was added after the rats had been on the basal ration for 3 weeks. Evidently the rats become depleted in factor W during this time.

A small sample of crude crystalline material, made from the lactone of the hydroxy acid portion of the pantothenic acid molecule and reconjugated with β-alanine gave a growth of 1.1 g per day. The material was fed at a level equivalent to 50 micrograms of pantothenic acid per rat per day. Thus none of the concentrates gave growth equivalent to that obtained with liver extract, due to the lack of factor W, especially in the case of more purified preparations. Experiments on the supplementary action of pantothenic acid and factor W will be published later.

It is evident that the rat required in addition to the crystalline factors supplied in our basal ration an alkali labile factor, which is probably pantothenic acid. This factor will not completely replace the original liver extract or crude filtrate preparations, showing further the need for factor W.

The rat apparently requires the intact pantothenic acid molecule since the alkali-treated concentrates contain both the β-alanine and dihydroxy acid fractions. Stimulation due to β-alanine as present in our alkali-treated concentrates was not significant.

## 10830

## The Metabolism of Human Spermatozoa.*

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Though several papers appear in the literature describing some aspects of the metabolism of mammalian spermatozoa, little information is available on the metabolic behavior of *human* spermatozoa. McCarthy, *et al.*,[1] investigated glycolysis in human semen by measuring the decrease in sugar concentration at various intervals after incubation of the semen at 38°C. After 24 hours of incubation, they found that 75 to 90% of the sugar had disappeared but that, in some cases, the amount of lactic acid formed was not enough to account for the sugar used. They concluded that lactic acid is not

* Aided by a grant from the National Committee for Maternal Health, Inc.

1 McCarthy, J. F., Stepita, C. T., Johnston, W. B., and Killian, J. F., PROC. SOC. EXP. BIOL. AND MED., 1927, **25**, 54.

the end product of glycolysis in human semen. Goldblatt,[2] using essentially the same technic, came to similar conclusions. In neither case was an investigation made of the amount of sugar lost by oxidation to $CO_2$ and $H_2O$.

The work to be described here is part of a program designed to study in detail the metabolism of human spermatozoa and the enzyme systems responsible for the maintenance of metabolic function. The technic used was that of Warburg.[3] All results were obtained manometrically at 37.5°C.

Fresh specimens of spermatozoa, obtained from young, healthy individuals, were received in the laboratory within one hour after ejaculation. Since, in these manometric experiments, it was desirable to measure acid production in a non-retentive bicarbonate medium, the spermatozoa were centrifuged slowly out of the seminal fluid and resuspended in Ringer's solution containing 200 mg % glucose. For glycolysis measurements, bicarbonate was added to make a final concentration of 0.03M. For measurement of respiration, M/15 phosphate was substituted for the bicarbonate to make a pH of 7.4. Cell counts and calculations of motility were then made upon these final suspensions before measured amounts were added to the Warburg vessels. Nearly maximal motility is retained in Ringer's solution. For aerobic glycolysis, the vessels containing the spermatozoa were equilibrated at 37.5°C with a gas mixture containing 95% $O_2$-5% $CO_2$ for 15 minutes and for anaerobic glycolysis, a gas mixture containing 95% $N_2$-5% $CO_2$ was used. Respiration measurements were made in air and 100% $O_2$ but within these tensions of $O_2$, no difference was found. Measurements of respiration and glycolysis were made over a 3-hour period and the results below are the average gas consumption and production over that period expressed as $mm^3$ of $O_2$ and $CO_2$ per million cells per hour. Examination of motility was made at the end of the experiment.

It should be said at the outset that human spermatozoa in Ringer-phosphate show practically no respiration, so little indeed, that figures for respiration will not be presented here. However, the aerobic production of lactic acid as measured by $CO_2$ displaced from bicarbonate is exceedingly high relative to anaerobic glycolysis. In 80 experiments, the mean figures for aerobic and anaerobic glycolysis are:

| $mm^3CO_2/10^6$cells/hour in $N_2$ | $mm^3CO_2/10^6$cells/hour in $O_2$ |
|---|---|
| 0.100 | 0.080 |

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

[3] Warburg, D., *The Metabolism of Tumors*, 1930, Constable & Co., Ltd., London.

It will be seen that aerobic glycolysis is 80% of the anaerobic. Since, under careful experimental conditions, normal tissue shows high respiration and little or no aerobic glycolysis, human spermatozoa must be considered anomalous in that nearly all of the energy required for normal function is derived from glycolysis and *not* from *respiration.* This is demonstrated clearly in motility measurements made under aerobic and anaerobic conditions. Even after 10 hours in $N_2$, maximum motility is maintained and the glycolysis over that period, in most cases, remains linear. The same cannot be said of motility and glycolysis under aerobic conditions. Aerobic glycolysis after 3 hours tends to fall off with time and, after 3 or 4 hours, motility in $O_2$ shows a corresponding decrease. There are definite indications that the presence of $O_2$ inhibits the normal behavior of human spermatozoa.

*Summary.* The metabolism of human spermatozoa in Ringer-glucose is almost exclusively glycolytic and not respiratory. Aerobic lactic acid production is 80% of the anaerobic and falls off with time whereas anaerobic glycolysis is linear over a period of many hours. Maximal motility is maintained for many hours under anaerobic conditions but shows a marked tendency to decrease in air or in pure $O_2$. This suggests that the presence of $O_2$ has an inhibiting effect on the normal function of human spermatozoa.

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## Relaxation of the Pelvic Ligaments of the Guinea Pig Induced by Progesterone.*

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Early in the search for the active principle of the corpus luteum, 2 methods of extraction were reported to yield substances differing somewhat in their physiological effects. The crude extracts of Corner and Allen[1] induced progestational changes in the test rabbit.

* This work was carried out at the Biological Laboratory, Cold Spring Harbor, L. I., N. Y.

† John D. Jones Scholar, the Biological Laboratory, 1939.

1 Corner, G. W., and Allen, W. M., *Am. J. Physiol.*, 1929, **88**, 326.