Letters to the Editor

Hypothesis: when is a seminoma not a seminoma?

In the February issue, Raghavan and his coworkers¹ reported a case of testicular "seminoma" with metastases to the para-aortic lymph nodes, which were treated by radiation; high concentrations of alpha-fetoprotein (AFP) were later detected in the serum. Fragments of subsequently biopsied persistent tumour in a lymph node were implanted in thymectomised, irradiated mice. The tumour grew in the form of solid sheets and trabeculae interspersed with microcystic areas containing Schiller-Duval bodies. The ultrastructural features of the xenograft tumour were typical of yolk sac carcinoma, and AFP was demonstrated in the blood of the mice by radioimmunoassay and in the cytoplasm of the tumour cells by the immunoperoxidase method. Re-examination of the slides from the primary and metastatic tumour then revealed "extracellular, eosinophilic, PAS-positive globules often related to minute cystic spaces."

The authors interpreted their findings as indicating a continuum of differentiation between seminoma and yolk sac tumour rather than the "mere" occurrence of a mixed germ cell tumour with seminoma and yolk sac elements. Although we agree with their conclusion that a solid form of yolk sac tumour exists^{2,3} and cannot exclude the possibility that a seminoma may differentiate into it, an alternative explanation for the findings appears equally plausible. In our opinion, both the primary and metastatic tumour were mixed germ cell tumours with predominant seminoma and a minor component of yolk sac tumour, an association that has been well documented.⁴ The yolk sac element of the neoplasm could easily have been transplanted and grown as a solid form of yolk sac tumour without necessarily having originated from transplanted seminoma. Only if exhaustive sectioning of the original tumour had shown no yolk sac elements, would the authors' conclusion have strong support from their experimental findings. We believe that the xenograft tumour, which they describe as consistent with an "anaplastic seminoma," has the appearance of a solid or hepatoid yolk sac tumour, which we have recently described in the ovary.^{2,3} No convincing photographic evidence for a seminoma-

tous component of the xenograft tumour is presented by the authors.

Each of our six hepatoid yolk sac tumours was composed of discrete solid masses or trabeculae of epithelial cells similar to those of the xenograft tumour; occasional very small glandular spaces were identified. Each tumour also contained numerous PAS-positive diastase-resistant intracellular and extracellular hyaline bodies. Ultrastructural examination of two specimens disclosed numerous free ribosomes, abundant rough endoplasmic reticulum and accumulations within dilated cisternae of electron-dense material corresponding to the PAS-positive bodies; intercellular canaliculi were lined by villi. Although bile was not identified, the appearance of the tumour cells was consistent with hepatic cells. In three tumours, both AFP and alpha-1-antitrypsin were identified in the cytoplasm and alpha-1-antitrypsin was also present in the hyaline globules. In only one of the tumours was a seminomatous component identified and it was distinct from the yolk sac component in that case.

It is well known that the human yolk sac in early embryonic life is in direct continuity with the primitive gut, which is sometimes referred to as the secondary yolk sac vesicle. In the polyvesicular vitelline pattern of yolk sac tumour (PVVT), distinctive vesicular structures with eccentric constrictions have been interpreted by Teilum⁵ as recapitulating the embryonic conversion of the primary yolk sac into the secondary yolk sac, reflecting an early differentiation of the tumour into primitive gut. Furthermore, Salazar and his coworkers⁶ have identified ultrastructurally cells in yolk sac tumours that resemble undifferentiated intestinal crypt cells and liver cells, indicating a more advanced embryonal differentiation.

In summary, we interpret the findings of Raghavan and his associates as indicating solid growth of the yolk sac component of a human mixed germ cell tumour in immunosuppressed mice. The evidence presented does not establish the ability of a seminoma to differentiate into a yolk sac tumour or to secrete AFP, nor does it support the concept that an anaplastic seminoma may be a solid form of yolk sac tumour. Indeed, AFP was undetectable in the serum in a recent series of 130 patients with pure testicular

seminomas, some of which were undoubtedly of the so-called anaplastic variety.⁷

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Professor Munro Neville replies as follows:

There appears to be agreement between the Boston group and ourselves that a solid form of yolk sac carcinoma exists. On the basis of our xenograft studies, we have interpreted this cellular pattern, at both the light and electron microscopic level, to be similar to that reported for anaplastic seminoma. Scully and his coworkers believe the pattern to be morphologically similar to one they have termed as "hepatoid" yolk sac carcinoma. Ultra-

structurally, this latter type of lesion contained cells with numerous ribosomes, abundant rough endoplasmic reticulum and canaliculi lined by villi. Such features are not shared by the solid cellular areas of the HX53 xenograft which resemble "seminoma cells" being regular in outline with rounded cell membranes and rare desmosomes and microvilli. The cytoplasm is "empty" with sparse organelles (including rough endoplasmic reticulum) apart from ribosomes, and prominent areas of glycogen.¹ These appearances then are totally unlike hepatic cells. Moreover, AFP is absent from the solid area of the xenograft but present in the hepatoid yolk sac lesions.

Since our paper was written, the HX53 xenograft has been passaged repeatedly with similar results. It has also been established in tissue culture for 14 passages and preliminary cloning studies have shown the evolution of two cell types. Moreover, it has been found to produce fibronectin of the yolk sac type.

The Boston group have only published their findings to date in abstract form so that we are unable to compare the morphology of "hepatoid" lesions. However, we continue at present to believe that a lesion morphologically resembling anaplastic seminoma is in reality a solid form of yolk sac carcinoma which may differentiate to produce AFP and bear the typical light and ultrastructural features of a yolk sac carcinoma. This seems a more feasible explanation than the continuing belief in a "mixed germ cell tumour" when such appearances are seen.

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Reference

¹Monaghan, *et al.* *Cancer* 1981; (in press).

A simple washing technique for solid-phase radioimmunoassays and enzyme-linked immunosorbent assays

The solid-phase radioimmunoassays and enzyme-linked immunosorbent assays (ELISA) utilise various forms of plastic solid-phase supports. Among currently available plastic solid-phase supports, the 96-well-microplates are perhaps most widely used for microassays, especially for

ELISA (known as microplate ELISA or micro-ELISA). The usual ELISA procedure includes three or four washing steps, each of which consists of multiple intermittent dispensing and aspirating of the washing solution.¹ Even with the use of semiautomated instruments, the washing is the most tedious part of the procedure. This letter describes a simple washing technique for such procedures utilising the previously described through-passage receptacles (TPR) as the solid-phase support.²

The TPR is made of polystyrene or other plastics. It has an upper portion, which serves as a funnel, and a lower portion, which has fin-like structures arising from the wall producing a large surface area (3.7 cm²) relative to the space (0.1 ml). Because of surface tension, the lower portion of the TPR retains approximately 0.1 ml of water against gravity. To facilitate washing, the TPR has a hole at the bottom. Twenty-four TPRs are assembled into a newly designed tray (Fig. 1). For simultaneous washing of the TPRs in a tray, I designed a 6 × 8.5 × 5 cm moulded plastic box ("wash box") having 24 holes (0.1 cm diameter) with short conical outlets at the bottom (Fig. 2). When the "wash box" is filled with water, approximately 10 ml of water flow through each hole for 30 seconds. The water drips from the conical outlets and each water drop is approximately 0.08 ml.

During the test, the bottom holes of the TPRs are sealed before adding 0.1 ml volumes of the specimen or reagent solutions. The sealing is done by pressing the TPR tray on to a sheet of Parafilm (American Can Company, Greenwich, CT, USA) overlying another tray which serves only as a support. After each incubation, the film is detached from the TPR tray, while the tray is in an inverted position. Most of the liquid content of the TPR is retained in the lower portion of the TPR. The inverted position is used to minimise the loss of the liquid during the detachment of the film. For each washing step, the TPR tray is placed on to a waste container and then overlaid by a "wash box," to which a predetermined volume of a washing solution is added (Fig. 3). When the liquid contents of the TPRs are identical, multiple TPR trays can be stacked and washed together using one "wash box" placed on to the top tray. Since only 0.1 ml liquid is retained in the TPR at any point of washing, each drop (0.08 ml) of the washing solution replaces a substantial portion of the TPR content.

Fig. 1 Twenty-four TPRs assembled into a tray.

Fig. 2 Two "wash boxes" with 24 holes at the bottom. The inverted box on the left shows the conical outlets of the holes.

Fig. 3 Simultaneous washing of TPRs. The washing solution is added to a "wash box" placed on to a TPR tray.

Fig. 4 Simultaneous emptying of TPRs by vacuum aspiration.