

devoid of both glucose and progesterone may not be able to mobilize enough of their own glycogen stores to supply energy for hatching and subsequent development. This could explain why hatching was markedly decreased and was the only developmental event occurring in glucose-free BSA-supplemented BME. Further investigation into the energy related mechanisms involved in post-blastocyst development is currently being undertaken.

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## Effect of human seminal plasma on tumour-associated immunity in prostatic cancer. A preliminary report

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**Summary.** Evidence of significant suppression of tumour-associated immunity in patients with prostatic cancer by human seminal plasma (HuSPI) has been observed. Collation of the immunosuppressive property of HuSPI in this and previous studies, together with recent studies demonstrating experimental induction of prostatic cancer by spermatozoa and the relationship of prostatic cancer to sexual activity are suggestive of an etiologic role for SPI in prostatic cancer.

In examining the natural history of adenocarcinoma of the prostate<sup>1,2</sup>, we, as others, have been intrigued by the high incidence of occult carcinoma and wide variation in the age of onset of clinical disease. As possible explanations for these enigmas, the existence of the prostate as an immunologically privileged site due to its lymphatic anatomy, i.e., afferent lymphatics<sup>3</sup>, or immunosuppressive properties of its hormonal and/or secretory milieu or tumour-elaborated factors (in the case of carcinoma)<sup>4</sup> has been hypothesized. In an attempt to elucidate the role of these factors as contributory to the privileged status of the prostate, the effect of human seminal plasma (HuSPI) on cell-mediated tumour associated immunity (TAI) in patients with prostatic cancer<sup>5-9</sup> has been evaluated and as such is the subject of this preliminary communication.

**Materials and methods.** Peripheral blood leukocytes (PBL) were obtained from 25 patients with a confirmed histological diagnosis of adenocarcinoma of the prostate by Ficoll-Paque (Pharmacia Fine Chemicals, Uppsala, Sweden) centrifugation using a modification of the method of Boyum<sup>10</sup> as recently described<sup>9</sup>. PBL at a concentration of  $1 \times 10^7$  cells/ml in RPMI 1640 medium (Grand Island Biological Company, Grand Island, New York) containing 100 IU penicillin G/ml 100 µg streptomycin/ml, untreated and treated with 280 µg/ml HuSPI (obtained from 14 healthy adult males), determined as the optimal inhibitory dosage

from a dose-response curve, were incubated at 37 °C for 50 min in a mixture of 5% CO<sub>2</sub> in air. After incubation, cells were washed twice in RPMI 1640 medium and viability assessed by trypan-blue dye exclusion. Employing a modification<sup>9</sup> of the tube leukocyte adherence inhibition method<sup>11</sup>, untreated and treated patients' leukocytes were reacted independently with 3M KCl-(NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> extracts of allogeneic malignant prostate and bladder, as sources of specific and non-specific antigens<sup>9</sup>, and the number of non-adherent cells counted in quadruplicate using a Standard Neubauer haemocytometer.

Delineation of specific reactivity of prostatic cancer patients' leukocytes with malignant prostate (specific antigen) was made by concomitant reaction of the patients' leukocytes with malignant bladder as a source of non-specific antigen.

**Results.** The effect of HuSPI on TAI to allogeneic extracts of malignant prostate in 25 patients with prostatic cancer is shown in the table. Comparison of the significance of the difference in responsiveness of the patients' leukocytes untreated and treated with SPI when reacted with malignant prostate indicated a highly significant difference ( $p < 0.01$ ).

As further shown in the table and, in agreement with previous studies of the tissue-specificity of TAI in prostatic cancer patients, the reactivity of the patients' leukocytes

Effect of human seminal plasma (HuSPI) on tumour-associated immunity in patients with prostatic cancer

Peripheral blood leukocytes	Non-adherent cells obtained with allogeneic extracts of malignant (mean $\pm$ SD %):		Significance (p)
	Prostate (specific antigen)	Bladder (non-specific antigen)	
Untreated	20.4 $\pm$ 13.1	5.4 $\pm$ 4.4	<0.01
Treated with 280 µg/ml HuSPI	11.7 $\pm$ 8.1	5.8 $\pm$ 3.9	<0.05
Significance (p)	<0.01	>0.05	

with malignant prostate differed significantly from the obtained with malignant bladder ( $p < 0.01$ ). A slight but non-significant ( $p > 0.05$ ) increase in reactivity to bladder was noted following treatment of leukocytes with SPL.

Suppression of TAI by SPL was not due to a cytotoxic effect of the extracts or SPL, as the viability of leukocytes (determined by trypan-blue dye exclusion) incubated for 50 min in culture medium alone and in that containing either of the extracts or 280 µg/ml SPL was essentially identical.

**Discussion.** These initial observations demonstrate a significant suppressive effect of HuSPL on TAI in prostatic cancer patients and are in consonance with observations by others demonstrating the suppressive activity of SPL on a range of in vitro immune responses of normal murine and human hosts<sup>12-16</sup>.

While delineation of the biological significance of the suppressive effects of SPL will require further study, it may be noted that among other possibilities, suppression by SPL may represent a means of preservation of the species, whereby under normal conditions tolerance to spermatozoa in the male and in the female, following coitus, are maintained.

By the same token, this suppressive property of SPL may contribute to the failure of the prostatic cancer patient to develop a substantial immune response to his malignancy in the early stages of disease, i.e., studies of serum antibody<sup>17,18</sup> and, as yet unpublished observations of cellular responsiveness (Bhatti, unpublished observations) have demonstrated greater levels of TAI in patients with metastatic (stage D) disease than in those with localized (stage A) disease. Therefore, on the basis of : a) the suppressive properties of SPL demonstrated in this and previous studies<sup>12-16</sup>, b) experimental induction of prostatic cancer from sensitization by spermatozoa penetrating into prostatic tissue<sup>19</sup> and c) the relationship of prostatic cancer to sexual activity<sup>20</sup>, it is further hypothesized that unejaculated spermatozoa principally under conditions (psychological or physiological) placing limitations upon sexual activity, penetrate and sensitize the prostate under suboptimal conditions, e.g., chronic prostatitis, inducing the neoplastic process which culminates in a silent (occult) asymptomatic

carcinoma with no substantial stimulation of host responsiveness, despite aberrant tissue antigens, because of the milieu, i.e., seminal plasma.

Most certainly, the above hypothesis is not an all or none phenomena. It may however, particularly with regard to the suppressive effect of SPL on TAI, offer some explanation for the high incidence of occult prostatic cancer and the wide variation in the age of onset of clinical disease.

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## Some ultrastructural observations on the denervated skeletal muscle of frog

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**Summary.** The frog skeletal muscle resists atrophy for a long time after denervation. If, however, the entry of the nerve is prevented for more than 3.5 months, small muscle portions, sometimes containing nucleus, are sequestered from the parent muscle fibre. The basal lamina does not dissociate from these detached muscle portions.

While working on the denervated skeletal muscle (Rectus internus major and Sartorius) of the frog, *Rana esculenta*, for studying changes in the neuromuscular junction after denervation, we observed the detachment of small muscle portions from the parent muscle fibre. However, such morphological observations were made only after very long durations of denervation – above 3.5 months or so. After this long isolation of the nerve by 2 successive operations, the muscle atrophies and reduces in volume. The muscular membrane is seen convoluting at this stage and some muscle portions are observed detached from the parent fibre (figure 1). This observation was confirmed by the study of semi-serial sections. The basal lamina is observed in contact with the convoluted sarcoplasmic membrane as well as with the detached portions. A large number of

vacuoles of different forms are present immediately beneath the convoluted sarcoplasmic membrane. At certain places, a portion of the muscle with the nucleus is detached from the parent muscle cell (figure 2).

Such detachment of muscle portions from parent fibre after denervation has already been reported by Miledi and Slater<sup>3,4</sup>. During the 4th month of denervation, these authors reported a similar kind of atrophy and sequestration of muscle parts in rat. They have given a hypothesis that the multiplication of satellite cells could play a role in the isolation of muscle portions. After denervation, these satellite cells may send slender processes which penetrate the muscle fibre and may eventually divide it. We, however, did not observe such satellite cell processes during our study. Besides, in *Rana esculenta* a multiplication of satel-