Effect of Mucus on the Swimming-Rate of Sperms

It has been shown that bull sperm swim more slowly in mucus from the cow's uterine cervix than they do in saline¹. This slowing-down might be an effect of hydroxyl ions, since the pH of mucus is rather high², and it has been claimed that media above pH 8.3 are very harmful to sperm³. Alternatively, it might be due to the presence of inhibitory substances in solution, or to the visco-elasticity imparted to the secretion by very large flexible molecules.

Our present experiments indicate that probably it is the last of these factors which is the most important.

We have carried out experiments on the effects of varying the pH of simple saline/phosphate mixtures on the swimming-rate and survival of bull sperm. In the majority of individual experiments, and in the mean for all experiments pooled, it appeared that initially both the swimming-rate and percentage of motile sperm were higher at pH 8.5 than at 7.5 and 6.5 or in unbuffered saline. Although this advantage was transitory, its existence precludes explanation of the slowing down of sperm by mucus as a simple effect of pH.

Estrous mucus may be readily separated into a gel and a supernatant by centrifugation at 44,000g for 1½ h; the viscous supernatant yields a further precipitate after 4 h, at 103,000g⁴. The final supernatant is essentially non-viscous, but contains the soluble material, mainly protein and low molecular weight substances, present in the native secretion⁴. The behaviour of spermatozoa in this supernatant was examined.

The pH, after addition of a suitable amount of semen, was in the range 7.4–7.7. Supernatants from two separate samples of mucus were tested. The first of these was used as diluent for three samples of semen, and the sperm in each case were found to swim at about four-fifths the saline rate at the same cell concentration, as compared with about two-fifths the saline rate in the whole mucus. In the second experiment two samples of semen from different bulls were made up each at two dilutions, 20,000 and 5,000 sperm per µl., in saline and mucus supernate. Rates were measured at the beginning and also at the end of 1 h, and at the end of 3 h. At the beginning of the experiment the rate was considerably higher in mucus supernate than in saline at both dilutions, about 20 per cent higher at 20,000 and 30–60 per cent higher at 5,000. This difference persisted at the end of an hour, but at the end of 3 h the rates in mucus supernate were similar to those in saline.

Finally the supernatant from a large pool of a number of separate samples of mucus was tested. The rates of sperm from five samples of semen from five different bulls were higher in this material than in saline, in one case nearly twice as high. Although the difference diminished at the end of an hour the rates in mucus supernate were all still higher than those in saline.

It appears, therefore, that neither the high pH nor the presence of soluble-inhibiting substances can be considered as an explanation of the slowing down of sperm by mucus. The gel which may be spun out of the native mucus contains, after repeated washing with saline, very little of the soluble substances in the original secretion, but after appropriate dilution with

saline retains the rheological properties of the mucus. It appears to be a complex of sialomucopolysaccharide and protein⁵. In this material the spermatozoa behave as they do in native mucus, both as regards their velocity¹ and their orientation along strain lines⁶.

This work forms part of a programme of research supported by the Population Council Inc., The Rockefeller Institute, New York.

D. TAMPION
R. A. GIBBONS

National Institute for Research in Dairying,
Shinfield,
Reading.

¹ Tampion, D., and Gibbons, R. A., *Nature*, **194**, 695 (1962).

² Lardy, H. A., Pounden, W. D., and Phillips, P. H., *Proc. Soc. Exp. Biol., N.Y.*, **44**, 517 (1940).

³ van Duijn, C., Jun., and Rikmenspoel, R., *J. Agric. Sci.*, **54**, 300 (1960).

⁴ Gibbons, R. A., *Biochem. J.*, **73**, 209 (1959).

⁵ Gibbons, R. A., and Roberts, G. P., *Proc. N.Y. Acad. Sci.* (in the press).

⁶ Tampion, D., and Gibbons, R. A., *Nature*, **194**, 381 (1962).

Abnormal Crystals in *Amoeba lescheræ*

For the elucidation of a problem that I was investigating, it was necessary for me to have the developmental stages of *Amoeba lescheræ*. A quick method of obtaining these is to use material from a culture grown under natural conditions¹ containing a large supply of mature amoebæ. It will be remembered that in such a culture, or in a catch procured from a natural source in summer, there will be found¹: (1) mature and ageing adults; (2) encysted developmental stages of every variety; (3) spores; (4) possibly actively moving developmental stages. In the mature individuals there will be spores and differentiating spores. A sudden change in the pH (ref. 2) brought about by any agent not detrimental to the culture will kill off all the adults and so release any spores present in those adults. A study of the phenomenon is best made in Petri dishes (4 in. × 1 in.). After the lapse of a varying period of time (a week, more or less) subsequent to the changing of the pH, a search with the No. 7 objective water immersion lens will reveal large numbers of hatching spores, and creeping amoebulae of various ages. But all these have a tendency to encyst, after a feed, especially at night, so the best time to search the Petri dish for active specimens is towards noon and early afternoon.

I chose for my source of material a set of three amoeba cultures grown in shallow Petri dishes (7 in. × 1 in.) which, with a hand lens, were seen to be luxurious. Because of what I found when I examined the adults under higher magnification, it will be necessary to state the history of these Petri dish cultures. The top culture was started on February 12, 1961, in the usual way by putting adult amoebæ plus culture fluid into Glasgow tap water containing boiled wheat grains. Food was renewed on June 12, 1961. On September 1, 1961, the culture was passed through a butter muslin net sieve to remove old grains and matted mould growth. The filtrate was so luxurious in adults that it was divided into three sub-cultures.

In January 1962 the phenomena to be described were discovered. I began by pipetting material from the top dish of the three stacked cultures for exam-