adrenal hormones without necessarily acting directly on the same renal tubular locus affected by the adrenocortical hormones. The results obtained with ACTH are different from those reported by Axelrod and MacLeod(1). Since no details regarding procedures and methods are available at this time, it is not possible to explain the differences in our results and those of Axelrod and MacLeod.

1. Axelrod, D. R., and MacLeod, J., *Fed. Proc.*, 1951, v10, 7.
2. Van Slyke, D. D., and Hiller, A., *J. Biol. Chem.*, 1947, v167, 107.
3. Farah, A., Cobbey, T. C., and Mook, W., *J. Pharm. Exp. Therap.*, 1952, v104, 31.

Received May 20, 1952. P.S.E.B.M., 1952, v80.

Pyruvate Metabolism and Assessment of Semen Quality. (19602)

D. R. MELROSE AND CHARLES TERNER. (Introduced by Gregory Pincus.)
From the Ministry of Agriculture and Fisheries, Reading Cattle Breeding Centre, and the National Institute for Research in Dairying, University of Reading, England.

During a study of the metabolism of pyruvate in mammary tissue it was observed that 2:4-dinitrophenol (DNP) stimulates the respiration without increasing the rate of utilization of the substrate, thus causing its more complete oxidation(1). Preliminary experiments in which the effect of DNP on the metabolism of pyruvate in spermatozoa was tested produced somewhat similar results(2). Since DNP is known to inhibit synthetic reactions(3) it was thought that it might be a useful reagent for an assessment of the quality of spermatozoa based on their synthetic power.

Experimental procedure. Washed bull spermatozoa(4) were suspended in phosphate saline(5), and incubated at 37° in the Warburg apparatus a) without addition, b) with pyruvate (0.005-0.01 M) and c) with pyruvate + DNP (10^{-4} M). All flasks also contained fumarate (0.002 M). The oxygen consumption was followed for one to 2 hours. Pyruvate and lactic acid were determined by chemical methods(6,7). Since pyruvate is rapidly reduced to lactic acid by spermatozoa under aerobic conditions(2), the amount of pyruvate metabolized by oxidative reactions is given by the difference between the amounts of pyruvate disappearing and lactic acid formed and is designated as "net pyruvate"(8).

Results. In early experiments in which semen from selected bulls of high fertility was used, the respiratory rate in the presence of pyruvate was often accelerated by DNP(2), but in samples from other equally fertile bulls, it remained unaltered or was slightly depressed. In all these experiments DNP increased the ratio O_2 consumed/net pyruvate. The fact that the observed ratios were relatively high and in the presence of DNP often exceeded the theoretical 2.5 suggested that the endogenous respiration was incompletely suppressed.

On addition of fluoride (0.02 M) the endogenous respiration of spermatozoa was reduced to a low level; although the level of respiration in the presence of pyruvate was also depressed, DNP now caused marked stimulation of the oxygen consumption in the presence of the substrate also in those samples in which, without fluoride, it was inhibitory (Table I). DNP did not stimulate the respiration in the absence of added substrates, or in the presence of small amounts of endogenous lactate (less than 5×10^{-4} M).

When the respiratory response to pyruvate and DNP of a larger number of samples was examined in the presence of fluoride, 5 groups could be distinguished. *A.* The respiratory rate in the presence of pyruvate was low, very little above the endogenous level; DNP caused large increases in O_2 consumption, often exceeding 100%. *B.* The rate of respiration in pyruvate was about twice the endogenous level and DNP caused little or no further increase. *C.* The respiratory rate in pyruvate was inter-

TABLE I. Effect of Pyruvate, Fluoride and 2:4-Dinitrophenol of Respiration of Spermatozoa. Washed bull spermatozoa incubated in phosphate saline at 37°; gas, air. Fumarate (.002 M) in all flasks. Pyruvate, .01 M; fluoride, .02 M; DNP, 10^{-4} M. Volume, 4 ml. Sperm count/flask: Exp. I, 9.7×10^8; Exp. II, 7.92×10^8; Exp. III, 12.6×10^8. Incubation period: I, 45 min; II, 70 min; III, 120 min.

Additions	Z_{O_2} (μl/10^8 spermatozoa/hr) I	II	III
0	−18	−11.7	− 6.8
Pyruvate	−25.9	−21	−17.5
" , DNP	−25.8	−19.2	−23.1
Fluoride	− 4.2	− 4.2	− 4.8
" , pyruvate	− 6	− 5.2	− 7.6
" " , DNP	−11.3	−11.8	−15.6

TABLE II.* Metabolic Response of Bull Spermatozoa in Relation to the Fertility of the Donors.

Avg fertility of bulls (as % non-returns at 16 wk after insemination)	No. of bulls	No. of samples tested	Type of metabolic response: Good (A)	Intermediate (C)	Poor (B,D,E)
>65	3	11	9	2	0
55-65	8	22	7	8	7
<55	5	27	4	2	21
Unknown	3	10	2	1	7
Total	19	70	22	13	35

* We wish to thank Miss Zena D. Hosking for the statistical analysis of the data.

At least 3 samples of semen from each bull were tested, except in 4 cases.

$\chi^2 = 27.4$ (4 d.f.) $P < .001$. If the data are grouped within two classes only—above or below 55, then $\chi^2 = 19.1$ (2 d.f.) $P < .001$.

mediate between the endogenous and the pyruvate-plus-DNP levels. *D.* Both pyruvate and pyruvate-plus-DNP caused only small increases of respiration above the normal endogenous level. *E.* In a few experiments the endogenous respiration was exceptionally low and the addition of substrate and DNP was without effect.

All samples were graded according to the usual criteria (sperm count and motility) at the time of collection, and the average fertility of each donor was computed from its breeding record in artificial insemination during the past 12 months. No connection could be found between the type of respiratory response and the motility of the spermatozoa, which in most cases was good. However, a correlation between the metabolic response of the individual samples and the known fertility of the donors is apparent from Table II.

Chemical estimation of the metabolites involved showed that spermatozoa of type A metabolized pyruvate with a low O_2/net pyruvate ratio (less than 1) which was increased by DNP to 2.0-2.5, a result expected in tissues of high synthetic capacity(1). Samples of type B, despite their high respiratory activity, revealed their low synthetic power by oxidizing pyruvate to completion in the absence of DNP. Spermatozoa of type C showed intermediate O_2/net pyruvate ratios. In samples of type D, pyruvate was metabolized at slow rates and oxidized to completion (Table III).

Conclusions. 1. The results described above suggest that manometric measurements of respiration in a system comprising fluoride, pyruvate and DNP may provide a measure of the metabolic activity and give an indication of the potential fertilizing capacity of spermatozoa. Although other factors outside the scope of the manometric test play a large part in determining fertility, it seems to be generally recognized that the metabolic vigor of the spermatozoa is an important property in relation to their capacity for survival and fertilization(9). It should be pointed out that, since the observed metabolic rates represent the mean of the metabolic activities of a large number of spermatozoa, samples classified as poor may yet contain a small proportion of spermatozoa of good quality and under favorable conditions effect fertilization. In fact none of the bulls tested was completely sterile, but those of an average fertility of less than 55% as computed for Table I, are considered of little value as donors of semen for artificial insemination. 2. Although the correlation between the respiratory response and the fertility of the sperm samples in the field could not be tested on a larger scale than that reported, the test is put forward at this stage to make it available for more extensive trials by other workers. Present experience suggests that the test may prove its usefulness in helping to detect bulls which produce semen of exceptionally high quality (type A), and also

TABLE III. Metabolism of Pyruvate in Spermatozoa. Washed bull spermatozoa incubated in phosphate saline at 37°; gas, air. Fumarate (.002 M) and fluoride (.02 M) in all flasks. Pyruvate (.01 M) unless otherwise stated. DNP, 10^{-4} M. Volume, 4 ml.

Type of metabolic response	Incubation period, min	Sperm count /flask × 10^8	Additions	Z (μl/10^8 spermatozoa/hr) O_2	Pyruvate	Lactic acid	Net pyruvate	Ratio O_2/net pyruvate
A	70	7.92	0	− 4.2	—	—	—	—
			Pyruvate (.005 M)	− 5.2	−25.3	+16.3	−9	.6
			" " , DNP	−11.8	− 7.7	+ 2.4	−5.3	2.2
B	90	10.2	0	− 8.6	—	—	—	—
			Pyruvate	−14.1	−30.5	+23.4	−7.1	2
			" , DNP	−16	−12.5	+ 4.4	−8.1	2
C	90	11.7	0	− 4.9	—	—	—	—
			Pyruvate	−11.5	−38.7	+29.6	−9.1	1.3
			" , DNP	−14.9	− 7.4	+ 1.2	−6.2	2.4
D	120	9.1	0	− 6	—	—	—	—
			Pyruvate	− 9.1	−12.8	+ 9	−3.8	2.4
			" , DNP	− 8.2	− 7	+ 3.6	−3.4	2.4

semen of poor fertilizing capacity (types B and D) which, because of its good appearance and motility, might otherwise be considered satisfactory. The test may also be used for a periodic check on the quality of semen produced by bulls of fluctuating fertility. 3. Fructose, glucose, or lactate are not suitable as substrates since their utilization is in all cases increased by DNP.

Summary. Bull spermatozoa were graded according to their metabolic behavior on incubation with pyruvate, fluoride and 2:4-dinitrophenol. A correlation was shown between the type of response of the samples *in vitro* and the fertility of the donors in the artificial insemination of cattle.

1. Terner, C., *Biochem. J.*, 1951, v50, 145.
2. Melrose, D. R., and Terner, C., *Biochem. J.*, 1951, v49, i.
3. Clifton, C. E., *Advanc. Enzymol.*, 1946, v6, 269.
4. Tosic, J., and Walton, A., *Biochem. J.*, 1950, v47, 199.
5. Krebs, H. A., and Eggleston, L. V., *Biochem. J.*, 1940, v34, 442.
6. Friedemann, T. E., and Haugen, G. E., *J. Biol. Chem.*, 1943, v147, 415.
7. Barker, S. B., and Summerson, W. H., *J. Biol. Chem.*, 1941, v138, 535.
8. Olson, R. E., Pearson, O. H., Miller, O. N., and Stare, F. J., *J. Biol. Chem.*, 1948, v175, 489.
9. Mann, T., *Advanc. Enzymol.*, 1949, v9, 329.

Received May 21, 1952. P.S.E.B.M., 1952, v80.

Method for Determination of Vitamin A in Stool.* (19603)

ELIZABETH KAISER AND B. M. KAGAN.

From the Department of Pediatric Research, Kunstadter Laboratories for Pediatric Research, Medical Research Institute of Michael Reese Hospital, Chicago, Ill. This department is supported in part by the Michael Reese Research Foundation.

Studies conducted in this laboratory on the metabolism of vit. A required a method for the quantitative determination of the concentration of this vitamin in stool. A search of the literature failed to reveal a satisfactory method. The procedure reported by Wendt (1) involves drying the stool with anhydrous sodium sulfate and subsequent extraction of vit. A with alcohol. This procedure is time consuming and may destroy some of the vitamin. Wendt did not report the degree of re-

* This study was aided by a grant from the National Vitamin Foundation.