

of necessity, variable in their maturation, further complicates the problem.

The purpose of this investigation was to bring into the liver particles of the approximate size of schistosome eggs, coupled to antigenic schistosome fractions. These particles had to be tolerated, stable for long periods and suitable for easy histological processing. Sepharose-CL-4B beads were eventually chosen after trials with several kinds of particles, e.g. PVC, polyacrylamide (Biogel P-150; Aminoethyl Biogel P-150) and Sepharose-4B, as they fulfilled the requirements most adequately. For the introduction of the particles into mice 2 routes were tried; the coecal vein and the splenic vein. The latter way has the disadvantage that splenectomy is unavoidable, with a risk of influencing the granulomatous reaction and disturbing the experiment. For this reason the injection in the coecal vein was used throughout. The loss of animals as a result of the procedure remained within reasonable limits, being less than 10%. Mortality within 48 h is proportional to the quantity of beads injected above a threshold of approximately 20,000 beads. It appears from the results that SEA is able to induce in mice livers granulomatous reactions with collagen deposition similar in aspect and evolution to periovular schistosomal granulomas. The initially loose collagen matrix later turns into a dense connective matrix which then remains histologically unchanged for the rest of the experiment. The simultaneous presence of type I and type III collagen in collagen deposits supports these data⁶. Administration of a suspension of spleen cells from syngeneic infected animals enhances the reaction, which also becomes more preco-

cious. The absence of neutrophils in the reaction around the beads, and the absence of any Splendore-Hoeppli phenomena, suggest that mainly cellular hypersensitivity reactions of type IV are involved and enhanced. The effect of immunization on collagen deposition appears even more striking, though it is not yet clear whether this difference in fibrogenesis between primed and unprimed animals is a qualitative one or merely quantitative, as a consequence of the more important granuloma formation in sensitized animals.

The absence of any collagen deposition around particles without SEA confirms that periovular fibrosis is not merely a foreign body reaction¹¹.

No lesions which could be traced to immune reactions have been found in the liver parenchyma. Early focal necrosis is observed but is certainly due to mechanical circulatory disturbances through embolization of the beads. The post-necrotic scarring which eventually ensues is unrelated to and clearly distinct from the collagen deposition around the beads. No significant presinusoidal thrombosis related to the beads was observed.

It appears that the model discussed is well suited to following the evolution of reactions around egg constituents in the course of time, from a well-defined moment onwards, including the sequence of the deposition of collagen types. Furthermore, it becomes possible to evaluate the participation in the granuloma formation and in collagen deposition of purified antigenic fractions, in combination or not with different modalities of immunization.

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The change in length and width of the Sertoli cell nuclei in cytologic smears of testes with depopulation of the seminiferous epithelium

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Summary. The appearance of the Sertoli cells in cytological smears of testes with depopulation of the seminiferous epithelium is described. The mean values of the lengths and widths of the Sertoli cell nuclei in smears differed significantly between the depopulation and the control group ($p < 0.01$).

Today it is generally considered that Sertoli cells regulate spermatogenesis. The data on the normal morphology of the Sertoli cells in testicular smears as well as on the changes of their morphology under different conditions of damage to the testes would allow insight into the state of spermatogenesis. The purpose of the present work was to describe the appearance of the Sertoli cells in cytological smears of testes with depopulation of the seminiferous epithelium (Sertoli cell only syndrome).

Material and methods. The material investigated comprised the cytological smears from biopsy material from 20 human testes in which the histological findings in the

seminiferous epithelium showed depopulation. The control samples were obtained from 10 testes of men whose seminiferous epithelium in histological sections showed a normal aspect and also slight exfoliation. Cytological smears were made by the imprint method and stained according to the May-Grünwald-Giemsa method. The measurement of the lengths and widths of the Sertoli cell nuclei in the smears was carried out using light microscope (C. Zeiss Jena) exclusively under immersion (magnification: $\times 1080$). In the smears of every testis the lengths and widths of 50 Sertoli cell nuclei were measured. In the investigated group (depopulation of the seminiferous epi-

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.¹. Only a few authors have concerned themselves up to now with the Sertoli cells in this condition²⁻⁷. 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 testis⁸. 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 nucleus⁸, is a reaction of the Sertoli cell to damage.

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Vasoactive intestinal peptide (VIP) occurs in nerves of the pineal gland¹

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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^{2,3}. VIP nerves also occur around cerebral blood vessels and in the choroid plexus⁴. In peripheral organs VIP nerves are generally associated with smooth muscle, blood vessels and exocrine glands⁵. 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°C ⁶ 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) technique⁷. The VIP antisera (code 5603 and 98 P) were raised against highly purified porcine VIP. They have been characterized

in detail elsewhere^{8,9} and have been used in several previous immunohistochemical studies^{2,4}. 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 findings¹⁰. 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