

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 and, as yet unpublished observations of cellular responsiveness (Bhatti, unpublished observations) have demonstrated greater levels of TAI in patients with metastatic (stage D) disease than in those with localized (stage A) disease.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

Vinod Verma<sup>1</sup>

Laboratoire de Cytologie, Université Pierre et Marie Curie, Paris (France), 9 October 1978

**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-



Fig. 1. Transverse section of the muscle, 124 days after denervation. The sarcoplasmic membrane is convoluted (arrows) and small muscle portions (P) are separated from the parent muscle fibre (Mu). The basal lamina is in contact with the convoluted sarcoplasmic membrane as well as with sequestered muscle pieces. Note the presence of vacuoles underneath the muscle membrane.  $\times 39,500$ .



Fig. 2. Transverse section of the muscle, 115 days after denervation. A part of the muscle (P) is separated with its nucleus (N) from the parent muscle fibre (Mu).  $\times 15,200$ .

lite cells is observed from the 2nd month onwards of denervation<sup>5</sup>, whereas the sequestration of muscle portions takes place only during the 5th month or later than that. Miledi and Slater<sup>3</sup> reported that denervated muscle of *Rana temporaria* atrophies after a longer period than that of rat. In *R. temporaria*, there is no sequestration of muscle portions, even after more than 4 months<sup>6</sup>. Thus, the results of present investigation show that the denervated muscle of *R. esculenta* atrophies earlier than that of *R. temporaria*. This observation is complimentary to our previous research where we have shown the electrophysiological and morphological differences between these 2 species of frog<sup>2</sup>. Birks and collaborators<sup>6</sup> in *R. temporaria* and Miledi and Slater<sup>4</sup> in rat have described the dissociation of basal lamina from sarcoplasmic membrane in the denervated muscle. They suppose that during muscular atrophy, the sarcoplasmic membrane retracts and detaches itself from

the basal lamina. We never observed dissociation of basal lamina in case of *R. esculenta* even after a period of 4 months or more. Even the detached muscle pieces were lined with the basal lamina. We do not know whether to attribute this difference to the difference in animal species or to the preparation of the tissue.

- 1 Present address: Dr V. Verma, Department of Pharmacology, Postgraduate Institute of Medical Education and Research, Chandigarh-160012, India.
- 2 For material and method see V. Verma and M. Pécot-Dechavassine, *Cell Tiss. Res.* 185, 451 (1977).
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## Comparative evaluation of 7 helminth antigens in the enzyme-linked immunosorbent assay (E.L.I.S.A.)

F. Speiser and N. Weiss

Swiss Tropical Institute, Department of Medicine, Socinstrasse 57, CH-4051 Basel (Switzerland), 2 April 1979

**Summary.** 112 sera from Europeans with parasitologically proven helminthiasis were tested in the enzyme-linked immunosorbent assay (E.L.I.S.A.) against 6 crude extracts of various helminths (2 of adult worms: *Dipetalonema viteae*, *Fasciola hepatica*; 3 of eggs: *Ascaris suum*, *Toxocara canis*, *Schistosoma mansoni*; and of *Echinococcus granulosus* scolices) and against bovine hydatid fluid. Each serum was tested simultaneously at a fixed dilution of 1:160 against all antigens. Extensive cross-reactions were observed, leading to the conclusion that non-purified helminth antigens, even in combination, are of limited value for reliable serodiagnosis in E.L.I.S.A.

The enzyme-linked immunosorbent assay (E.L.I.S.A.) has already been used for serological investigations of several helminth diseases<sup>1,2</sup>. In most cases sera from people living in endemic areas and control sera from nonparasitized individuals have been evaluated. The specificity of E.L.I.S.A. as a tool for individual serodiagnosis has not yet been adequately analyzed.

In this study various non-purified helminth antigens were evaluated in E.L.I.S.A. using sera from Europeans who had been carefully examined for parasitic diseases. Multi-parasitic infections were rarely encountered. As crude helminth

antigens were used cross-reactions were to be expected. It was however of interest to determine whether characteristic reaction patterns of sera in this multi-antigen test system could be of help in the orientation of further clinical and serological examinations in cases of unexplained blood eosinophilia.

**Materials.** 112 sera were obtained from Europeans, most of whom had visited tropical countries. On the basis of parasitological findings, 96 sera were divided into 9 groups (table 2). In addition, 16 sera with positive toxocarosis serology, as well as 60 sera from people parasitologically