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The Inhibitory Influence of a Metal–Plastic Implant on Cellular Proliferation Patterns in an Experimental Tumour Compared with Normal Tissue

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Abstract—*A simple metal–plastic implant was found to inhibit the growth of Walker* 256 *carcinoma in* 200 *out of* 230 *animals. In* 126 *animals no macroscopically identifiable tumour mass was evident and in* 74 *animals a very inhibited tumour nodule was found. In the remaining* 30 *animals tumour growth was observed around the implant.*

Detailed histological studies revealed that the implant had a profound inhibitory influence on the proliferative behaviour of the tumour. Where only partial inhibition of tumour growth occurred, distinctive patterns of tumour cell distribution and cellular proliferation were observed suggesting that the implants established a field effect in the tumour tissue.

INTRODUCTION

THE AUTOREGULATORY system which governs the size of mitotic cell populations in non-malignant tissue has been widely investigated [1–5] and it has been postulated that failure of this system results in neoplasia. However, there is evidence to suggest that some tumour cells retain a latent ability to communicate with each other, and that this ability may be influenced by the host environment [6]. Previously we have indicated that the presence of a platinum–silicone rubber implant may alter the ionic microenvironment of tumour cells so as to inactivate the division mechanism, thus resulting in inhibition of tumour growth [7].

These findings suggest that the platinum–silicone rubber implant is capable of regulating the usually uncontrolled cellular proliferation in tumour tissue. There are a number of examples of normal tissue which also undergo a high rate of continuous cellular replication but the size of the total cell population is maintained within specified limits by the tissue's inherent autoregulatory system [1, 4, 5]. The thymus is such a tissue and, advantageously, has a non-systematically mediated homeostatic mechanism [8]. In addition it is a discrete, well-defined organ which is readily transplantable between syngeneic animals. When neonatal thymus tissue is transplanted it follows a characteristic regenerative pattern. The typical thymic architecture is completely broken down and the lymphocyte population destroyed; but the cells of the reticular-epithelial framework survive. A blood supply to the graft is established three days after transplantation, the cell debris cleared and epithelial framework repopulated by host lymphocytes [9]. The characteristic appearance of a normal young thymus is regained by the 10th day [10]. For these reasons the thymus was utilized to assess the influence of the platinum implant on the controlled proliferation of normal tissue.

The present paper presents further details on the role of the implant during the initial interaction between tumour and host tissue as well as presenting some preliminary findings on the influence of the implant on the growth of transplanted neonatal thymus tissue.

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MATERIALS AND METHODS

Animals

Twenty-week-old male rats of an inbred WAB substrain were used as hosts throughout

the following experiments. The animals were maintained in a constant temperature, with alternating 12 hr periods of light and dark, and were supplied with a commercial pellet diet and tap water *ad libitum*.

Surgical procedures

Details of the construction and insertion of the platinum–silicone rubber implant have been described elsewhere [7].

Thymus tissue was obtained from 0-day-old syngeneic donors. The gland was inserted in the centre of the platinum loop which lay in a subcutaneous, mid-dorsal position. Control thymus glands were transplanted freely into a similar position in animals without implants.

Walker 256 carcinoma was obtained from 20-week-old donor rats bearing a solid subcutaneous tumour. A 5 mg fragment of a tissue fragment alone, from the same donor, was transplanted into a similar mid-dorsal position in control animals.

Animals bearing thymus grafts were killed 3 weeks after surgery, whilst animals bearing tumour grafts were killed 1, 2, 3, 4 and 6 days after transplantation.

Colchicine administration

Colchicine (0.2 mg/100 g body wt) was administered by i.p. injection 4 hr prior to death.

Histology

In animals bearing either thymus or tumour grafts, the tissue surrounding the loop and control tissue was removed and fixed in Bouins fluid for 36 hr, washed in alcohol, dehydrated and embedded in paraffin wax. Sections were cut at 5 μm. The tumour tissue was stained with Mayers haematoxylin and the thymus by the periodic acid leucofuchsin method [11].

Cell counts

In thymus tissue the total cell fragment numbers in 200 microscope areas (180 μm dia) were counted and the number of mitotic figures and cells with PAS-positive cytoplasm, excluding macrophages and neutrophils, were scored.

The tumour transplant, together with surrounding connective tissue, was examined and the results reported are examples of these sections. The total number of tumour cell fragments, together with the number of arrested mitotic figures were counted on the entire microscope section. The mitotic rate and cell density of each standard area counted were categorised in relation to the mitotic cell numbers and densities observed during the developmental stages of the control tumour. These mitotic rates and cell densities were then superimposed on camera lucida drawings of the sections counted.

The actual whole cell numbers for both types of tissue were obtained by application of the Floderus correction formula [12].

RESULTS

Walker 256 carcinoma has been utilized in over 1500 experimental inoculations in this laboratory and the tumour has never failed to "take" after 6 days. However, in a preliminary paper we reported inhibition of tumour in the presence of the platinum–silicone rubber implant in 92 out of 114 animals. Currently we have found tumour inhibition in 200 out of 230 animals, 6 days after tumour transplantation. In 126 animals the implant was encapsulated in connective tissue but no macroscopically identifiable tumour tissue was evident. However, in 67 of these 126 animals a very small population of organized tumour cells was found on microscopic examination of the tumour site; no such population was observed in the remaining 59. In a further 74 animals a small macroscopically visible nodule was present. Nevertheless, these nodules were greatly inhibited compared with free growing, matched control tumours. In the remaining 30 animals tumour growth was observed around the platinum loop.

In a freely growing Walker 256 carcinoma little growth occurs during the initial adaptive phase which lasts for approximately 3 days, but thereafter the tumour cell population grows exponentially until the time of death. It was found that the implant had a profound influence on the proliferative behaviour of the tumour cell population throughout this developmental period. Figure 1 shows the mean overall mitotic rates and tumour cell densities of tissue associated with the implants, compared with their matched control tumours, at stages up to 6 days after transplantation. It can bee seen that, in implant bearing animals, cell proliferation was significantly suppressed, compared with control levels, at all stages of tumour development. Two days after transplantation the rate of mitosis as assessed

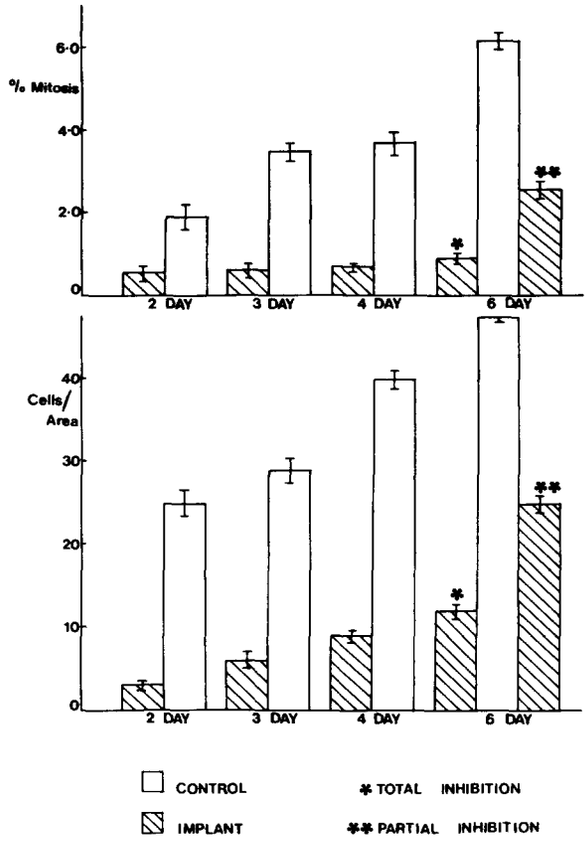

Fig. 1. Tumour cell mitotic rates and cell densities in control compared with inhibited tumours at intervals after transplantation.

by mitotic index was more than 300% higher in control tumours than in tumour tissue from implant bearing animals. From this stage, there was no significant increase in the rate of cellular proliferation in inhibited tumour tissue, whereas in freely growing tumours the mitotic index increased throughout the experimental period to attain a peak 6 days after transplantation. At this time it was inferred from the mitotic index that control tumour cells were dividing at a rate which was 5 times higher than that of inhibited tumour cells. Even in situations where tumour tissue was only partially inhibited, the rate of cell division was less than half that observed in control tumours.

These comparatively low rates of cell division were paralleled by similarly low tumour cell densities in the inhibited tumours, with tumour cells sparsely distributed amongst the connective tissue surrounding the implant loop (Fig. 1).

In order to obtain more precise information on the pattern of mitotic cell inhibition in the presence of an implant, the levels of mitoses and tumour cell densities were categorised for standard areas and superimposed on a camera lucida drawing of the section.

Twenty-four hours after transplantation both the control and implant sites consisted mainly of cell debris, infiltrated with host lymphocytes and leucocytes. A few viable tumour cells were present but little proliferative activity was observed. No difference was detectable between the two sites at this stage.

Forty-eight hours after transplantation host capillaries had begun to penetrate the control tumour fragments. The site had been almost cleared of cell debris and host cells. The tumour cells were evenly distributed throughout the control tissue mass (Fig. 2a). Although the proportion of cancer cells in division was low at the periphery of the tumour, adjacent to the discrete connective tissue capsule, in the major control area of the tumour a high mitotic index was observed (Fig. 2b).

Vascularization of the tissue surrounding the platinum loops of the implants was also well under way at this stage. No viable cells were found in the centre of the loop and the tissue around the loop consisted mainly of collagen interspersed with host leukocytes with few viable tumour cells. This pattern was reflected in the cell count analysis where the tumour cells were absent from the major

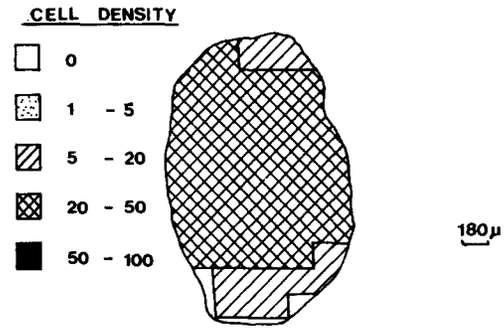

Fig. 2a. Cell density across a control tumour section 2 days after transplantation.

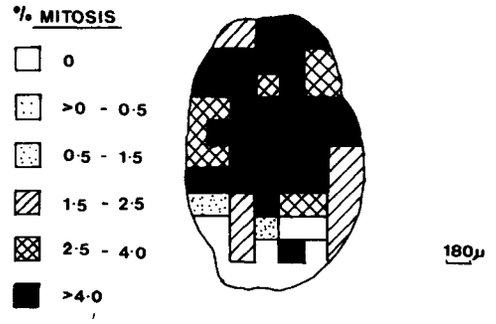

Fig. 2b. The distributions of cells undergoing division in control tumour tissue 2 days after transplantation.

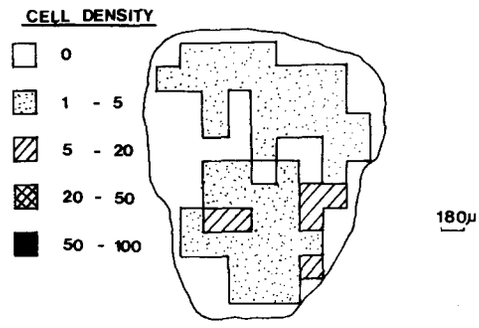

Fig. 3a. The cell density distribution of tumour cells in the tissue surrounding the platinum loop 2 days after transplantation.

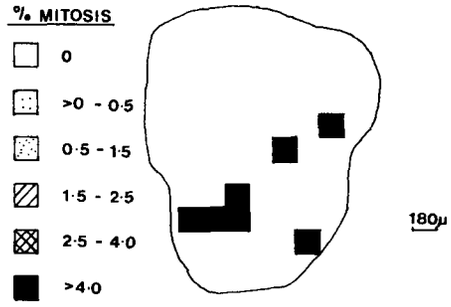

Fig. 3b. The distribution of dividing tumour cells adjacent to the platinum loop 2 days after transplantation.

portion of the tissue around the loop (Fig. 3a). Cellular proliferation was low in this small population of tumour cells and dividing cells were not found adjacent to the loop (Fig. 3b).

Three days after transplantation a thicker connective tissue capsule surrounded the control tumours, with low cell densities and few mitoses immediately beneath the capsule. Central to this region tumour cells were closely packed and appeared to be rapidly proliferating. In contrast the area in the immediate vicinity of the implant loop was devoid of tumour cells; the cells which were found outside the loop being dispersed between the bands of connective tissue around the loop. The overall tumour cell density of 6.65 ± 0.71 cells/area was significantly lower than the 29.50 ± 1.25 cells/area observed in the control tissue ($P < 0.001$). The majority of the tumour cells were situated at the periphery of the tissue furthest from the loop. The overall mitotic rate in inhibited tumours of $0.67 \pm 0.14\%$ was also significantly lower than the level of $3.49 \pm 0.26\%$ observed in control tumours ($P < 0.001$).

The distribution of the cell population of control tumours at four days was similar to that observed at three days (Fig. 4a). However, there was some evidence of necrosis.

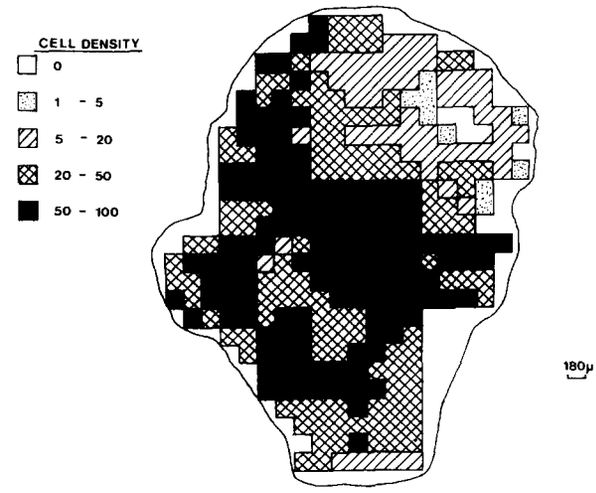

Fig. 4a. The cell density distribution of control tumour cells 4 days after transplantation.

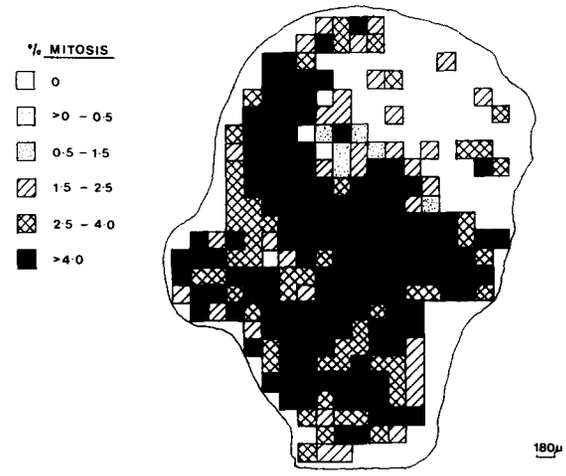

Fig. 4b. The distribution of mitotic tumour cells in control tissue 4 days after transplantation.

This was reflected in the distribution of mitotic cells, with areas of low mitosis located amongst areas undergoing rapid proliferation (Fig. 4b). At this stage marked differences were observed between the macroscopic appearance of the control tumour sites where the tumour measured about 150 mm^3, compared with the implant sites where there was no macroscopic evidence of tumour tissue. Microscopic assessment of the inhibited tissue, however, showed a sparsely distributed tumour cell population (Fig. 5a). The pool of proliferative cells was not evenly distributed throughout the inhibited tumour but was concentrated in small discrete areas of intense mitotic activity (Fig. 6b).

Between 4 and 6 days after transplantation the control tumours grew exponentially. The tissue consisted of densely packed tumour cells undergoing rapid proliferation (Plate 9, Figs. 6a and 6b) but with an increasing amount of

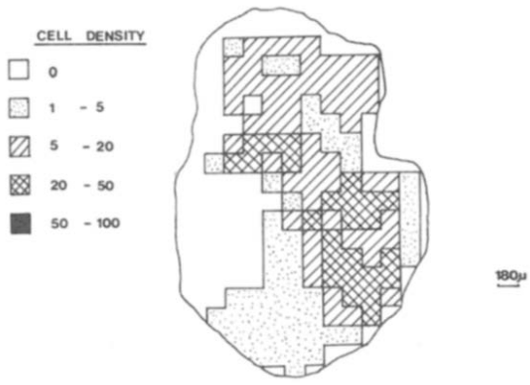

Fig. 5a. The distribution of inhibited tumour cells around the platinum loop 4 days after transplantation.

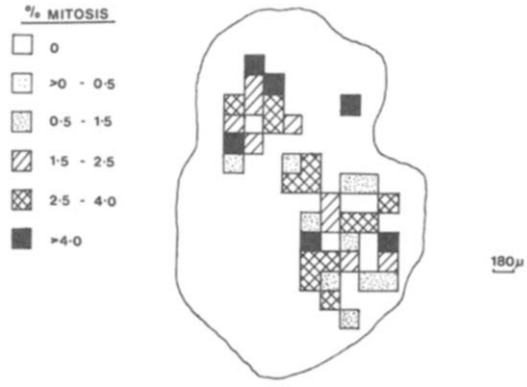

Fig. 5b. The mitotic activity of tumour cells around the platinum loop 4 days after transplantation.

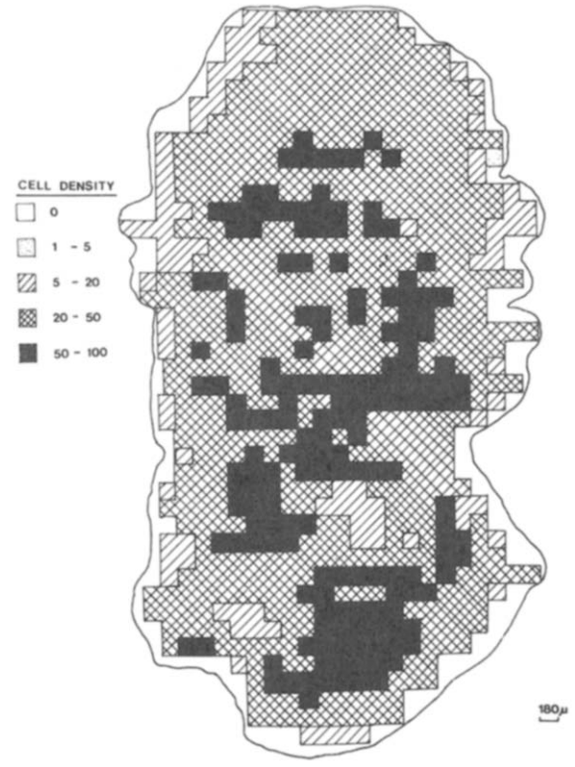

Fig. 6a. The cell density distribution in control tumours 6 days after transplantation.

necrotic tissue (Plate 10). In animals where tumour growth was totally inhibited by the implant the cells were sparsely, but evenly, distributed throughout the connective tissue (Fig. 7a). Cell division appeared to be totally inhibited in the areas immediately adjacent to the platinum of the loop (Fig. 7b).

It appeared, therefore, that there was a pattern of mitotic cell distribution in the presence of the implant. At all stages after transplantation mitotic cells were never found in close proximity to the wire of the loop, but tended to be grouped together into small isolated pockets of cell proliferation beyond the loop. This pattern is further demonstrated in cases of partial inhibition. In these situations microscopic examination showed very small inhibited tumour nodules, characterized by a low mitotic index, encapsulated in thick connective tissue which surrounded the loop. The tumour cell densities and mitotic indices observed near the loop do not resemble areas of cell necrosis but have the appearance of a totally inhibited tumour (Figs. 8a and 8b). Further out from the loop, regions of high tumour cell density and cell division, ap-

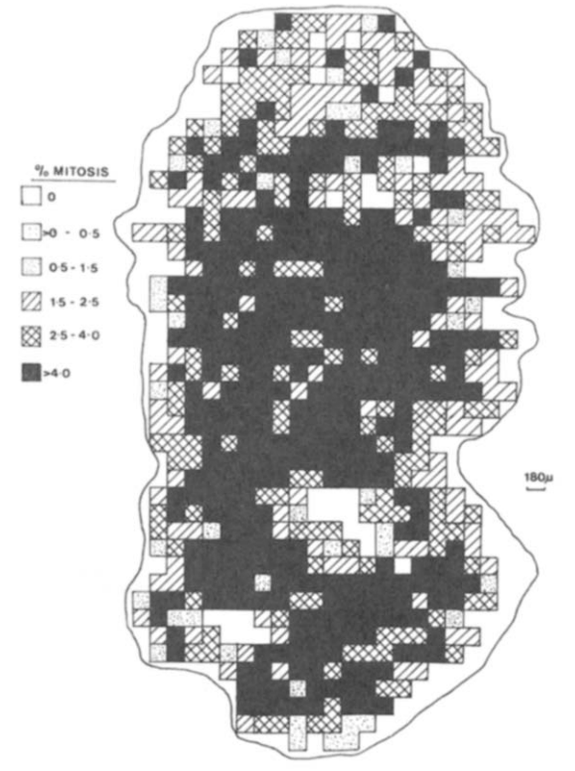

Fig. 6b. The distribution of mitotic cells in a control tumour 6 days after transplantation.

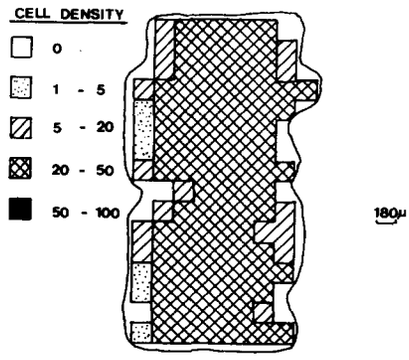

Fig. 7a. The distribution of tumour cells in a tumour totally inhibited by the implant 6 days after transplantation.

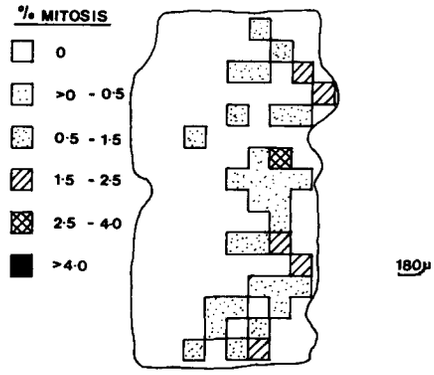

Fig. 7b. The distribution of mitotic cells around the platinum loop 6 days after transplantation.

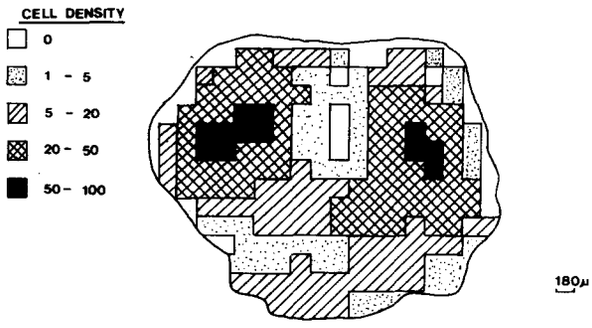

Fig. 8a. The cell density distribution of a partially inhibited tumour 6 days after transplantation.

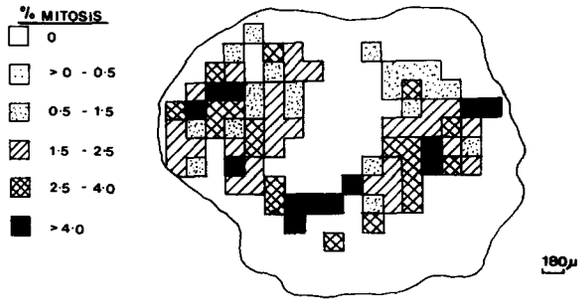

Fig. 8b. The distribution of mitotic cells in a partially inhibited tumour 6 days after transplantation.

proaching those found in control tissues, were observed (Figs. 8a and 8b). These distinctive patterns of tumour cell distribution and cellular proliferation have never been observed in control tissue.

Three weeks after grafting the cell density, mitotic cell and PAS-positive cell distribution patterns of thymic tissue are identical to those described for an intact thymus (Fig. 9a, b and c). When neonatal thymus tissue was transplanted within the loop of the implant the graft followed an identical pattern of regeneration to that observed in control transplants. Three weeks after grafting no significant difference was found in PAS-positive cell numbers, cell density or mitotic rate between controls and grafts within implant loops. In addition the cell distribution patterns were very similar between the two groups (Fig. 9). It would appear, therefore, that, despite the profound effect of the implant on tumour tissue, it had no influence on the cellular population of normal proliferating tissue, as exemplified by the transplanted growing thymus.

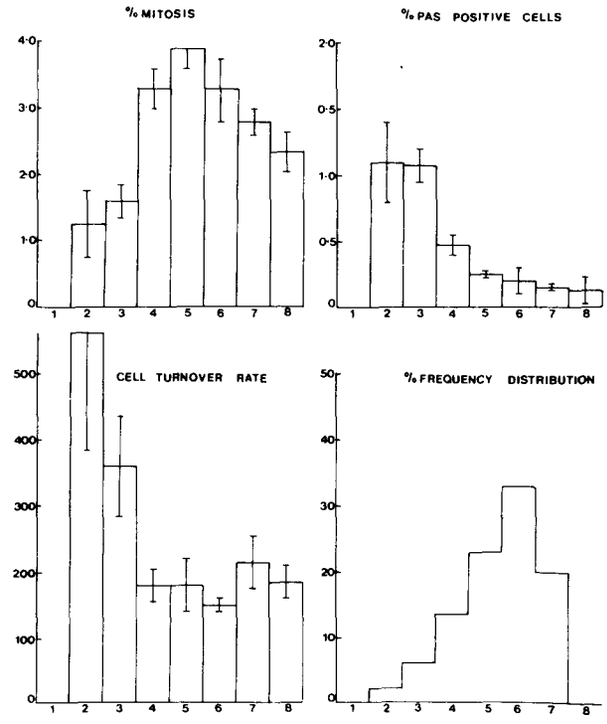

Fig. 9. Cell density, mitotic lymphocyte, PAS-positive cell and frequency distribution in relation to cell density categories in neonatal thymus transplanted 3 weeks previously within a platinum loop. (x-axis obtained by categorizing standard microscope areas according to the total number of cells counted, category 1 consisted of between 81 and 100 cells per area through to category 8 with between 221 and 240 cells per area.)

DISCUSSION

One immediate explanation for the inhibitory effects of the implant on tumour growth is the restriction of blood supply. However, it was clear that numerous blood capillaries had quickly infiltrated the tissue surrounding the loop and there was no microscopic evidence that the blood supply to the region had been affected. Certainly the physical constrictions of the loop did not affect angiogenesis since we have previously shown that implants consisting of a platinum loop with a silicone rubber tube alone do not inhibit tumour growth [7]. In addition the very labile lymphocyte population of thymus grafts were unaffected by the implant.

Previous work has shown that the distinctive architecture of the thymus is paralleled by an equally distinctive cell density distribution pattern [1]. The rate of cellular proliferation bears a specific relationship to the cell density distribution. The mitotic rate increases with increasing cell density to attain a peak at the mean cell density and thereafter declines. It has been suggested that PAS-positive cells, located mainly in the medulla, produce inhibitors and stimulators of mitosis and that it is these internally produced factors, in relation to cell density, that play a vital role in the autoregulatory mechanism which specifies tissue mass [13]. This complex relationship between the cellular components of the thymus is maintained throughout the life-span of the tissue, as well as after transplantation. The presence of the implant had no significant effect either on graft size or the cellular components of thymic tissue. It would appear therefore that the implant may only inhibit growth in malignant tissue.

When considering the mechanism of action of the implant on tumour tissue it must be borne in mind that the present findings suggest that the implant inhibits tumour cell proliferation rather than destroying the tumour cells. In our preliminary report on the retardation of tumour growth we suggested that tumour cell proliferation was inhibited by the implant inducing an alteration in the ionic environment within the tumour inoculum. It was postulated that the potential difference which is usually established between tumour and host tissue is prevented by the implant acting as a conducting pathway for electrons. Thus the tumour site may be maintained at an electrochemical potential similar to that found in healthy tissue.

If this explanation is correct then an electrical field would be established in the tumour tissue around the loop. The distribution of proliferative tumour cells in the region of the loop reported here supports the existence of such a field. In the cases of nodule formation it may be that cells which infiltrate into connective tissue migrate beyond the confines of this field and thus are able to establish a localised population of tumour cells within a microenvironment distant from the field effect which is favourable to tumour cell proliferation.

The question of how this change in the ionic environment influences tumour cell division remains unresolved. Experiments are being undertaken to establish the precise ionic changes which take place around the loop in an attempt to further elaborate this mechanism.

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