sensitization[5] and since we were studying laboratory workers with frequent exposure to *Salmonella*, we were probably obtaining an in vitro secondary response by 'memory' B cells.

The differences in incorporation of ^{3}H-CdR and ^{3}H-TdR into DNA in human cells may be due to differences in the intracellular transport systems of the 2 nucleosides as has been shown in pigs[6], but there are also potential differences in enzyme and metabolic pathways. The nucleosides are substrates for different kinases[7] and deaminases[6,8]. Thus our results may be due to potentially identifiable enzyme differences in T and B cells.

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Alpha-fetoprotein in tumor-bearing mice assayed by particle agglutination inhibition

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Summary. Serum alpha-fetoprotein (AFP) in hepatoma BW7756-bearing mice was measured by a new particle agglutination inhibition test employing AFP adsorbed to charcoal particles. The AFP levels and tumor weights showed nearly parallel increases to means of 2633 µg/ml and 5.2 g, respectively, 28 days after implantation.

In investigations of human neoplasms and of experimental animal tumor models, an increasingly important assay is the measurement of AFP in biological fluids (serum, urine, ascites fluid). The highly sensitive immunoenzymatic and radioimmunoassays have proven most effective for humans, whose normal adult blood AFP concentrations range from 1 to 30 ng/ml[1,2]. However, a slightly less sensitive procedure is appropriate for certain strains of mice, whose normal adult concentrations range from 100 to 400 ng/ml[3]. Accordingly, we have developed a rapid, simple charcoal particle card test to detect and monitor serum AFP in tumor-recipient and pregnant mice.

Materials and methods. The sera tested were from 26 Nya:NYLAR and 30 C57L/J normal male and female adult mice, from 13 Nya:NYLAR 15–18-day pregnant mice, and from 57 C57L/J mice with hepatoma BW7756 implants. Sera were collected from tumor-bearing mice sacrificed on days 3, 6, 10, 21 and 28 after implantation. The sera were examined by radial immunodiffusion in agar and by particle agglutination-inhibition tests. The AFP was isolated from the amniotic fluid by DE52 anion exchange chromatography, G-100 Sephadex gel filtration and concanavalin A affinity chromatography[4,5]. Monospecific anti-AFP serum in rabbits was produced as previously described[6]. The purified AFP and the monospecific antiserum were used to prepare a radial immunodiffusion (RID) reference curve[7]. The charcoal particle-AFP (C-AFP) suspension was prepared by admixture of 1 vol of aqueous charcoal (2.5 mg/ml; Hynson, Westcott and Dunning, Baltimore, Md.) with 5 vol of purified AFP (2 µg/ml) in 0.85% NaCl solution and 4 vol of 0.1 M glycine solution (pH 8.2) containing 5 g NaCl and 1 g bovine serum albumin (GBS/BSA). The C-AFP agglutination, using anti-AFP rabbit antiserum, was characterized by dense particle aggregates against a clear background (figure 1). In contrast, C-AFP with control mixtures (normal rabbit serum and GBS/BSA diluent) were a uniform gray.

For the inhibition test, antiserum at 1/100 dilution was mixed with an equal amount of purified AFP or of a heated (56 °C for 30 min) serum specimen. The mixture was incubated at 45 °C for 60 min and then added to C-AFP on a pasteboard card, which was mechanically rotated for 8 min. The minimum detectable concentration of AFP was established by inhibition tests of dilutions of the purified AFP.

Results and discussion. Agglutination-inhibition titrations of purified AFP with antiserum dilutions showed that 0.2 µg AFP completely inhibited agglutination at the 1/100 anti-

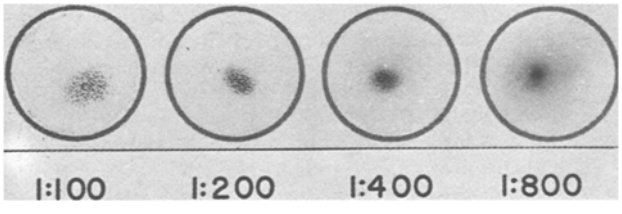

Fig. 1. Titration of rabbit anti-mouse AFP with the C-AFP suspension. 2-fold (1:100–1:800) serial dilutions of antiserum were mixed with the C-AFP suspension. The results were read as 3+, 2+, 1+, and no agglutination.

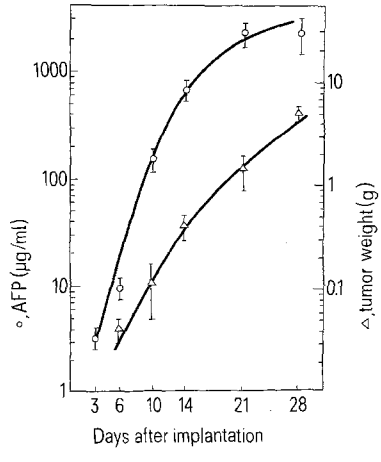

Fig. 2. AFP serum concentrations by charcoal particle test of C57L/J mice after implantation of hepatoma BW7756. Data are shown as mean ± SEM. From 5 to 16 animals were used for each determination.

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.