same antigen as an immunogenetic marker. There was no difference in the frequencies of the other antigens examined between Japanese patients with JOD and controls.
A comparison was made by dividing the JOD patients into those with and those without a family history of MOD (table 2). Obviously the number of the cases examined was too small to enable us to drow any conclusion. However, BW54 was still significantly increased (corrected $p < 0.002$) in the negative family history group as compared with controls, whereas in the positive family history group this was not the case.
This suggests a possible distinction between JOD with a positive family history of MOD and JOD without such a family history. This, however, has to be examined with a much larger number of patients.

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cAMP in spermatozoa taken from different segments of the rat epididymis[1]

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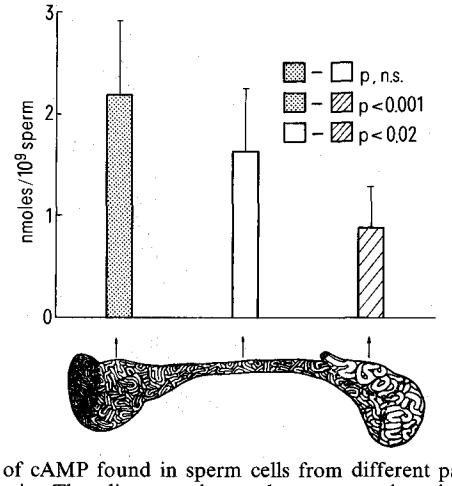
Summary. Experimental evidence conclusively indicates that the epididymis is under endocrine control and plays an active role in the process of spermatic maturation.

The mechanism by which the luminal fluid in the mammalian epididymis confer fertilizing capacity and maintain the viability of spermatozoa, is not known. Experiments performed in guinea-pigs[2,3], mice[4] and presently in rats[5] show that the epididymis is an active secreting organ of macromolecules which, once liberated into the duct lumen, specifically bind to spermatozoa. On the basis of these results, we assume that these proteic molecules synthesized in the epididymal tissue would account for the triggering of the spermatic maturation process.
Any approach to this mechanism requires a detailed knowledge of the changes which the sperm undergoes during its transit through the epididymis. Assuming that the spermatic maturation process is mediated by cAMP, we decided to evaluate the intracellular content of this nucleotide in spermatozoa obtained from different epididymal levels of the rat. Although the presence of cAMP has been reported for several mammalian spermatozoa, very little work has been devoted to the study of the sperm cyclic nucleotide under basal conditions during the epididymal transit[6,7].



Values of cAMP found in sperm cells from different parts of the epididymis. The diagram shows the punctured regions (head, corpus and tail).

Material and methods. 20 mature rats, weighing approximately 200 g, were used. Animals were anesthetized with sodium pentobarbital (Nembutal 30 mg/kg), i.p. The testis and epididymis were approached as 1 unit through a scrotal incision and the epididymis was not separated from the testis in order to maintain the blood supply. The field was bathed with prewarmed saline solution to prevent dehydration. We followed Levine and Marsh's technique for micropuncture. This method permits the extraction of spermatozoa of defined areas of the epididymal duct, assuring the possibility of working with pure samples, not contaminated with blood or epididymal tissue[8].
Immediately after collection, the samples were placed in saline (NaCl 0.96% at 0 °C). After being gently shaken, aliquots were extracted for sperm count (0.1 ml) volume which was replaced by TCA concentrated solution in order to obtain a final 8% concentration. Sperm counts were made in triplicate with a haemocytometer. After a sonic irradiation for 60 sec at maximum intensity, the sample was centrifuged at $2000 \times g$ during 15 min. Previous work has shown that, under our working conditions, spermatic rupture was almost complete. Cyclic AMP was determined by the method of Gilman[9], except that commercial bovine heart cAMP dependent protein kinase was purchased from Sigma Chemical Co. and used as the cAMP-binding protein. All reagents were of the best grade available commercially and were used without further purification. Data obtained in the experiments were analyzed by a Student's t-test for no pair samples.
Results and discussion. The cAMP levels found in spermatozoa obtained from different epididymal segments of the rat, as well as the puncture areas chosen, are shown in the

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 compaired, 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 active[5].

Hoskins, Hall and Munsterman[10] and Del Río[5] 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 spermatozoan.

Investigations are in course to correlate the secreting activity of the epididymis with spermatic cAMP content, in order to shed some light on the spermatic maturation phenomena.

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PRO EXPERIMENTIS

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

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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-wheel[1], photoswitch[2], tambour trace[3], pedal mechanism[4], seesaw[5], activity cage[6], force recorder[7], electromechanical force transducer[8], and microphone[9], 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 $10 \times 10 \times 10$ cm^3 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 automat-

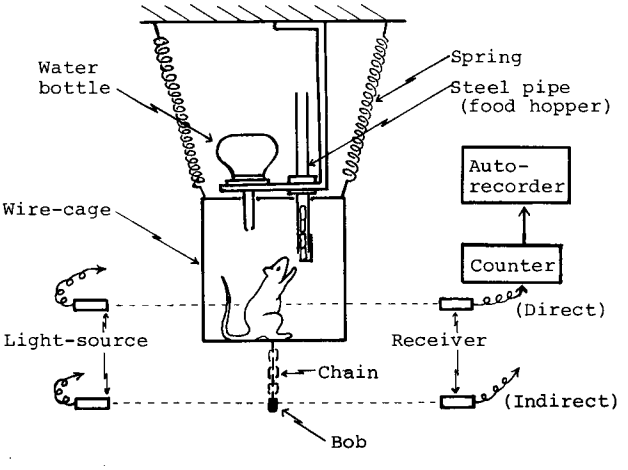

Fig. 1. Diagram of apparatus used in this experiment. When the mouse moves, the bob oscillates and intercepts the beam of photoswitch. The pulses generated by the interception are cumulated by the electromagnetic counter which is photographed by an automatic camera.