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Sperm agglutinins in seminal plasma and serum after vasectomy

CORRELATION BETWEEN IMMUNOLOGICAL AND CLINICAL FINDINGS

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SUMMARY

The development of sperm agglutinins in serum and seminal plasma in relation to vasectomy was studied in forty-seven men by testing samples taken before vasectomy and on five occasions during the first year after vasectomy. Thirty additional patients were tested only 1 year after vasectomy.

One year after vasectomy, sperm agglutinins in the serum in titres from 4 to about 4000 had developed in 62% of the entire group, while antibodies in the seminal fluid detectable by the gelatin agglutination test were present in only 4% of the group, and apart from one unusual case the titres were low here (either 4 or 8).

Analysis of the modes of agglutination revealed changing patterns in several patients during the observation period, with a predominance of tail agglutinins after 1 year. In some cases, mixed agglutination was seen with serum but pure tail-to-tail agglutination with seminal plasma.

The total number of spermatozoa in a pre-vasectomy ejaculate was found to be correlated with an early immune response and with the titre values after 1 year.

The group of patients in whom agglutinins had developed 1 year after vasectomy were found to have significantly larger nodules at the sites of operation than those without sperm agglutinins.

INTRODUCTION

In their efforts to clarify the genesis of sperm agglutinins, Rümke & Hellinga (1959) demonstrated a correlation between 'occlusion of the efferent ducts on both sides with normal spermatogenesis' and the presence of sperm agglutinins in serum. This correlation was later confirmed by Phadke & Padukone (1964), and several prospective studies have subsequently described the development of circulating sperm agglutinins in vasectomized men (Shulman *et al.*, 1972; Ansbacher, 1973; van Lis, Wagenaar & Soer, 1974).

It now seems established that sperm agglutinins in serum develop in about 60% of vasectomized men within 1 year of vasectomy. The agglutinins are apparently persisting, and there are even indications of an increase in titres through the first decade (Gupta *et al.*, 1975). Immunization may occur either in the epididymis—due to extravasation of spermatozoal antigens or escape of whole spermatozoa into the interstitial tissue (Rümke, 1972)—or be caused by penetration of spermatozoa at the site of operation, leading to the formation of a spermatic granuloma (Schmidt & Morris, 1973). To what extent each of these pathogenic mechanisms is operative remains unsettled.

While sperm antibodies in serum have been extensively studied, little information is available on the occurrence of anti-spermatozoal antibodies in seminal plasma after vasectomy: apparently, only Ansbacher (1973) has investigated this and briefly reported totally negative findings of sperm agglutinins and immobilizing antibodies in thirty-nine ejaculates, tested 1 year after vasectomy. However, the occurrence

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of sperm antibodies in seminal plasma after vasectomy is a rather important problem, not only from a basic immunological point of view in the investigation of the origin of sperm antibodies in seminal plasma, but also from a clinical point of view, since any anti-fertility effects of sperm agglutinins in the male seem to be displayed in the ejaculate (Rümke, & Hellinga, 1959; Fjällbrant, 1968; Husted & Hjort, 1975), and successful restoration of fertility in vasectomized men by vasovasostomy could therefore be expected to depend on the levels of sperm agglutinins in seminal plasma.

The principal objective of the investigation presented below was to compare the levels of sperm agglutinins in seminal fluid with those in serum, and also to take into account the different specificities of the sperm agglutinins as recorded by the different modes of agglutination (head-to-head, tail-to-tail or mixed). At the same time, the correlation between immunological findings and some clinical parameters was analysed.

MATERIALS AND METHODS

Patients. A total of fifty-three men undergoing vasectomy agreed to take part in this prospective study, but three withdrew after a few weeks, and three were excluded because of post-operative complications. The remaining forty-seven men donated blood and semen samples immediately before vasectomy, 9 days, 1, 2 and 3 months, and 1 year after the operation. The vasectomy was performed under local anaesthesia with resection of approximately 1 cm of the vas deferens and single ligatures on both the epididymal and vesicular ends.

In addition, a group of thirty men was examined retrospectively. Blood and semen samples were here collected only at 1 year after vasectomy.

Clinical examinations. Clinical examinations of the forty-seven patients were performed 3 months and 1 year after the operation. The sites of operation were palpated in order to detect granulomata, and at the 1 year examination attempts were made—by the same investigator examining all patients—to estimate the actual diameter of the nodules at the sites of operation, using the normal parts of the vas deferens as a reference set at 2·0 mm. Furthermore, the consistency of the epididymis was characterized—as normal or distended—and unusual tenderness was noted.

Sera. These were stored at −20°C and inactivated at 56°C for 30 min before being tested.

Semen samples. These were procured by masturbation and subjected to the usual semen analyses, including counting of the spermatozoa (Linnet, 1977). Seminal plasma was obtained by centrifugation and stored at −20°C. After thawing, before being tested, it was again centrifuged (10 min at 2800 *g*), giving a clear fluid of low viscosity.

Sperm agglutinins. These were detected and characterized by means of the gelatin agglutination test (GAT) (Kibrick test) and slide agglutination test (SAT) respectively, carried out as described by Rose *et al.* (1976). Fresh ejaculates from selected sperm donors were used for both tests.

In the GAT, samples of serum and seminal plasma were screened in dilutions of 1:4 and 1:16, and positive samples were subsequently titrated in two-fold dilutions, starting with 1:4. For each patient, positive samples up to 3 months after operation were titrated in the same batch. In subsequent testing of the 1 year samples, the 3 month samples were included as controls to make sure that the sensitivity of the test system had not undergone unacceptable changes.

Samples positive in the GAT were tested for the mode of agglutination in the SAT. The agglutinates observed were recorded as head-to-head agglutinates (H), tail-to-tail agglutinates (T) or mixed agglutinates (M) (with both head-to-head and tail-to-tail agglutination at the same time).

RESULTS

Sperm agglutinins in serum and seminal plasma

Sperm agglutinins were detected in at least one of the serum samples in thirty-two (68%) out of the forty-seven patients in the prospective group, and among the thirty men examined only at 1 year after vasectomy, eighteen (60%) had sperm-agglutinating activity in serum. 1 year after the operation, the prevalence in the total group of seventy-seven men was 62%, which is similar to observations reported in the above-mentioned studies.

The detailed findings for each of the thirty-two patients in the prospective group in whom sperm agglutinins developed are given in Fig. 1. None of these patients had agglutinins before vasectomy; nor had any of them a positive GAT at day 9 after vasectomy. Fig. 1 shows that there was no uniform pattern for the appearance of sperm antibodies. In most cases, agglutinins would appear after 1–3 months, but in six cases they were not detectable until 1 year after vasectomy (Nos 5, 40, 31, 42, 3 and 4). In general, titres increased during the observation period, but decreases and even disappearances were also seen (Nos 35, 37, 33 and 36).

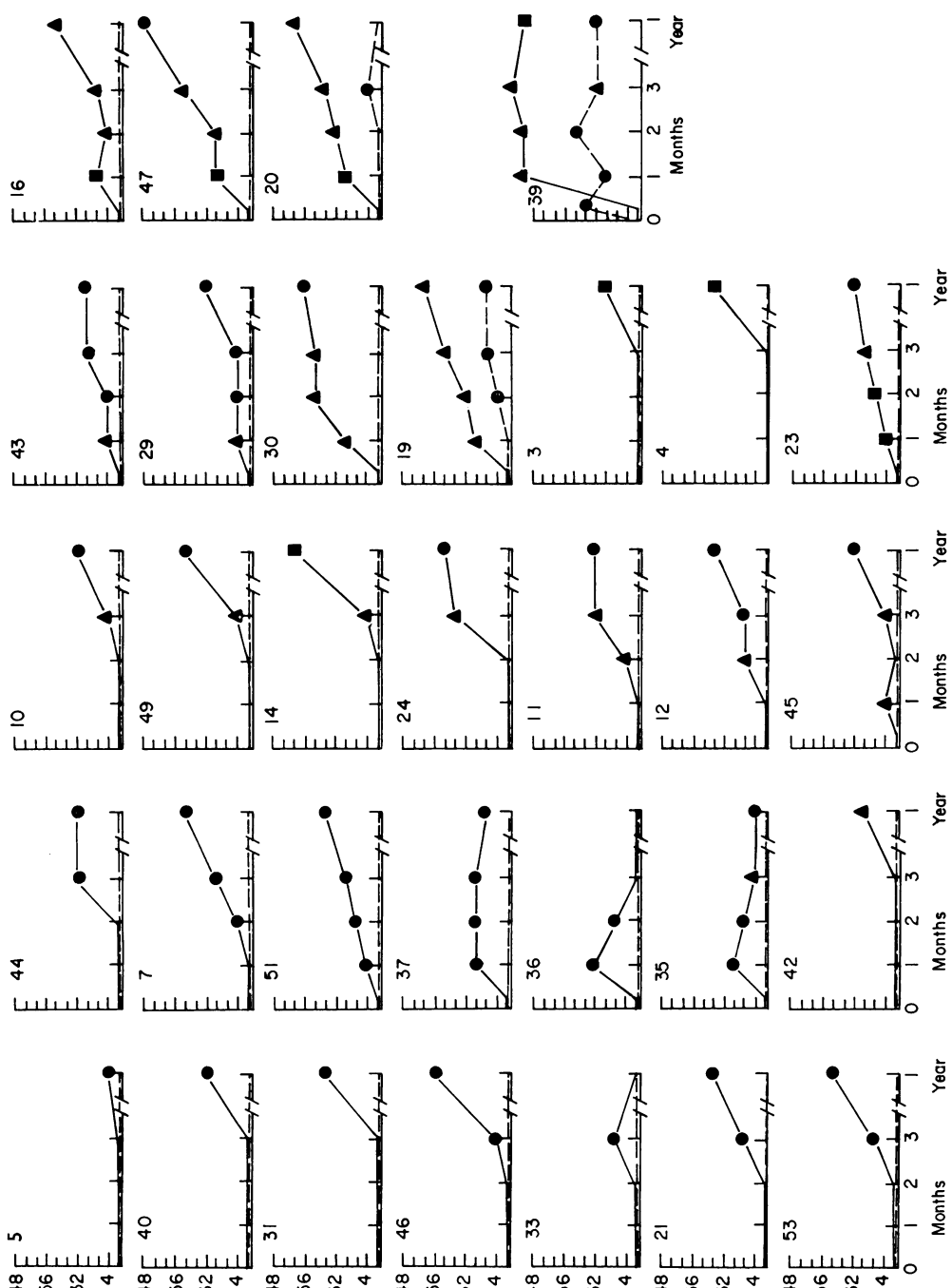

FIG. 1. Titres and modes of agglutination of sperm agglutinins in serum and seminal plasma in thirty-two patients in whom such antibodies could be detected during the first year after vasectomy (out of a total of forty-seven patients studied). (——) Serum; (- - -) seminal plasma. (■) Head-to-head agglutination, (●) tail-to-tail agglutination, and (▲) mixed agglutination.

Antibody findings in seminal plasma were scarce compared with those in the serum. Only four patients—three from the prospective group and one from the retrospective group—revealed definitely positive reactions in the GAT. Furthermore, two patients in the latter group gave doubtful reactions in the 1:4 dilution, and testing in the SAT disclosed typical agglutinates. Including the two weak reactions, sperm agglutinins in seminal plasma were found in 7·8% of the seventy-seven men, the prevalence at 1 year as determined by the GAT being 3·9%.

The detailed findings in the three patients belonging to the prospective group appear from Fig. 1. In two cases (Nos 19 and 20), seminal plasma titres were low and were several titre steps lower than in the serum. Similar findings were made in the three patients in the retrospective groups (with titres in the serum of 128, 2048 and 128, and titres in seminal plasma of 8, <4 and <4, respectively). Patient No. 39 (Fig. 1) presented an exceptional case with titres up to 128 in seminal plasma—as compared with titres of 4000 to 8000 in serum—and sperm agglutinins were detected in seminal plasma as early as 9 days after vasectomy, when the serum still gave negative results.

In the testing of the seminal plasma, atypical cloudiness was observed in the GAT in a few cases, and in the SAT the donor spermatozoa appeared to be immobilized. The use of a condom was admitted by four of the five men with such reactions. The disapproval of the use of a condom was emphasized, and later seminal samples were negative in the GAT.

Modes of agglutination

Registration of the modes of agglutination in the SAT may be easy and clear-cut—particularly in cases with pure head-to-head agglutination—but may in other situations be a question of evaluating the predominant mode in a more complex picture. However, in the blind reading of the tests, characteristic reaction patterns for the individual patients were generally observed, thereby providing evidence of the reliability of the testing.

TABLE 1. Number of patients with the various modes of agglutination observed with their sera at different times after vasectomy

Modes of agglutination	Prospective group (time after vasectomy)				Retrospective group (1 year after vasectomy)
	1 month	2 months	3 months	1 year	
Head-to-head	4	1	0	4	3
Mixed	6	8	14	4	4
Tail-to-tail	4	7	11	22	11
Total	14	16	25	30	18

The recorded modes of agglutination for each serum or seminal plasma sample giving a positive GAT are listed in Fig. 1, and Table 1 gives the findings with sera. Although all different modes of agglutination could be seen initially in serum, mixed agglutination (M) was the predominant mode during the first 3 months after vasectomy. During this period, the number of sera with head-to-head agglutination (H) decreased (from four after 1 month to none after 3 months), whereas tail-to-tail agglutination (T) was observed in an increasing number of sera (from four to eleven). 1 year after vasectomy, tail-to-tail agglutination was recorded in nearly 75% of all positive sera.

Looking at the individual patients—as indicated by the symbols in Fig. 1—it appears that patients in whom T agglutinins were first recorded continued to have T agglutinins (columns 1 and 2 in Fig. 1), whereas in patients starting with H agglutinins in serum a gradual change to M agglutination, and sometimes further to T agglutination, could often be seen (last two columns in Fig. 1).

All three patients with sperm agglutinins in the ejaculate were found to have T agglutinins in seminal plasma. In one case (No. 39) only four out of the five samples tested were recorded as giving T agglutina-

tion, while the mode of agglutination with the sample after 3 months was listed as M agglutination (which may be due to the difficulties involved in the reading of the SAT). In comparing the modes of agglutination in the serum and seminal plasma, respectively, it should be noted that the sera of the three patients caused mixed agglutination. Thus there are indications that if both H and T agglutinins are present in the serum, they do not necessarily occur in the seminal plasma in the same proportion. However, H agglutinins may also occur in seminal plasma after vasectomy, since the only patient in the retrospective group with a definitely positive GAT showed this mode of agglutination.

Correlation between clinical and serological findings

The total number of spermatozoa in the pre-vasectomy ejaculates (sperm concentration × volume) ranged from 4 to 1600×10^6 with a median of $1{\cdot}22 \times 10^8$. Elicitation of an immune response with temporary or continued production of sperm agglutinins in the serum within the given span of time was, after 1 month, 2 months and 3 months, found to be correlated with the total number of spermatozoa in the pre-vasectomy ejaculate (Table 2, column a). Since this number must be considered the best estimate of the production of spermatozoa in the individual patients, the observed correlations indicate the significance of the 'antigen load' for an early response. In a similar analysis of the antibody findings after 1 year, no correlation could be demonstrated, apparently because sperm agglutinins had now developed in some of the patients with low sperm numbers. However, it should be stressed that a large number of spermatozoa before vasectomy does not necessarily lead to production of sperm agglutinins. This is illustrated in Fig. 2a, which gives the exact findings for each patient 1 year after vasectomy.

TABLE 2. Statistical analysis of correlation between sperm agglutinins and the number of spermatozoa in a prevasectomy ejaculate, as well as between sperm agglutinins and the size of nodule at the site of operation 1 year after vasectomy (forty-seven patients)

	Mann–Whitney test			Spearman rank correlation test, corrected for ties		
					Titres (positive sera only) correlated to:	
Time after vasectomy	Positive reaction in GAT before or at time indicated	Ranking according to total number of spermatozoa (one-sided testing)	Ranking according to size of nodules (two-sided testing)	Positive GAT at time indicated	Total number of spermatozoa	Size of nodules
		(Column a)	(Column b)		(Column c)	(Column d)
1 month	14	$P < 0{\cdot}025$	$P < 0{\cdot}01$	14	$r_s = 0{\cdot}0260$*	$r_s = 0{\cdot}0110$*
2 months	17	$P < 0{\cdot}025$	$P < 0{\cdot}02$	16	$r_s = 0{\cdot}343$*	$r_s = 0{\cdot}646$†
3 months	26	$P < 0{\cdot}025$	$P < 0{\cdot}01$	25	$r_s = -0{\cdot}049$*	$r_s = 0{\cdot}240$*
1 year	32	$P > 0{\cdot}1$*	$P < 0{\cdot}01$	30	$r_s = 0{\cdot}428$‡	$r_s = 0{\cdot}163$*

* Not significant; † $P < 0{\cdot}02$, two-sided testing; ‡ $P < 0{\cdot}025$, one-sided testing.

In the testing for a quantitative relationship between the number of spermatozoa in the pre-vasectomy ejaculate and the titre in serum, taking into account only patients with sperm agglutinins, the picture was reversed, since a significant correlation was found only for the titre values at 1 year (Table 2, column c, and Fig. 2a).

At the clinical examination 1 year after vasectomy some patients were found to have definite nodules at the sites of operation. The diameters of these nodules were estimated, and that of the largest nodule was used in the statistical analysis. In patients without nodules, the sites of the ligatures could always be identified by a just-perceptible thickening. In such cases, the diameter of the vas deferens was rather arbitrarily set at 2·2 mm (compared with the normal diameter set at 2 mm). The diameters recorded in this way ranged from 2·2 to 9 mm with a median of 3 mm.

Statistical analysis (here double-sided testing, but otherwise similar to the testing for the total sperm

number) revealed that the group of patients responding with production of sperm agglutinins within a given period had significantly larger nodules (at 1 year) than patients without sperm agglutinins (Table 2, column b).

Considering the titre values in the group of patients with antibodies at a given time, the patients with the largests nodules tended to have the highest titres on all occasions, but only the titres obtained at 2 months after vasectomy showed a significant correlation with the size of the nodules (Table 2, column d). The exact data for this comparison are given in Fig. 2b.

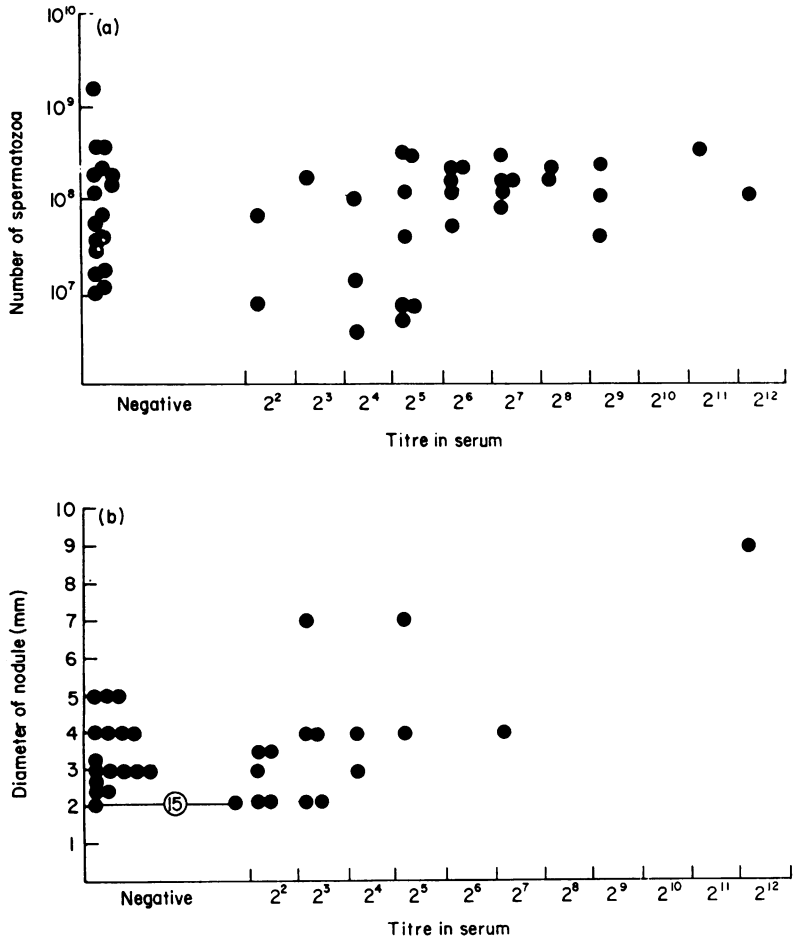

FIG. 2. (a) Titres of sperm agglutinins in serum 1 year after vasectomy compared with total number of spermatozoa in a prevasectomy ejaculate. (b) Titres of sperm agglutinins in serum 2 months after vasectomy compared with the diameter of the largest nodule at the site of operation recorded in each patient 1 year after vasectomy. Number in ring shows number of points in that group.

The mode of agglutination was also correlated with the size of the nodules, at least at 3 months, when the number of sera giving mixed agglutination reached a maximum. At this time, ten (83%) out of the twelve patients with sperm agglutinins and a nodule greater or equal to 4 mm after 1 year showed mixed agglutination with their serum as against only four (31%) out of the 13 with agglutinins and a nodule less than 4 mm (Fisher's exact test: $P_1+P_2 = 0{\cdot}0154$).

Distension of the epididymis, observed in seventeen patients at 3 months and in twenty-seven patients at 1 year after vasectomy was unrelated to the antibody findings.

DISCUSSION

In recent years, the immunological consequences of vasectomy have been widely investigated, primarily to clarify if this experimentally induced autoimmunity in men may involve any risks. The interest has mainly concentrated on sperm-specific antibodies in serum (Shulman *et al.*, 1972; Ansbacher, 1973; van Lis, Wagenaar & Soer, 1974), non-organ-specific autoantibodies in serum (Crewe *et al.*, 1976; Mathews *et al.*, 1976) and cell-mediated immunity to spermatozoa (Nagarkatti & Rao, 1976). None of these studies have revealed any findings to indicate that a more restrictive view on vasectomy is justified at the present, but further studies on the long-term effects of vasectomy are still needed, particularly after the demonstration of complex-mediated glomerular nephritis in vasectomized rabbits with high levels of sperm antibodies (Bigazzi *et al.*, 1976).

Another aspect is now coming more into focus, perhaps particularly from the patient's point of view: can the immune phenomena prevent or complicate re-fertilization by vasovasostomy? The answer to this question should be based on studies of the levels of sperm antibodies in the genital tract.

While Ansbacher (1973) was unable to detect sperm agglutinins in seminal plasma in vasectomized patients, the present investigation clearly demonstrates that such antibodies can appear in seminal plasma after vasectomy, although rarely, usually in low titres and sometimes apparently only temporarily (No. 20 in Fig. 1).

In comparison with previous findings with the same techniques in infertile males (Husted & Hjort, 1975), the results in vasectomized patients show a completely different pattern with much lower ratios between titres of sperm agglutinins in seminal plasma and serum. Thus tail-to-tail agglutinins could, for example, be detected in the seminal plasma of all infertile males with this antibody in a titre of 16 or more in the serum, whereas among the vasectomized patients tail-to-tail agglutinins in titres of 128 or more in the serum were found in several cases without detectable agglutinins in the seminal plasma.

There is good evidence that the greater part of the immunoglobulins (IgG and possibly also IgA) enters the semen by transudation via the prostate, reaching a level of only about 1% of the concentration in serum (Rümke, 1974). The relatively high ratios between the titres in seminal plasma and serum (much higher than 1%) in infertile males have therefore—together with differences in physical characteristics of sperm antibodies in serum and seminal plasma—been interpreted as evidence of a local production of sperm antibodies, probably of the IgA class (Friberg, 1974; Husted & Hjort, 1975; Leslie, Quinlivan & Sullivan, 1976). Among the vasectomized patients, on the other hand, the levels of sperm agglutinins in seminal plasma do not generally exceed about 1% of the concentration in serum, and there is therefore here no indication of a local production of sperm agglutinins, at least not from the seminal vesicles and the prostate.

As far as re-fertilization by vasovasostomy is concerned, the rather negative antibody findings in seminal plasma are, of course, essential. Among the total of seventy-seven men investigated, the antibody concentration in seminal plasma did only in one case (No. 39) reach a level at which—based on experience from infertile patients—it might be expected to interfere with fertility. However, it should be borne in mind that a study of vasectomized patients cannot disclose antibodies entering the upper part of the genital tract (rete testis and epididymis), and the present findings should not give rise to a too optimistic view. Gupta *et al.* (1975) were able to demonstrate sperm autoagglutination in the ejaculates of as many as five out of thirteen patients who had undergone vasovasostomy. All five patients had sperm agglutinins in serum, four of them in titres of 64 or more, and none of them appeared to be fertile. It should therefore be realized that negative findings in seminal plasma before vasovasostomy may be of little prognostic value for the restoration of fertility.

The analysis of the relationships between clinical and immunological findings revealed a significant correlation between an early immune response to spermatozoa and the total number of spermatozoa in in the pre-vasectomy ejaculate, which was used as a measure of the production—and consequently also resorption—of spermatozoa in the individual patients. Similar observations have been made in Rhesus monkeys (Alexander, 1977). In the long run, the sperm count played a minor role since a number of patients with low counts would gradually also elicit an immune response. However, there was in the

group of patients with sperm antibodies after 1 year a significant correlation between the sperm count before vasectomy and the titre of sperm agglutinins. That the amount of antigen produced—and under the given circumstances the amount of antigen to be disposed of—might play a role for the immune response would not seem surprising. It might rather be wondered why any immune response did not develop in the other patients with equally high sperm counts—not even in the one with the highest count.

Although biopsies of the nodules at the sites of operation were not studied, it would be reasonable to assume that these nodules—at least the larger ones—represented true spermatic granulomata which would form an extra focus for immunological stimulation. This was also indicated by the correlation which was observed between the size of the nodules and the tendency to the development of sperm agglutinins. However, the basic mechanisms behind these observations remain unknown. It might be that spermatozoa are always treated as foreign material, and that the size of the granulomata would therefore simply depend on the amount of spermatozoa leaking into the surrounding tissue. But it should be kept in mind that the nodules might also mainly reflect the immunological reactivity of the patient, and that true granulomata would consequently develop only in patients in whom the capability of responding to spermatozoal antigens is already present.

The significantly increased score of mixed agglutination (both H and T agglutination) among patients with a nodule greater or equal to 4 mm and sperm agglutinins after 3 months might—as a preliminary hypothesis—point to a better immunogenecity of the head antigen when whole spermatozoa gain access to the interstitial tissue (as in the nodule) than when resorption of material from degenerated spermatozoa in the epididymis is supposed to play a principal role in immune stimulation (as in patients with smaller nodules). In the latter situation, antibodies to the tail seem to predominate, as also in the longer run, when the immunological reactivity in the nodules has ceased (Schmidt & Morris, 1973).

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