isolated and innervated gracilis muscles of the recipient dogs were perfused with blood derived from a normal donor animal (R_s-innervated GM). The results of this study indicated a progressive fall in both gracilis muscle blood flows (GMF) and gracilis muscle vascular conductances (GMVC) through stage IIb (figure). However, as the experiment progressed into stages IIc and III there was a significant increase in both GMF and GMVC which we interpret as vascular decompensation.

In the 2nd group of experiments the innervated gracilis vascular beds of the recipient animals were perfused with blood derived from the shocked donor animals (D_s-innervated GM). Again the initial response was vasoconstriction in stages IIa and IIb; however, no vasodilation occurred in the subsequent stages IIc and III.

The 3rd group of experiments was similar to the 2nd except that the gracilis muscle was denervated (D_s-denervated GM). The results obtained from these 7 experiments also indicated persistent vasoconstriction which was not followed by a loss of vascular tone.

Conclusion. The data acquired from these 3 groups of experiments suggests that initial vasoconstriction is the result of both increased sympathetic nervous activity and elevated plasma levels of vasoconstrictor agents. In addition, since the vasodilation (vascular decompensation) occurred only in the hemorrhaged animals with intact sympathetic nerves (group 1), and not in either group 2 or 3 the possibility that a local neural reflex plays a significant role in the peripheral vascular failure is unlikely.

1 This work was supported in part by grants from the American Heart Association, Missouri Affiliate, Inc. and the American Osteopathic Association. Presented at the Spring AOA Research Conference, Chicago, Ill., March 15-17, 1979.
2 Acknowledgments. The authors wish to thank Mrs Gertrude Krueger and Mrs Carol Bond for their valuable assistance in typing and editing this manuscript, and to Mr Robert May and his staff for the graphics presented.
3 C.J. Wiggers, Am. J. Physiol. *144*, 91 (1945).
4 R.F. Bond, E.S. Manley, Jr and H.D. Green, Am. J. Physiol. *212*, 488 (1967).
5 R.F. Bond, E.S. Manning and L.C. Peissner, Circulation Shock *4*, 115 (1977).
6 R.F. Bond, L.C. Peissner and E.S. Manning, Circulation Shock *4*, 327 (1977).
7 C.F. Rothe and E.E. Selkurt, Am. J. Physiol. *207*, 203 (1964).
8 R.F. Bond, E.S. Manning and L.C. Peissner, Circulation Shock *6*, 43 (1979).

Effect of p-nitrophenylglycerol on motility of rat epididymal spermatozoa

Wung-Wai Tso[1] and Wai-Ming Lee[1]

Department of Biochemistry, Chinese University of Hong Kong, Shatin (N. T., Hong Kong), 2 April 1979

Summary. Using a convenient capillary tube assay, the antiswarming agent acting on *Proteus,* p-nitrophenylglycerol (PNPG), was found to have produced an antimotility effect in sperms from rat cauda epididymides.

The normal motility pattern in some simple procaryotic flagellates, such as *Escherichia coli* and *Salmonella typhimurium,* can be altered by the presence of many chemicals. These chemicals may exert their effects at any one of the following levels: behaviourally active compounds, including attractants and repellents, act by stimulating chemotactic orientation in the organism[2,3]; uncouplers inhibit motility by shutting off the energy source[4], while other compounds, though not toxic enough to cause an immediate lethal effect to the organism, may disintegrate the coordinated function of the flagella[5]. In addition to these compounds, p-nitrophenylglycerol (PNPG) has recently been reported to be an effective antiswarming agent in preventing *Proteus* contamination[6,7]. Kopp et al.[7] observed PNPG had no effect on the morphology or function of *Proteus* flagella and suggested that it may interfere with the mechanism of negative chemotaxis. Similar studies testing chemicals which might alter the motility of spematozoa were also reported[8]. It appears now that a list of motility-altering chemicals is available for a practical as well as a mechanistic investigation of motility.

The fertilization of eggs in higher organisms requires an aim-at-target directional swimming performed by the randomly distributed spermatozoa. It has also been reported that chemotaxis is essential for fertilization[9]. Various attempts have been initiated to identify effective male contraceptives which act in one way or another to stop fertilization[10-17]. While many of these chemicals have been investigated for their antispermatogenic effects, the approach of inhibiting fertilization at the level of disrupting the directional swimming should not be ignored. This report describes our study on the effect of PNPG in reducing spermatozoa motility.

Materials and methods. Spermatozoa were collected from cauda epididymides of rats weighing 270-320 g (Sprague-Dawley) and suspended in an isotonic pH 7.5 Tris buffer solution[18]. Cell debris was removed by passing the suspension through sterilized cotton cloth and the final concentration of spermatozoa was adjusted to 10 ± 2 million spermatozoa per ml. The quality of the spermatozoa was monitored microscopically, so that only samples from batches with 40% or more spermatozoa showing rapid forward motion were employed.

Quantitative assay of spermatozoal motility was done using a method similar to those employed in the study of bacterial motility[19]. This is a more convenient assay than that using flat capillary tubes[20]. The capillary tubes that contained the test medium were 75 mm long with an internal diameter of 1.1-1.2 mm (Kimble Co., Toledo, Ohio, USA). The capillaries were handled with forceps. One end was sealed in a flame to minimise physical disturbance due to convention and gravity. The capillary was then quickly passed several times through the flame and immediately plunged open end down into a small beaker (10-25 ml) containing about 5 ml of PNPG dissolved in buffer medium. As the capillary cooled, liquid was drawn in about 25-35 mm. This capillary was ready then to be inserted (without rinsing) open end first into a small test tube (10×75 mm) containing the spermatozoa suspension (10 ± 2 million spermatozoa per ml) to a depth of approximately 2 mm.

The temperature at which the motility was measured was kept at 24.5 ± 0.5 °C, which has been reported to be within the optimal temperature range for spermatozoa motility[21].

At the end of the experiment, the capillary tubes were carefully removed from the cell suspension. Their contents were all emptied and thoroughly mixed and aliquots were counted microscopically.

Chemicals used were of analytical grade and used without further purification. p-Nitrophenylglycerol was obtained from Sigma Chemical Co. and glycerol from Ajex Chemi Unilab. In all cases calculated weights of the chemicals were dissolved in the Tris buffer.

Results and discussion. The capillary tube method of assaying motility has been widely used in studies of bacterial motility and chemotaxis[3,19]. In an altered form, it has also been applied to the study of sperm penetration in cervical mucus[21,22]. Our results obtained by the modified capillary assay indicate a linear proportional relationship of sperm entry during the first hour (figure 1). This measurement presumably correlates with the total number of forwardly progressive spermatozoa and of slowly progressive ones[23]. A background entry number of 2000-3000 spermatozoa apparently came from some physical disturbance produced when the capillary tubes were inserted. A subtraction of this background number from the total gives the absolute number of spermatozoa that entered by progressive swimming. At longer incubation periods a reduction in the total number of spermatozoa presumably indicates a sedimentation effect.

For comparison, the number of spermatozoa entering in 30 min was recorded at various chemical concentrations (with a control). This is expressed in figure 2. PNPG has a noticeable inhibiting effect at about 10^{-4} M. The half motility inhibition dosage (ID_{50}) is 10^{-3} M; and at 10^{-1} M, essentially all spermatozoa were nonmotile. An experiment with glycerol, which protects sperms from cold damage[24], shows no inhibition at concentrations up to 10^{-1} M (data not shown). Because PNPG is known to be nontoxic to microorganism[6] and we observed no dysfunctional effects on the morphology of the spermatozoa at these concentrations, it is concluded that it has a specific inhibiting effect on spermatozoa motility.

Our preliminary study indicates the applicability of the capillary tube assay in a general survey of potential male contraceptive chemicals. We have shown that PNPG has an antimotility effect on spermatozoal motility. Kopp suggested that PNPG acts by interfering negative chemotaxis[7]. In spite of our finding that spermatozoal motility is inhibited by PNPG, there is no evidence, however, that spermatozoa repel each other. Whether PNPG is in fact operating in an identical manner to that seen in *Proteus* to inhibit motility awaits further investigations.

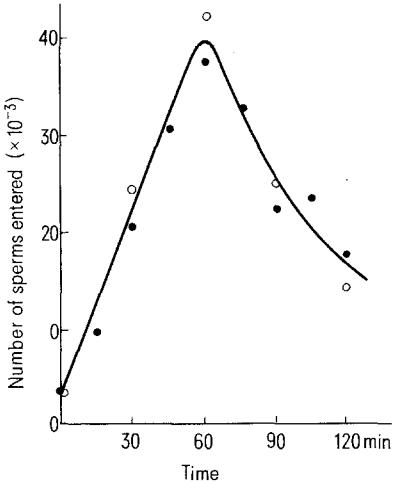

Fig. 1. Total number of sperms which entered capillary tubes at various time period. The results from 2 experiments were plotted (● and ○). Assay conditions were as described in Materials and methods.

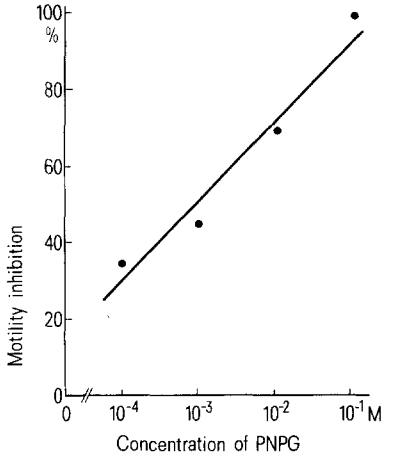

Fig. 2. Motility inhibition curve. The percentage was obtained from dividing the total number of sperms entering the capillary tube in 30 min in a uniform PNPG concentration by that in a control medium.

1 Acknowledgment. The authors are grateful to K.C. Tsui and Y.F. Lam of the Hong Kong Agriculture and Fisheries Department for valuable discussion.

2 J. Adler, J. gen. Microbiol. *74*, 77 (1974).
3 W.-W. Tso and J. Adler, J. Bacteriol. *118*, 560 (1974).
4 S.H. Larsen, J. Adler, J.J. Gargus and R.W. Hogg, Proc. nat. Acad. Sci. USA *71*, 1239 (1974).
5 M.L. DePamphilis and J. Adler, J. Bacteriol. *105*, 384 (1971).
6 B.W. Senior, J. med. Microbiol. *11*, 59 (1978).
7 R. Kopp, J. Muller and R. Lemme, Appl. Microbiol. *14*, 873 (1966).
8 L. Nelson, in: Fertilization, p. 27, Ed. C.B. Metz and A. Monroy. Academic Press, New York 1967.
9 Z. Dickmann, J. exp. Biol. *40*, 1 (1963).
10 T.J. Lobl and S.E. Porteus, Contraception *17*, 123 (1978).
11 J. Mathews and T.J. Lobl, Micron *1*, 297 (1976).
12 G. Corsi, G. Palazzo, C. Germani, P.S. Barcellona and B. Silvestrini, J. med. Chem. *19*, 778 (1976).
13 T.J. Lobl and S.E. Porteus, J. Reprod. Fert. *50*, 371 (1977).
14 B. Silvestrini, S. Burberi, B. Catanese, V. Cioli, F. Couston, R. Liscianni and P.S. Barcellona, Exp. molec. Path. *23*, 288 (1975).
15 M.S. Cohen, M.J. Colin, M. Golimbu and R.S. Hotchkiss, Fert. Steril. *28*, 78 (1977).
16 S.P. Lorton and N.L. First, Fert. Steril. *28*, 1295 (1977).
17 P. Talbot and L.E. Franklin, J. exp. Zool. *203*, 1 (1978).
18 D.J. Hanahan and J.E. Ekholm, in: Methods in enzymology, p. 168. Ed. S. Fleischer and L. Packer. Academic Press, New York 1974.
19 J. Adler, J. gen. Microbiol. *74*, 77 (1974).
20 R.N. Mills and D.F. Katz, Fert. Steril, *29*, 43 (1978).
21 R.A. Appell and P.R. Evans, Fert. Steril. *28*, 1329 (1977).
22 M. Ulstein, Acta obstet. gynec. scand. *51*, 287 (1972).
23 E.A. de Turner, N.J. Aparicio, D. Turner and L. Schwarzstein, Fert. Steril. *29*, 328 (1978).
24 W.C. Becker, P.L. Senger, E.P. Aalseth and C.E. Marshall, J. Anim. Sci. *44*, 1067 (1977).