Fatty Acids in Semen

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(Received 23 May 1960)

Only one paper seems to have appeared on the presence of free fatty acids in semen, namely that of Flipse & Potter (1955), who identified acetic acid and formic acid in bull semen. We now report the results of investigations on the concentrations of volatile fatty acids and free fatty acids (long-chain) in the semen of bull, ram, rabbit, fowl, dog, stallion and man. In addition, volatile fatty acid concentrations in the semen and blood of rams have been compared, and their concentration determined in ram-epididymal fluid.

EXPERIMENTAL

Materials. Ram semen was collected by electrical stimulation by the bipolar electrode of Blackshaw (1954). An artificial vagina was used for collecting bull, stallion and rabbit semen, with White's (1955) apparatus for the last named. Dog semen was obtained by manipulation and fowl semen by abdominal massage (Burrows & Quinn, 1939). In each experiment, the semen was frozen in solid CO₂ and ejaculates were usually pooled for analysis. Normal human semen was obtained from a sterility clinic.

Testes were dissected from rams immediately on slaughter and packed in an ice-salt mixture during transport to the laboratory. Testicular fluid was aspirated from the rete testes and fluid taken from the tail of the epididymis after cutting with a scalpel. Pooled material from 10 rams was used in the analysis.

Blood samples were obtained from the jugular vein, with heparin as anticoagulant.

Determination of volatile fatty acids. Volatile fatty acids

were estimated by the method described by Annison (1954). Blood or semen (1 vol.) was mixed with 3.5 vol. of water and deproteinized with 0.5 vol. of freshly prepared 25% (w/v) metaphosphoric acid. The precipitate was separated by centrifuging and washed with 2.5 vol. of 2.5% (w/v) metaphosphoric acid. The supernatant and washings were adjusted to pH 8.0–8.5, with 10N-NaOH, by using phenolphthalein as indicator, and concentrated where necessary *in vacuo* to 3–4 ml. at 40° in a rotary evaporator. A few drops of octan-2-ol were added to prevent the frothing of semen samples, which became excessive if the temperature rose above 40°. The concentrated solution was adjusted to pH 2.5–3.0 with 10N-H₂SO₄ (thymol blue indicator) and transferred to a Markham still for distillation. Two successive 100 ml. distillates were collected (the second served as a blank) and titrated with 0.01N-NaOH (phenolphthalein indicator) under CO₂-free conditions.

The volatile fatty acids in the neutralized distillates from the semen samples were analysed by gas-liquid chromatography (James & Martin, 1952) according to the general procedure outlined by Annison (1954).

Determination of free fatty acids. Semen was diluted with 2 vol. of water, and portions (2 ml.) were examined for free fatty acid (long-chain) by the method of Dole (1956), with modifications described by Annison (1960). Dilution of semen before extraction with organic solvents was necessary to avoid the formation of stable emulsions.

RESULTS

Values for the volatile fatty acid content of the semen of the bull, ram, rabbit, man, stallion, fowl and dog are given in Table 1. Since many of the

Table 1. *Volatile fatty acid content of the semen of various species*

Analyses for individual acids were usually made on single pooled samples.

Animal	No. of samples	Total (m-equiv./l.)	Individual acids (molecular percentages)	
			Formic acid	Acetic acid
Bull	17	0.82 (0.20–1.43)	27	73
Ram	22	1.63 (0.68–2.73)	0	100
Rabbit	2	1.34 (1.08–1.80)	4	96
Man	3	0.73 (0.50–0.95)	0	100
Stallion	4	0.77 (0.67–1.00)	0	100
Fowl	2	0.51 (0.37–0.64)	0	100
Dog				
(1) Prostatic fluid	4	0.11 (0.06–0.15)	—	—
(2) Spermatozoa fraction	2	0.10 (0.08–1.20)	0	100
(3) Pre-spermatozoa fraction	1	0.90	—	—

samples were pooled ejaculates, the normal range of values for individuals in any one species is probably greater than the data indicate. However, values as high as those recorded by Flipse & Potter (1955) in bull semen (i.e. up to 25.0 m-equiv./l.) were never encountered.

Table 2 shows a comparison of the volatile fatty acid concentration in the blood and semen of 20 rams. Blood samples were taken at the same time as the semen from each animal and four animals from each of five strains were used.

Analyses of variance for the blood and semen data did not reveal any statistically significant differences in volatile fatty acid concentration between strains. In all animals the concentration of these acids in semen was higher than in the blood (t 18.2, $P < 0.01$). There was, however, no significant correlation between the two (r 0.112; D.F. 18).

The volatile fatty acid content of testicular and tail fluids, pooled in each instance from 10 ram epididymes, was 1.93 and 1.70 m-equiv./l. respectively.

The free fatty acid (long-chain) content of the semen of the bull, ram, stallion, rabbit and dog is shown in Table 3. Plasma-free fatty acid analyses

were not undertaken, but reported values for the dog, sheep and rabbit (cf. Annison, 1960) suggest that the semen values were lower than those of plasma. One sample of dog prostatic fluid contained considerably less than did the spermatozoa fraction from the same ejaculate (Table 3).

DISCUSSION

The results reported here for bull semen are difficult to reconcile with those of Flipse & Potter (1955), who found volatile fatty acid concentrations in bull semen of 7.9–25.0 m-equiv./l., i.e. approximately ten times those found in the present investigation. The discrepancy cannot be explained in terms of the age of the semen samples before analysis since Flipse & Potter (1955) found no consistent differences in total or constituent acids when semen samples were analysed 5 and 180 min. after collection. Further, many of the samples analysed in the present study were cooled immediately with solid carbon dioxide, so that, in these instances, there could be no question of a breakdown of the volatile fatty acids before analysis. The relative concentrations of acetic acid and formic

Table 2. *Comparison of the volatile fatty acid concentration (m-equiv./l.) in the blood and semen of 20 merino rams (four animals of each strain)*

Mean values are given, with the range for each strain and the overall population S.D. The residual mean square is shown in *italics*.

Strain	Blood		Semen	
Peppin A	0.84	(0.63–1.27)	1.71	(1.04–2.36)
Peppin B	0.91	(0.72–1.03)	1.66	(1.03–2.25)
Non-peppin medium	0.67	(0.59–0.76)	1.06	(0.68–1.48)
Fine wool	0.68	(0.62–0.77)	1.40	(1.10–1.75)*
S.A. strong	0.60	(0.52–0.68)	1.67	(1.44–2.23)
Overall	0.74	± 0.17	1.50	± 0.46

<i>Analysis of variance</i>				
Source of variation	Blood		Semen	
	D.F.	Variance ratio	D.F.	Variance ratio
Between strains	4	2.5	4	2.7
Residual	15	0.264	14†	0.118

* Three animals only.

† Decreased by 1 to compensate for the insertion of a missing value, by the method of Cochran & Cox (1950).

Table 3. *Free fatty acids in secretions of several species.*

Each sample usually consisted of several ejaculates.

Animal	Secretion	No. of samples	Concentration (m-equiv./l.)
Bull	Semen	3	0.13 (0.06–0.24)
Stallion	Semen	2	0.10 (0.10–0.09)
Ram	Semen	6	0.21 (0.15–0.27)
Rabbit	Semen	1	0.01
Dog	Spermatozoa fraction	1	0.18
Dog	Prostatic fluid	1	0.02
Fowl	Seminal plasma	1	0.08

acid in bull semen in the present studies were similar to those reported by Flipse & Potter (1955).

There is evidence that bull spermatozoa are able to oxidize acetic acid (Lardy & Phillips, 1944), and if this was present in seminal plasma in the relatively high concentrations reported by Flipse & Potter (1955) it could clearly be an important source of energy for the spermatozoa after ejaculation. The concentrations found here would, however, be of little metabolic significance in comparison with the large quantities of fructose that are known to be present normally in bull semen (Mann, 1946, 1954).

Much of the carbohydrate ingested by herbivores is converted into volatile fatty acids by micro-organisms in the alimentary tract, and, in ruminants at least, volatile fatty acid, rather than sugar, is the chief form in which carbohydrate is absorbed (Annison & Lewis, 1959). It was thought therefore that acetate concentrations in the semen of herbivores (particularly the ram and bull) might be higher than those of other species such as man, dog and fowl. There are, however, no striking differences between species.

The fact that the concentration of volatile fatty acids is consistently higher in semen than in blood suggests that the accessory organs may be capable of concentrating or producing the acids.

The concentrations of volatile fatty acids in the epididymal fluids examined were of the same order as that of whole semen. However, the volatile fatty acids of the epididymal tail may well be in a dynamic state, with a constant interchange between blood, epididymal plasma and spermatozoa. These substances might still therefore be important in the economy of the spermatozoa despite their low concentration in the epididymal fluid.

An unexpected finding was the absence of formate from the semen of all species examined except bull and rabbit. Formate is present in the blood of sheep, goats, oxen, rabbits, horses, dogs and in the human blood (Annison, 1954).

Free fatty acids have been shown to be the metabolically active fraction of circulating lipids (cf. Frederickson & Gordon, 1958). Although they are present in low concentrations in blood (0.3–0.8 m-equiv./l.), rapid turnover rate is an indication of their metabolic importance. The concentrations found in semen (Table 3) are such that free fatty acids can hardly be important as an energy source to spermatozoa after ejaculation. It is possible, however, that they are involved in the metabolism of spermatozoa in the epididymis.

SUMMARY

1. The concentrations of volatile fatty acids and free fatty acids (long-chain) in bull, ram, rabbit, fowl, dog, stallion and human semen were determined. The volatile fatty acid concentration ranged from 0.08 to 2.73 m-equiv./l. and there were no outstanding differences between ruminants and non-ruminants. Values for free fatty acid content ranged from 0.01 m-equiv./l. for dog prostatic fluid to 0.21 m-equiv./l. for ram semen.

2. Chromatographic evidence indicated that acetic acid and formic acid were present in bull and rabbit semen, but in the other species examined acetic acid was the only volatile fatty acid detected.

3. No significant correlation was found between the volatile fatty acid concentrations in the semen and blood of rams.

4. The volatile fatty acid concentration of ram epididymal fluid was similar to that of whole semen.

5. It is concluded that volatile fatty acid and free fatty acid (long-chain) concentrations in the semen of the species examined is too low to be of importance for the spermatozoa as a substrate after ejaculation.

We are indebted to Professor C. W. Emmens for his interest and advice, to Mr P. Bryden of the N.S.W. State Abattoirs for the supply of ram testes, and to the Wool Research Committee for financial assistance. The work of two authors (I.G.W. and T.W.S.) has also been aided by grants from the Planned Parenthood Federation of America and the Rural Credits Development Fund of the Commonwealth Bank of Australia.

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