Clin. exp. Immunol. (1974) **17**, 287–297.

THE ORIGIN OF IMMUNOGLOBULINS IN SEMEN

P. RÜMKE

The Netherlands Cancer Institute, Sarphatistraat 108, *Amsterdam, The Netherlands*

(*Received* 15 *October* 1973)

SUMMARY

IgG and IgA, albumin and lactoferrin as well as the semen compartment parameters acid phosphatase, fructose and spermatozoa were determined in separately collected fractions of the same ejaculate of some normal donors. The distribution over the fractions per ejaculate of IgG, IgA and albumin was generally more or less similar to the distribution of acid phosphatase indicating that the bulk of these proteins enters the semen via the prostate and not via the vesicles or testis and epididymis. The distribution of lactoferrin unexpectedly was not clearly related to fructose. IgM could not be detected.

The concentrations in the (eight) total ejaculates expressed as percentages of the serum concentrations were for albumin slightly higher than for IgG, both in the order of 1% and moreover correlated with each other, indicating that IgG reached the seminal fluid in general by transudation from the circulation. The relative IgA concentrations could not be measured exactly but seemed to be slightly higher than of albumin, and not correlated to albumin concentrations, suggesting that local production of IgA may occur also.

INTRODUCTION

Antibodies against spermatozoa have occasionally been found in serum as well as in seminal plasma of infertile men. They are the primary cause of the infertility if they agglutinate or immobilize the spermatozoa in an otherwise normal ejaculate (Wilson, 1954; Rümke & Hellinga, 1959; Fjällbrant, 1968). In a study of 133 men in whom both serum and semen samples were titrated, it was found that in general the titres in serum were much higher than in semen (Rümke & van Amstel, in preparation). Diffusion of immunoglobulins from blood to semen should therefore be considered as a possible source of the antibodies in semen. The more so since blood plasma proteins, such as albumin, transferrin and complement factor C3, have been detected in small amounts in seminal plasma (Herrmann, 1969). On the other hand the fact that in 3 of the 133 above mentioned men the titres were

Correspondence: Dr P. Rümke, The Netherlands Cancer Institute, Sarphatistraat 108, Amsterdam, The Netherlands.

higher in seminal plasma than in serum and also the occurrence of the secretory piece of IgA in seminal plasma (Ueling, 1971) strongly suggests that local production in the genital tract may also be possible. Evidence for local IgA sperm antibody production was found by Coombs, Rümke & Edwards (1973).

The possibility that prostate secretory epithelial cells are involved in the accumulation and discharge of immunoglobulins was suggested by Ablin, Gonder & Soanes (1971).

In order to investigate from which organ of the genital tract different substances in semen originate, one can make use of the phenomenon that different compartments of semen follow one another in a definite order of sequence. This has been demonstrated by the split ejaculate method which depends on the collection and analysis of separate fractions of the same ejaculate (Mann, 1964). Since acid phosphatase in semen is produced by the prostate and fructose chiefly by the seminal vesicles, determination of these components provides a useful guide to the composition of the compartments in the different fractions of a split ejaculate. Usually the first fraction consists mainly of prostatic, and the last of vesicular secretion, while spermatozoa are ejaculated in between. If therefore, the acid phosphatase-rich first fraction also contains the bulk of albumin, as has been found by Estborn & Smedin (1959), one may draw the conclusion that albumin in seminal plasma is chiefly derived from the compartment supplied by the prostate.

It is the aim of this study to compare the patterns of occurrence in the fractions of split ejaculates of the immunoglobulins IgG and IgA, albumin and lactoferrin with those of the compartment parameters acid phosphatase, fructose and spermatozoa in order to investigate from which organs, i.e. prostate, vesicles or testis and epididymis these proteins originate. To that purpose the ratios of the concentrations in sequential fractions were used for comparison. In the hypothetical case of two similar ratios the conclusion can be drawn that the two components have the same source. Another aim of the study was to obtain an impression about the amounts of albumin and IgG that diffuse from blood to semen.

MATERIALS AND METHODS

Split ejaculates

The fractions of the split ejaculates were kept at +0°C immediately after liquefaction. After measuring the volume and counting the spermatozoa in a Bürker counting chamber, they were centrifuged for about 10 min at 650 ***g***. A minimal amount of the plasma samples was used fresh for acid phosphatase determinations and the rest was stored frozen and used later for the other determinations. Acid phosphatase was determined in the fresh fractions according to the method of Bessey, Lowry & Brock (1946). Fructose was determined according to the indol method according to Karvonen & Malm (1955).

IgG, IgA, IgM, albumin and lactoferrin were determined by the radial immuno-diffusion technique (Mancini, Carbonara & Heremans, 1965). The antisera against the immunoglobulins and anti-albumin were commercial monospecific antisera. In the first experiment IgG levels were expressed in percentages of a standard serum. In the second experiment a standard serum was used with known amounts of the immunoglobulins and albumin, which made it possible to express the concentrations in mg/ml. Anti-lactoferrin and lactoferrin were earlier prepared in this laboratory (Rümke *et al.*, 1971).

RESULTS

First experiment

Five donors provided split ejaculates, three of them in two fractions (A, B and C) and one (D) in three fractions and one (E) in four fractions. Donor E was an infertile man with strong auto-agglutination of his spermatozoa due to spermagglutinins. Because of the agglutination the spermatozoa could not be counted. The other donors were normal volunteers. Table 1 shows the concentrations of the compartment parameters acid phosphatase, fructose spermatozoa and of IgG in the different fractions. If IgG is mainly supplied by one of the compartments the ratio of the concentrations of IgG in two fractions should be comparable to the ratio of the concentrations of the parameter of that compartment. The comparison is performed by calculating the difference between the logarithm of the IgG ratio and the logarithm of the ratio of each of the parameter concentrations. The distribution of IgG resembles most strongly that parameter for which this figure is closest to zero. These figures are shown in Table 1 and also in Fig. 1 where the concentrations are plotted logarithmically. Parallelism of lines connecting the concentrations means similar ratios. The differences of the logs of the parameter ratios with the log of the IgG ratio indicate the degree to which the two lines deviate from parallelism.

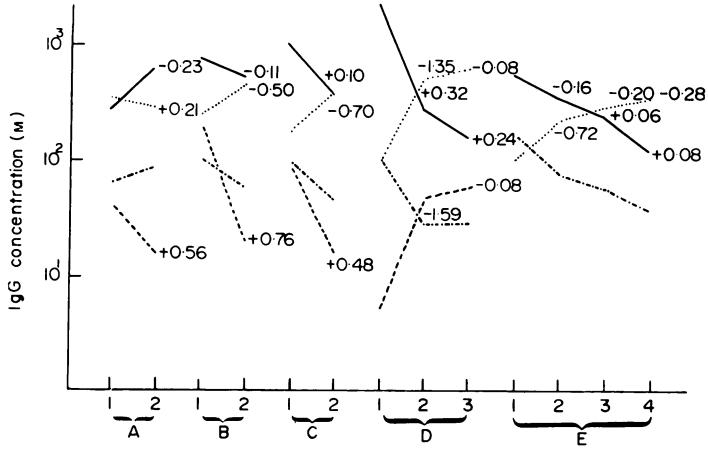

FIG. 1. Relation of IgG concentration, indicated by thick lines ($\times 10^4$ of standard) with concentrations of (———) acid phosphatase (prostate, $\times 10^3$ iu), (. . . .) fructose (seminal vesicle, mg/ml) and (- - -) sperm (epididymis + testis, $\times 10^6$/ml) in fractions of split ejaculates. Lines connect the logarithmically plotted concentrations of the compartment parameters and IgG in the sequential fractions of the five split ejaculates (Table 1). The differences of the log ratios of IgG with those of the parameters are indicated. A hypothetical similar distribution of IgG over two fractions with a parameter is thus indicated by parallel lines and the figure 0.

The accuracy of the different techniques was not estimated, but it has to be expected that due to the complexity of the calculations the standard deviation of these end-figures might be rather large. In spite of this lack of information the results show clearly that IgG is divided over the split fractions of the donors B, C, D and E more or less in the same way as acid phosphatase. The changes of IgG are highly dissimilar compared to those of fructose

TABLE 1. Concentrations of acid phosphatase, fructose, spermatozoa and IgG in fractions of five split ejaculates. A, B, C and D are normal donors, E is a patient with strong auto-agglutination of his spermatozoa (spermatozoa therefore not counted). To compare the distributions of IgG over two sequential fractions with the distribution of the parameter, the log of the ratios of the concentrations are given and also the difference of log ratio IgG with each of the log ratios of the parameters (see also Fig. 1)

	Donor A		Donor B		Donor C		Donor D			Donor E			
Split fractions	I	II	I	II	I	II	I	II	III	I	II	III	IV
Volume (ml)	1·0	0·6	2·5	1·6	2·0	3·8	0·6	1·2	0·4	1·0	1·1	0·5	0·9
Acid phosphatase ($\times 10^3$ iu/l)	264	616	760	566	1020	368	2280	276	160	549	350	242	125
Log ratio	−0·3680		+0.1280		+0·4428		+0·9170		+0·2368	+0·1955		+0·1603	+0·2869
Log ratio—log ratio IgG	−0·23		−0·11		+0·10		+0·32		+0·24	−0·16		+0·06	+0·08
Fructose (mg per 100 ml)	340	285	245	450	175	400	90	510	615	100	230	290	345
Log ratio	+0·0767		−0·2640		−0·3591		−0·7534		−0·0813	−0·3617		−0·1007	−0·0754
Log ratio—log ratio IgG	+0·21		−0·50		−0·70		−1·35		−0·08	−0·72		−0·20	−0·28
Spermatozoa ($\times 10^6$ per ml)	40	15	200	20	100	15	5	50	60				
Log ratio	+0·4260		+1·0000		+0·8239		−1·0000		−0·0792				
Log ratio—log ratio IgG	+0·56		+0·76		+0·48		−1·59		−0·08				
IgG ($\times 10^{-4}$ of standard)	61	63	99	57	99	45	110	28	28	168	74	59	37
Log ratio	−0·1338		+0·2397		+0·3424		+0·5942		0·0000	+0·3561		+0·0983	+0·2027

spermatozoa concentrations. Only in donor A the IgG ratio lies between those of acid phosphatase and fructose. These results strongly suggest that the bulk of IgG in seminal plasma is derived from the same source that supplies acid phosphatase, and indicate that IgG enters the seminal fluid mainly via the prostate.

It was also attempted to determine IgM in these fractions. In none of the cases, however, could IgM be detected.

Second experiment

The split ejaculates, each in two fractions, of eight normal donors were investigated. In this experiment not only IgG, but also IgA, albumin and lactoferrin were investigated and again acid phosphatase, fructose and spermatozoa concentrations were used as parameters for the different compartments of seminal fluid. Albumin was included since this blood plasma protein is not synthesized in the genital organs and its concentration in seminal plasma can therefore only be an expression of the degree of transudation from blood to semen. This cannot be said for certain of IgG since plasma cells in the prostate could presumably be the source of this immunoglobulin in semen.

Lactoferrin was studied because this protein of the secretory fluids, which with the present technique is not detectable in serum, was earlier found in semen to be a product of the seminal vesicles (Hekman & Rümke, 1969).

IgA was estimated because of the possibility that this secretory immunoglobulin could be locally produced in the genital tract. Its relative concentration compared to IgG could therefore be higher than it is in serum. A disadvantage of the method used for the determination of IgA was that no discrimination could be made between secretory and monomeric (free) IgA, since the antiserum used in the radial immunodiffusion test was against free (myeloma) IgA, and also the standard used for the reference curve was serum IgA. According to studies where pure secretory IgA was estimated with the same techniques, the figures found had to be multiplied by approximately 3 to account for the different behaviour of secretory IgA in the antibody-containing agar plates (Tomasi & Bienenstock, 1968; Braendtzaeg, 1970; Munster, 1972).

Since seminal plasma might contain both free and secretory IgA in unknown ratios in the different fractions the real concentrations probably lie between the figure found and 3 times this figure. But since the main purpose of our study was to compare ratios of concentrations in two fractions, the factor was neglected, assuming that the ratio of free and secretory IgA in the two fractions was more or less the same. Keeping in mind that this might be incorrect, the results of the IgA determinations should be taken with a certain reserve.

Also albumin, IgG and IgA were estimated in serum of blood drawn on the same day as the donation of the semen samples. Table 2 gives the results of the quantitative determination together with the volumes of the fractions. IgM could never be detected and is therefore not mentioned in the table. As in the first experiment the ratios of the four proteins are compared to the ratios of the parameter concentrations by calculating the differences between the logarithms of the ratios. These figures of seven donors are given in Table 3. They show as a general trend that most of the albumin, IgA and IgG enter the seminal fluid together with acid phosphatase. It is not excluded that smaller amounts of the proteins are delivered with the other compartments.

The distribution of lactoferrin over the two fractions did not show a clear resemblance to fructose. In some cases it was nearer that of acid phosphatase than fructose.

TABLE 2. Concentrations of acid phosphatase, fructose, spermatozoa, albumin, IgA, IgG and lactoferrin in each of the two fractions of seven split ejaculates of normal donors

Donor	Fraction	Volumes (ml)	Acid phosphatase ($\times 10^4$ iu/l)	Fructose (mmol/l)	Spermatozoa ($\times 10^6$/ml)	Albumin (mg/ml)	IgA* (mg/ml)	IgG (mg/ml)	Lactoferrin (mg/ml)
F									
	I	1·6	83	9·1	140	1·06	0·014	0·180	0·95
	II	1·8	50·4	20·4	22	0·38	0·007	0·100	0·75
G									
	I	1·3	30	7·2	25	0·43	0·017	0·095	0·20
	II	2·1	20·5	13·5	21	0·30	0·013	0·080	0·19
H									
	I	1·1	37	11·8	28	0·76	0·035	0·065	0·88
	II	0·9	27·5	17·5	2	0·30	0·011	0·050	1·05
I									
	I	1·5	20·5	5·1	63	1·00	0·011	0·170	0·58
	II	0·7	10·7	16·8	21	0·48	0·002	0·100	0·82
J									
	I	0·5	16·9	3·1	27	0·76	0·070	0·355	0·55
	II	3·2	7·5	4·3	51	0·59	0·064	0·285	0·70
K									
	I	0·3	44·5	4·9	130	0·44	0·0108	0·070	0·50
	II	0·45	48·0	11·5	36	0·30	0·0093	0·075	0·75
L									
	I	0·9	45	7·1	45	0·27	0·018	0·035	0·29
	II	1·1	10·6	19·6	35	0·21	0·0074	0·025	0·59
M									
	I	0·2	52	—	75	0·92	0·0063	——	0·73
	II	2·2	22·5	14·2	10	0·32	0·0054	0·025	0·88

* Expressed as if semen contained monomeric serum IgA only.
When all semen IgA are in the secretory form these figures should be multiplied by a factor of three.

TABLE 3. The data of Table 2 were used to calculate the log of the ratios of the concentrations of the proteins and the parameters in the two fractions of the seven split ejaculates. The differences of the log ratios of the proteins with each of the parameters are given in the table. If a protein and a parameter are derived from the same source, their distribution over the two fractions should be the same and the log ratio difference should then be zero. The table shows the trend of lower differences between albumin, IgA and IgG log ratios with acid phosphatase log ratios than with the log ratios of the other parameters, indicating that the bulk of these proteins stems from the prostate. The expected relation of lactoferrin with fructose is not found

Donor	Log $\frac{CI}{CII}$ of: Acid phosphatase $-\log \frac{CI}{CII}$ of albumin	Fructose $-\log \frac{CI}{CII}$ of albumin	Spermatozoa $-\log \frac{CI}{CII}$ of albumin
F	−0·23	−0·80	+0·36
G	+0·01	−0·43	−0·08
H	−0·28	−0·57	+0·74
I	−0·04	−0·84	+0·16
J	+0·24	−0·25	−0·39
K	−0·20	−0·54	+0·39
L	+0·52	−0·55	−0·00
Mean (irrespective of sign)	0·22	0·57	0·30

Donor	Log $\frac{CI}{CII}$ of: Acid phosphatase $-\log \frac{CI}{CII}$ of IgA	Fructose $-\log \frac{CI}{CII}$ of IgA	Spermatozoa $-\log \frac{CI}{CII}$ of IgA
F	−0·08	−0·65	+0·50
G	+0·05	−0·39	−0·04
H	−0·38	−0·67	+0·64
I	−0·46	−1·26	−0·27
J	+0·31	−0·18	−0·32
K	−0·10	−0·44	+0·49
L	+0·24	−0·83	−0·28
Mean (irrespective of sign)	0·23	0·63	0·36

Donor	Log $\frac{CI}{CII}$ of: Acid phosphatase $-\log \frac{CI}{CII}$ of IgG	Fructose $-\log \frac{CI}{CII}$ of IgG	Spermatozoa $-\log \frac{CI}{CII}$ of IgG
F	−0·04	−0·61	+0·55
G	+0·09	−0·35	+0·00
H	+0·01	−0·29	+1·03
I	+0·05	−0·75	+0·25
J	+0·26	−0·24	−0·37
K	−0·00	−0·34	+0·59
L	+0·48	−0·59	−0·04
Mean (irrespective of sign)	0·13	0·45	0·40

Donor	Log $\frac{CI}{CII}$ of: Acid phosphatase $-\log \frac{CI}{CII}$ of lactoferrin	Fructose $-\log \frac{CI}{CII}$ of lactoferrin	Spermatozoa $-\log \frac{CI}{CII}$ of lactoferrin
F	+0·12	−0·45	+0·70
G	+0·14	−0·29	+0·05
H	+0·20	−0·09	+1·22
I	+0·43	−0·37	+0·63
J	+0·46	−0·04	−0·17
K	+0·14	−0·19	+0·73
L	+0·94	−0·03	+0·42
Mean (irrespective of sign)	0·35	0·21	0·56

TABLE 4. Concentrations of albumin, IgA and IgG in serum and semen of seven normal donors as calculated from the data of Table 2. The volumes of serum containing the same amount of the protein as in the total ejaculate express the degree of the hypothetical transudation from blood to semen. The equivalent volumes of albumin and IgG are correlated (Spearman's rank correlation test, $P = 0{\cdot}04$), those of albumin and IgA are not correlated ($P > 0{\cdot}5$), suggesting that IgG like albumin enters the seminal fluid by transudation, whereas IgA is likely to be also produced locally

Donor	Concentration (mg/ml) in blood serum			Concentration (mg/ml) in seminal plasma (calculated from Table 2)			Concentration in seminal plasma expressed as percentage of the serum concentration			Amount (μl) of serum equivalent to total amount in seminal plasma		
	Albumin	IgA	IgG	Albumin	IgA*	IgG	Albumin	IgA*	IgG	Albumin	IgA*	IgG
F	33·9	1·72	17·3	0·7	0·01–0·03	0·14	2·1	0·6–1·8	0·8	70	20–61	27
G	33·5	3·54	10·2	0·35	0·02–0·05	0·09	1·1	0·4–1·3	0·8	36	14–41	29
H	43·5	3·22	10·5	0·55	0·02–0·07	0·06	1·3	0·8–2·3	0·6	25	15–45	11
I	40·5	1·68	15·2	0·84	0·01–0·03	0·15	2·1	0·5–1·5	0·9	45	11–32	21
J	36·8	2·32	12·2	0·61	0·06–0·19	0·29	1·7	2·8–8·4	2·4	62	103–310	89
K	38·5	2·24	15·0	0·36	0·01–0·03	0·07	0·9	0·4–1·3	0·5	69	3–10	24
L	37·2	0·91	8·7	0·24	0·01–0·04	0·03	0·6	1·3–4·0	0·3	13	26–80	7
M	38·0	0·50	7·7	0·37	0·01–0·02	(0·06)†	1·0	1·1–3·3	(0·7)†	23	11–33	(8–9)†

* The range covers the possibilities of IgA being only in the serum monomeric form to IgA being only in secretory form.
† Estimated values.

Transudation of albumin and immunoglobulin from blood to seminal plasma

Serum albumin, IgA and IgG were determined in order to obtain an impression of the degree of transudation of albumin and the maximal possible degree of transudation of IgA and IgG. The degree to which the immunoglobulins transudate, especially IgA, cannot be determined since the presence in seminal plasma can also be due to local production. Moreover, as mentioned earlier, the IgA levels in seminal plasma could not be determined exactly since the ratio of secretory IgA to free IgA was an unknown factor. Nevertheless the figures allow a calculation of the maximal possible hypothetical transudation.

Table 4 shows the concentrations in serum, the concentrations in seminal plasma as calculated from the data of Table 2, the concentrations in seminal plasma expressed as the percentage of the serum concentration, and the volumes in microlitres of serum possessing the same amount as present in the seminal plasma. The figures for IgA show the ranges of possibilities, the first figure in case IgA was present only in free form, the latter if all IgA was in the secretory form, in which case the concentration was multiplied by 3.

It is clearly shown that the levels of the three proteins are relatively low in seminal plasma, compared to serum, in the order of 1% for IgG, a little higher for albumin and probably still a bit higher for IgA. The volumes of serum containing the same amount of the particular protein as present in the total ejaculate quantitate the degree in which these proteins hypothetically transudate from blood to semen. The volumes for IgG are significantly correlated with those for albumin, however the volumes for IgA are not correlated with those for albumin (Spearman's rank correlation test, P respectively 0·04 and $>0·50$).

DISCUSSION

The distribution of IgG over the different fractions of the split ejaculates in the first and second experiment most closely resembles the distribution of acid phosphatase. The distributions are compared with each other by calculating the differences between the logarithms of the ratios of the concentrations in two sequential fractions. As shown in Tables 1 and 3 they are in general closer to zero when IgG is compared to acid phosphatase than to fructose or spermatozoa concentrations.

Fig. 1 visualizes the parallelism of IgG distribution to that of acid phosphatase. The distribution of albumin and of IgA in the second experiment also shows in general a greater resemblance to acid phosphatase than to the other parameters (Table 3). These findings indicate that generally the bulk of these proteins enters the seminal fluid via the prostate. Albumin is for obvious reasons not produced locally in the prostate, but it transudates; its gradient can therefore be used as a parameter for transudation of plasma proteins from blood to semen. When concentrations of albumin and IgG are compared in semen and serum, it is shown that in most cases the degree of transudation of albumin is a little higher than that of IgG (Table 4), probably due to the differences in mol. wt. Moreover the degree of transudation of albumin correlated significantly with the hypothetical transudation of IgG. It is therefore likely that IgG does enter the seminal fluid by transudation from the blood.

The determinations of IgA were hampered by the fact that the method used was insufficient to distinguish between secretory and monomeric (serum) IgA. If a factor of 3 is used to multiply the IgA levels found in seminal plasma in order to correct for the possibility that all IgA is in the secretory form, the levels in seminal plasma, though higher than those of IgG and albumin, are still relatively low. Since the serum volume equivalents for IgA

were likely to be higher than those of albumin, and since they were also not correlated with those for albumin, it can be concluded that, apart from transudation, local production of IgA may also occur, although in relatively low amounts.

One donor (J) had an IgG serum volume equivalent higher than that of albumin. Since he also had an IgA concentration higher than the other donors, it might tentatively be concluded that this donor had an insipient focal infection giving rise to both local IgA and IgG antibody production.

Lactoferrin is a protein produced locally. The levels in serum are too low (in the order of 1 μg/ml according to Rümke *et al.* (1971)) to be of importance for contribution by transudation. Hekman & Rümke (1969) found lactoferrin to be a product of the seminal vesicles. Hekman could not detect any lactoferrin in three ejaculates of men with congenital absence of the vasa deferentia and the vesicles, whose ejaculates consist of prostate fluid only (personal communication). The present findings show that only in three cases lactoferrin runs parallel with fructose, indicating that the vesicles were the source. In the other four cases there was as much indication that lactoferrin originates from other sources as from the vesicles.

If the prostate is excluded, it should be considered that such smaller accessory glands as the ampullar gland, Cowper's bulbo-urethral and Littré's urethral glands deal in the production of lactoferrin. These organs are likely to be absent in men with congenital absence of the vasa deferentia and the vesicles, since this syndrome is due to a congenital defect of the development of the Wolffian ducts, therefore, the previously mentioned observation that ejaculates of men with this syndrome lack lactoferrin, does not exclude the possibility that one of these organs produces lactoferrin, which would then imply that lactoferrin does not run parallel with fructose. Another explanation for the lack of parallelism with fructose could be that the production of lactoferrin and fructose in the vesicles is unequally divided in the time course, that the vesicles discharge their contents and that thus in the split fractions the distribution might be disproportional. It should be considered that lactoferrin sticks easily to albumin (Hekman, 1971) and that therefore its determination might be disturbed. Also it adheres to membranes, for instance to spermatozoa, where lactoferrin was found to be an important sperm-coating antigen (Hekman & Rümke, 1969). It might therefore be that lactoferrin is partly 'lost' to cells, though the figures do not indicate that adsorption to spermatozoa could be held responsible for the discrepancy between the lactoferrin and fructose distribution. Since granulocytes contain lactoferrin it has also to be considered that lactoferrin partly stems from sites with local inflammations.

ACKNOWLEDGMENTS

The author gratefully acknowledges Nanda van Amstel, Dick Visser, Margreet Luning and Henny Buter for skilful technical assistance, Dr K. Kok (University of Amsterdam) for carrying out the fructose determinations, Mr A. A. M. Hart for statistical help and Dr Annemarie Hekman for fruitful discussions.

REFERENCES

ABLIN, R.J., GONDER, M.J. & SOANES, W.A. (1971) Localization of immunoglobulins in human prostatic tissue. *J. Immunol.* **107,** 603.

BESSEY, O.A., LOWRY, O.H. & BROCK, M.J. (1946). A method for the rapid determination of alkaline phosphatase with five cubic millimeters of serum. *J. biol. Chem.* **164,** 321.

BRAENDTZAEG, P. (1970) Unfolding of human secretory immunoglobulin A. *Immunochemistry*, **7**, 127.

COOMBS, R.R.A., RÜMKE, P. & EDWARDS, R.G. (1973) Immunoglobulin classes reactive with spermatozoa in the serum and seminal plasma of vasectomized and infertile men. *Immunology of Reproduction* (Ed. by K. Bratanov), p. 354. Sofia, Bulgarian Academy of Science.

ESTBORN, B. & SWEDIN, B. (1959) Localisation of acid phosphatase by starch-gel electrophoresis of fractionally collected human semen. *Scand. J. clin. Lab. Invest.* **11**, 235.

FJÄLLBRANT, B. (1968) Interrelation between high levels of sperm antibodies, reduced cervical mucus penetration by spermatozoa, and sterility in men. *Acta Obstet. Gynec. scand.* **47**, 102.

HEKMAN, A. (1971) Association of lactoferrin with other proteins, as demonstrated by changes in electrophoretic mobility. *Biochim. biophys. Acta*, **251**, 380.

HEKMAN, A. & RÜMKE, P. (1969) The antigens of human seminal plasma; with special reference to lactoferrin as a spermatozoa-coating antigen. *Fertil. Steril.* **20**, 312.

HERRMANN, W.P. (1969) Immunologische Charakterisierung menschlicher Spermaplasmaproteine. *Andrologie*, **1**, 11.

KARWONEN, M.J. & MALM, M. (1955) Colorimetric determination of fructose with indol. *Scand. J. clin. Lab. Invest.* **7**, 305.

MANCINI, G., CARBONARA, C.O. & HEREMANS, J.F. (1964) Immunochemical quantitation of antigens by single radial immunodiffusion. *Immunochemistry*, **2**, 235.

MANN, T. (1964) *The Biochemistry of Semen and of the Male Reproductive Tract.* Methuen and Company Ltd, London.

MUNSTER, P.J.J. VAN (1972) *De secretoire component.* Thesis, University of Nijmegen.

RÜMKE, P. & HELLINGA, G. (1959) Autoantibodies against spermatozoa in sterile men. *Amer. J. clin. Path.* **32**, 357.

RÜMKE, P., VISSER, D., KWA, H.G. & HART, A.A.M. (1971) Radio-immuno assay of lactoferrin in blood plasma of breast cancer patients, lactating and normal women; prevention of false high levels caused by leakage from neutrophile leucocytes in vitro. *Folia Med. Neerl.* **14**, 156.

TOMASI, T.B. JR & BIENENSTOCK, J. (1968) Secretory immunoglobulins *Advanc. Immunol.* **9**, 1.

UEHLING, D.T. (1971) Secretory IgA in seminal fluid. *Fertil. Steril.* **22**, 769.

WILSON, L. (1954) Spermagglutinins in human semen and blood. *Proc. Soc. exp. Biol. (N.Y.)*, **85**, 652.