	Sham	Vasectomized	Vas resected
3	88.9±4.8	94.2±5.5	95.7±3.9
6	95.3±5.1	68.8±3.2	71.8±5.6
12	100.1±4.7	72.0±4.3	77.2±6.2
18	93.3±2.9	70.8±4.9	65.9±6.7

Migration index ±SD. Migration inhibition of more than 20% was considered significant.

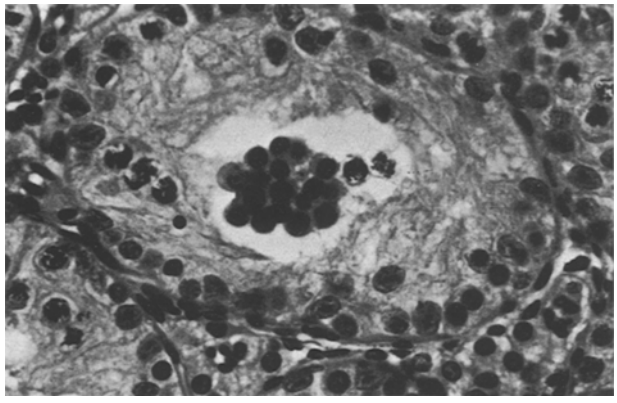

Fig. 1. Section of seminiferous tubules from testis of rabbit conventionally vasectomized 18 months previously (H and E, × 400).

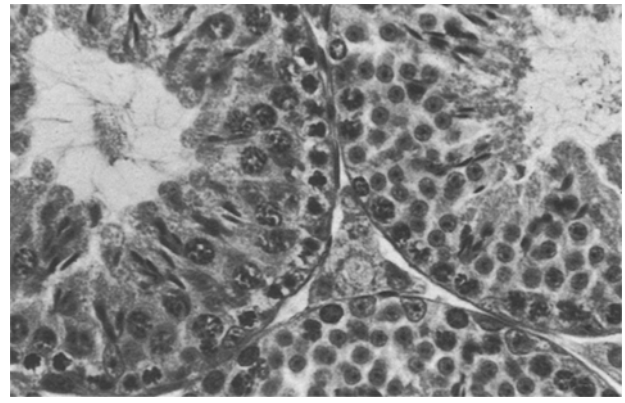

Fig. 2. Section of seminiferous tubules from testis of rabbit sham-vasectomized 18 months previously (H and E, × 400).

stained with haematoxylin and eosin and examined by light microscopy.

Results and discussion. CMI to spermatozoa in vitro was detected in the vasectomized and vas resected rabbits 6, 12 and 18 months after surgery while CMI was not detected in sham-vasectomized rabbit throughout the experimental period and in the vasectomized and vas resected animals 3 months after surgery (table). 18 months after surgery, the caudae epididymides from the vasectomized and vas resected rabbits appeared distended, without cysts and granulomata. The testes on the other hand were reduced in weight, were tender and pale whitish. By light microscopy, the caudae epididymides were aspermatogenic. A few macrophages were found in the yellowish fluid obtained from this part of the tract. In the testes, the seminiferous tubules were aspermatogenic, and desquamation was accompanied by inter- and intratubular mononuclear infiltration (figure 2). The epididymides and testes from the sham-vasectomized animal appeared normal. No differences in morphology could be found in the tracts of vas resected and conventionally vasectomized rabbits.

Detected CMI in vitro correlated with the histopathology of the testes 18 months after vasectomy although this autoimmunity was probably not the only cause of the observed orchitis since humoral antibodies and immune complexes have been shown to be elevated in the rabbit 6 months after vasectomy[2,3]. Due to the small number of the animals studied here, these observations need to be confirmed in larger numbers of rabbits. The role of each of the factors that have been identified by this and other studies in vivo, needs to be defined. Further, due to species response differences to vasectomy, care needs to be taken in efforts to relate animal model findings to the situation in man.

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Properties of vascular permeability factor in human sera for guinea-pig skin

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Summary. Properties of vascular permeability factor in native human sera (PF/Nat) showed close similarities with those of necrotizing factor. Time course studies revealed that skin necrosis could be initiated by enhanced vascular permeability.

Since the original description by Edler and Wilhelm in 1958[1] on PF/Nat in native human sera which induced enhanced vascular permeability (P) for guinea-pig skin, its properties and/or significance had been scarecely investigated. In this paper, the studies of the properties of PF/Nat or P factor are described, which show a close similarity with those of necrotizing(N) factor[2-9], inducing local inflammation up to necrosis in the guinea-pig skin after intradermal injections of the native sera. However, the differences between the P factor and the N factor in the initial phase of the activity and in the distribution among human sera might suggest that enhanced P activity initiated in the induction of skin necrosis.

Materials and methods. Sera: Sera were obtained from healthy individuals and patients with collagen diseases, and stored at −70 °C.

The P and N factor: 0.1 ml of serum specimens was injected in the shaved dorsal skin of Hartley strain guinea-pigs weighing 250–300 g and 1 ml of 0.5% Evans blue solution (Daiichi-kagaku Ltd, Tokyo) was injected i.v. 45 min after. For a time study, the specimens were injected at various intervals prior to the dye injection. The P activity