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Relative Metabolic Rates of Semen, Seminal Plasma, and Bacteria in Semen of the Boar.*

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It is generally assumed that seminal plasma is an inert fluid incapable of respiration, and that the gaseous exchange of semen quantitatively represents that of the sperm.† However, in the words of Thornton and Wood,[1] "the modern conception of organic complexes in solution is that they respire, consuming oxygen." This suggested that seminal plasma may be capable of extra-cellular respiration. Seminal plasma is a relatively complex fluid as is indicated by the work of Goldblatt,[2] who has shown that seminal plasma of man contains various proteins as well as diastase and thrombokinase. The presence of catalase in semen of man was reported by Kurzrok and Miller.[3] Likewise, McKenzie, Miller, and Bauguess[4] have shown that seminal plasma of the boar contains an appreciable amount of nitrogenous material and some glucose.

Extra-cellular respiration in seminal plasma could constitute a source of error in measurements of sperm metabolism. To eliminate the possibility of an unrecognized error from this source, we have measured the relative metabolic rates of semen and seminal plasma of the boar. In addition, we have investigated the possibility that bacteria‡ may constitute a source of error in measurements of metabolism of boar's semen.

Semen was obtained from 2 normal boars, and from one double cryptorchid animal, with the artificial vagina (McKenzie[5]). Differences in concentration, motility rating, and metabolic rate of the

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† "Sperm" is used here as a contraction of "spermatozoa."

1 Thornton, H. R., and Wood, F. W., *Canad. J. Res.*, 1935, **12**, 295.

2 Goldblatt, M. W., *Biochem. J.*, 1935, **29**, 1346.

3 Kurzrok, R., and Miller, E. G., *Am. J. Obstet. and Gynec.*, 1928, **15**, 56.

4 McKenzie, Fred F., Miller, J. C., and Bauguess, L. C., *Mo. Agr. Exp. Sta. Res. Bul.* 279, 1938.

‡ No attempt at identification of the microörganisms found in boar's semen was made. The term "bacteria" is employed here to include all of the microorganisms present.

5 McKenzie. Fred F., *The Cattleman*, Sept., 1939.

ejaculates of the 2 normal boars were not greater than the day to day variations in the semen of either animal. Sperm were separated from seminal plasma by centrifugation at gravity × 1400. Metabolism of the ejaculates was measured at 37°C with a modified Barcroft-Warburg respirometer§ (Dixon[6]).

Seminal plasma of the boar consumed oxygen in definitely measurable amounts which varied from 5 to 22% of that of the whole semen from which it was obtained. A white precipitate, which appeared when carbon dioxide-free air was passed over seminal plasma and into $Ba(OH)_2$, was considered qualitative evidence of CO_2 production. This precipitate was obtained even after the plasma had been held at 100°C for 5 minutes. The R.Q. of plasma, measured with the respirometer, was unity. Oxygen consumption of boar's semen and seminal plasma is given in Table I.

It is unlikely that the figures for oxygen consumption of plasma

TABLE I.

Relative Metabolic Rates of Semen and Seminal Plasma of the Boar, and of Seminal Plasma Before and After Treatment to Kill Microörganisms

	Semen		Seminal plasma before treatment		Seminal plasma after treatment			
No. of observations	O_2 consumption* per cc/hr, mean, mm^3	Bact.†	O_2 consumption* per cc/hr, mean, mm^3	Bact.†	Treatments used to kill microorganisms	Bact.†	O_2 consumption* per cc/hr, mean, mm^3	Change in O_2 consumption following treatment, %
1	72.7	—	8.9	—	Formaldehyde, 1	—	9.2	+ 3.4
3	126.2	+	10.0	+	drop 10% solution in 3 cc plasma	—	10.7	+ 7.0
1	64.3	—	13.5	—	Merthiolate, 1 cc	—	8.1	—40.0
1	69.7	—	7.5	—	0.1% solution in	—	4.6	—38.7
5	93.5	+	10.4	+	2 cc plasma	—	6.4	—38.5
1	71.7	+	9.1	+	Mercuric chloride,	—	7.5	—17.6
1	144.3	+	7.2	+	0.1 cc 10% solution in 1 cc plasma	—	6.1	—15.3
1	76.2	+	9.6	+	100°C for 5 min	—	5.2	—45.8
1	30.5	+	6.6	+		—	3.8	—42.4
3	—	—	11.5‡	—	—	—	—	—

*Volumes were measured at 37°C, and have been reduced to standard conditions of temperature and pressure.

†The + sign indicates that microörganisms were present, — that they were absent.

‡Ejaculate of a bilaterally cryptorchid boar.

§ Accuracy of the apparatus was determined by simultaneous measurements of O_2 consumption of a given sample of semen in each of the 6 manometer units. The mean deviation was less than 5%.

[6] Dixon, M., Manometric Methods, Cambridge University Press, London, 1934.

have been influenced significantly by passage of air into solution in the plasma during the measurements. In each case the materials were exposed to air for an hour, the last quarter of which was at the temperature of the experiment, before the first readings were made. Any tendency for air to dissolve in the fluids in the reaction vessels during the experiments should be balanced by a similar tendency for it to dissolve in the water in the compensation vessels. After 10 minutes in a vessel in which the pressure was reduced with a powerful filter pump, the R.Q. of seminal plasma was the same as that of a part of the sample which had not been subjected to this treatment, namely, unity.

In contrast with the sperm-free ejaculate of a human, reported by Shettles,[7] which exhibited "no measurable respiration," the sperm-free ejaculate of a double cryptorchid boar consumed about the same amount of O_2 as did normal seminal plasma (Table I).‖ That portion of the seminal plasma which passed through a porcelain filter failed to consume oxygen in each of 12 trials. This filtrate was sterile and yielded a very slight precipitate, or none at all, with sodium tungstate and H_2SO_4 (Peters and Van Slyke[8]). Thus it appears that the filtrate was free, or nearly free, of protein and of such other materials, particularly enzymes, as may have been attached to the protein molecules.

Bacteria are usually present in semen obtained from farm animals. Even though the ejaculate may be free of bacteria as it leaves the urethra, some contamination from the external genitals appears to be almost inevitable.

Measurements of errors in glycolysis determinations of ram's semen due to bacteria were made by Comstock[9] who concluded that "the effect of bacteria is negligible for at least 4 hours" as a source of error in measurements of glycolysis. We have attempted to measure errors in seminal metabolism determinations due to bacteria by comparing oxygen consumption of untreated plasma with that of plasma in which the bacteria had been destroyed. Bacteria in the plasma appeared to be approximately as numerous as those in the semen from which it was separated as judged by the number of colonies which appeared on beef-infusion medium after smears of undiluted ejaculate had been incubated for 24 hours at 37°C. Treatments

[7] Shettles, L. B., *Am. J. Physiol.*, 1940, **128**, 408.

‖ The direct method of Warburg was used in Shettles' experiments. This technic and that employed by us are considered equally accurate (see Dixon[6]).

[8] Peters, John P., and Van Slyke, Donald D., *Quantitative Clinical Chemistry*, Vol. 2, Williams and Wilkins, Baltimore, 1932, p. 682.

[9] Comstock, R. E., *J. Exp. Zool.*, 1939, **81**, 147.

used to destroy bacteria in plasma are given in the sixth column of Table I. It appears that the treatments used to destroy bacteria directly influenced the metabolism of plasma, as was indicated by the fact that the effects characteristic of given compounds appeared even when bacteria were not present in the samples, for example, those indicated in the first, third, and fourth data-lines of Table I. Since the changes in plasma metabolism following bactericidal treatments appear to be independent of effects attributable to bacteria, but characteristic of the treatments employed, it seems logical to conclude that the quantity of oxygen consumed by those bacteria present was too small to be measured by our method, and therefore completely negligible. The data given in Table I deal with the second hour after ejaculation. Some of these measurements were continued through the sixth hour after ejaculation, but the results indicate that possible errors due to bacteria were negligible even during this longer period.

Conclusions. 1. Extra-cellular respiration was exhibited by seminal plasma of the boar. Oxygen consumption of seminal plasma ranged from 5 to 22% of that of the whole semen. The R.Q. was unity. After the plasma had been held for 5 minutes at 100°C respiration at reduced intensity was observed. Respiration was not exhibited by that fraction of the seminal plasma which passed through a porcelain filter. 2. The metabolic rate of the ejaculate of a cryptorchid boar was of the same order as that of seminal plasma. 3. No errors in measurement of oxygen consumption were detected which could be attributed to the presence of bacteria.

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Intraperitoneal Administration of Sulfanilamide; Concentration in Peripheral Blood in Dogs.

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The reports of encouraging results following the use of sulfanilamide administered subcutaneously in the treatment of peritonitis of appendiceal origin[1, 2] have prompted the use of this drug in

[1] Ravdin, I., Rhoads, J. E., and Lockwood, J. S., *Ann. Surg.*, 1940, **111**, 53.

[2] Corry, D. C., Brewer, A. C., and Nicol, C., *Brit. M. J.*, 1939, **2**, 561.