

figure. The cAMP content of sperm taken from the epididymal caput and corpus are statistically higher than that found in the cauda of the organ. However, when the two first segments are compared, the difference is non-significant. In our observations, the highest contents of the cyclic nucleotide were found in areas where the secreting activity of the organ was found to be more active⁵.

Hoskins, Hall and Munsterman¹⁰ and Del Río⁵ proved the existence of a factor of epididymal origin which activates mammalian spermatozoa. The cells are known to be released immature from the testes, morphologically, physiologically and biochemically and then to acquire, during their transit through the caput and corpus of the organ, the fertilizing capacity observed in the cauda. Our hypothesis is that the epididymal secretion acts in the distal caput and body, and that this action is mediated through the cAMP. Although inhibitors of cAMP degradation have not been used (theophylline for instance) having worked at 0°C and processed samples immediately after extraction, allow us to disregard the possibility that the low levels found in the cauda are due to a hydrolysis process of the nucleotide resulting from an active breakdown of the already mature spermatozoon.

Investigations are in course to correlate the secreting activity of the epididymis with spermatogenic cAMP content, in order

to shed some light on the spermatogenic maturation phenomena.

- 1 The authors thank Dr F. Barbieri and E. Rothe for reviewing the manuscript, and R. Inés Ramos for his assistance in this study. The work was supported in part by Grant from the Programa Latinoamericano de Investigaciones en Reproducción Humana. P.L.A.M.I.R.H. (grant No. 34.95.2.75), Consejo Nacional de Investigaciones Científicas y Técnicas (Argentina) and the Fundación Lucio Cherny (Argentina). A.G.D.R. is Research Career member, National Research Council, Argentina.
- 2 A. G. Del Río, M. Gerrity, R. N. Peterson and M. Freund, 57th Annual Meeting of the Endocrine Society, 1975 (abstract).
- 3 A. G. Del Río, M. Gerrity, M. Couture, R. N. Peterson and M. Freund, 15th Annual Meeting American Society of Cell Biology, 1975 (abstract).
- 4 V. Kopečný, J. Reprod. Fert. 47, 403 (1976).
- 5 A. G. Del Río, in press (1977).
- 6 D. D. Hoskins, D. T. Stephens and M. L. Hall, J. Reprod. Fert. 37, 131 (1974).
- 7 D. L. Garbers, N. L. First, J. J. Sullivan and H. A. Lardy, Biol. Reprod. 5, 336 (1971).
- 8 N. Levine and D. J. Marsh, J. Physiol. 213, 557 (1971).
- 9 A. G. Gilman, Proc. natn. Acad. Sci. USA 67, 305 (1970).
- 10 D. D. Hoskins, M. L. Hall and D. Munsterman, Biol. Reprod. 13, 168 (1975).

PRO EXPERIMENTIS

An improved method for estimating the activity of a mouse with the photoswitch

H. Murakami and K. Kinoshita

Department of Hygiene, Faculty of Medicine, Kobe University, Kobe 650 (Japan), 29 August 1977

Summary. A new method for estimating the activity of a mouse by means of the photoswitch is contrived. This device can follow the activity with high fidelity and notable sensitivity, as compared with the conventional method.

Various devices, such as running-wheel¹, photoswitch², tambour trace³, pedal mechanism⁴, seesaw⁵, activity cage⁶, force recorder⁷, electromechanical force transducer⁸, and microphone⁹, have been applied to a method for estimating the general activity of the mouse or the rat. Of them, the photoswitch is one of the most widely used equipments, because it is very simple and comparatively inexpensive. This paper describes an improved method with the photoswitch by which the activity of an animal is recorded with higher fidelity and sensitivity than by any conventional method.

Apparatus. As shown in figure 1, a wire-mesh cage 10 × 10 × 10 cm³ in size is suspended from the ceiling of a shelf with a spring attached to each of the upper 4 corners of the cage. A steel pipe is used as a food hopper, in which pelleted food is supported by bent metal rods in order that a mouse can take the food between the pipe and the rods. Together with a water bottle, the pipe is attached to a clamp-shaped plate fixed to the ceiling, with the spouts of the bottle and the pipe inserted loosely into the cage. For this method, the cage is allowed to swing without hindrance whenever the animal moves inside of the cage. 2 pairs of light-source and receiver are provided for examining the activity simultaneously by 2 different methods. The infrared beam of a photoswitch is so adjusted as to pass the point 2 cm right above the centre of the floor of the cage. So the activity of the animal is detected directly through the interception of the beam by the body (the D-method). As the target for the beam of the other photoswitch, a small cylindrical bob with a chain is hung from the centre of the

floor of the cage. This photoswitch works by oscillation of the bob, whereby the activity of the mouse can indirectly be detected (I-method). The pulses generated by working of both photoswitches are recorded by the respective electromagnetic counters which are photographed by an automa-

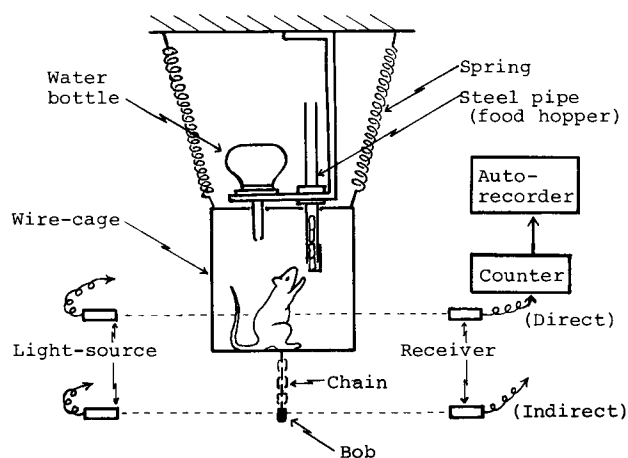


Fig. 1. Diagram of apparatus used in this experiment. When the mouse moves, the bob oscillates and intercepts the beam of photoswitch. The pulses generated by the interception are cumulated by the electromagnetic counter which is photographed by an automatic camera.

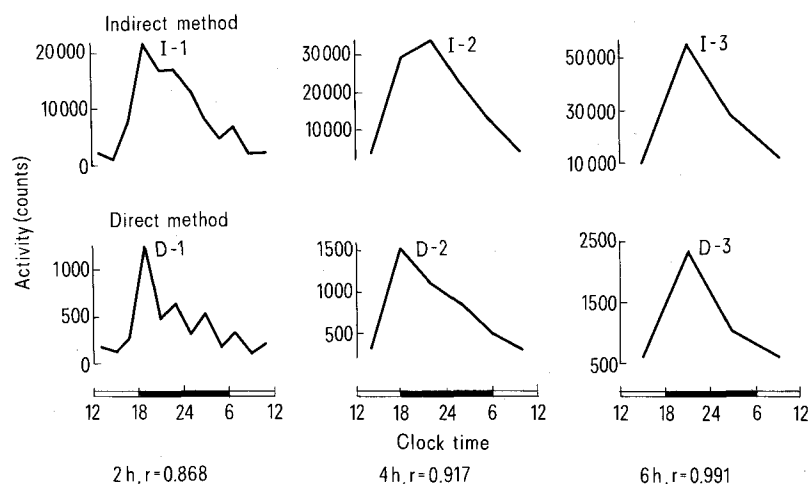


Fig. 2. The same mouse's activity plotted in increments varying from 2 h to 6 h. Top 3 curves show activity detected through the oscillation of the bob hung from the cage as shown in figure 1; Bottom 3 curves, activity detected directly through the interception of the beam of photoswitch by the body of the mouse.

ic camera every 2 h. The mechanism of this recording system is described in detail elsewhere¹⁰.

Results and discussion. Figure 2 shows an example of the same activity of a mouse observed simultaneously by the I- and D-methods. The count recorded by the former method is more than 10 times as high as that by the latter. The sensitivity to the activity of the mouse can be regulated by changing the length of the chain or the diameter of the bob. As shown in I-1 and D-1 of figure 2, there is a difference in the pattern of activity observed every 2 h between the I- and D-methods, although a significant correlation is proved between the results of the 2 methods ($r = 0.868$, $p < 0.01$). This discrepancy of pattern between both methods is caused by the fact that the D-method fails to record the activity of a mouse when the animal moves out of the beam. When recorded every 6 h, however, patterns presented by both methods are similar (I-3 and D-3), and the coefficient of correlation is 0.991. Figure 2 suggests that the

shorter the observation period, the less reliable the data obtained from the D-method. Conversely, the I-method is superior to the D-method in recording the activity for a short period.

- 1 M.F.W. Festing and R. Greenwood, *Lab. Anim.* 10, 81 (1976).
- 2 W. Poley and J.R. Royce, *J. abnorm. Psychol.* 72, 195 (1972).
- 3 L.E. Mount, *J. Physiol., Lond.* 190, 371 (1967).
- 4 E. Erkinaro, *J. Zool., Lond.* 168, 433 (1972).
- 5 M. Ohashi, S. Kurisu, H. Imai and H. Murakami, *Physiol. Behav.* 13, 321 (1974).
- 6 F.R. Hainsworth, *Am. J. Physiol.* 212, 1288 (1967).
- 7 A.A. Borbély and J.P. Huston, *Physiol. Behav.* 13, 795 (1974).
- 8 V.H. Denenberg, J. Gartner and M. Myers, *Physiol. Behav.* 15, 505 (1975).
- 9 L.E. Scheving, J.E. Pouly and T.-H. Tsai, *Am. J. Physiol.* 215, 1096 (1968).
- 10 H. Murakami and H. Imai, *Lab. Anim. Sci.* 25, 634 (1975).

Infusion at constant rate in vivo^{1,2}

J. K. Gong and C. DeLuca

State University of New York/ Buffalo, School of Dentistry, Department of Oral Biology, 4510 Main Street, Buffalo (N.Y. 14226, USA), 19 July 1977

Summary. Infusion can be maintained at a constant rate over an extended period of time in vivo by the use of an implanted diffusion chamber. Plasma ^{59}Fe was maintained at a constant level for 10 days when infused from a s.c. implant. Injected isotope was cleared exponentially with a half-clearance time of about 8 h.

The uptake of administered substances by cells or tissues in vivo occurs, usually, over relatively short periods of time due to rapid clearance from the circulation. It may be advantageous at times to maintain constant plasma levels of a metabolite or drug for a more extended period. It was found that infusion from an implanted chamber could serve this purpose.

Chambers used in this study were constructed with a Lucite ring, 0.6 cm thick, sectioned from tubing, 2.5 cm in diameter³. Type GS Millipore filters (0.22 μm pore size) in double layers were bonded to the ring to form a drum-shaped chamber. The use of GS filters prevented entry of host cells and minimized inflammatory response of the host⁴. Chambers were sterilized with dry heat at 80 °C for 48 h, then loaded through a hole pre-drilled radially at 1 point on the ring and sealed with a nylon screw. ^{59}Fe was placed into prepared chambers; these were then implanted either s.c. or i.p. For comparative purposes, the isotope was injected i.p.

7.5 μCi (as Fe Cl_3) were administered in 1 ml cell culture medium to young adult (ca 100 g) male rats of the ACI/f inbred strain. Blood samples were taken from the tail vein during the interval from 1 h to 18 days after implantation or injection of the isotope. Duplicate samples of 80 μl were taken with precalibrated microhematocrit tubes. Samples were centrifuged; the tubes were scored with a file and broken at the interface between the plasma and the red blood cells (RBC). Each fraction was counted in a well-type crystal scintillation spectrometer. Activity was expressed as the percentage of total dose (% TD) per ml of plasma or RBC, respectively.

Figure 1 shows ^{59}Fe profiles in the plasma following various routes of administration. Within 1 h, plasma levels in the injected animal rose to 30–100 times that in animals into which the isotope was implanted. This fell very rapidly in the injected animal so that 90% of the circulating isotope was cleared within the 1st day. Much lower, but much more