by our present data showing a small stimulatory, rather than inhibitory, effect of oestrogen-progesterone treatment on isoproterenol-induced NAT. The very low doses of isoproterenol (5 nmoles/l) which we have used when compared with the stimulatory dose of norepinephrine (10^{-4} moles/l)[5] could have different responses. Though in our hands, this concentration of norepinephrine gave identical results (in terms of NAT activation) in both treated and control animals (results not shown).

We attempted to study the role of progesterone in the above experiments by isolating its effect in vitro, i.e. by the addition of progesterone to cultures of pineals. Our preliminary experiments show clear differences in melatonin production when the culture media were assayed following stimulation. An analysis of the time course of this effect showed that progesterone exerts an inhibition of melatonin output (figure). A reduction in activity of pineal hydroxyindole-O-methyl transferase (HIOMT), the enzyme necessary for melatonin biosynthesis has been reported[12] in castrate female rats, chronically treated with progesterone. Also, it is known[13] that in the female rat HIOMT activity is depressed during pseudo-pregnancy, a period when progesterone levels are high. Experiments are in progress to further investigate, in terms of melatonin secretion, the effects of different concentrations of oestrogen/progesterone.

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HLA BW54 and B5 in Japanese diabetics with juvenile-onset and insulin-dependency (with special reference to the family history)

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Summary. The frequency of HLA BW54 and B5 in Japanese patients with JOD is increased and decreased, respectively. In JOD patients without a family history of MOD, the frequency of BW54 is significantly increased, whereas in JOD patients with a positive family history the frequency was not increased in a statistically significant manner.

There are some indications of a relationship between HLA, which is hereditary, and various diseases, including neoplastic diseases, viral infections and conditions with abnormal immune responses[1]. In recent publications the evidence that viral infection may be involved in the pathogenesis of juvenile-onset diabetes with insulin-dependency (JOD) has been presented[2,3]. Abnormalities in cellular immunity are also reported[4,5].

These findings suggest that there might be a possible relationship between at least some forms of diabetes mellitus and HLA. The increased frequency of HLA-B8 and/or BW15 in JOD among Caucasians was reported[6-9]. We reported a significant increase of BW54, previous J-1 or BW22-2, and a decrease of B5 in Japanese patients with JOD[10,11].

Diabetes mellitus is generally regarded as a genetically predisposed disease[12]. This appears to be obvious in maturity-onset diabetes (MOD)[13]. But the genetic contribution, as judged by family histories, to JOD seems to be less clear, because only less than half of the patients examined have a family history of diabetes mellitus[13,14]. JOD might be related to the genetic susceptibility to viral infection and/or abnormal immune responses.

In this study 27 Japanese patients with JOD were HLA typed according to NIH method[15], and these results were also compared to the absence or presence of a positive family history of MOD; 106 healthy subjects were also HLA typed as a control. All the subjects were typed for antigens A1, A2, A3, A9, A10, B5, B7, B8, B12, B13, B14, BW16, BW17, BW21, BW22, BW27, BW35, BW37, BW40 and BW54.

As clearly seen in table 1, JOD among Japanese is associated with an increased frequency of of BW54 (corrected $p < 0.002$) and a decreased frequency of B5 (corrected $p < 0.002$).

Our observation also revealed an increased frequency of BW35 and a decreased frequency of B5 in Japanese patients with Graves' disease[16]. Thus a decreased frequency in B5 appears to be a common characteristic in HLA phenotypes among Japanese patients with Graves' disease and JOD, while these two diseases among Japanese do not share, as far as an increased frequency is concerned, the

Table 1. Phenotype frequencies of HLA in Japanese JOD patients

	BW54		B5		B12	
	Control	JOD	Control	JOD	Control	JOD
(+)	22	17	58	3	8	1
(−)	84	10	48	24	98	22
χ^2	16.5		14.8		0	
p	0.0001		0.0001		NS	
Corrected p	0.0019		0.0019			

Table 2. Relation between BW54 and JOD with special reference to absence or presence of a family history of maturity-onset diabetes

	Control	Family history (−)	(+)
(+)	22	12	5
(−)	84	3	7
χ^2		20.1	1.4
p		0.0001	NS
Corrected p		0.0019	

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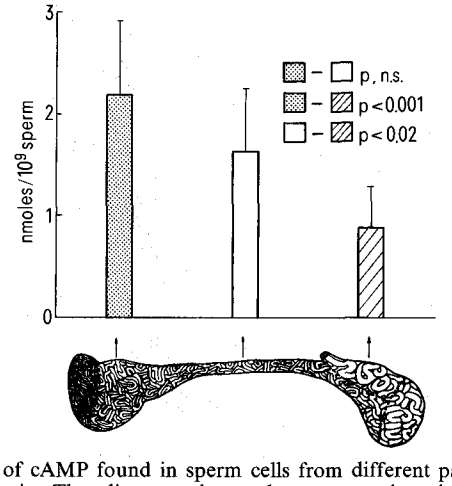
Summary. Experimental evidence conclusively indicates that the epididymis is under endocrine control and plays an active role in the process of spermatic maturation.

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