A Comparison of Blood Chemistry, Reproductive Hormones, and the Development of Antisperm Antibodies After Vasectomy in Men

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Serum chemistry parameters as well as levels of follicle-stimulating hormone (FSH), luteinizing hormone (LH), testosterone, and sperm-immobilizing and sperm-agglutinating antibodies were measured prior to vasectomy and at 1.5, 3, 6, 9, and 12 months afterwards in 99 men. We did not, however, acquire a sample from every man for every point in time. Since the development of antibodies to sperm is a well-documented change that occurs in about half of vasectomized individuals, we investigated whether men who develop circulating antibodies exhibit any changes in serum chemistry and/or hormone levels when compared to those who do not. Although no men had antisperm antibodies at the time of vasectomy, 40.5% of the study population subsequently developed them. The number of men with circulating antisperm antibodies increased significantly for the first 3 to 6 months and then remained stable for the remainder of the study period. Some individuals had only agglutinating or immobilizing antibodies, but more commonly both types were found. The group of men who exhibited early antibody formation may have had slightly higher mean counts of spermatozoa before vasectomy, but there was no difference in counts between those men who never exhibited antisperm antibodies and those that did. Furthermore, there was no difference in ages between those that did and did not exhibit antibodies to sperm. All blood values were within the normal range. Values for FSH were consistently, albeit slightly, lower for those individuals who developed circulating antisperm antibodies. No differences were found in LH and testosterone levels.

Key words: vasectomy, antibody response, testosterone, luteinizing hormone, follicle-stimulating hormone.

Among the requirements for a male contraceptive are reliability, ease of application, absence of significant adverse reactions, and reversibility. Vasectomy meets many of these qualifications (it is reliable; it is a single event, thus eliminating the need for constant precautions; it has a low surgical risk) and has become a popular method of male fertility control. Whether vasectomy has any detrimental consequences has been extensively investigated over the past 10 years. Most studies on

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the physiologic effects of vasectomy have measured short-term changes and indicate no significant adverse side effects.

Numerous studies on pituitary-gonadal axis function after vasectomy have revealed no significant change in sex hormone levels (Wieland et al, 1972; Rosemberg et al, 1974; Johnsonbaugh et al, 1975; Smith et al, 1975; Varma et al, 1975; Kobrinsky et al, 1976; Naik et al, 1976; Purvis et al, 1976; Skegg et al, 1976; Smith et al, 1976; Whitby et al, 1976). Results of biochemical blood analyses have not been reported for vasectomized men. We wished to determine if vasectomy could result in subtle metabolic or endocrine changes.

Antisperm antibodies develop in a large percentage of vasectomized men (Ansbacher, 1973; Alexander et al, 1974) and experimental animals (for listing of studies, see Alexander, 1977). The present study was undertaken to determine whether the presence of antisperm antibodies in vasectomized men was correlated with other changes in blood chemistry and hormone levels. Subtle systemic changes resulting from or occurring in conjunction with these antibodies may have been masked in previous studies because antibody responders were not considered as a separate group. We performed semen analyses, measured serum chemistry parameters, and determined circulating levels of follicle-stimulating hormone (FSH), luteinizing hormone (LH), testosterone, sperm-immobilizing antibodies, and sperm-agglutinating antibodies in 99 men prior to vasectomy, and intermittently for up to 12 months afterwards. We compared data from those individuals that developed free circulating antisperm antibodies with results from those who did not within the experimental period.

Materials and Methods

The Study Population

Two groups of volunteers were obtained from patients requesting vasectomy for contraceptive purposes. One group of 60 men was from the Harborview Medical Center (HMC), Seattle, Washington, and another group of 39 men was from the Madigan Army Medical Center (MAMC), South Tacoma, Washington. The two groups were studied independently. The hospitals doing the vasectomies also performed the follow-up and blood chemistry assays; the endocrine and antibody assays were performed in separate and independent laboratories (CAP and NJA, respectively). In addition, two analyses of semen samples from the HMC patients were done preoperatively.

We were unable to obtain data on all of the patients at each of the time intervals due to some follow-up problems. During the initial sampling we obtained serum data on more than 90% of the patients. Time intervals for postvasectomy samplings were 1.5 (HMC only), 3, 6, 9, and 12 months. Thereafter, follow-up losses increased markedly, and less than 20% of the patients were available at various intervals up to the 44th month. This high attrition rate was primarily due to the transient nature of the population rather than refusal to participate. This report will cover only data collected in the first year after vasectomy.

Surgical Procedures

Vasectomies were performed on an outpatient basis with local anesthesia. Standard surgical procedures, including ligation and removal of a section of the vas deferens, were employed.

Assay Procedures

Venous blood for hormone determinations was collected at the various time intervals indicated in Table 1, allowed to clot, centrifuged, and then stored at −20 C until assayed. Serum LH, FSH, and testosterone were measured by specific radioimmunoassay as described previously (Capell, 1973). Our normal adult male range for LH is 2.4−27.0 mIU/ml. Normal adult male values for serum FSH range from 46−460 ng LER 907/ml using batch #3 first antibody and LER 1366 purified FSH for labeling. Normal serum testosterone levels range from 0.28−1.53 μg/dl. Standard SMA 12/60 assays were done.

Seminal fluid analyses were performed prior to vasectomy. The seminal fluid was obtained by masturbation and delivered to the research laboratory within four hours. The analyses included measurement of volume, sperm concentration and morphology (Gordon et al, 1965; Gordon et al, 1967; Ulstein et al, 1975).

Sperm Antibody Tests

We used the macroscopic sperm-agglutination technique of Kibrick et al (1952) and the sperm-immobilization procedure of Isojima et al (1968) to detect antibodies to spermatozoa. Pooled normal serum samples and a known positive sample provided negative and positive controls in each assay. Pooled normal guinea pig serum was used as the source of complement. All test sera were inactivated at 56 C for 30 minutes. All antibody assays were done twice. A titer greater than the first dilution (1:10) for the sperm-agglutination test and a sperm-immobilization value of 5.0 or more were considered positive. A patient was classified as a positive if any sperm antibody measurement made after vasectomy was positive.

Statistical Methods

Initially, we analyzed the raw data by comparing the means between study populations and between time intervals within each population and the combined population. The incidence of the development of free

TABLE 1. Antisperm Antibodies, HMC Group

Months after Vasectomy	0	1.5	3	6	9	12
Number of positives/negatives	0/57	25/27	22/23	25/22	22/24	22/30
Percent of men with detectable levels of:						
Sperm agglutinating antibody alone	0	8	11	11	11	8
Sperm immobilizing antibody alone	0	2	7	4	7	10
Sperm agglutinating plus immobilizing	0	4	13	28	28	23
Percent of men positive for antibodies	0	14	31	43	46	41

circulating antisperm antibodies in time was analyzed by frequency analysis using two-by-two contingency tables; the derived statistic is then distributed as a chi-square with 1 degree of freedom.

To determine whether the men who developed circulating sperm antibodies in response to vasectomy were significantly different in any way from those that did not exhibit antibody levels, we divided each study population into men in whom free serum antibodies developed (positives) and those in whom they did not (negatives).

Data from negative and positive antibody responders were examined at each sampling period to determine whether there were any significant differences between the two groups. Because the number of men sampled varied considerably between sampling times, a fairly large amount of variation would be expected from one measurement time to the next. We used the two-tailed t-test to determine whether the means for positives and negatives were similar.

The final stage of statistical analyses involved selection of patients from whom we had obtained prevasectomy blood samples and postvasectomy samples at 1.5, 3, 6, 9, and 12 months. This group came entirely from the HMC since only two patients from the MAMC qualified. We analyzed this group both with and without regard to circulating sperm antibody (positives/negatives) status.

In the text, a statistic is presented as the mean ± the standard error of the mean, followed by the sample size (N); when pertinent, the level of significance is indicated.

Results

Two groups of men from different locations were used in this study. Different hospital laboratories ran the blood chemistry analyses. In addition, the two populations varied significantly in age; therefore, the two groups were not combined for analysis. No men from either population had agglutinating or immobilizing antibodies at the time of vasectomy. However, measurable levels of one or both types of antibodies developed in 29 HMC men and 13 MAMC men within one year after vasectomy. The incidences of sperm antibodies for each time interval are shown in Tables 1 and 2. The number of men with circulating antisperm antibodies increased significantly with time after vasectomy for the first three to six months and then remained stable for the remainder of the 12-month test period. The prevasectomy comparison to all other time periods yielded a probability value of ≤ 0.001, except for the 1.5-month period. The 1.5-month period versus all other periods demonstrated a value of $P \leq 0.001$, except for the 3.0-month period. There were no significant differences in incidence of antibodies when the 3-, 6-, 9-, and 12-month periods were compared to each other. Although some individuals had only agglutinating or immobilizing antibodies, more commonly we found both types.

The mean total sperm count ($\times 10^6$ sperm) of HMC men in whom free antisperm antibodies developed within six weeks was 381.7 ± 120.9 (N = 7); for men in whom antibodies were not found, it was 232.8 ± 172.3 (N = 27). These figures indicate that men who exhibited early antibody formation may have had a slightly higher mean sperm count. A comparison of the mean total sperm count of all men in whom antibodies ever developed (231.5 ± 212.5, N = 28) with that of men without antibodies (above) showed no significant difference.

As we mentioned, more members of the HMC group developed antibodies. Furthermore, the HMC group was significantly ($P < 0.001$) younger. The HMC group was 29.1 ± 0.68 (N = 60) years old, and the MAMC group was 36.0 ± 1.33 (N = 39) years old. Therefore, we evaluated

TABLE 2. Antisperm Antibodies, MAMC Group

Months after Vasectomy	0	3	6	9	12
Number of positives/negatives	0/35	8/15	11/22	6/19	8/18
Percent of men with detectable levels of:					
Sperm agglutinating antibody alone	0	0	6	8	0
Sperm immobilizing antibody alone	0	4	3	0	8
Sperm agglutinating plus immobilizing	0	17	12	12	19
Percent of men positive for antibodies	0	21	21	20	27

whether younger men were more likely to develop antisperm antibodies. We found no significant differences in the ages of positives (MAMC: 32.3 ± 2.33 years (N = 13), HMC: 28.4 ± 1.04 years (N = 29)) and negatives (MAMC: 37.8 ± 1.41 years (N = 26), HMC: 30.4 ± 0.70 years (N = 31)).

No significant differences in testosterone or prevasectomy LH values were found between the groups positive or negative for antibodies in either the HMC and MAMC populations. However, the positives in both the HMC and MAMC (Fig. 1) groups consistently had lower FSH values than the negatives at all time intervals (even through 36 months in the MAMC group and 42 months in the HMC group). This finding suggests that positives and negatives differed with respect to circulating FSH levels, even prior to vasectomy, and implies that the presence of free antisperm antibodies may be more likely after vasectomy in men with lower circulating FSH levels.

No consistent pattern of differences between positives and negatives was evident for any blood chemistry parameter measured in this study. Serum potassium levels tended to be higher in positives than in negatives from nine to 12 months after vasectomy, and there was a pattern of low probability values, particularly in the larger and more balanced HMC population (9-month potassium = 4.19 ± 0.07 (N = 25) vs 4.37 ± 0.08 (N = 20), $P = 0.096$; 12-month potassium = 4.20 ± 0.06 (N = 31) vs 4.39 ± 0.07 (N = 21), $P = 0.05$).

The data on the HMC group also indicated lower serum sodium levels in the positive population both before (0-time sodium = 141.3 ± 0.40 (N = 31) vs 139.8 ± 0.46 (N = 29), $P = 0.015$) and after (9-month sodium = 141.2 ± 0.48 (N = 25) vs 139.7 ± 0.63 (N = 20), $P = 0.065$) vasectomy. Apart from a slight suggestion of lowered serum chloride levels in the positive population in the first three months after vasectomy (3-month chloride = 104.0 ± 0.46 (N = 27) vs 101.9 ± 1.23 (N = 14), $P = 0.057$), none of the other blood chemistry parameters showed remarkable differences between positive and negative individuals. Data for total protein, blood urea nitrogen (BUN), and uric acid are shown in Fig. 2; no pattern of shifts was obvious. Albumin and bilirubin values are depicted in Fig. 3; creatinine and cholesterol values are presented in Fig. 4.

To remove the distortion resulting from the fluctuating population at different time intervals, we selected out of the HMC group all of the men on whom data were available at all or most post-vasectomy intervals. Means from these 29 men (21 negatives and eight positives) were plotted over time for each blood value assayed. At no time did a mean value for any parameter fall outside the range for normal adult men. Additionally, analysis of variance revealed no significant differences between any mean within each parameter.

Discussion

Antisperm antibodies developed after vasectomy; by 6 weeks 14% of the men in the HMC group were positive and by 3 months 31% of the HMC group and 21% of the MAMC group had circulating antibodies against sperm. By one year 41% of the HMC group and 27% of the MAMC group had antibodies. Some individuals exhibited only immobilizing and some only agglutinating antibodies, although the majority had both. These data argue in favor of both agglutinating and immobilizing antigens on the surface of the spermatozoon. We found that those individuals that

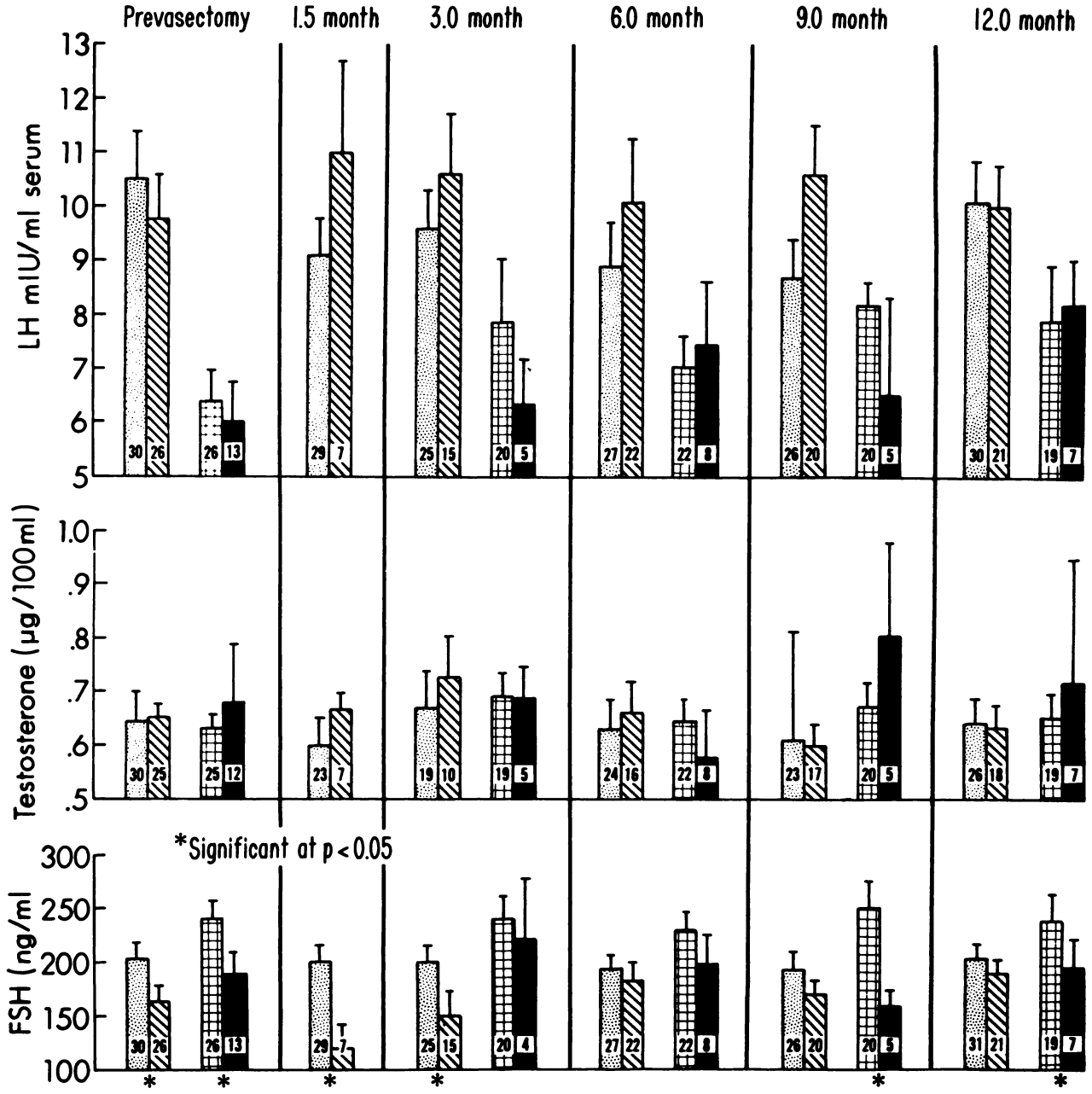

Fig. 1. LH, testosterone, and FSH prevasectomy, 1.5, 3, 6, 9, and 12 months postvasectomy values for the HMC group positive and negative and the MAMC group positive and negative for circulating antisperm antibodies. The mean ± the standard error is shown. The numbers within the bars are the number of men in the group tested for that time interval.

developed antibodies generally retained circulating levels throughout the period of testing.

Comparisons of the mean pre- and postvasectomy blood values in this study may be of somewhat limited value, since they do not take into consideration changes occurring in the nonvasectomized population throughout the period of study. In the absence of a control population of nonvasectomized men, we had no way of assessing the impact of aging factors as well as seasonal and other environmental factors on blood chemistry values or reproductive hormone levels. Furthermore, transient changes over time may have been distorted or obscured by the variable follow-up losses at each time interval. Consequently, only dramatic changes resulting from

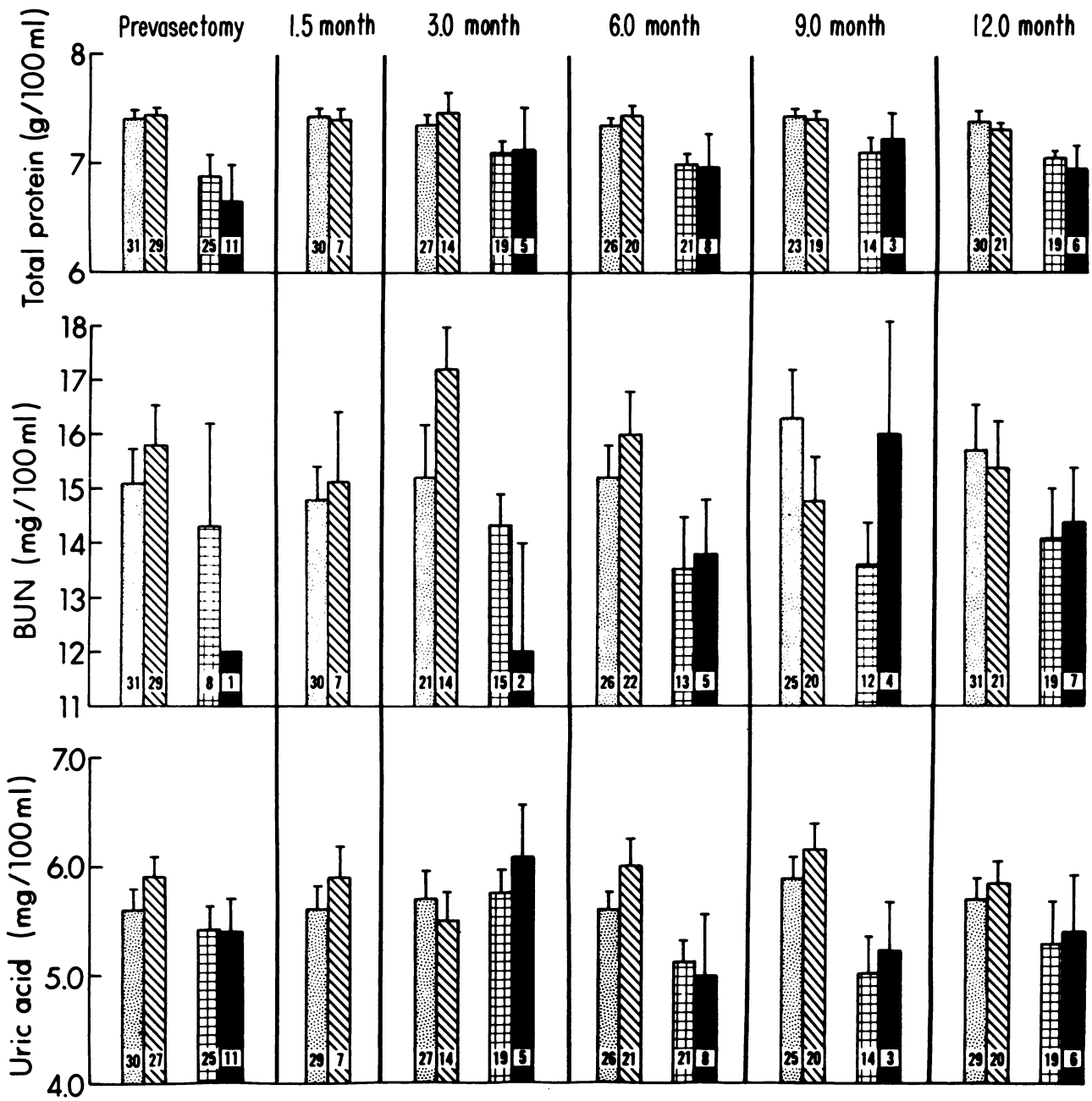

Fig. 2. Total protein, blood urea nitrogen (BUN), and uric acid levels prevasectomy, 1.5, 3, 6, 9, and 12 months postvasectomy for the HMC group positive and negative and the MAMC group positive and negative for circulating antisperm antibodies. The mean ± the standard error is shown. The numbers within the bars are the number of men in the group tested for that time interval.

vasectomy would be evident from analyses of this type. However, the scope of the parameters we studied offers a more comprehensive picture of the systemic physiologic events occurring after vasectomy than had been available heretofore. Our study substantiates previous ones that suggested no dramatic changes in the gonadal-pituitary axis after vasectomy, and further suggests that no significant changes in blood chemistry parameters occur as a result of this surgical procedure.

Our study offers a new perspective, i.e., a com-

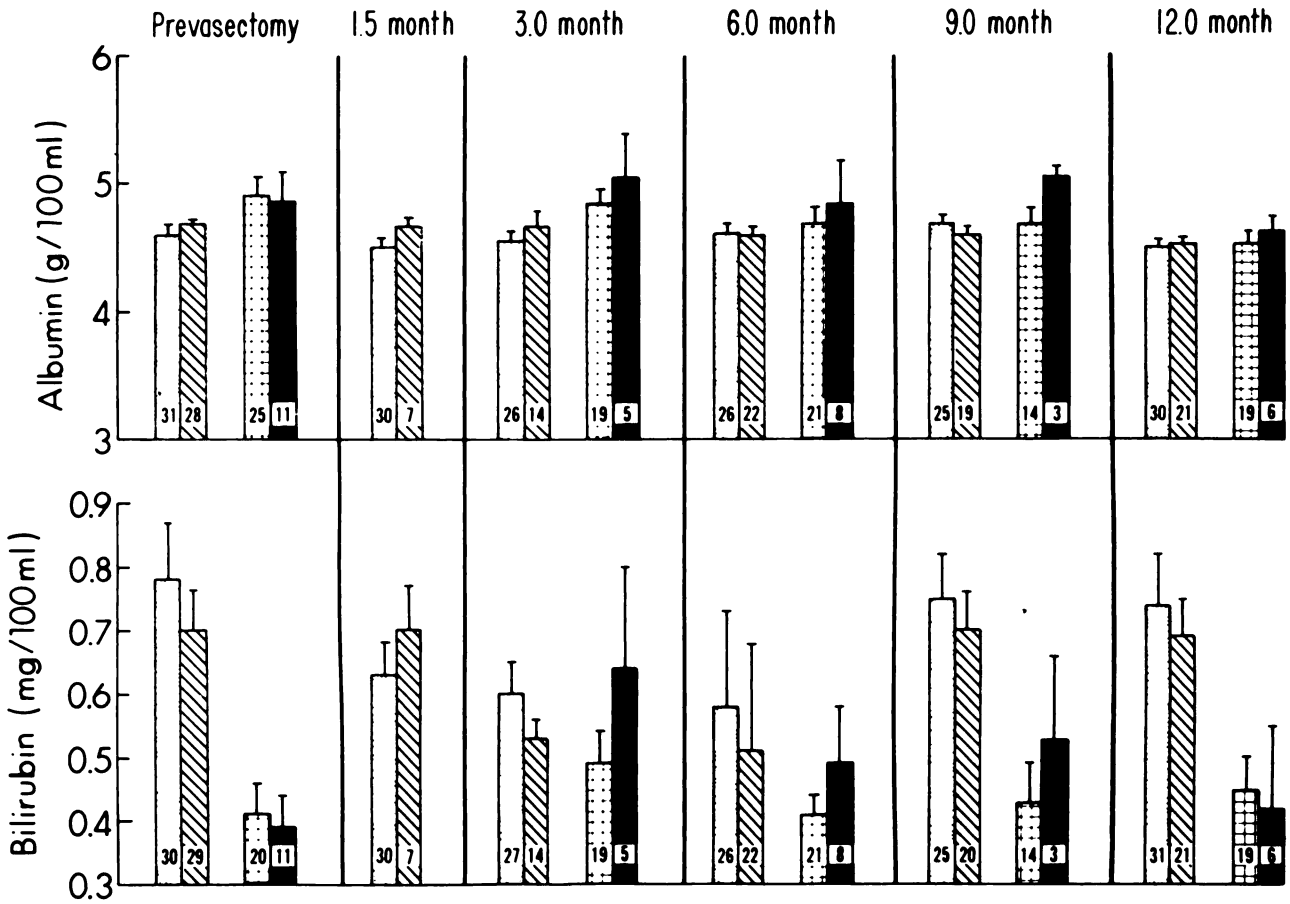

Fig. 3. Albumin and bilirubin values prevasectomy, 1.5, 3, 6, 9, and 12 months postvasectomy for the HMC group positive and negative and the MAMC group positive and negative for circulating antisperm antibodies. The mean ± the standard error is shown. The numbers within the bars are the number of men in the group tested for that time interval.

parison of the blood values before and after vasectomy in those men with and without circulating antisperm antibodies. The finding that the group of men who exhibited free antisperm antibodies after vasectomy tended to have lower FSH levels before vasectomy than the group in whom such antibodies are not detected was of interest. Even after vasectomy, for the first three months in the HMC group the FSH values were significantly lower in the group with detectable antibodies (Fig. 1). The fact that a similar phenomenon was observed in two groups of men vasectomized at different centers and otherwise very different with respect to their blood chemistry values provides additional support for the veracity of the FSH finding. FSH levels by themselves do not appear to have predictive value for free antisperm antibody formation, since only 54% of the HMC men with antisperm antibodies after vasectomy had prevasectomy FSH levels below the median value. Conversely, 53% of men in whom antibodies were not detected had prevasectomy FSH levels above the median value. Most studies on FSH after vasectomy have shown no changes (Johnsonbaugh et al, 1975; Skegg et al, 1976; Naik et al, 1976; Smith et al, 1976). On the other hand, Kobrinsky et al (1976) did find a significant fall in the FSH level during the first week after vasectomy and low FSH levels thereafter. The relationship between lower FSH levels and postvasectomy antisperm antibody formation is not obvious. It does not appear to correlate with sperm output since the prevasectomy total sperm counts did not differ significantly between men with postvasectomy antibodies and men without them. This finding raises some intriguing questions about both the role of FSH in the adult male and the cause of antisperm antibody formation. For example, one

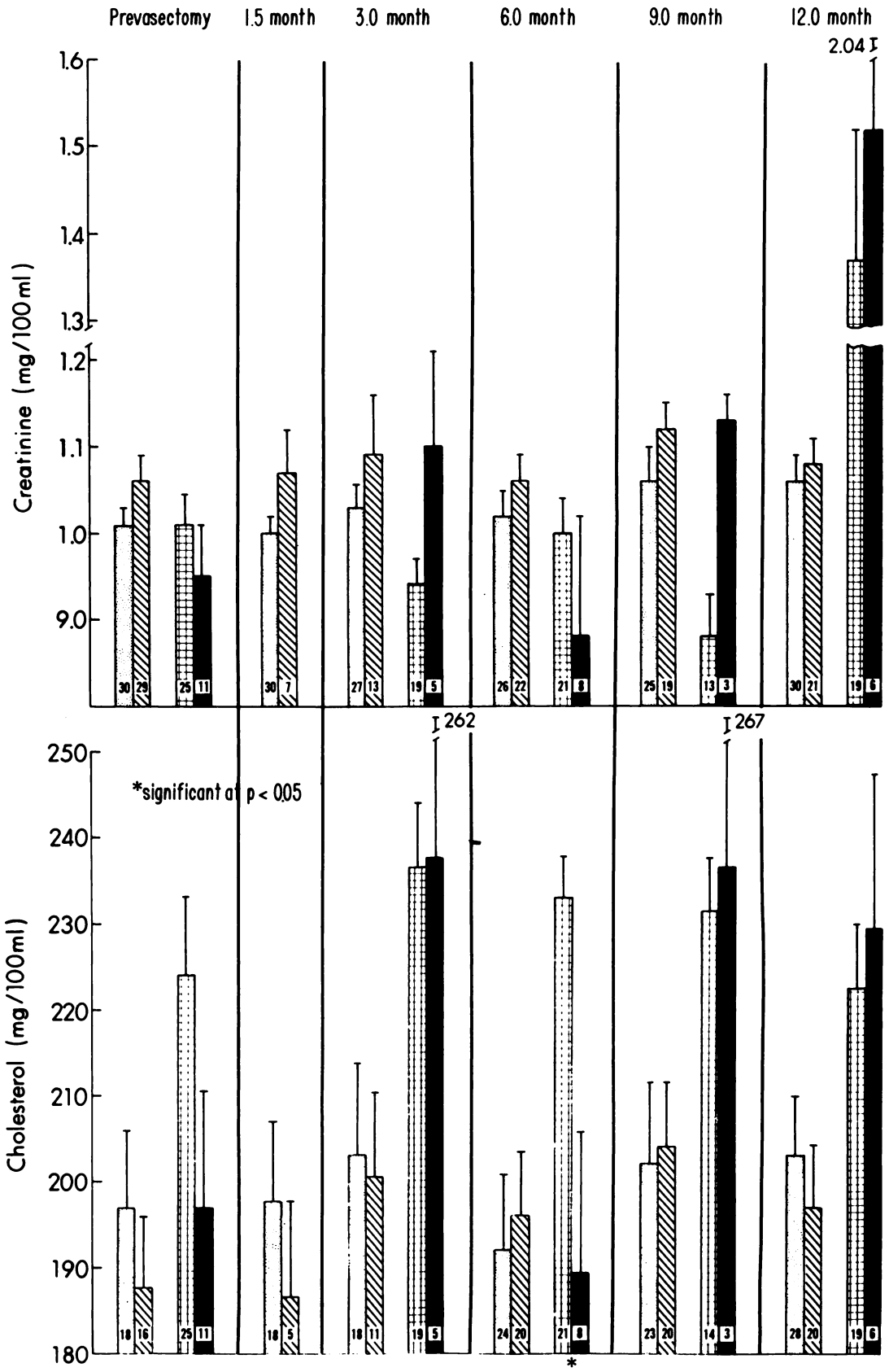

Fig. 4. Creatinine and cholesterol values prevasectomy, 1.5, 3, 6, 9, and 12 months postvasectomy for the HMC group positive ▨ and negative ▩ and the MAMC group positive ■ and negative ▦ for circulating antisperm antibodies. The mean ± the standard error is shown. The numbers within the bars are the number of men in the group tested for that time interval.

could speculate that perhaps lower FSH levels are somehow genetically associated with the antisperm antibody immune response.

If FSH levels increased markedly after vasectomy, autoimmune orchitis might be suspected. But such a disease state is not generally found after vasectomy. However, evidence of alterations to the seminiferous tubules is accumulating. For example, immune complex deposition in the basal lamina surrounding the tubules (Bigazzi et al, 1976; Alexander and Tung, 1977) has been found in rabbits, and reduced spermatogenesis with patchy areas where tubules are devoid of the germinal epithelium has been found in guinea pigs (Alexander, 1973). Lesions characterized by lymphocytic infiltration, a hallmark of classic orchitis, have also been observed in monkeys (Tung and Alexander, in preparation). In some vasectomized men, testicular changes, including thickening of the basement membrane (Bigazzi et al, 1979), increased phagocytosis by Sertoli cells (Hagedoorn and Davis, 1974), and degeneration of spermatids (Kubota, 1969) have been observed. Such changes generally do not seem to be of a magnitude to induce large shifts in FSH levels. This is not to say that some individuals will not be more severely affected than others.

The LH values for the total data set were not significantly different for those men negative and positive for antibodies. However, the LH levels of negative men were slightly higher than those of men positive for antibodies at 6 weeks and 12 months. Purvis et al (1976) found a similar rise in LH levels one month after vasectomy in a sample of 20 men. Smith et al (1975) observed that LH levels were higher at 1 and 6 weeks after vasectomy and that they dropped again at 3 months and later rose at 6 months and 1 year. However, these investigators have cautioned that all the LH values were in the normal range for men in their laboratory, as were those of the present study, and they have attributed the variation to diurnal variation, psychological distress, and surgical stress.

There were no significant differences in testosterone levels between those men with and without circulating antibodies. Although vasectomy may cause alterations in the germinal epithelium, it does not result in significant intertubular testicular damage or changes to the androgen-producing cells.

There was some indication of higher potassium levels nine to 12 months after vasectomy and lower chloride levels within the first three months after vasectomy in men in whom free antisperm antibodies developed than in those without antisperm antibodies. The pattern of lower serum sodium levels, seen both before and after vasectomy in men who develop sperm antibodies after vasectomy, is difficult to interpret. Studies in rhesus monkeys showed no significant changes in sodium, potassium, or chloride levels when vasectomized animals were compared to age-matched controls or when those with and without antisperm antibodies were compared (Alexander and Tung, 1979).

In a previous article, one of us (Alexander, 1977) suggested that total antigen available to the body might partially determine whether or not free circulating levels of antisperm antibodies develop in an individual. In a study in rhesus monkeys, the monkeys with the highest initial total sperm count had the highest sustained free antisperm antibody levels. To test this hypothesis, we obtained total sperm counts for individuals from the HMC group prior to vasectomy. Although initial sperm counts were somewhat higher in the early-positive group, there was no significant difference in the mean total counts of positives and negatives. We found no relationship between the development of antibody titers and age. Such a correlation might have been expected, since Lucas and Rose (1978) have reported higher antisperm antibody titers in men under 30 than in older age groups.

We compared cholesterol levels of those men that developed antibodies with those that did not for two reasons: 1) studies indicate that circulating lipids can affect immune responses (Curtiss and Edgington, 1976), and 2) recent studies in monkeys have shown an association between vasectomy and atherosclerosis (Alexander and Clarkson, 1978; Clarkson and Alexander, in press). We found no differences in cholesterol levels between men positive and those negative for antibodies. Alexander and Tung (1979) have reported a similar finding in vasectomized rhesus monkeys. It seems fair to assume that vasectomy causes no changes in cholesterol synthesis or catabolism.

Since it has been suggested that vasectomy could result in higher uric acid levels due to increased resorption of nucleic acids (Johnson, 1972), we checked for postvasectomy changes but found none. This result substantiates those of Ansbacher (1973) and Alexander and Tung (1979),

who also found no shifts in uric acid levels in men and monkeys, respectively. No significant shifts in albumin, bilirubin, BUN, total protein, and creatinine support the results of studies in rhesus monkeys (Alexander and Tung, 1979).

We have discussed the various blood parameters in terms of whether an individual was positive or negative for free antisperm antibodies. The tests used to determine antibody levels measure only antibody that is unbound. Thus it is possible that some men in our study population mounted an immune response but, because there was excess sperm antigen present, immune complexes were immediately formed and little or no free antibody was available for detection with our assay system. On the other hand, it is possible that a percentage of men do not mount any immune response to sperm. Further work is necessary in order to understand the development, maintenance, and implications of antisperm antibody levels after vasectomy.

In summary, we found no significant changes in the blood chemistry and hormone levels after vasectomy. However, 48% and 37% of the vasectomized men in the two groups developed antisperm antibodies postvasectomy and these men generally had lower FSH levels and lower sodium concentrations both before and following vasectomy.

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