Some factors affecting nerve regeneration in relation to radiotherapy

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Summary: The effect of delay and irradiation, both separately and combined, on regeneration of rat sciatic nerve was evaluated in adult Wistar rats. Those animals which had received irradiation at some stage fared significantly worse than those which had immediate division and reanastomosis of the sciatic nerve without irradiation. The possible relevance of these findings to nerve regeneration in patients treated by surgery and radiotherapy for parotid cancer is discussed.

Introduction
About 3% of all head and neck tumours occur in the salivary glands, and of these about 80% are found in the parotid (Batsakis 1979). According to Eneroth (1964), about 70% of parotid tumours are benign, of the pleomorphic adenoma type. The majority of the remainder are malignant tumours. The incidence of facial nerve palsy as one of the presenting symptoms in parotid malignancy appears to be about 12–14% (Conley & Hamaker 1975, Eneroth & Hamberger 1974).

A small number of patients will have had radiotherapy prior to surgery. Eneroth & Hamberger (1974) recommend this for poorly-differentiated adenoid cystic carcinoma and for undifferentiated carcinoma. Certain cases have further surgery for local recurrence after primary surgery and radiotherapy, and may also need a nerve graft.

The close temporal relationship of irradiation, tumour resection, and facial nerve grafting which may occur in patients with parotid malignant tumours has engendered a series of studies on the effect of irradiation on nerve grafting. A review of the literature to date does not provide much information about the effect of irradiation on a regenerating nerve. Fletcher & Jesse (1977) suggested that facial nerve regeneration may be reduced after irradiation.

Conley (1961) and Miehlke et al. (1972) suggested in their paper on the subject that postoperative irradiation had little effect on a previous nerve graft. McGuirt (1976), using the cat as an experimental model, confirmed Conley’s and Miehlke’s impressions. However, Lathrop (1963) suggested that postoperative irradiation had a detrimental effect on nerve regeneration.

Spiess (1972) exposed intact rat sciatic nerve to high doses of irradiation. He did not demonstrate any functional impairment, but did show axonal degeneration and capillary endothelial damage on histological examination. Stearns (1982) has shown that preoperative irradiation markedly reduces the functional repair in regenerated nerve. Clinically, it was felt that there were two situations where a nerve graft might be used: (1) when an intact nerve was divided electively to allow for adequate tumour resection; (2) when the facial nerve had been disrupted by tumour growth or iatrogenically during a biopsy procedure. Thus, two categories of facial nerve grafting may be envisaged: division of the nerve and immediate anastomosis, or division of the nerve and delayed grafting.

The object of the experiments to be described was to examine the effect of irradiation and delay on nerve regeneration. Three groups of experiments were performed. In one group, irradiation was administered, the nerve was divided and immediately reanastomosed. In another, irradiation was administered before division, as in the first group, but

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reanastomosis was delayed. In a third group, irradiation was administered after division of the nerve, but before anastomosis. Two control groups were set up in which either immediate or delayed reanastomosis was carried out on non-irradiated nerves.

Methods

Five groups of ten adult Wistar rats were used, each containing equal numbers of males and females. The right sciatic nerve was used by convention as the experimental nerve, in each animal, and the left sciatic nerve as the control nerve.

All procedures were performed under general anaesthesia. Those procedures involving irradiation were performed under intraperitoneal pentobarbitone anaesthesia. Those involving surgical manipulation of the nerves were performed under oxygen, nitrous oxide and halothane anaesthesia. The electrophysiological experiments were performed under ether anaesthesia. These variations were solely for the sake of expediency in the different situations.

The following procedures were carried out for each group:

**Group 1:** The right sciatic nerve was transected and reanastomosed in the thigh. The division of the nerve was done using a scalpel blade and reanastomosed with four 10/0 virgin silk sutures.

**Group 2:** The same procedure was followed as for Group 1, but with a delay of four weeks between division and reanastomosis of the nerve.

**Group 3:** The right sciatic nerve was transected and four weeks later a single dose of 2000 rad delivered to the area of division from a 250 KV machine. Four weeks after this the nerve was reanastomosed.

**Group 4:** A dose of 2000 rad was delivered to the right sciatic nerve. Four weeks later the nerve was divided and then reanastomosed immediately.

**Group 5:** A dose of 2000 rad was delivered to the right sciatic nerve. Four weeks later the nerve was divided and four weeks after division the nerve was reanastomosed.

Each animal was subjected to *in vivo* studies of the action potential in the experimental and control sciatic nerves eight weeks after neural reanastomosis. The animals were then sacrificed.

The sciatic nerves were exposed under anaesthesia. Each nerve was isolated from surrounding muscle and other tissue by means of a waxed paper well filled with liquid paraffin at 37°C. A pair of stimulating electrodes was placed just proximal to the anastomosis. A pair of recording electrodes was placed 1 cm distal to the anastomosis and a ground electrode placed between the two. A stimulus of 2 volts for 0.5 msec was used. The resultant mass action potential was recorded on an oscilloscope after due amplification and filtration. The trace obtained was photographed using an oscilloscope camera (Figure 1).

The amplitude of the action potential was measured in µV. The amplitude of the experimental nerve was expressed as a percentage of the amplitude of the control nerve. These results were then recorded for each animal.

![Figure 1](image_url)
Table 1. Amplitude of experimental nerve in five groups of rats following nerve division and reanastomosis with and without delay and irradiation

<table>
<thead>
<tr>
<th>Animal no.</th>
<th>Group 1 (n = 9)</th>
<th>Group 2 (n = 8)</th>
<th>Group 3 (n = 10)</th>
<th>Group 4 (n = 10)</th>
<th>Group 5 (n = 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>46.7</td>
<td>13.6</td>
<td>23.5</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>20.8</td>
<td>87.5</td>
<td>0</td>
<td>10.2</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>93.7</td>
<td>25.0</td>
<td>5.45</td>
<td>62.5</td>
<td>8.3</td>
</tr>
<tr>
<td>4</td>
<td>18.4</td>
<td>30.0</td>
<td>25.0</td>
<td>17.3</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>53.8</td>
<td>11.8</td>
<td>0</td>
<td>19.2</td>
<td>0</td>
</tr>
<tr>
<td>6</td>
<td>47.0</td>
<td>12.5</td>
<td>0</td>
<td>20.0</td>
<td>19.0</td>
</tr>
<tr>
<td>7</td>
<td>80.0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>40.0</td>
</tr>
<tr>
<td>8</td>
<td>86.9</td>
<td>80.0</td>
<td>0</td>
<td>20.0</td>
<td>82.0</td>
</tr>
<tr>
<td>9</td>
<td>61.0</td>
<td>(died)</td>
<td>0</td>
<td>3.2</td>
<td>0</td>
</tr>
<tr>
<td>10</td>
<td>(died)</td>
<td>(died)</td>
<td>0</td>
<td>18.2</td>
<td>0</td>
</tr>
<tr>
<td>Mean</td>
<td>56.5</td>
<td>32.5</td>
<td>5.4</td>
<td>17.0</td>
<td>14.1</td>
</tr>
</tbody>
</table>

Results
A value $X$ was calculated for each animal where

$$X = \frac{\text{Amplitude of mass action potential in } \mu \text{V of experimental nerve}}{\text{Amplitude of mass action potential in } \mu \text{V of control nerve}} \times 100$$

This enabled comparisons of each group to be made. The individual and mean $X$ values for each group are given in Table 1. The results were assessed using a Krauskal-Wallis multiple comparison analysis (Table 2). It had been shown in a pilot study that there was no significant difference in the action potentials of contralateral sciatic nerves in individual rats. A total of three animals died before completion of the experiment.

Discussion
An analysis of the five groups of animals can be undertaken in a variety of ways. The raw mean scores gave results much as might be expected.

The best results were obtained in non-irradiated nerves where there was division followed by immediate reanastomosis (Group 1) ($\bar{X} = 56.5\%$). In this procedure, few variables were introduced, and it was possible to reanastomose the nerves without tension and in an accurate anatomical position with relation to the fasicles.

Group 2, in which there was a delay between division and reanastomosis in non-irradiated nerves, had the next best result ($\bar{X} = 32.5\%$). Here, however, a variable was introduced because the nerve ends were sutured under a little more tension than those in Group 1 due to contraction of the nerve. It was also difficult to obtain accurate anatomical anastomosis.

Table 2. Differences between groups at the $P = 0.05$ level

<table>
<thead>
<tr>
<th>Group</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>NS</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>S</td>
<td>NS</td>
<td>NS</td>
<td></td>
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<tr>
<td>3</td>
<td>NS</td>
<td>NS</td>
<td></td>
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<td>4</td>
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<tr>
<td>5</td>
<td></td>
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</tbody>
</table>

NS, not significant; S, significant
because of tissue distortion due to fibrosis. However, McCabe (1970) found that, in man, the proteosynthetic ability in nerve cells is at a maximum some 4–8 weeks after division, and this increased activity may compensate to some extent for the factors hindering regeneration.

All the groups which were irradiated had much lower mean amplitudes, and frequently no action potential was recorded in Groups 3 and 5. The group which fared the worst was Group 3 (\(X = 5.4\%\)) where the nerve was divided, then irradiated, and finally reanastomosed after a delay of four weeks. In this group an action potential was recorded in three nerves only.

Analysis of these results using the Kruskal-Wallis multiple comparison analysis suggests that at the \(P = 0.05\) level there is no significant difference between the nerves which were reanastomosed immediately after transection, and those in which there was a delay of four weeks before reanastomosis. It is also apparent that all the nerves which had been irradiated fared significantly worse than those which had not been irradiated.

The effect of irradiation on the nerve and its environs is to greatly reduce the local blood supply. Presumably, regenerating nerves, like other regenerating tissues, require a good blood supply and are thus severely handicapped in its absence. Irradiation may also directly damage the Schwann cells.

Although this study was highly experimental, it suggests that the results of a nerve grafting procedure in patients with parotid tumours that have undergone radiotherapy are likely to be poor. If this is so, it may be worthwhile considering the alternative method of active reanimation of the face, such as a muscle pedicle from the masster or other form of dynamic sling.

References

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