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Growth and Histology of Four Canine Mammary Tumour Lines Established in Nude Mice*

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Abstract—*A colony of nude (BALB/c/Nu/Nu) mice has been established and used to produce and study transplantable tumour lines derived from spontaneously occurring canine mammary neoplasms. Four primary tumours of differing morphological types were studied. Two were adenocarcinomas (MS306 and PD6014) with varying degrees of differentiation, the third (F5010) was a complex adenocarcinoma composed of both stromal and epithelial elements and the fourth (V5500) was a fibrosarcoma. Tumour fragments were implanted into the subcutaneous tissue of* 4- *to* 6-*week-old mice and resulted in tumour growth in each case. There was a latent period of* 1 month *for tumours PD6014, F5010 and V5500 and of* 3 months *for tumour MS306 before tumour growth ensued. After serial transplantation this period decreased to* 1–2 weeks *for the first* 3 *tumours and to* 6 weeks *for the last. After the initial lag period, tumour volume increased logarithmically in all cases with doubling times of* 6–20 days. *Each tumour line has been passaged through* 3 *serial transplantations with* 3 *of the tumours retaining their original histological appearance whilst the fourth became slightly more dedifferentiated.*

INTRODUCTION

THE DISCOVERY of the nude mouse [1] with its thymic deficiency [2] has provided an animal in which the consequent lack of cell-mediated immunological activity could be exploited for the heterologous transplantation of tissues without subsequent rejection. Model systems are required for the effective screening of anticancer agents and, as the development of new drugs is a lengthy and costly process, it is important that the behaviour of tumours in these systems resembles those spontaneously occurring in man and domestic animals. This is particularly relevant in the case of the hormone-dependent tumours such as those of the mammary gland, where chemically or virally induced tumours may not behave or respond to therapy as those occurring in nature do. Canine and human mammary cancers have a close resemblance in their behaviour, presenting with similar histological types [3–5] and also possessing oestrogen, progesterone and androgen receptors [6–8]. The transplantation of human solid breast tumours has met with little success [9–13], most progress being achieved with carcinoma cell lines [14–18].

This communication recounts the successful transplantation of canine malignant mammary tumours into nude mice, which provides a convenient *in vivo* system for the study of rapidly growing neoplasms that maintain their cellular morphology and receptor content and which can be used to screen possible anticancer agents.

MATERIALS AND METHODS

Materials

Heterozygous BALB/c Nu/$^+$ mice were obtained from Orlac Ltd., Oxford, U.K. Cage covers were supplied by Protoplastic Productions, Llanharran, Mid Glamorgan, U.K. Glass fibre filters were purchased from Evans & Adlard & Co. Ltd., Gloucs, U.K. Irradiated diet (Pilsbury modified rat and mouse diet) was kindly donated by the M.R.C. Pneumoconiosis Research Unit, Llandough, South Glamorgan, U.K. Dulbecco's modified Eagle's medium (DME) was bought from Gibco-Biocult Ltd., Paisley, U.K.

Animals

BALB/c Nu/Nu mice were bred in the Tenovus Institute Animal Husbandry Unit from heterozy-

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gous BALB/c Nu/$^+$ females and Nu/Nu males. Mice were kept under pathogen-limited conditions in covered filtered cages containing autoclaved sawdust and received a diet of irradiated pellets and acidified water (pH 2.8) *ad libitum*. No antibiotics were used. Under these conditions mice survived for 12–16 months. All animals were used for experimentation at 4–6 weeks of age.

Implantation of tumours

Canine mammary tumours were obtained with the cooperation of local veterinary surgeons and, after removal, were transported to the laboratory on ice for immediate use. Tissue diagnosed histologically as malignant was minced in DME and the resulting fragments (approx. 2 mm in diameter) injected subcutaneously on both flanks of male and female nude mice using a trocar.

Tumour volume was determined at weekly intervals by caliper measurements. When tumours achieved a diameter of 1.5–2 cm the host animal was killed by cervical dislocation and the tumours removed using aseptic techniques in a laminar air flow unit. Lungs, livers, kidneys, spleen and regional lymph nodes were also removed. After removal of samples for histological studies, the remainder of the tumour was divided into two; one part was used for the determination of receptors [19] and the other was minced in DME and transplanted into recipient mice. Each tumour removed was compared histologically with the original canine mammary tumour and the relative degree of differentiation and cellular necrosis noted.

Histological techniques

Wax and frozen sections were prepared and stained with eosin and haematoxylin.

RESULTS

Of 11 canine malignant tumours transplanted into athymic mice 8 showed growth, and of these 4 contained oestrogen receptors [19]. Subsequent studies have concentrated on these tumours.

It was possible to transplant these tumours serially into their third generation in nude mice. There was an initial latent period of 3 months before growth ensued with tumour MS306 but this decreased in subsequent generations to 6 weeks. Tumours V5500, PD6014 and F5010 had a shorter initial latent period of 1 month, which was reduced to between 1 and 2 weeks in subsequent generations. The doubling times of tumour V5500 (Fig. 1) was 11 days in the first generation and decreased to 8 days by the third generation. The initial doubling time of tumour MS306 was 20 days, which decreased to 10 days by the third generation. PD6014 had a doubling time of 18 days, decreasing to 6 days, whilst the equivalent reduction for tumour F5010 was from 12 to 6 days. This trend towards successively shorter doubling times with each serial passage is clearly illustrated by comparison of the time required for tumour growth to attain a volume of 1000 mm^3 in serial passages (Fig. 2). The 4 lines all showed 56–66% reductions in growth time between the first and third generations. The take rate and growth rates of all tumour lines were similar in both male and female hosts.

Tumours grew as discrete spherical or ovoid

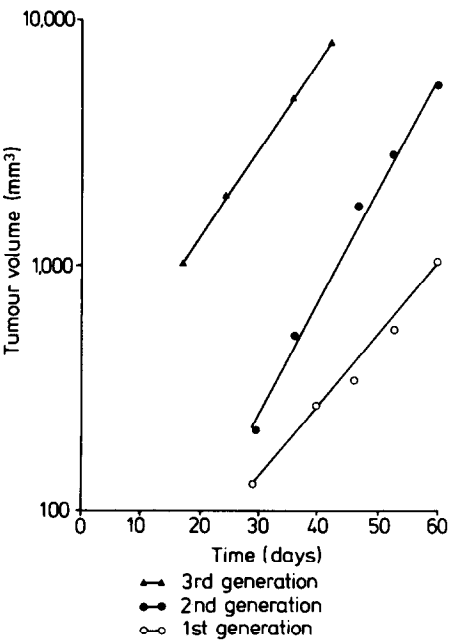

Fig. 1. Typical growth kinetics for tumour line V5500 in serial passages. Tumour volumes were determined by caliper measurements at the times shown for the growth of V5500 in first (○), second (●) and third (▲) generation tumours.

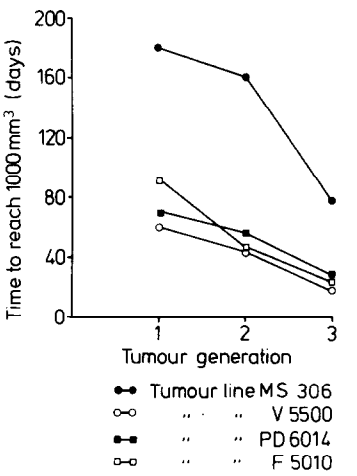

Fig. 2. Increase in growth rate with successive passages. The time taken for tumour growth to 1000 mm^3 was measured for implants of (●) MS306, (○) V5500, (■) PD6014 and (□) F5010.

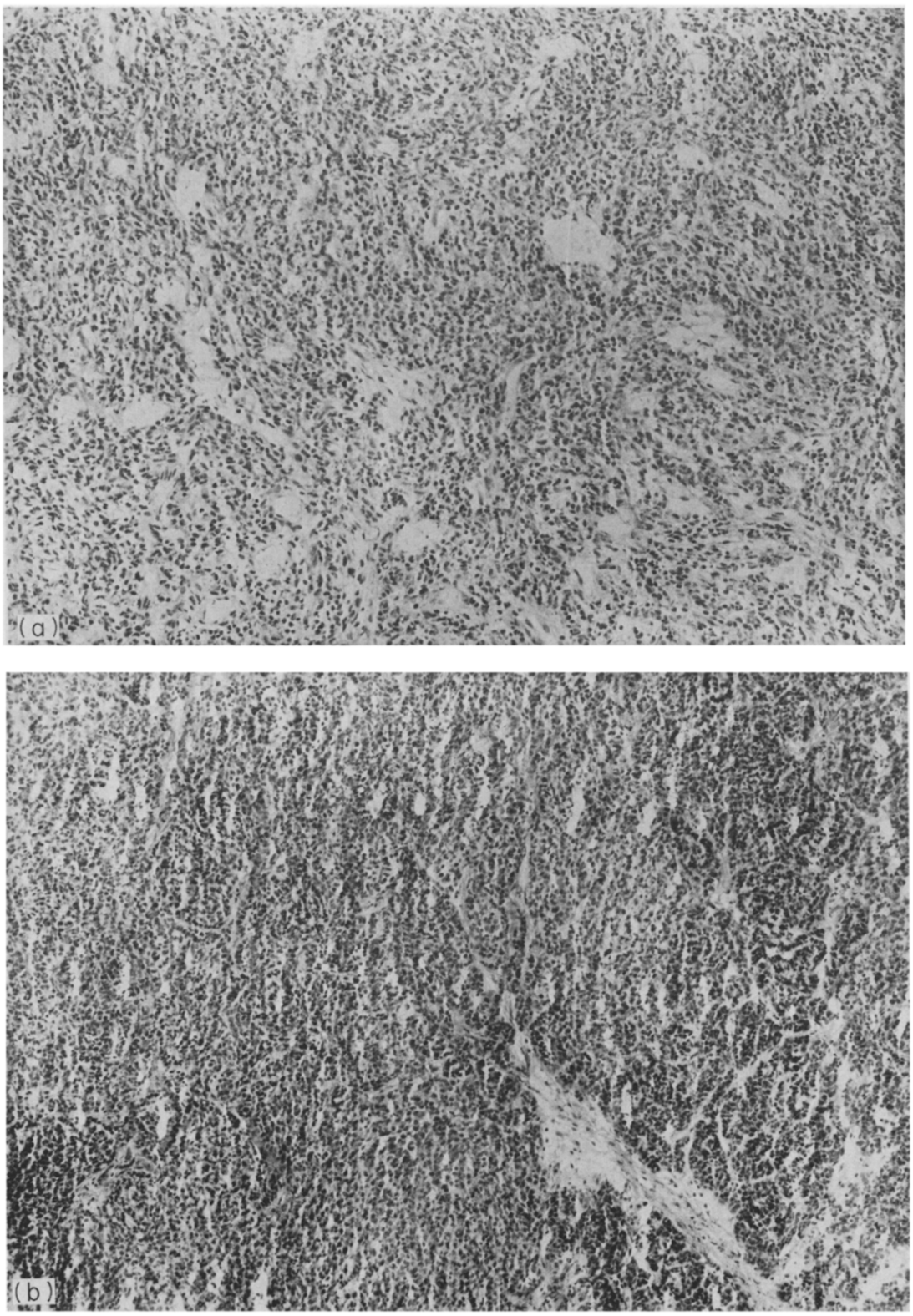

Fig. 3. Morphology of the MS306 tumour line derived from a canine mammary solid adenocarcinoma. (a) Primary tumour; (b) third-generation tumour line.

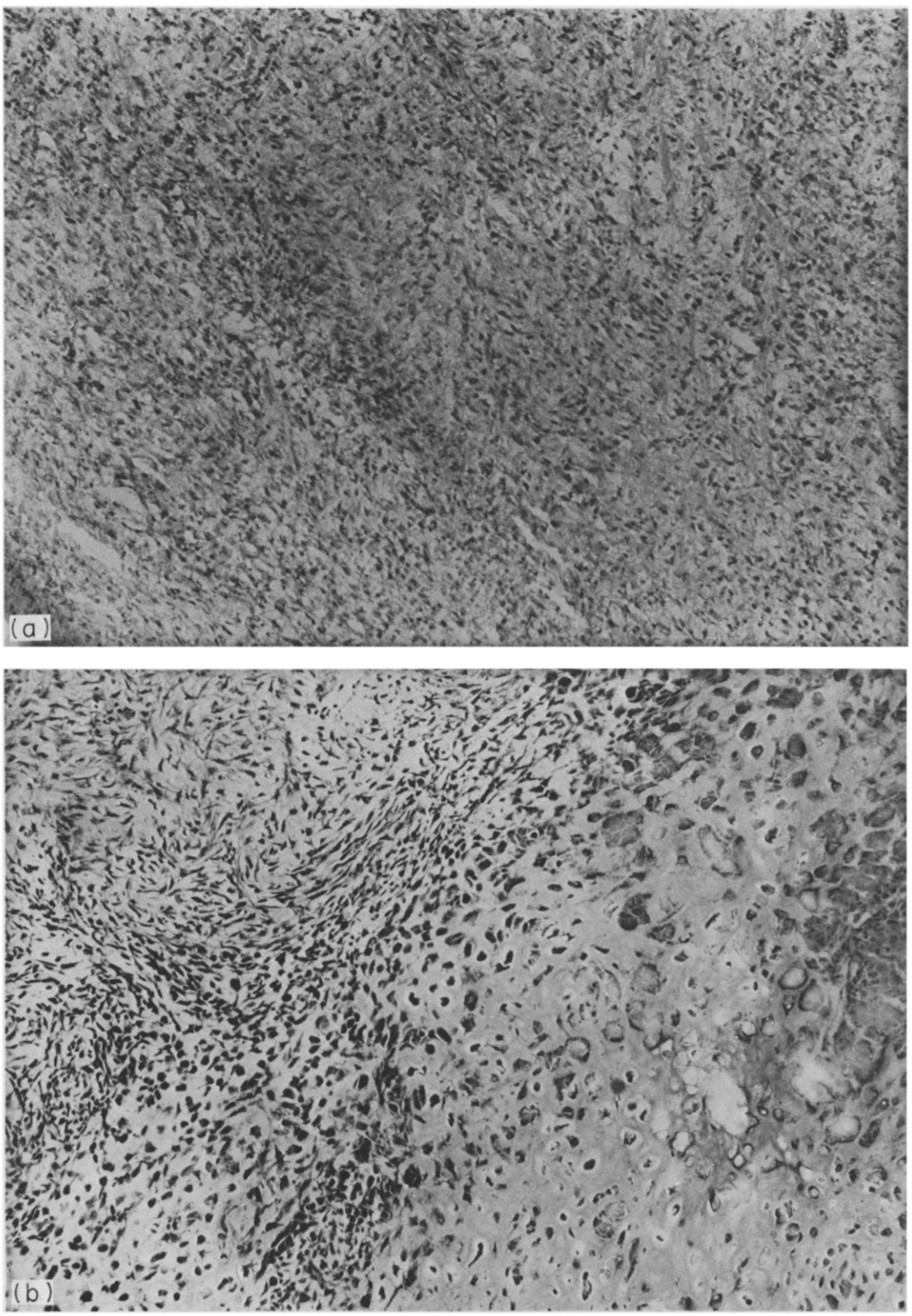

Fig. 4. Morphology of V5500 tumour line derived from a canine mammary fibrosarcoma. (a) Primary tumour; (b) third-generation tumour line.

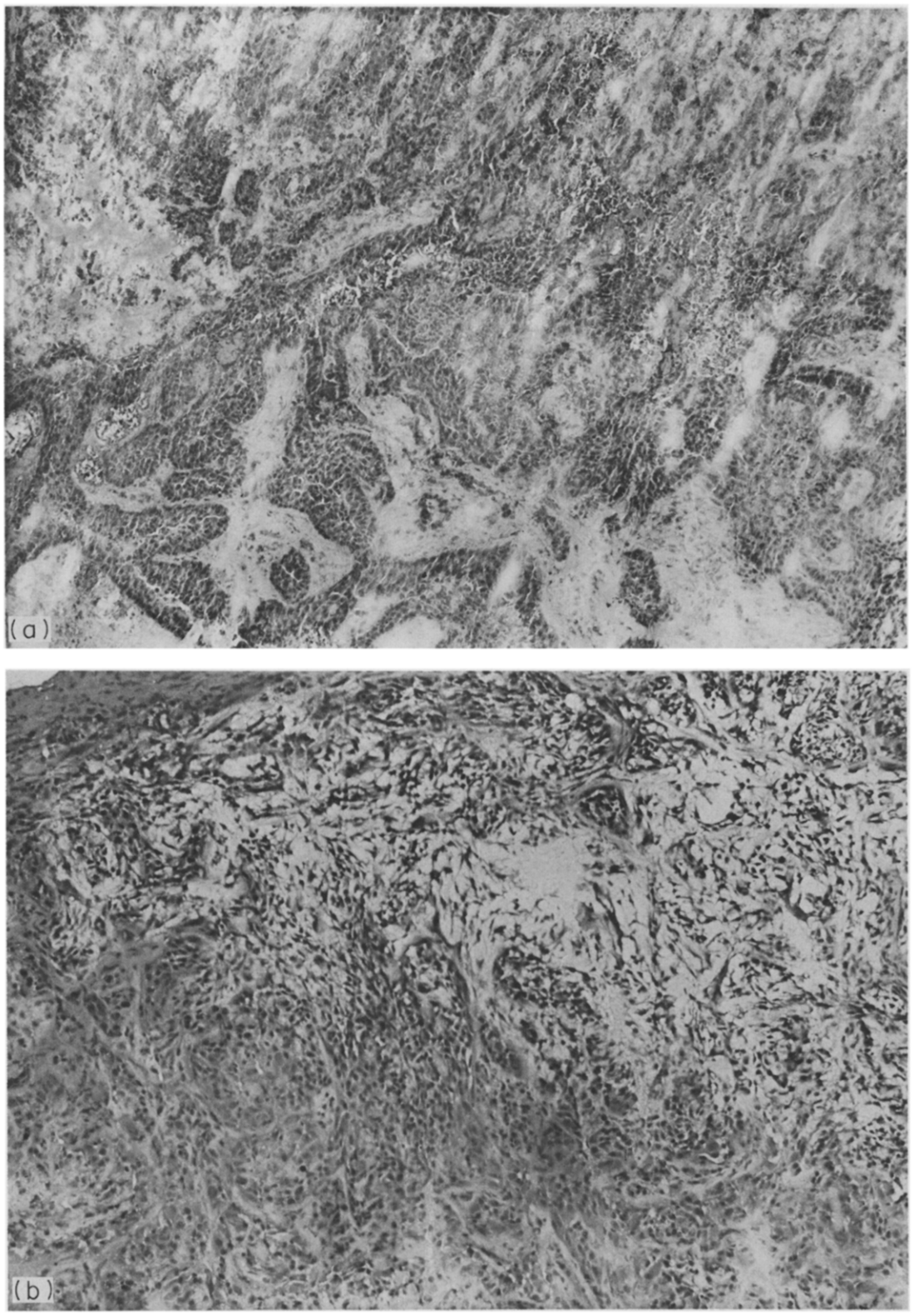

Fig. 5. Morphology of PD6014 tumour line derived from a canine mammary advanced adenocarcinoma. (a) Primary tumour; (b) third-generation tumour line.

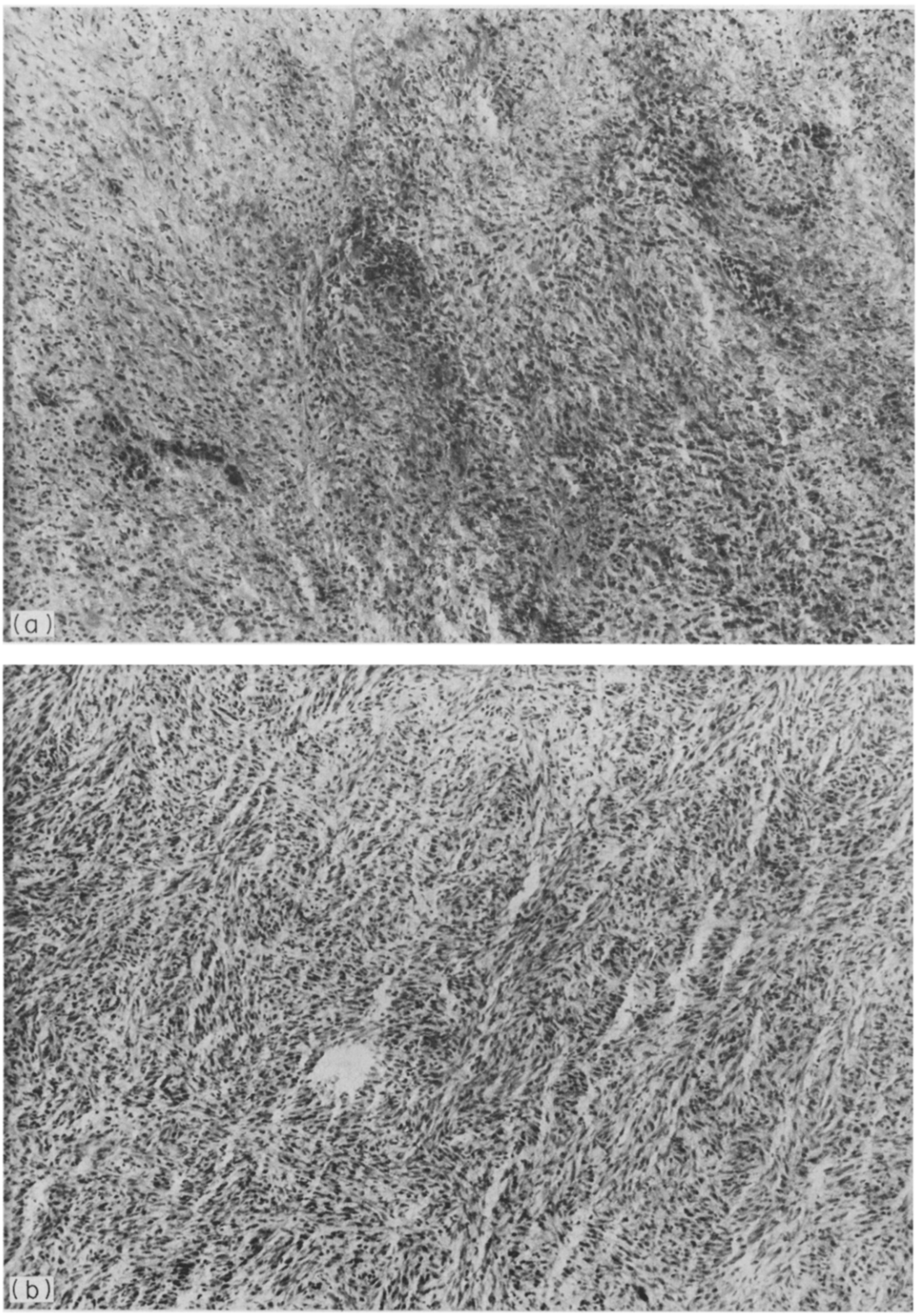

Fig. 6. Morphology of F5010 tumour line derived from a canine mammary complex adenocarcinoma. (a) Primary tumour; (b) third-generation tumour line.

masses at, or very near to, the point of implantation. Infiltration of surrounding tissue or the peritoneal cavity was only observed at advanced stages of growth (tumour volume $>3000\ mm^3$).

The cellular morphology and tumour growth pattern of each tumour line has remained essentially the same as that observed in the primary canine tumour. Tumour line MS306 was derived from a solid adenocarcinoma (Fig. 3a) displaying a poorly differentiated growth pattern. The cells were uniformly epithelial and generally cuboidal, arranged in solid masses supported by a diffuse fibrous stroma. Tumour cells did not exhibit cribriform or other pseudoglandular growth patterns. The primary tumour was vascular and well circumscribed, although local invasion was apparent in some areas. None of these characteristics had appreciably altered by the third generation (Fig. 3b) in nude mice. The tumour line V5500 was derived from a fibrosarcoma (Fig. 4a) in which the dominant cell type exhibited 'spindle' or fibroblastoid morphology. Cells were rapidly dividing and supported in a stromal matrix, the density of which varied between a loose fibrous and a dense chondroid consistency. Widespread pervasive infiltration of the surrounding tissue was a major feature of the primary tumour. Tumours removed at the third generation in nude mice showed identical morphology (Fig. 4b). The third tumour line, PD6014, originated from an advanced adenocarcinoma (Fig. 5a) with ductal or lobular origins, displaying infiltrating and lobular growth patterns. Foci contained closely packed cells and lacked pseudoglandular or other differentiated features. The predominant cell type was epithelial with some degree of pleomorphism. Histological examination of this tumour in the third generation in nude mice revealed a transition in tumour morphology to an exclusively solid, infiltrative growth pattern (Fig. 5b). The final line, F5010, was established from a complex adenocarcinoma (Fig. 6a) composed of epithelial and connective tissue elements. The primary tumour was highly anaplastic and locally infiltrative. Serially transplanted tumours (Fig. 6b) were identical in morphology to the original tumour.

DISCUSSION

The four solid cell tumour lines MS306, V5500, PD6014 and F5010, derived from canine mammary tumours, have been shown to be serially transplantable for up to three generations in non-immune-suppressed, non-hormone-treated nude mice. The take rate of all four tumours was 100% in both the initial implantation and in subsequent passages. This contrasts with that of previous studies of xenografts of human breast tumours in nude mice, which varied from 0 [20] to 13% [21, 22].

The original xenographs did not show any growth until 1–3 months after implantation, after which rapid growth ensued. This seemingly inactive period may be due to the apparent latency of breast tumour metastases [22] or to the dormancy of tumour cells [21]. It may also reflect the time required to establish vascular continuity with the host.

There was a marked increase in the growth rate with each successive passage, in compliance with reports by other workers [23], which is likely to be due to the adaptation of the tumour to the host animals. Each passage of the tumour provides an opportunity for the selection of the fastest growing cells, hence leading to a reduction in the lag time following implantation and to an increase in overall growth rate.

Microscopic examination of organs removed from tumour-bearing mice showed no evidence of metastatic growth from any of the four tumour lines. Metastatic rates of tumours in adult nude mice appear to be very slow [25–27], and due to the rapidity of growth of the tumour lines reported here it is likely that animals were killed before metastases were apparent. The low metastatic rate may reflect special growth requirements of the tumour lines. Subcutaneous tissue is known to contain only low concentrations of oestradiol [28] and, once established in such a region, an oestrogen-dependent tumour would be totally reliant upon vascularisation for growth. This situation might produce a growth pattern which would severely limit the local invasion of surrounding tissue [15]. In this study large-scale vascularisation was seen only after long-term (2 months) tumour growth, and only then was invasion of the surrounding tissue found.

The cellular morphology of all four tumour lines has remained unaltered through serial transplantation with the minor exception of the PD6014 line, which became slightly more dedifferentiated. These findings contrast with those of Ozzello and Sordat [16] working with tumour lines CaMa15 and MaMo4, which underwent gross dedifferentiation during passage.

The oestradiol receptor content has also remained constant through serial passages (subject of a further report) [19] and receptors have remained fully functional in terms of specific saturable binding of oestrogens.

This system offers great potential for the study of the effects of oestrogens and antioestrogens on tumour growth and of the mechanisms of action

of such compounds. Studies of this nature are now in progress.

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