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Inhibition of the Development of Walker 256 Carcinoma with a Simple Metal–Plastic Implant

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Abstract—*Walker 256 carcinoma was inoculated into male rats within a platinum wire loop joined to a platinum disc by a length of silicone rubber and left for 8 days. In 92 out of 114 experiments tumour development was greatly inhibited. Of the 92 animals where there was inhibition, 52 cases had no macroscopically visible tumour although dividing tumour cells were present at the inoculation site. There was no necrosis. A firm connection between the silicone rubber and the platinum disc appeared to be essential for the effect.*

Cell division in completely inhibited tumours was equivalent to about 1% cells in mitosis compared with about 7% for the normally proliferating solid tumour. There was also a change in tumour cell morphology.

The conclusions are that the implant was probably altering the charge distribution around the tumour inoculum producing a microenvironment favourable for controlled proliferation.

INTRODUCTION

THE INITIAL interaction between the microenvironment of the host and the cancer cell inoculum is critical to the future development of transplantable tumours. It has been shown that the efficiency of inocula varies among differing neoplastic cell lines [1–3] and, furthermore, although tumour embolization occurs throughout the vascular system, metastases only develop in a small proportion of possible sites. There is other evidence to suggest that host tissue can influence tumour growth [4, 5] and, in particular, local factors in the host tissue appear to be important in the establishment of metastases [6]. Nevertheless there is an uncomfortable gap in our knowledge concerning events during the initial stages of tumour establishment, when relatively few malignant cells are present, and thus a successful host to tumour cell interaction is more probable.

The initial basic cellular reaction to a cancer inoculum involves the establishment of a localized population of host cells, mainly arising from the lymphomyeloid complex. Host cells also react to the implantation of foreign bodies. In the latter situations the cellular population involved is dependent upon the type of material utilized [7]. For example in the case of metal electrodes the host cells are thought to respond to changes in charge distribution close to the polarised metal surface [8]. It is also inferred from the action of applied and induced electrical fields in a variety of biological and medical models [9–12] that local charged elements may actually control the course of cellular proliferation. Many workers have reported beneficial effects of electrotherapy on the treatment of bone defects, fractures [12–15] and wound healing [16–18]. In addition, electrical forces have been shown to have general biological effects on the migration of cells, morphogenesis, regeneration, growth and behaviour [19]. It is possible that localized changes in extracellular ions are the common factors which underlie all of these changes in cell behaviour and proliferation. The present paper gives the preliminary results of experiments with a simple metal plastic implant where the aim was to create a local charged field and thereby influence

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the development of cancer cells at the implantation site.

MATERIALS AND METHODS

Animals

Twenty week-old male rats of a WAB substrain were used throughout the following experiments. Tumour tissue was obtained from 20 week-old donor rats bearing a solid, subcutaneous Walker 256 carcinoma which was maintained in the Department of Zoology by serial transplantation.

Electrodes

Most implants consisted of a loop of platinum wire, 0.5 cm diameter sealed with silicone adhesive to 8 cm of silicone rubber tubing, 2 mm diameter, filled with silicone rubber which was in turn sealed to one surface of a thin platinum disc, 1 cm diameter. Control units were identical to the electrode assembly but with the platinum disc omitted. In one series the implants consisted of a length of platinum wire (0.5 cm diameter) looped at one end with the other end spot welded to the platinum disc. The whole assembly was about 8 cm long.

In most experiments the units were implanted subcutaneously by securing the plate at the base of the skull and feeding the connector tubing and loop through a subcutaneous pocket of connective tissue so that the loop was secured in a dorsal position, immediately above the pelvic girdle. A 5 mg fragment of tumour tissue was placed in the centre of the loop. A tissue fragment alone, from the same donor, was transplanted into a similar position in control animals to check the viability and growth pattern of the donor tumour tissue. In one series of experiments the implant was placed in the reverse position and the tumour inoculum placed in the loop positioned at the base of the skull.

Colchicine administration

Two tenths of a milligram of colchicine, per 100 g body wt, was administered by intraperitoneal injection, 4 hr prior to death.

Histology

Groups of electrode unit, placebo and control animals were killed at intervals between 2 and 8 days after implantation. The tissue surrounding the loop and control tumour tissue was removed and fixed in Bouin's fluid for 36 hr, washed, dehydrated and subsequently embedded under vacuum. Sections were cut at 5 μm and stained with Mayers haemotoxylin.

Cell counts

The total number of tumour cell fragments, together with the number of arrested mitotic figures, were counted in 200 microscope areas (180 μm diameter) for each tissue sample. The actual whole cell numbers were obtained by application of the Floderus correction formula [20].

Potential difference

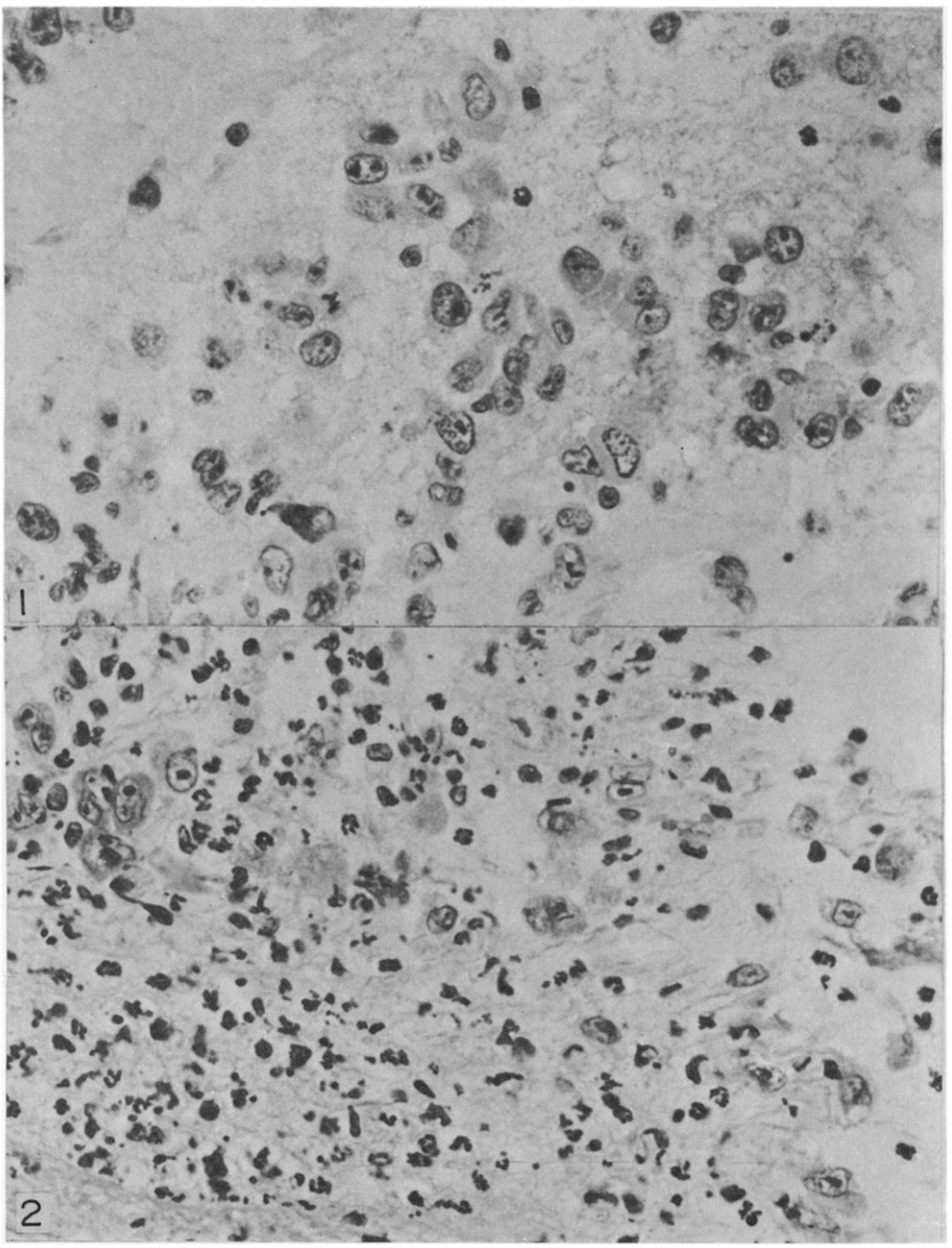
The potential difference between the tumour site and the position taken up by the platinum disc was measured by the technique of Woodrough [19].

RESULTS

In the freely growing subcutaneous tumour, the initial 4 day establishment phase of the inoculum was followed by exponential growth of the cell population until the time of death. In 1400 experimental inoculations the tumour never failed to reach a weight of about 22 g after 6 days. An identical growth pattern was observed for tumour cells inoculated in matched controls where the implant either had no disc or consisted of a platinum loop only (50 animals). However, when the complete implant was utilized, tumour development at 6 days was inhibited in 92 out of 114 animals (matched controls mostly without implants, Table 1).

Subjective categories of inhibition were used because of the impossibility of obtaining meaningful weights of tissue in and around the loop. There was no macroscopically identifiable tumour tissue in 55 animals. However, microscopic examination showed a small population of organized tumour cells in 26 of these 55 animals. In 15 animals a small macroscopically visible nodule was present outside the loop. This probably represents growth of cells displaced from the loop by movement of the animal. Where normal tumour growth occurred in 22 out of 114 animals the tumour surrounded the loop.

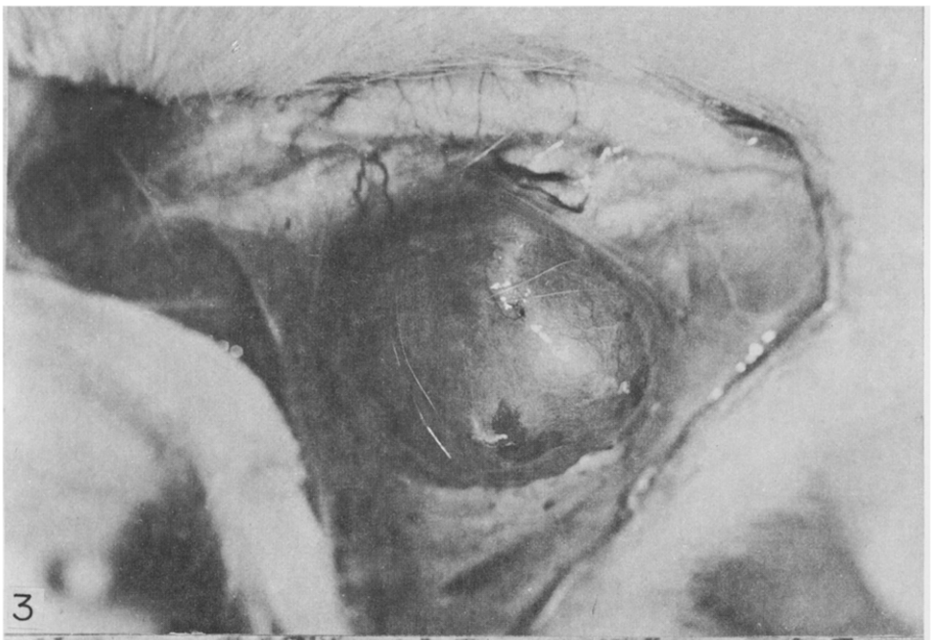
Microscopic examination of control sites, 2 days after implantation, showed viable tumour cells to be present, some of which were proliferating, interspersed amongst host neutrophils and some cell debris (Fig. 1).



Figs. 1 *and* 2. *Solid fragment of Walker* 256 *carcinoma during the establishment phase,* 2 days *after transplantation.*

1—*Control tissue.*

2—*Tissue within a platinum implant.* (×560.)



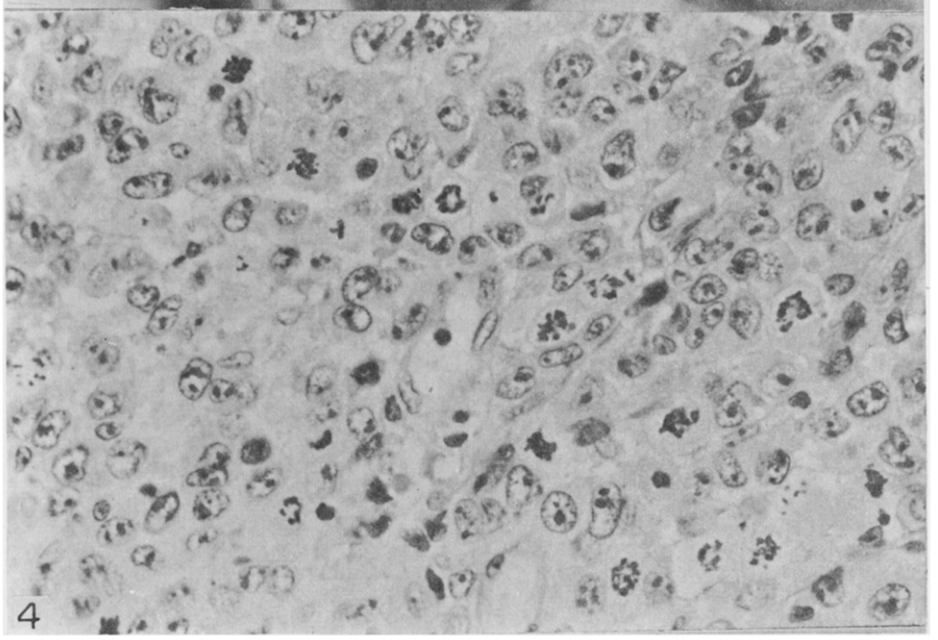


Fig. 3. *Macroscopic view of a large, encapsulated control tumour,* 6 days *after transplantation.*

Fig. 4. *Microscopically this tumour consisted of closely packed cells undergoing a high rate of proliferation.* (×560.)

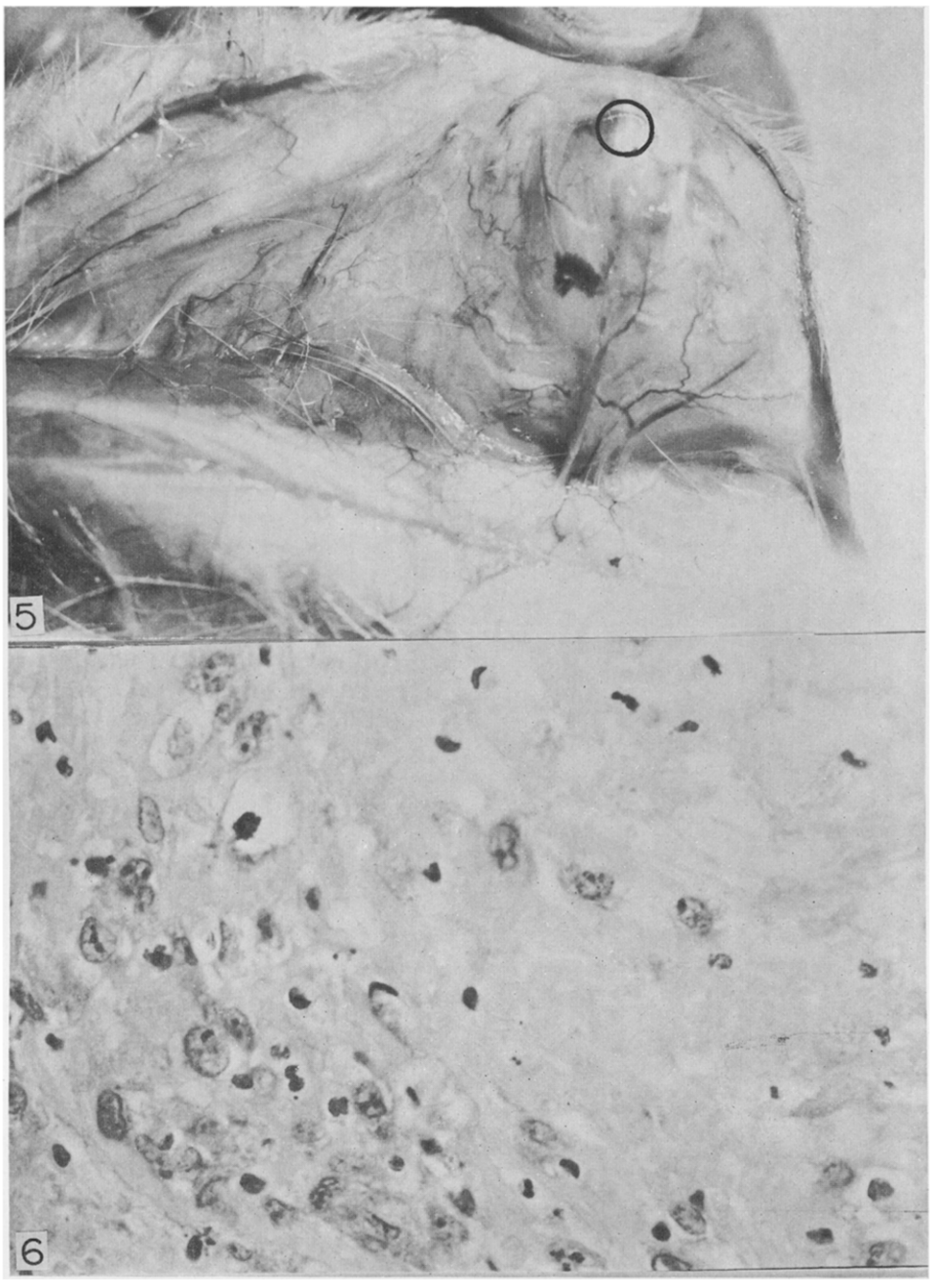


Fig. 5. *Macroscopic view of a platinum implant which had included viable tumour tissue. Tumour growth was not observed* 6 days *after implantation, but the implant was surrounded by connective tissue.*

Fig. 6. *Viable tumour cells were observed sparsely distributed within this connective tissue.* (×560.)

Table 1. Inhibition of tumour growth by platinum implants

Total No. implants	*		Degree of tumour inhibition	
	Complete		Partial	None
	§	‡	†	F
114	29	26	37	22

*§Macroscopically, no observable tumour but, microscopically, tumour cells sparsely distributed in the connective tissue.
‡Macroscopically, no observable tumour but, microscopically, organised tumour cells present.
†Macroscopically visible nodule, but at least 50% smaller in size than the smallest equivalent control.
F—definite tumour mass present, larger than those classified†.

When the tissue surrounding the platinum loop of the implants was examined, at the same stage, a few viable tumour cells were found amongst a mass of cell debris and host neutrophils (Fig. 2).

Six days after transplantation, large, encapsulated tumours were found in control animals (Fig. 3). These tumours consisted of closely packed cells, undergoing rapid proliferation. Very little connective tissue was found (Fig. 4). In contrast, tumours were not apparent in the majority of animals bearing implants (Fig. 5).

As may be anticipated the implantation of the assembly unit resulted in connective tissue growth around the unit. However, the degree of connective tissue formation showed a great deal of variability. Tumour inhibition tended to be associated with a poor connective tissue reaction. In the cases of tumour inhibition, tumour cells were found sparsely distributed amongst the connective tissue (Fig. 6). Tumour cells present in cases classified as completely inhibited differed from those in control tumours in that the mitotic index was reduced by about 76% (from $1.96 \pm 0.18\%$ in control tumours to $1.20 \pm 0.25\%$ in implant inhibited cells 2 days after implantation $P < 0.001$). The mean tumour cell densities were not significantly different from each other at this stage. However, 6 days post-operatively control tumour cell density had increased to 51.2 ± 0.5 cells/area which was 145% higher than the inhibited tumour level of 20.9 ± 0.6 cells/area ($P < 0.001$). The mitotic index of completely inhibited tumours was reduced by 700% compared with control values 6 days post-operatively ($6.93 \pm 0.16\%$ in control tissue and $0.85 \pm 0.11\%$ in inhibited tissue $P < 0.001$). It can be seen, therefore, that there was no increase in the proportion of cells in mitosis within inhibited tumours after the establishment phase and up to 6 days post-operatively, whereas the mitotic index of control tissue increased between 2- and 3-fold for the same time period.

We have previously shown that cell division in a non-cancerous tissue is markedly related to cell density, with inhibition occurring at extreme high and low densities [21]. In the present study, it was found that, during the initial stages of establishment, in the control tumours, cell density covered a fairly wide range with an even distribution of cell division throughout these densities (Fig. 7A). In contrast, mitotic figures of implant inhibited tumours were observed over a narrower range of very low cell densities. However, during the most rapid growth phase of the control tumour, from 4 to 6 days, the tissue maintained a fairly uniform cell density throughout its cellular mass with the majority of the proliferating cells occurring in a narrow range of cell densities (Fig. 7B). In the situation of tumour arrest, 6 days after implantation, a wider range of cell densities was observed with marked tendency towards low cell densities (Fig. 7B). An even distribution of proliferating cells was observed throughout these cell densities. A comparison of the cell density–mitosis relationships for control and experimental tumours indicates that the fall in cell density was not sufficient to account for the drop in cell division within the tumour implant population assuming a fixed relationship between mitotic index and cell density.

In a later series of experiments the implant was removed after 4 days, leaving the connective tissue sheath and tumour cells *in situ* until the eighth day. At this time tumour nodules were observed in all 10 experimental

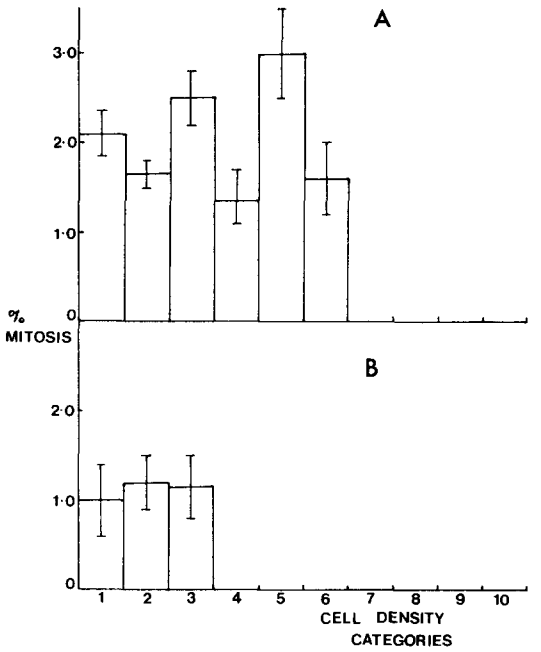


Fig. 7A. The mitotic rate of control tumour cells, in relation to cell density 2 days *after transplantation.*

Fig. 7B. The mitotic rate of tumour cells within a platinum implant, 2 days *after transplantation. (Results expressed as mean* ± S.E. % *mitosis for each category,* n *overall* = 200.)

animals. From these experiments it was apparent that the continued presence of the implant was necessary to maintain tumour inhibition and that removal of the implant resulted in tumour cell proliferation at control values.

There was a small potential difference between the site of implantation and the position of the platinum disc (Table 2). This was slightly reduced when the implant was present. A well developed tumour reversed this site potential. Tumour inhibition by the implants was associated with potentials that were closer to normal (Table 2).

In one series of experiments, the loop and disc were connected with platinum wire. These implants produced a similar inhibition to those based on the silicone tube (18 animals with 13 tumours inhibited). In another series (10 animals) the position of the implant was reversed and the tumour inoculum was placed in loops situated in the anterior position normally occupied by the disc. The change in orientation relative to the axis of the body did not alter the inhibitory action of the implants.

DISCUSSION

An explanation for the inhibition of Walker cell tumours by the platinum–silicone rubber implant involves considering the special physical properties of the assembly and the dynamics of the cellular populations enclosed within the micro-environment created by the platinum loop.

From the present study, the basic requirement for inhibition of tumour development is the establishment of a direct low impedence connexion between disc and loop. In the silicone rubber implants this was presumably provided either by the body fluids and tissues associated with the plastic surface or by water and ions that are known to be absorbed by silicone materials implanted in the body. An implication is that inhibition is caused by a flow of electrons between the two pieces of metal because a rubber tube without a disc

Table 2. Potential differences between the tumour site and the position of the implant disc

Experiment	(mV)
No implant	6.42 ±0.72
Normal solid tumour (6 days)	−2.21* ±2.19
Inhibited tumours (6 days) Partial inhibition	0.91† ±1.86
Complete inhibition	2.62† ±1.68

Potential differences were measured by the technique of Woodrough [19] and have been expressed as the potential of the loop relative to the disc.
*Significantly different from normal.
†Not significantly different from normal.
($n = 20$).

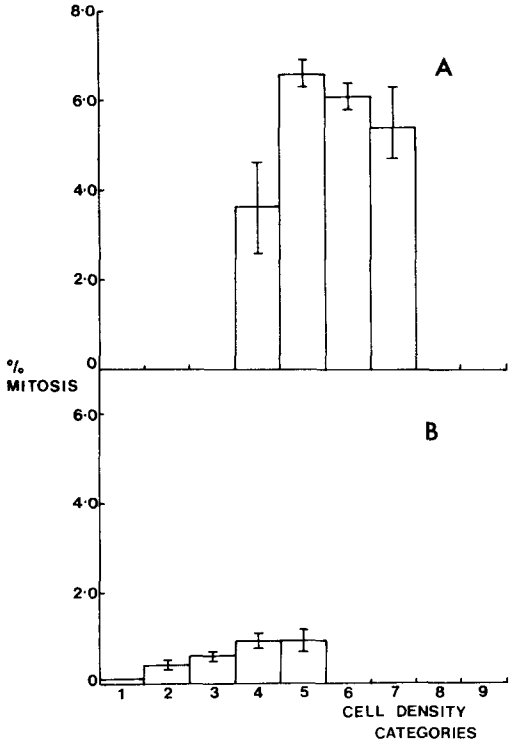


Fig. 8A. The mitotic rate of control tumour cells, in relation to cell density, 6 days *after transplantation.*

Fig. 8B. The mitotic rate of tumour cells within a platinum implant, 6 days *after transplantation. (Results expressed as mean* ± S.E. % *mitosis for each category,* n *overall* = 200.)

was ineffective. Since platinum in contact with biological fluids behaves passively to electron flow, the driving force is likely to reside in the cellular elements of the tissue in contact with the platinum.

It has been shown that spontaneous tumours set up a potential difference relative to adjacent healthy tissue [21]. Also, in the present work, a potential difference of a few millivolts was detected between established solid tumours and the normal site of the implant disc. By providing a specific conducting pathway the implant would prevent a potential being established and maintain the inoculum site at an electrochemical potential similar to that of normal tissue. If this interpretation is correct, the tumour cells should show a definite pattern of division in relation to the electrical field established around the loop. Preliminary histological studies indicate that there is a definite pattern of cell division in partially inhibited tumours, indicative of such a field.

Many questions surrounding the mechanism of inhibition remain. First and foremost, is the inhibition due to a direct action on the tumour cells or do they respond to a primary local action on the host? With regard to the latter possibility, the blood supply to the region enclosed by an active platinum loop was not obviously affected, although a localised population of host neutrophils was a characteristic feature of the loop area.

There was a major effect on tumour cell division without necrosis and the other notable histological feature was the appearance of a large proportion of very elongated tumour cells which were not seen in the normal solid tumour. On the whole, the results are in keeping with some unique ionic features of the tumour micro-environment being involved with the phenomenon of uncontrolled proliferation. Problems of measurement technique are currently being pursued which prevent us from measuring the impedence of the silicone implants within the body and the ionic composition of individual cells within the platinum loop.

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