probably arise from fibrillary movements of muscle and from external electrical phenomena; experiments to determine their nature and to eliminate them are under way.

The technic permits the prompt differential diagnosis of pregnancy from other abdominal tumors, it is free from false positive results, and it is less time consuming than biological tests for pregnancy. A positive result is certain proof of life of the fetus. Finally, the detection of twin pregnancy becomes possible earlier than by any other method of examination.

We believe that clinical fetal electrocardiography is now a practical laboratory accomplishment and represents a useful clinical aid.

We are grateful to Mr. H. Harwell and Mr. R. K. Hellman of the Connecticut Telephone and Electric Corporation of Meriden, Conn., for the amplifier employed in these experiments.

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Influence of Cell Concentration on Respiration Rate of Sperm.*

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Numerous experiments have demonstrated that as the density of a population within a limited, closed system increases, the physiologic limitations imposed upon the individuals making up the population progressively increase. Thus, it has been shown that egg production of Drosophila confined within a limited space declines with increasing density of population.[1] Sea urchin sperm, inactive in the testis, have been shown to become very active at low concentrations in seminal plasma, and to exhibit a progressively increasing respiration rate accompanying increasing dilution with sea water.[2] Marine biologists are familiar with this phenomenon. A large increase in respiration rate of sperm of the fowl on reduction of concentration to 1/5 the original level has been reported.[3]

If the respiration of mammalian sperm cells similarly varies with

* Contribution from the Department of Animal Husbandry, Missouri Agricultural Experiment Station. Journal Series No. 778.

1 Pearl, Raymond, *J. Exp. Zool.*, 1932, **63**, 57.

2 Gray, J., *Brit. J. Exp. Biol.*, 1928, **5**, 337.

3 Winberg, Hakan, *Arkiv. f. Zool.*, 1939, **32A**, No. 7.

concentration, serious errors could result when different samples are compared, since the sperm concentration of semen varies within wide limits. To test this point, we carried out, during the past year, measurements of the O_2 consumption rates of identical sperm, of either swine or sheep, suspended at various concentrations in identical media. The mean volume of boars' ejacula is around 200 cc with sperm concentrations commonly ranging from 2.5 million to over a billion per cc.[4] The mean volume of (Shropshire and Hampshire) rams' ejacula is 2 cc, with mean concentration in the neighborhood of 3 billion sperm per cc of semen during the months of greatest sexual activity.[5] These figures correspond closely to those for semen used in this study.

Nine purebred Ramboullet rams 3 to 4 years of age were used, the total ejacula of 5 to 7 was combined to obtain conveniently large samples. The boars used were: one Poland China and 2 Chester White boars one year of age, and one Hampshire boar 2 years of age. The ejaculum of one boar was sufficient for each trial.

Semen samples were obtained with the artificial vagina[6] by student assistants. Sperm concentration was increased by centrifugation at gravity $\times$ 1400. Any possible injuries caused by centrifugation probably were consistent for all dilutions. Respiration was measured at 37°C, for one hour, with a modified Barcroft-Warburg respirometer.† [7] Sperm concentration was determined with a hemocytometer.

In the first series, boar semen with an initial concentration of one billion, or less, sperm cells per cc was diluted with seminal plasma. This apparently had no influence on respiration rates (broken lines in Fig. 1). Later, sperm were concentrated by centrifugation, and in some trials concentrations as great as 6 billion sperm per cc (upper limit of concentration of untreated ram sperm) were obtained.

In the second series, each semen sample was divided, by centrifugation and subsequent dilution with seminal plasma, into fractions

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

[5] McKenzie, Fred F., and Berliner, Victor, *Mo. Agr. Exp. Sta. Res. Bul.* 265, 1937.

[6] McKenzie, Fred F., *The Cattleman*, Sept., 1939.

† Accuracy of the apparatus was determined periodically by simultaneous respiration measurements of yeast suspension in each of the 6 manometer units. The error (mean deviation $\times$ 100/mean) was 2%. An O_2 consumption rate so high as to exceed the capacity of the respirometer ([7], p. 48) was never approached in these experiments.

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

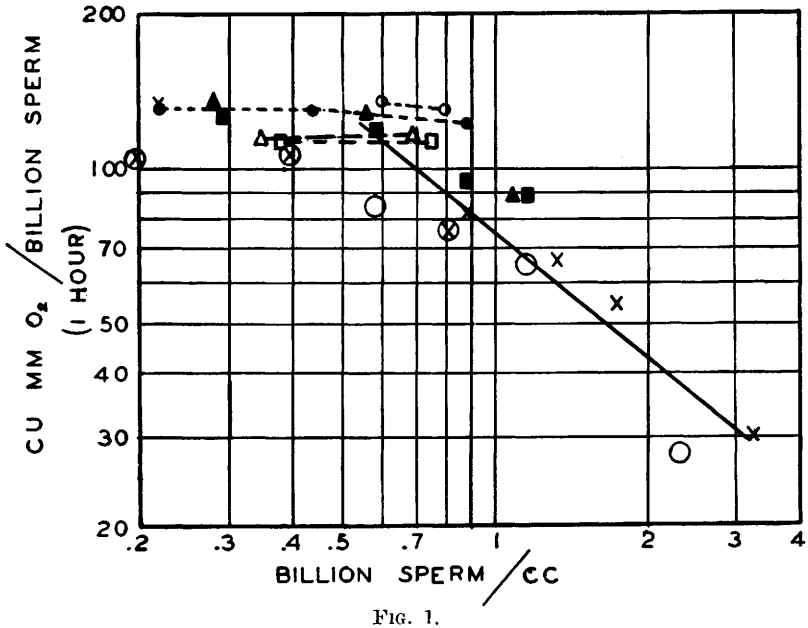

FIG. 1.
Oxygen consumption of boar sperm. (The symbols identify individual trials.)

which varied in concentration from 0.2 to 6 billion sperm per cc. While errors as large as 16% may have occurred in the initial determinations of sperm concentration,[8] the errors in relative concentrations of the sperm suspensions were merely the minute ones introduced in pipetting. Any effect of bacterial contamination was probably negligible.[9] In some trials the O_2 consumption of seminal plasma was determined[9] and subtracted from the seminal O_2 consumption to give that of the sperm. In the others, respiration of seminal plasma was estimated. In Fig. 1 are given the results of these trials with boar sperm, and in Fig. 2 with ram sperm. These figures show that, except at lower concentrations, a decrease in concentration was invariably accompanied by an increase in O_2 consumption of individual cells.‡

Any possible effect of pH as a variable was reduced, in the last

[8] Berkson, Joseph, Magath, Thomas B., and Hurn, Margaret, *Am. J. Physiol.*, 1940, **128**, 309.

[9] Winchester, C. F., and McKenzie, Fred F., PROC. SOC. EXP. BIOL. AND MED., 1941, **46**, 455.

‡ The numerical data are plotted on logarithmic paper, the numbers shown being the original data while the relationships shown are those existing between the logarithms of these numbers. The use of this method in biological work has been discussed by Brody and Kibler.[10]

[10] Brody, S., and Kibler, H. H., *Mo. Agr. Exp. Sta. Res. Bul.* 328, 1941.

series of trials, by the addition of phosphate buffer to the seminal plasma used in dilution, or suspension of washed sperm in phosphate buffer. The initial pH of all portions for any one trial was thus the same, and the final pH values were sometimes as close as 0.1 pH unit in the separate portions. The results are given in Figs. 3 and 4. The computed slopes of the various curves accompany the figures. Due to the lack of uniformity in slope, the respiration rate of a given sperm sample cannot be computed reliably at some theoretical concentration on the basis of data obtained at another concentration. *For comparison of respiration rates, therefore, semen samples must be diluted to a standard level of concentration.* The pH should also be controlled and seminal respiration rates corrected for O_2 consumption of seminal plasma.[9]

Why does seminal respiration decline with increasing sperm concentration? Gray[2] concluded that sperm cells exercise a mutual

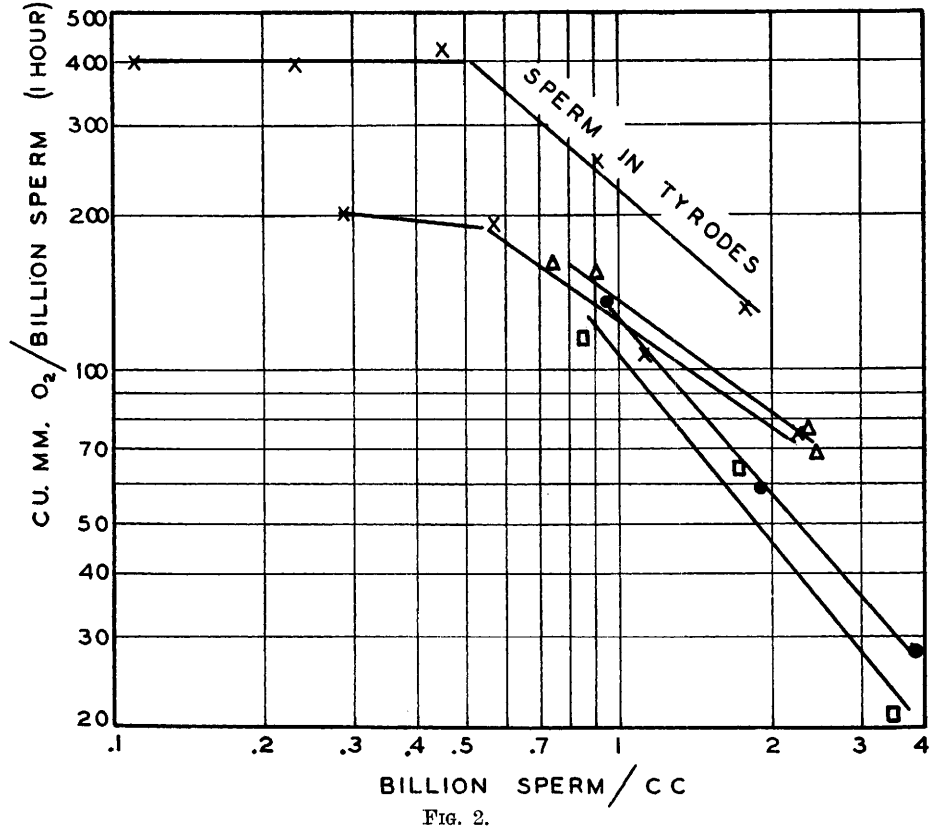

FIG. 2.
Oxygen consumption of ram sperm. (The symbols identify individual trials.)

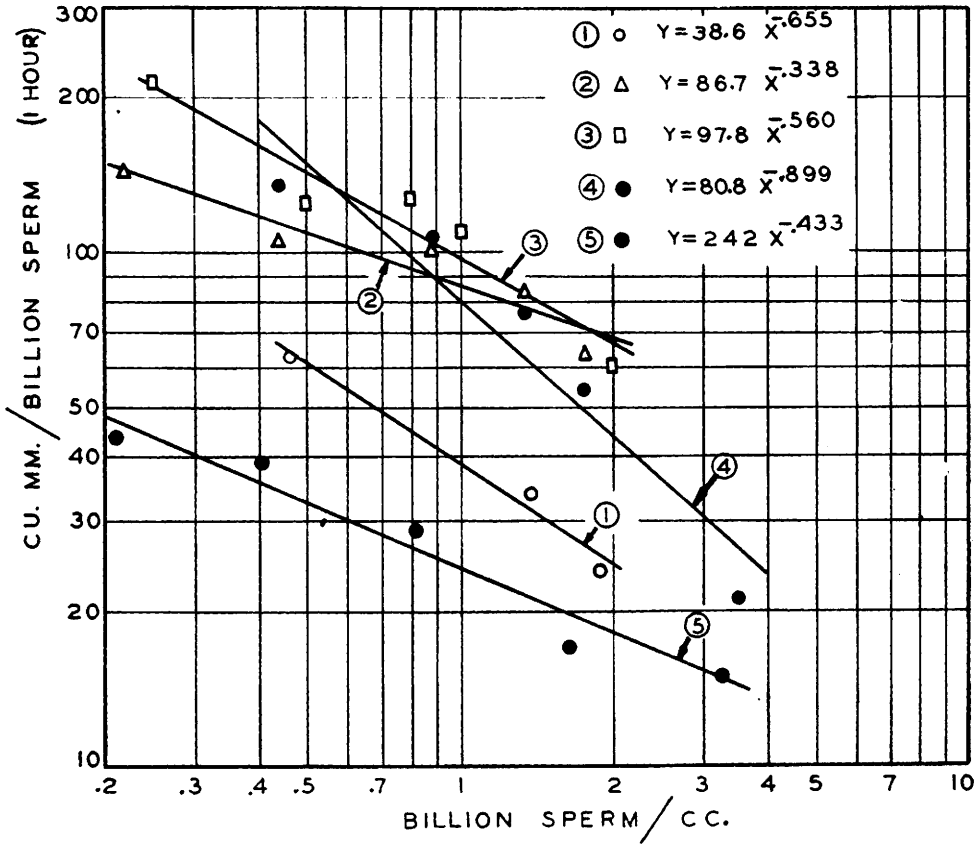

FIG. 3.
Oxygen consumption of boar sperm in buffered seminal plasma.

restraint, and showed that the effect probably was not due to reduction of O_2 available to individual cells since *more* O_2 sometimes was consumed by unit volume of suspension at relatively low concentration than at higher concentration. Our results confirm this observation. It may be significant that the respiration rate of leucocytes[11] is influenced by cell concentration in a manner analogous to that of sperm, while respiration rates of 2 non-motile organisms, yeast (Fig. 4) and bacteria,[12] are not. The fact that sperm in buffered media (Fig. 3 and 4) behaved similarly to those in seminal plasma (Figs. 1 and 2) appears to exclude differences in pH as a factor. The clumping together of cells cannot be regarded as the cause since this was observed as frequently at low as at high concentrations.

[11] Barron, E. S. G., and Harrop, G. A., Jr., *J. Biol. Chem.*, 1929, **84**, 89.
[12] Gerard, R. W., *Biol. Bul.*, 1931, **60**, 227.

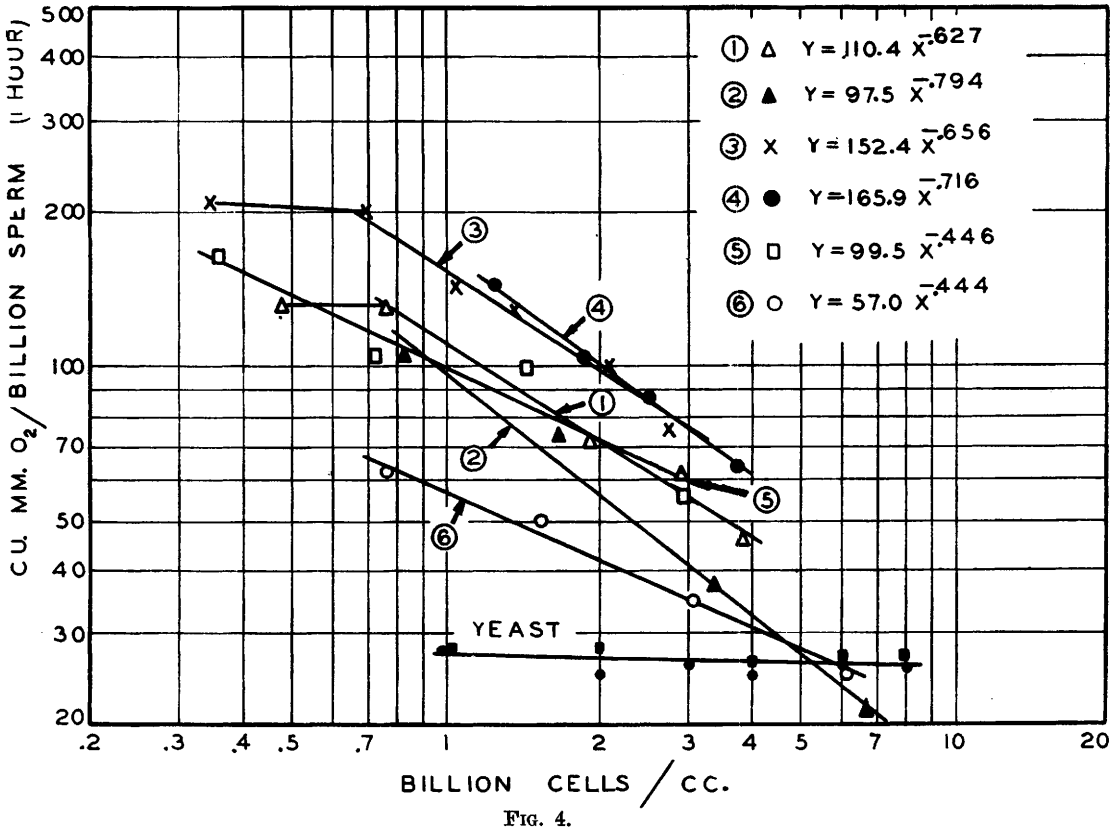

FIG. 4.
Oxygen consumption of ram sperm, and yeast cells, in buffered media.

Oxygen consumption curves of washed ram sperm in buffered solutions were about the same as those of normal semen; this is evidence that neither a seminal enzyme nor a metabolite is responsible.

Conclusions. 1. At sperm concentrations from 1 to 6 billion cells per cc an increase in concentration was accompanied by a decrease in sperm respiration rate. At concentrations of 0.2 to 1 billion sperm per cc this effect of concentration was not invariably observed. 2. In contrast with the behavior of sperm cells, respiration rates of yeast cells remained constant at concentrations from 1 to 8 billion cells per cc. 3. The effect of sperm concentration on sperm respiration rate does not appear to be due to changes in pH, diminished supply of O_2 to individual cells, an enzyme, or products of metabolism. 4. When comparisons are to be made of relative respiration rates of semen samples, the samples must be adjusted to the same level of sperm concentration.