

thelium) 1000 nuclei were measured. In the control group 500 Sertoli cell nuclei were measured. The data obtained were worked out statistically.

**Results.** In cytological smears from control testes, the Sertoli cells were sometimes found individually, but most frequently in clusters. In their cytoplasm were embedded germ cells. In the smears from the testes with depopulation of the seminiferous epithelium there were clusters of Sertoli cells, as well as individual cells, but they were without germ cells. The Sertoli cell nuclei from this group showed a special morphological shape reminiscent of small fishes. They seemed somewhat elongated. The average length of the 1000 Sertoli cell nuclei measured in smears taken from 20 testes with the damage mentioned, was  $15.5 \mu\text{m}$  with a standard deviation (SD) of  $\pm 2.1 \mu\text{m}$ , and the width of these nuclei was  $10.5 \pm 1.9 \mu\text{m}$ . The average length of the 500 Sertoli cell nuclei measured in smears from 10 control testes was  $13.7 \pm 1.0 \mu\text{m}$  and the width of these nuclei was  $12.5 \pm 1.6 \mu\text{m}$ . The mean values of the lengths and widths of the Sertoli cell nuclei in smears differed significantly between depopulation and control groups ( $p < 0.01$ ).

**Discussion.** The depopulation of the seminiferous epithelium, i.e. the condition in which the seminiferous tubules are lined by Sertoli cells exclusively, was first described by Del Castillo et al.<sup>1</sup>. Only a few authors have concerned themselves up to now with the Sertoli cells in this condition<sup>2-7</sup>. In the present work we have described a special

morphological appearance of the Sertoli cell nuclei, which are reminiscent of small fishes. The measuring of the Sertoli cell nuclei in smears showed that they are elongated and narrower in the smears of the testes with depopulation of the seminiferous epithelium. This finding is in accord with the observation of Dym and Ray in the rat testis<sup>8</sup>. They described the disappearance of the nuclear envelope infoldings of Sertoli cells as a response to depletion of luteinizing hormone and testosterone. The change in the shape of the Sertoli cell nuclei in the direction of elongation may be explained by the disappearance of nuclear envelope infoldings. This change, which denotes de-differentiation of the nucleus<sup>8</sup>, is a reaction of the Sertoli cell to damage.

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## Vasoactive intestinal peptide (VIP) occurs in nerves of the pineal gland<sup>1</sup>

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**Summary.** Nerves staining with antibodies against vasoactive intestinal peptide (VIP) were detected in the pineal gland of the rabbit, cat and pig. VIP nerves were numerous in the cat but few in the rabbit and pig. A particularly rich VIP nerve supply was noted in the pineal stalk of the cat. The nerves were predominantly located around small blood vessels. Occasionally, nerve fibres were seen in the glandular parenchyma without obvious relation to blood vessels.

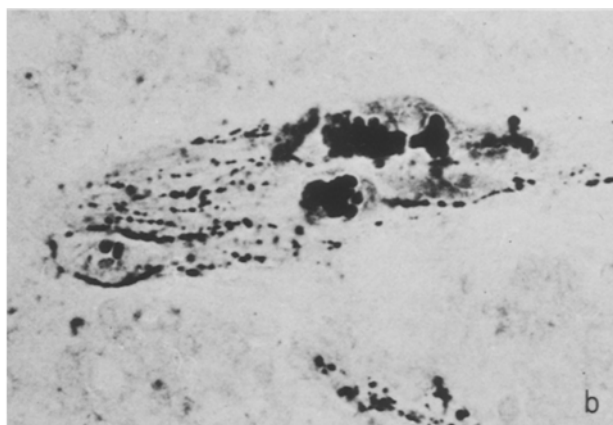
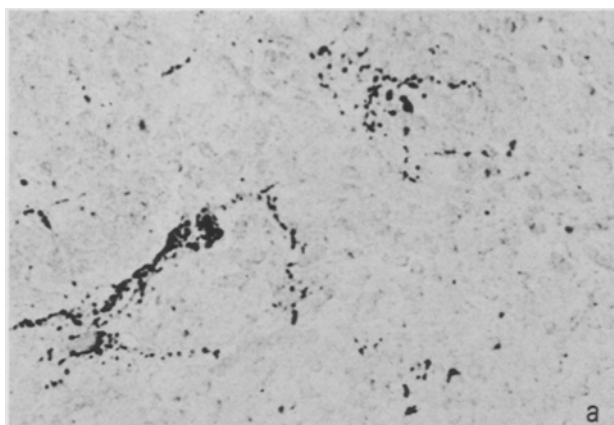
A number of putative neurotransmitter peptides have been identified during the last few years. Among such peptides are substance P, somatostatin, enkephalin and vasoactive intestinal peptide (VIP). VIP-containing neuronal elements occur throughout the central nervous system but predominate in the cortex and certain limbic structures<sup>2,3</sup>. VIP nerves also occur around cerebral blood vessels and in the choroid plexus<sup>4</sup>. In peripheral organs VIP nerves are generally associated with smooth muscle, blood vessels and exocrine glands<sup>5</sup>. We now wish to report that VIP nerves occur in the pineal glands of several mammals.

Pineal glands were collected from 4 rats, rabbits, cats and pigs. The rats were killed by decapitation under diethylether anesthesia and the rabbits and cats by bleeding under sodium pentobarbitone anesthesia. Porcine material was obtained from a local abattoir. The glands were frozen to the temperature of liquid nitrogen in a mixture of propane and propylene and freeze-dried. They were then exposed to diethylpyrocarbonate vapour for 3 h at  $55^\circ\text{C}$ <sup>6</sup> and embedded in paraffin in vacuo. Sections were cut at  $6 \mu\text{m}$  and processed for the immunohistochemical demonstration of VIP using the peroxidase-antiperoxidase (PAP) technique<sup>7</sup>. The VIP antisera (code 5603 and 98 P) were raised against highly purified porcine VIP. They have been characterized

in detail elsewhere<sup>8,9</sup> and have been used in several previous immunohistochemical studies<sup>2,4</sup>. Antiserum 5603 (provided by J. Fahrenkrug, Bispebjerg Hospital, Copenhagen, Denmark) was used in dilution 1:5280. Antiserum 98 P (provided by S.I. Said, University of Texas, Dallas, Texas, USA) was used in dilution 1:320. PAP complex (Cappel Labs, Downingtown, Pa, USA) was used in dilution 1:320. Control sections were processed with antisera inactivated by addition of excess antigen (30 nmoles of highly purified porcine VIP per ml diluted antiserum).

Both VIP antisera demonstrated immunoreactive nerves in the pineal glands of the rabbit, cat and pig. VIP nerves were numerous in the pineal gland of the cat, but were less numerous in the pineal gland of the rabbit and pig, and were not seen in the rat. The absence of VIP nerves in the rat pineal gland is in accordance with previous radioimmunological findings<sup>10</sup>. A particularly rich VIP nerve supply was noted in the pineal stalk of the cat. The majority of the pineal VIP nerves was associated with small blood vessels. In addition, scattered immunoreactive nerves occurred within the glandular parenchyma without relation to blood vessels. In the pig a few VIP nerves were seen also in the ensheathing pia.

VIP-containing nerves have a wide-spread distribution in



Cat pineal gland. PAP-procedure. Delicate varicose VIP immunoreactive nerves in the pineal parenchyma (a) and in the wall of small blood vessels (b). Unspecific staining over red blood cells  $\times 360$ .

the body. In the brain they are mainly located in cerebral cortical areas, hippocampus and hypothalamus<sup>11</sup>. VIP has a synaptic vesicular localization and can be released by a calcium-dependent, potassium-evoked mechanism<sup>12</sup>. Among known effects of VIP are relaxation of smooth muscle, dilatation of blood vessels and stimulation of intestinal and pancreatic secretion<sup>13-15</sup>. Its neuronal localization and strong biological actions make it tempting to assume that VIP exerts a neurotransmitter function.

The distribution of VIP immunoreactive nerves in the pineal gland suggests that they participate in the regulation of local blood flow. In addition, they probably influence glandular secretory activity.

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## The control of melanoblast differentiation in the periodic albino mutant of *Xenopus*

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**Summary.** Studies on the incidence of melanophores in older ventral trunk tissues and in isolated regions of periodic albino embryos of *Xenopus* suggest that melanin granule formation in mutant melanoblasts depends on an environmental contribution which arises at stage 43 in the endodermal tissues.

Periodic albinism is a mutation ( $a^p/a^p$ ) of *Xenopus laevis* which affects melanin synthesis. Mutant oocytes and embryos completely lack melanin. However during larval stages the pigmented eye epithelium and melanoblasts undergo a limited elaboration of melanin granules. Such granules later degenerate and post-metamorphic animals possess a typical albino phenotype<sup>1</sup>. Embryonic transplant and explant studies<sup>2-4</sup> have shown that the developmental action of the mutant gene is intrinsic in the neural crest derivatives and does not affect the ability of environmental tissues to support melanoblast differentiation. The mutant

gene appears to act at the level of melanosome assembly<sup>5</sup> and does not affect either the migration of melanoblasts or the numbers of these cells colonising the tissues (unpublished results). The present work attempts to clarify the circumstances surrounding the transitory ability of mutant melanoblasts to overcome their deficiency and synthesize melanin during larval stages. Melanin synthesis in mutant melanoblasts commences at stage 43 compared with stage 33/34 in wild-type cells<sup>1</sup> (staging according to Nieuwkoop and Faber<sup>6</sup>). This delay in differentiation of mutant melanoblasts may reflect changes in intrinsic melanoblast prop-