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COMPONENTS OF HUMAN SPLIT EJACULATES

I. SPERMATOZOA, FRUCTOSE, IMMUNOGLOBULINS, ALBUMIN, LACTOFERRIN, TRANSFERRIN AND OTHER PLASMA PROTEINS

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Summary. The concentrations of spermatozoa, fructose, IgG, IgA, albumin, lactoferrin, transferrin, secretory piece of IgA, β_1C/β_1A-globulin (C'3-component of complement), ceruloplasmin and fibrinogen were evaluated in human split ejaculates and/or in whole human seminal plasma. The concentrations of spermatozoa, IgG, IgA, albumin and transferrin decreased from the first portion of the split ejaculate to the last, indicating that these proteins originate mostly from secretions other than the seminal vesicles. By contrast, the highest amounts of fructose and lactoferrin were present in the final portion of the split ejaculates, showing their seminal vesicle origin. No secretory piece, IgM, β_1C/β_1A-globulin, ceruloplasmin or fibrinogen could be detected in human semen. An unidentified antigen was found that has a relatively high molecular weight and shows β_1-mobility on immunoelectrophoresis.

INTRODUCTION

During ejaculation, the secretions of the human reproductive tract are released in a sequential manner (MacLeod & Hotchkiss, 1942; Lundquist, 1949; Farris, 1950; Mann, 1964; Amelar & Hotchkiss, 1965). The first portion of the ejaculate contains mainly spermatozoa and fluids originating from the Cowper's and prostate glands, whereas the final portion consists mostly of seminal vesicle fluid. Ejaculates collected in several portions are called 'split' (or 'partitioned') ejaculates (MacLeod & Hotchkiss, 1942; Lundquist, 1949; Harvey, 1956). Studies on the distribution patterns of certain components in split ejaculates provide information on the origin of semen constituents in the male reproductive tract. Fructose, which is produced almost solely by the seminal vesicles, is found

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in highest concentration in the final portions of the split ejaculate. Citric acid, which originates from the prostate gland, is found in highest concentration in the first portion (MacLeod & Hotchkiss, 1942; Lundquist, 1949; Amelar & Hotchkiss, 1963; Mann, 1964; Molnar, Biro & Berenyi, 1971). Recent studies in our laboratory revealed that the initial portion of the split ejaculate either did not coagulate, or liquefied immediately after coagulation. The final portion usually coagulated and lysis took place rather slowly (Tauber, Propping, Zaneveld & Schumacher, 1973). Addition of the first portion of the split ejaculate to the last portion increased the speed of liquefaction significantly, indicating that the first portion contained a lytic factor (possibly an enzyme) in higher amounts as compared to the final portion of the split ejaculate. Ultrastructural studies on the human seminal plasma coagulum have shown that the third fraction consists of a tight network of small fibres that are subject to the liquefaction process (Zaneveld, Tauber, Port, Propping & Schumacher, 1974).

It was therefore of interest to elaborate baseline information on the distribution pattern of proteins, enzymes and inhibitors in human split ejaculates. In this communication, the results of studies on non-enzymatic components are presented.

MATERIAL AND METHODS

Split ejaculates were obtained from ten healthy donors aged from 22 to 34 years, after 2 to 3 days of sexual abstinence. Two of the donors were of proven fertility. Owing to the volume requirements for these studies, the donors were asked to split their ejaculates only into three portions. The donors each received three jars and were advised to place the discharged material of the first two orgasmic contractions into the first jar (split Fraction I). The subsequent two contractions were to be deposited into the second jar (split Fraction II) and the material released during the final contractions into the third container (split Fraction III). Following liquefaction, the volumes of the samples were measured with a calibrated pipette and sperm counts were determined with a haemocytometer. The specimens were centrifuged at 1100 ***g*** for 10 min to separate the seminal plasma from the spermatozoa. The supernatant plasma was stored at −20°C in aliquots of 0·1 to 0·2 ml that were used as needed.

The following techniques were employed to determine the concentrations of non-enzymatic components.

Fructose

Colorimetric measurements of fructose were carried out according to the method described by Mann (1964) after deproteinization with $ZnSO_4$ and 0·3 N-$Ba(OH)_2$, and addition of the 0·1% resorcinol-ethanol/30% HCl reagent. The tests were carried out with 20 μl seminal plasma. The required agents were added to the test system in proportional amounts, giving a final dilution of the seminal plasma sample of 1:40. Pure fructose (D-laevulose: DIFCO) was used for the preparation of the standard solutions.

Other tests

Measurements of IgG, IgA, the secretory piece of IgA, albumin, transferrin,

and lactoferrin were achieved by using the radial immunodiffusion technique (Mancini, Carbonara & Heremans, 1965). The tests were performed with the LKB microimmunodiffusion equipment (Gelman Instruments, Ann Arbor, Michigan) as previously described in detail by Schumacher (1968) and Schill & Schumacher (1973). Monospecific antisera were incorporated on a 5 to 6% v/v basis in 1% agarose (Behring Diagnostics, Somerville, N.Y.) containing 1/15 M-phosphate buffer, pH 7·0, and 0·01% Merthiolate (Eli Lilly, Indianapolis, Ind.). For each side of an LKB frame holding three slides, 11·5 ml melted solution were used. Wells of 1·5 mm diameter were punched into the gel layer and the plugs were removed by a small suction needle, carefully preserving the shape of the well. The wells were filled with drawn-out disposable capillary pipettes so that the surface of the fluid was even with the surface of the gel, showing just the beginning of a slight convexity. The exact amount deposited in this fashion was 2 μl. This method applied by an experienced person is accurate and could not be improved by depositing the samples by a microlitre Hamilton syringe (see also Schill & Schumacher, 1972).

After 2 to 4 days of diffusion in a moist chamber, the diameters of the rings were measured and the concentrations of the antigen were calculated from reference curves obtained with standard solutions. The measurements were repeated after washing (2 days in physiological saline, 1 day in distilled water) and staining with 0·5% amidoblack/methanol-acetic acid solution of the dry slides. The mean values of both measurements were used for evaluation.

Monospecific rabbit antisera against human IgG, IgA, transferrin, albumin and secretory piece (IgA free) were purchased from Behring Diagnostics, Somerville, New Jersey. Human lactoferrin rabbit antiserum was obtained from Nordic Pharmaceuticals (P.O.B. 22, Tilburg, The Netherlands). A standardized and stabilized human serum preparation (Behring Diagnostics) with known antigen concentrations was used as standard. Since purified human lactoferrin was initially unavailable, dilutions of a 2·5-fold concentrated seminal plasma pool served as reference. For this preparation, 50 ml seminal plasma from fifteen healthy donors were dialysed against distilled water for 24 hr, then freeze-dried and redissolved in 20 ml physiological saline. When a highly purified human lactoferrin preparation was obtained, the lactoferrin concentrations of the concentrated seminal plasma pool and its dilutions were determined and the amounts of lactoferrin in the split ejaculates were computed accordingly.

Testing for the presence of fibrinogen, β_1C/β_1A-globulin (C'3-component of complement) and ceruloplasmin was done by the double immunodiffusion technique. Normal serum and plasma served as controls.

It should be noted that before testing the split ejaculates, ten to twelve whole seminal plasma samples were first evaluated for the presence of these components. No further experiments were performed with a component if it was absent from whole seminal plasma. Each determination was performed twice.

Computations and statistical evaluations

For each component, three criteria were recorded (Tables 1 to 8): (1) the measured concentrations ('conc.'), (2) the amount computed from the volume

('amount'), and (3) the percentages that these amounts represented if all three fractions were combined ('% of total'). The tabulated values represent averages of the data obtained for all tested split ejaculates from a particular donor. The number of samples evaluated is marked on the left-hand side of each column. Due to the varied volumes of the split ejaculate fractions, their use for other purposes, or for validation of results, the number of samples from a particular donor was not always the same from fraction to fraction, or from criterion to criterion. The recorded amounts were computed from the actual concentrations and volumes of the split fractions that were tested. These volumes obviously differed at times from those recorded in Table 1 since the latter represent all of the split ejaculate fractions collected, whereas the former represent only the number of fractions tested for a certain component. The actual volumes used may be obtained by dividing the concentrations by the amounts. For simplicity, they were not recorded separately.

The amount in the whole ejaculate ('calc. amount') was calculated by adding the amounts obtained in each fraction. The concentration in the whole ejaculate ('calc. conc.') was obtained by dividing the 'calc. amount' by the sum of the volumes of each fraction. The overall percentages were calculated from the mean amounts and not by averaging the % values of each column. The overall mean concentrations of a certain criterion were subjected to the *t* test in order to determine if significant differences ($P<0{\cdot}05$) were present between the fractions.

Table 1. Volume measurements of human split ejaculates*

	Fraction I			*Fraction II*			*Fraction III*			*Calculated total vol./whole ejaculate (ml)*
Donor	*No. of samples evaluated*	*Vol. (ml)*	*% Total vol.*	*No. of samples evaluated*	*Vol. (ml)*	*% Total vol.*	*No. of samples evaluated*	*Vol. (ml)*	*% Total vol.*	
1	5	0·61	27	5	0·90	40	5	0·76	33	2·27
2	5	1·24	26	5	2·24	47	5	1·28	27	4·76
3	4	0·48	24	4	0·53	26	4	1·03	50	2·04
4	3	0·40	17	3	0·57	24	3	1·37	59	2·34
5	3	1·45	51	2	0·50	18	3	0·87	31	2·82
6	3	0·67	32	3	0·83	39	3	0·60	29	2·10
7	10	0·43	19	10	1·03	46	9	0·77	35	2·23
8	3	0·53	22	3	1·00	42	3	0·87	36	2·40
9	3	0·38	12	3	0·60	18	3	2·27	70	3·25
10	2	0·18	4	3	0·77	17	3	3·67	79	4·62
Mean		0·64	22		0·90	31		1·35	47	2·89
S.D.		0·39			0·50			0·90		1·01

* See text for experimental detail and computations.

RESULTS

Volumes

The mean$\pm$S.D. of the computed total volume per ejaculate in ml was $2{\cdot}89\pm1{\cdot}01$, range 2·04 to 4·76 ml (Table 1). An increase of the mean volume from Fraction I to Fraction III was found. The difference between I and III was statistically significant ($P = 0{\cdot}025$). Four of the donors (Nos 3, 4, 9 and 10)

Table 2. Sperm concentrations and total number of spermatozoa*

Donor	*Fraction I*				*Fraction II*				*Fraction III*				*Whole ejaculate*	
	No. of samples evaluated	*Conc.* ($\times 10^6$/*ml*)	*Amount* ($\times 10^6$)	*% of total*	*No. of samples evaluated*	*Conc.* ($\times 10^6$/*ml*)	*Amount* ($\times 10^6$)	*% of total*	*No. of samples evaluated*	*Conc.* ($\times 10^6$/*ml*)	*Amount* ($\times 10^6$)	*% of total*	*Calc. amount* ($\times 10^6$)	*Calc. conc.* ($\times 10^6$/*ml*)
1	4	86·25	55·20	60	5	33·10	29·79	31	5	11·08	8·42	9	93·41	40·61
2	5	188·60	233·86	75	5	24·84	55·64	18	5	17·12	21·91	7	311·41	65·42
3	4	316·25	151·80	42	4	312·25	165·49	45	4	44·75	46·09	13	363·38	178·13
4	3	102·00	40·80	27	3	150·00	85·50	57	3	17·10	23·43	16	149·73	63·99
5	3	131·00	86·46	41	2	139·00	69·50	33	3	63·83	55·53	26	211·49	104·18
6	3	641·33	423·28	76	3	144·83	120·21	22	3	43·66	13·10	2	556·59	310·94
7	10	233·30	100·32	55	9	68·60	70·66	39	7	14·07	9·99	6	180·97	83·40
8	3	184·67	97·88	33	3	143·33	143·33	49	3	62·00	53·94	18	295·15	122·98
9	3	93·33	35·47	33	3	62·00	37·20	35	3	14·67	33·30	32	105·97	32·61
10	2	57·50	10·35	2	3	271·00	208·67	42	3	77·30	282·92	56	501·94	108·88
Mean		203·42	123·54	45		134·90	98·60	35		36·56	54·86	20	277·00	111·11
S.D.		172·77				95·79				24·85			160·00	82·21

* See text for experimental detail and computations.

Table 3. Fructose levels in human split ejaculates*

	Fraction I				*Fraction II*				*Fraction III*				*Whole ejaculate*	
Donor	*No. of samples evaluated*	*Conc. (mg/ml)*	*Amount (mg)*	*% of total*	*No. of samples evaluated*	*Conc. (mg/ml)*	*Amount (mg)*	*% of total*	*No. of samples evaluated*	*Conc. (mg/ml)*	*Amount (mg)*	*% of total*	*Calc. amount (mg)*	*Calc conc. (mg/ml)*
1	3	0·84	0·52	10	3	2·34	2·50	46	3	2·66	2·39	44	5·41	2·09
2	2	3·39	4·24	18	3	4·69	10·46	44	3	7·06	8·97	38	23·67	4·98
3	2	2·08	1·35	66	4	0·21	0·11	5	4	0·58	0·59	29	2·05	0·93
4	1	0·64	0·38	4	3	2·26	1·29	14	3	5·35	7·33	82	9·00	3·54
5	2	0·52	0·47	27	1	0·46	0·37	21	3	1·02	0·89	52	1·73	0·67
6	3	1·19	0·79	11	3	5·30	4·17	60	2	4·90	1·96	29	6·92	3·66
7	9	0·82	0·36	3	8	4·04	3·92	37	5	7·96	6·37	60	10·65	4·82
8	3	1·69	0·90	10	3	4·09	4·09	44	3	5·03	4·34	46	9·33	3·89
9	3	1·22	0·47	5	3	1·51	0·91	10	3	3·58	8·13	85	9·50	2·92
10	1	0·84	0·25	2	3	0·94	0·72	6	3	3·25	11·90	92	12·87	2·72
Mean		1·32	0·97	11		2·56	2·85	31		4·14	5·29	58	9·11	3·02
S.D.		0·87				1·79				2·40			6·25	1·47

* See text for experimental detail and computations.

Table 4. Levels of IgG in human split ejaculates*

Donor	*Fraction I*				*Fraction II*				*Fraction III*				*Whole ejaculate*	
	No. of samples evaluated	*Conc. (mg/ml)*	*Amount (mg)*	*% of total*	*No. of samples evaluated*	*Conc. (mg/ml)*	*Amount (mg)*	*% of total*	*No. of samples evaluated*	*Conc. (mg/ml)*	*Amount (mg)*	*% of total*	*Calc. amount (mg)*	*Calc. conc. (mg/ml)*
1	3	0·11	0·07	35	4	0·07	0·07	35	4	0·07	0·06	30	0·20	0·08
2	3	0·13	0·17	41	4	0·07	0·16	39	4	0·06	0·08	20	0·41	0·08
3	3	0·18	0·10	37	4	0·15	0·08	30	4	0·09	0·09	33	0·27	0·13
4	1	0·12	0·07	39	3	0·06	0·03	17	3	0·06	0·08	44	0·18	0·07
5	2	0·10	0·09	50	1	0·07	0·06	33	3	0·04	0·03	17	0·18	0·07
6	3	0·09	0·06	40	3	0·07	0·06	40	2	0·08	0·03	20	0·15	0·08
7	9	0·23	0·10	37	8	0·11	0·11	41	7	0·09	0·06	22	0·27	0·13
8	3	0·15	0·08	26	3	0·13	0·13	42	3	0·11	0·10	32	0·31	0·13
9	3	0·12	0·05	20	3	0·07	0·04	16	3	0·07	0·16	64	0·25	0·08
10	1	0·18	0·05	16	3	0·11	0·08	26	3	0·05	0·18	58	0·31	0·07
Mean		0·14	0·08	32		0·09	0·08	32		0·07	0·09	36	0·25	0·09
S.D.		0·04				0·03				0·02			0·08	0·03

* See text for experimental detail and computations.

exhibited the same trend of increasing volume in the fractions, whereas five donors (Nos 1, 2, 6, 7 and 8) delivered the highest volume with the second fraction. Donor 5 ejaculated the highest volume with the first fraction.

Spermatozoa

The computed mean ± S.D. value for the sperm count of the whole ejaculate $\times 10^6$/ml was 111·11 ± 82·21, range 32·61 to 310·94 $\times 10^6$/ml (Table 2). Both the count/ml and the total number of spermatozoa decreased from Fraction I to III with statistically significant differences between Fractions I and III ($P = 0{\cdot}005$), and between Fractions II and III ($P = 0{\cdot}005$). The decreasing trend in the number of spermatozoa/ml ejaculate was seen in seven donors. Three donors (Nos 4, 5 and 9) delivered the main number of spermatozoa in the second fraction.

Fructose

The computed mean ± S.D. concentration of fructose in the whole ejaculate in mg/ml was 3·02 ± 1·47, range 0·67 to 4·98 (Table 3). Fructose concentrations and amounts increased from Fraction I to Fraction III. Statistically significant differences existed between Fractions I and II ($P = 0{\cdot}05$) and between Fractions I and III ($P = 0{\cdot}0025$). Seven donors (Nos 1, 2, 4 and 7 to 10) showed the increasing pattern from Fractions I to III. Donor 3 exhibited the highest concentration in Fraction I, Donor 6 in Fraction II. Donor 5 possessed the highest concentration of fructose in Fraction III and the lowest in Fraction II. The total concentration of fructose per ejaculate for two donors (Nos 3 and 5) was much lower than for the others.

Immunoglobulins IgG and IgA

The computed mean ± S.D. concentrations in the whole ejaculate in mg/ml were as follows: IgG, 0·09 ± 0·03, range 0·07 to 0·13 mg/ml (Table 4); IgA, 0·03 ± 0·01, range 0·02 to 0·06 (Table 5). The total concentration of immunoglobulin (IgG and IgA combined) in mg/ml was 0·19 for Fraction I, 0·12 for Fraction II, 0·10 for Fraction III, and 0·12 for the whole ejaculate.

All the donors showed higher IgG and IgA levels in Fraction I than in Fraction III. For both immunoglobulins, the differences in mean concentration between Fractions I and II (IgG, $P = 0{\cdot}005$; IgA, $P = 0{\cdot}025$) and between Fractions I and III (IgG, $P < 0{\cdot}0005$; IgA, $P = 0{\cdot}005$) were statistically significant. Such distinct variations were not observed between the actual amounts of the immunoglobulins in each fraction, since the volume of the ejaculates increased from Fraction I to III. The IgG/IgA ratio was virtually identical in all three fractions (Fraction I, 2·8:1; Fraction II, 2·0:1; Fraction III, 2·33:1). In approximately 15% of the samples, IgA was not detectable at all.

Transferrin and albumin

The computed mean ± S.D. concentration of transferrin in the whole ejaculate in mg/ml was 0·07 ± 0·05, range 0·02 to 0·19 (Table 6). The levels of albumin were much higher (Table 7), the computed mean concentration in the whole

Table 5. Levels of IgA in human split ejaculates*

Donor	*Fraction I*				*Fraction II*				*Fraction III*				*Whole ejaculate*	
	No. of samples evaluated	*Conc. (mg/ml)*	*Amount (mg)*	*% of total*	*No. of samples evaluated*	*Conc. (mg/ml)*	*Amount (mg)*	*% of total*	*No. of samples evaluated*	*Conc. (mg/ml)*	*Amount (mg)*	*% of total*	*Calc. amount (mg)*	*Calc. conc. (mg/ml)*
1	3	0·03	0·02	25	4	0·02	0·02	25	3	0·02	0·04	50	0·08	0·02
2	3	0·03	0·04	31	3	0·02	0·06	46	3	0·02	0·03	23	0·13	0·02
3	3	0·08	0·05	42	4	0·06	0·03	25	4	0·04	0·04	33	0·12	0·06
4	1	0·04	0·02	29	2	0·03	0·02	28	2	0·02	0·03	43	0·07	0·03
5	2	0·04	0·04	50	1	0·02	0·02	25	3	0·02	0·02	25	0·08	0·03
6	3	0·07	0·05	56	3	0·04	0·03	33	1	0·03	0·01	11	0·09	0·05
7	9	0·12	0·05	46	8	0·04	0·04	36	6	0·03	0·02	18	0·11	0·05
8	3	0·04	0·02	18	3	0·03	0·03	27	1	0·03	0·06	55	0·11	0·03
9	1	0·03	0·01	17	1	0·02	0·01	17	2	0·02	0·04	66	0·06	0·02
10	1	0·05	0·02	20	3	0·03	0·02	20	1	0·02	0·06	60	0·10	0·02
Mean		0·05	0·03	30		0·03	0·03	30		0·03	0·04	40	0·10	0·03
S.D.		0·03				0·01				0·01			0·02	0·01

* See text for experimental details and computations.

Table 6. Transferrin levels in human split ejaculates*

Donor	*Fraction I*				*Fraction II*				*Fraction III*				*Whole ejaculate*	
	No. of samples evaluated	*Conc. (mg/ml)*	*Amount (mg)*	*% of total*	*No. of samples evaluated*	*Conc. (mg/ml)*	*Amount (mg)*	*% of total*	*No. of samples evaluated*	*Conc. (mg/ml)*	*Amount (mg)*	*% of total*	*Calc. amount (mg)*	*Calc. conc. (mg/ml)*
1	3	0·05	0·03	38	4	0·03	0·03	37	4	0·02	0·02	25	0·08	0·03
2	3	0·13	0·17	19	4	0·21	0·46	52	4	0·23	0·25	29	0·88	0·19
3	3	0·20	0·11	38	3	0·20	0·11	38	4	0·07	0·07	24	0·29	0·13
4	1	0·05	0·03	38	3	0·02	0·01	12	3	0·02	0·04	50	0·08	0·03
5	2	0·13	0·12	57	1	0·09	0·07	33	3	0·02	0·02	10	0·21	0·08
6	3	0·02	0·01	25	3	0·03	0·02	50	2	0·03	0·01	25	0·04	0·02
7	8	0·10	0·05	50	8	0·04	0·04	40	7	0·02	0·01	10	0·10	0·05
8	3	0·10	0·05	26	3	0·08	0·08	42	3	0·07	0·06	32	0·19	0·08
9	3	0·12	0·05	36	3	0·04	0·02	14	3	0·03	0·07	50	0·14	0·04
10	1	0·12	0·04	27	3	0·09	0·07	46	3	0·01	0·04	27	0·15	0·03
Mean		0·10	0·07	32		0·08	0·09	41		0·05	0·06	27	0·22	0·07
S.D.		0·05				0·07				0·065			0·24	0·05

* See text for experimental procedure and computations.

Table 7. Albumin levels in human split ejaculates*

Donor	*Fraction I*				*Fraction II*				*Fraction III*				*Whole ejaculate*	
	No. of samples evaluated	*Conc. (mg/ml)*	*Amount (mg)*	*% of total*	*No. of samples evaluated*	*Conc. (mg/ml)*	*Amount (mg)*	*% of total*	*No. of samples evaluated*	*Conc. (mg/ml)*	*Amount (mg)*	*% of total*	*Calc. amount (mg)*	*Calc. conc. (mg/ml)*
1	3	0·61	0·38	34	4	0·44	0·44	40	4	0·34	0·29	26	1·11	0·47
2	3	1·00	1·33	51	4	0·39	0·85	32	4	0·43	0·45	17	2·63	0·58
3	2	0·91	0·64	33	4	1·41	0·75	39	4	0·52	0·54	28	1·93	0·85
4	1	0·35	0·20	24	2	0·37	0·30	37	3	0·23	0·32	39	0·82	0·30
5	2	0·98	0·88	61	1	0·39	0·31	21	3	0·30	0·26	18	1·45	0·56
6	2	0·34	0·31	38	3	0·41	0·34	42	1	0·53	0·16	20	0·81	0·40
7	7	1·09	0·53	38	8	0·52	0·50	36	6	0·48	0·36	26	1·39	0·63
8	3	0·98	0·52	22	3	1·08	1·08	47	3	0·84	0·73	31	2·33	0·97
9	2	2·24	0·96	31	3	1·52	0·91	30	3	0·52	1·18	39	3·05	0·92
Mean		0·94	0·64	37		0·73	0·61	35		0·47	0·48	28	1·73	0·63
S.D.		0·56				0·47				0·17			0·80	0·23

* See text for experimental procedure and computations. Donor 10 was not tested because his Fraction I was depleted.

Table 8. Lactoferrin levels of human split ejaculates*

Donor	*Fraction I*				*Fraction II*				*Fraction III*				*Whole ejaculate*	
	No. of samples evaluated	*Conc. (mg/ml)*	*Amount (mg)*	*% of total*	*No. of samples evaluated*	*Conc. (mg/ml)*	*Amount (mg)*	*% of total*	*No. of samples evaluated*	*Conc. (mg/ml)*	*Amount (mg)*	*% of total*	*Calc. amount (mg)*	*Calc. conc. (mg/ml)*
1	3	0·48	0·30	15	4	0·91	0·91	45	4	0·93	0·79	40	2·00	1·24
2	3	0·74	0·98	17	4	1·26	2·75	47	4	2·00	2·10	36	5·83	1·28
3	2	0·91	0·64	16	4	1·96	1·04	25	4	2·31	2·38	59	4·06	1·80
4	1	0·33	0·20	8	2	1·02	0·82	33	3	1·07	1·47	59	2·49	0·90
5	2	0·96	0·86	52	1	0·33	0·26	16	3	0·62	0·54	32	1·66	0.65
6	2	0·23	0·21	46	3	0·20	0·17	37	1	0·27	0·08	17	0·46	0·23
7	8	1·19	0·54	22	8	1·01	0·98	41	6	1·17	0·89	37	2·41	1·11
8	3	2·04	1·08	16	3	3·13	3·13	48	3	2·76	2·40	36	6·61	2·75
9	2	0·38	0·16	8	3	0·17	0·10	4	3	0·81	1·84	88	2·10	0·64
Mean		0·81	0·55	18		1·11	1·13	37		1·33	1·39	45	3·07	1·18
S.D.		0·56				0·94				0·84			2·02	0·74

* See text for experimental details and computations. Donor 10 was not tested because his Fraction I was depleted.

ejaculate being 0·63±0·23, range 0·30 to 0·97 mg/ml. Both proteins showed a decreasing distribution from Fractions I to III, albumin both in amount and concentration and transferrin only in concentration. The mean amount of transferrin was highest in Fraction II and lowest in Fraction I. The concentration differences between Fractions I and III were statistically significant for both proteins (albumin, $P = 0{\cdot}01$; transferrin, $P = 0{\cdot}05$). Only Donor 2 possessed a higher concentration of transferrin in Fraction III than in Fraction I. Donor 6 possessed virtually identical concentrations in these fractions, and an opposite pattern of albumin concentrations, that in the third fraction being higher than that in the first fraction.

Lactoferrin

In contrast to the other proteins, lactoferrin showed a different distribution pattern (Table 8). The concentration and the amount increased from Fractions I to III, but the difference in concentration between Fractions I and III was not statistically significant. Donors 1 to 4 showed this increasing pattern, whereas Donors 6 and 7 possessed identical concentrations in all three fractions. Donors 5 and 9 had the lowest concentration of lactoferrin in the second fraction, while Donor 8 had the highest concentration in this fraction. Except for Donors 5 and 7, the concentration of lactoferrin in Fraction III was always higher than that in Fraction I. The computed mean±S.D. concentration of lactoferrin in the whole ejaculate was 1·18±0·74 mg/ml, range 0·23 to 2·75.

Secretory piece, β_1C/β_1A-globulin, ceruloplasmin and fibrinogen

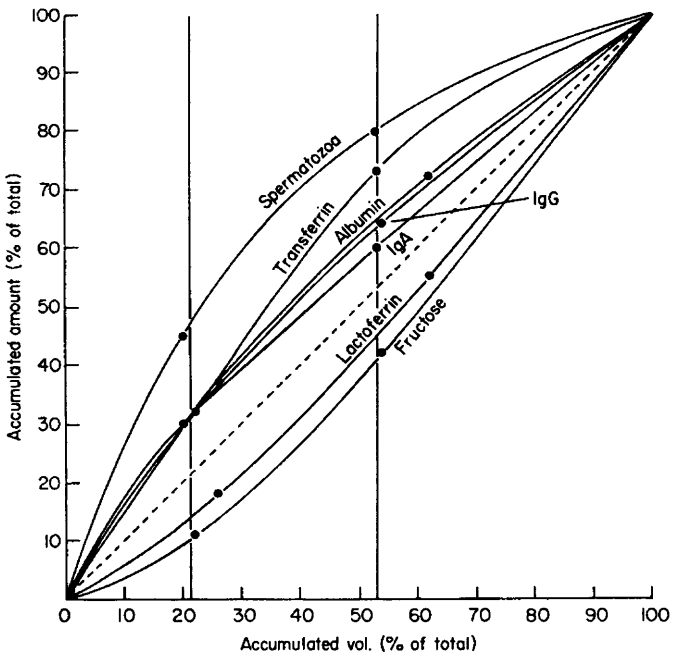
Secretory piece, fibrinogen, and ceruloplasmin were not found in twelve different whole ejaculates. Only one ejaculate out of twelve showed a weak specific reaction for β_1C/β_1A-globulin.

DISCUSSION

The collection of split ejaculates can at best be considered a rather crude method of separating the various components of semen in regard to their glandular origin (Eliasson & Lindholmer, 1972; Molnar *et al.*, 1971). The results frequently depend on the donor, and cannot be repeated precisely in the same manner from donation to donation. There is often an overlap between the different fractions that explains the wide individual variation of many parameters among the donors. To gain insight into the distribution pattern of certain proteins or other components, split semen samples have to be obtained from a multitude of donors. The mean results may then be regarded as representative of the true situation, as in our case where the computed total volume (2·9 ml), sperm concentration (111×10^6/ml) and fructose concentration (3·0 mg/ml) were found in agreement with the values quoted in the literature (Mann, 1964).

The tables indicate distinct differences in distribution patterns among the various components. A visual comparison of the data may be obtained by plotting the accumulated amounts of the components (as a percentage of the total) against the accumulated volumes (as a % of the total) corresponding to

these amounts (Text-fig. 1). The resulting curve then reflects the concentration changes that occur during the ejaculation process. The higher the rate of increase at the beginning of such a curve, the higher the input of that component in the semen at the early stages of ejaculation. A lower rate of increase at the beginning followed by a higher rate of increase toward the end characterizes a higher input at later stages of ejaculation. A straight line drawn between 0 and 100% separates these two types of curves. Typical examples for both types are the curves for the number of spermatozoa and for the amount of fructose. Spermatozoa are ejaculated very early and their curve shows a rapid initial increase, levelling off toward the end of the ejaculation. In contrast, fructose known to originate from the seminal vesicles increases slowly in the beginning but more rapidly toward the end (Text-fig. 1). Almost identical distribution curves for spermatozoa and fructose were obtained by Harvey (1956). These results indicate that the data obtained for the other components are reliable.



TEXT-FIG. 1. Distribution profile of non-enzymatic components in human ejaculates. The specimens were obtained from ten healthy donors. The curves reflect the concentration changes of the components during the ejaculation process. Curves below the dotted line represent contributions mainly from the seminal vesicles, curves above the line from other parts of the male genital tract. The two vertical lines indicate the average accumulated volumes (see Table 1) of all split ejaculate Fractions I and II, respectively. (For further details see text.)

The immunoglobulins (IgG and IgA), transferrin and albumin are highest in the early stages of ejaculation (Text-fig. 1), although not to the extent that would suggest that they are solely contributed by the prostate gland. Albumin, IgG and IgA are ejaculated at almost identical rates (43 to 45% of the total appears in the first third of the semen sample). Transferrin enters the ejaculate

at an even faster rate, 49% being present after the first third of the semen has been ejaculated. By contrast, lactoferrin appears to originate mostly from the seminal vesicles since its curve runs almost parallel to fructose (42% appears in the final third of the ejaculate).

The presence of IgG, albumin and transferrin in semen has been described previously, although these proteins have never been examined in split ejaculates (Goldblatt, 1935; Gray & Huggins, 1942; Hermann, 1959; Licht & Keutel, 1963; Quinlivan, 1968). Traces of IgA have been reported (Herrmann & Hermann, 1969b) and the low amounts of IgA in our study (0·03 mg/ml) agree with these observations.

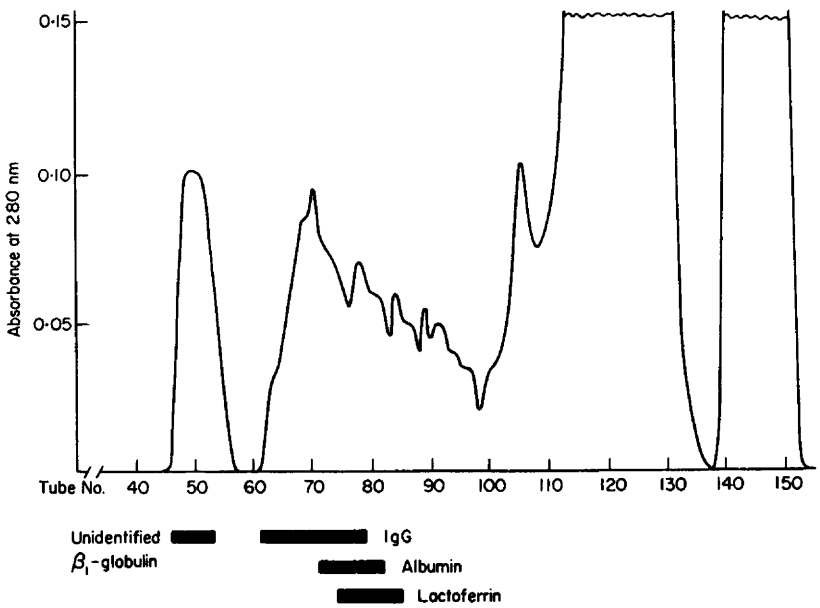
It is known that IgA is the predominant immunoglobulin in the majority of external fluids, such as saliva, urine, tears and secretions from the tracheobronchial and intestinal tracts (for review of the literature see Tomasi & Bienenstock, 1968). Immunoglobulin A may enter these secretions in various forms. One is the basic IgA unit (7S molecule with a molecular weight of ~170·000) representing free IgA which is immunochemically identical to serum IgA. Although higher polymers (16 to 20S) do also occur, the principal IgA form found in seromucous secretions occurs as an 11S molecule with a molecular weight of ~400,000 (secretory IgA) that has two 7S units conjugated through an interconnecting protein with a molecular weight of ~50,000. This protein is called the 'secretory piece'. Since free secretory piece could not be detected in the semen samples in our study, IgA is probably derived as a serum transudate.

Lactoferrin is an iron-binding protein with bacteriostatic properties (Masson & Heremans, 1966; Masson, Heremans, Prignot & Wauters, 1966). It was first identified in human seminal plasma by Masson, Heremans & Dive (1966), but is absent from serum. Patients with congenital aplasia of the vasa deferentia and the seminal vesicles have neither fructose nor lactoferrin in their semen. This indicates that lactoferrin is secreted in the vesicular glands (Hekman & Rümke, 1968). Our data corroborate these conclusions since lactoferrin showed a 'fructose-like' distribution (Text-fig. 1). It is dependent on the chelation of iron ions, and it has been suggested that lactoferrin is involved in the defence mechanisms against micro-organisms (Masson, 1970). Its concentration in semen is higher than in cervical mucus where it reaches a maximum only before the onset of menstruation (Schumacher, 1973). Lactoferrin has also been shown to be present in semen as a surface antigen on spermatozoa (Hekman & Rümke, 1968). Such sperm-coating antigens (SCA) were first described by Weil & Rodenburg (1960).

Transferrin is the iron-binding protein in serum and its presence in semen was first described by Quinlivan (1968). Transferrin may have similar functions to those of lactoferrin in the reproductive tract, i.e. protection against bacterial infection. The concentration of transferrin in semen is approximately one-third of that in serum, but is eight to ten times higher than in cervical mucus where it shows increasing levels in the late luteal phase and exhibits a peak shortly after the menstruation (Schumacher, 1973). The transferrin in semen probably derives from the serum, although the possibility of local production in the genital tract remains to be investigated.

The split ejaculates were also tested for the presence of immunoglobulin M,

using a rabbit antiserum that reacted monospecifically against serum IgM. With the radial immunodiffusion technique, strong precipitin rings were observed in all split ejaculate fractions from each donor using a certain batch of antiserum (Behring Diagnostics, New Jersey). These results could not be reproduced, however, when three other antisera batches from the same manufacturer were used. The presence of IgM in seminal plasma is, therefore, highly questionable. Other investigators, also using monospecific antisera directed against serum IgM, came to a similar conclusion (Quinlivan, 1968; Herrmann & Hermann, 1969a, b). It is conceivable, however, that alteration of the tissue through such aetiology as chronic prostatitis or adenoma of the prostate gland may cause the input of high molecular weight proteins from serum to semen as was reported for lipoproteins, α_2-macroglobulin and fibrinogen (Nylander, 1955; Leithoff & Leithoff, 1961). At least occasionally, IgM seems to be present in prostatic tissue. Ablin, Soanes & Gonder (1972) found IgM along the cyto-



TEXT-FIG. 2. Elution profile of centrifuged (130,000 *g* for 4 hr) human seminal plasma after Sephadex G-200 gel filtration (fraction volume, 3·0 ml). Albumin, lactoferrin and IgG were determined by radial immunodiffusion using monospecific antisera. The β_1-globulin in the excluded fraction was detected with antiserum against serum IgM that cross-reacted with this component in human seminal plasma.

plasmatic membrane of secretory epithelial cells in two patients with benign hypertrophy of the prostate. Further investigation in our laboratory as well as by the Behringwerke (Marburg, West-Germany; S. Baudner, personal communication) showed that the first antiserum batch cross-reacts with a β_1-globulin which can be found in large quantities in semen, but is virtually absent from serum. Sephadex G-200 gel filtration (0·1 M-tris-HCl buffer, containing 0·05 M-NaCl, pH 7·0) of pooled, centrifuged (130,000 *g*, 4 hr) seminal plasma resulted in the

presence of this component in the excluded fraction (void volume peak) indicating its high molecular weight (Text-fig. 2). This β_1-globulin is unidentified and is under further investigation.

A β_1C/β_1A-globulin (C'3 component of complement) was found in seminal plasma by Herrmann & Hermann (1969a, b) using the double immunodiffusion technique with a monospecific goat antihuman β_1C/β_1A-globulin antiserum. Based on these findings, the authors attempted to explain the occasional occurrence of immunological phenomena in seminal fluid which may be the cause of sub- or infertility. The consistent presence of this component in semen could not be confirmed in our study, however, since only one out of twelve whole ejaculates showed a weak precipitin line with a monospecific rabbit antiserum. At present, there is no explanation for this discrepancy.

The presence of the immunoglobulins IgG and IgA deserves special attention because of the increasing interest in the immunological approach to contraception as well as in the elucidation of male infertility problems connected with immunological phenomena. The concentrations of IgG and IgA in seminal plasma are lower than in cervical mucus (Schumacher, 1973). The IgG:IgA ratio was calculated to be between 2:1 and 3:1 for all split-ejaculate fractions and for the total concentration. This ratio is about half of that in serum (6:1) and similar to that in cervical mucus (Schumacher, 1973), but differs remarkably from that in other seromucous secretions where it is commonly less than 1:1 (Tomasi & Bienenstock, 1968). This suggests that the presence of immunoglobulins in semen is more likely to be due to transudation from the serum than to local production. Although the secretory piece of IgA could not be demonstrated in whole ejaculates, the existence of a local immune system in the male reproductive tract should not be excluded. It is possible that secretory IgA may enter the semen in such small quantities that it is not detectable by our immunodiffusion method. It is additionally conceivable that the 7S IgA form found in the split ejaculates may represent degradation products of the secretory 11S IgA molecule. Ablin *et al.* (1972), for instance, showed the presence of serum IgA in secretory granules of human prostatic tissue.

Little is known concerning the function of these proteins in the reproductive process. The immunoglobulins are probably a part of the antibody system of seminal plasma and may be partly responsible for the occasional agglutination of spermatozoa in semen samples. Albumin may protect the motility of spermatozoa (Eliasson & Lindholmer, 1974). Lactoferrin and transferrin could be involved in the defence mechanism against micro-organisms. The higher amounts of IgG, IgA, albumin and in particular transferrin in the initial portion of the ejaculate compared to the final portion, suggests that they may be involved in the liquefaction process of the human coagulum. Experiments aimed to evaluate whether these proteins do indeed play a rôle in this process will be discussed in a future communication.

Note added in proof

The recent results of P. H. Rümke (*Clin. exp. Immun.* (1974) **17,** 287–297) on the distribution of immunoglobulins in semen compare favourably with those presented in this paper.

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