SALIVARY GLAND FUNCTION AFTER RADIOTHERAPY FOR HEAD AND NECK CANCER

A comparison of accelerated and conventionally fractionated treatment

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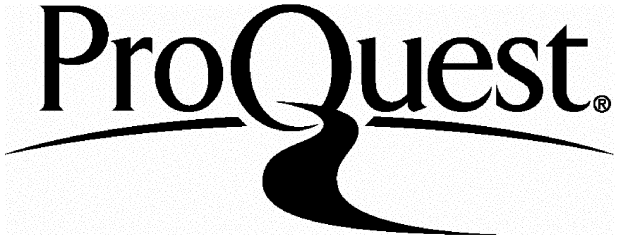
Thesis for Doctor of Medicine (MD) degree



ABSTRACT

In the treatment of head and neck cancer by radiotherapy the major salivary glands are often inevitably included in the treatment volume. The impairment of salivary gland function that can result is an important cause of late morbidity following treatment. Clinical experience has suggested that impaired salivary gland function after radiotherapy is less following treatment using the CHART regime as compared with conventionally fractionated treatment.

The salivary gland function of 73 patients treated by radiotherapy (CHART or conventionally fractionated treatment) for head and neck tumours has been studied. 26 were tested nine months to nine years after treatment and 47 were studied serially before, during and for up to 2 years after treatment. In 41 patients serum amylase was monitored before and during radiotherapy.

Pronounced falls in salivary flow and pH are seen once radiotherapy has commenced when significant amounts of salivary tissue are included in the treatment volume. Marked rises in the serum amylase accompany these very early changes. The parotid glands show the greatest sensitivity to radiotherapy compared to the other salivary glands. The early changes are as marked for patients receiving either CHART or conventionally fractionated treatment but a greater recovery of function after CHART results in the improved function seen in patients nine months or more following treatment with CHART.

The reduction in the late impairment of salivary gland function following radiotherapy with CHART is the result of the low dose per fraction employed combined with the reduced total dose. Patient comfort is greater and quality of life improved.

Salivary gland function has proved measurable and is a valuable system for the study of human radiobiology. The evidence from this study gives support for the concept of reduced late change resulting from a reduction in dose per fraction, an area of importance to the development of improved radiotherapy treatment schedules.

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CHAPTER 1
INTRODUCTION

1) Radiotherapy in the treatment of head and neck cancer:

The term head and neck cancer embraces malignant tumours arising in all parts of the head and neck except for the skin and central nervous system [56]. The vast majority of malignant tumours arise from the surface epithelium and are therefore squamous cell carcinoma or one of its many variants [82]. Head and neck cancer accounts for approximately 4% of all malignancies in Britain. Improvements in dental and oral hygiene coupled with reductions in intake of spirits and tobacco consumption have led to a falling incidence of cancers of the head and neck [114]. The male to female ratio is 3 or 4:1 and most patients are greater than 40 years of age [82].

For a large proportion of patients with head and neck cancer both surgery and radiotherapy hold out possibilities of cure [56]. Radiation is the most commonly used modality in the treatment of head and neck cancer [81]. In a study from the United States it was found that approximately 50% of all new cases of invasive head and neck cancer received radiation therapy, either as a primary treatment, as palliation, or as an adjunct to surgery or chemotherapy [93].

Most radiotherapy for head and neck cancer is given by high energy (megavoltage) external beam irradiation with photons or electrons. The advantages of megavoltage irradiation in the treatment of head and neck cancer as compared to lower energy irradiation are : reduced absorption in bone, greater skin sparing and more sharply defined beam edges. This results in a decrease in incidence and severity of normal tissue complications [62,73,108]. Neutrons have been employed but so far no advantage over treatment with photons has been demonstrated and the late normal tissue effects are greater [56]. Interstitial radiotherapy has the advantage of delivering a high dose of irradiation to the tumour without a high dose to the surrounding normal tissues and can be used for the entire treatment or in combination with external beam irradiation [95].

The main variables in a course of external beam radiotherapy are the number of fractions, the dose per fraction, the total dose given and the overall duration of treatment. Radiotherapists endeavour to employ a combination which will achieve the maximum tumour control with the minimum normal tissue damage. At each treatment centre regimes are employed that are based on clinical experience, practical considerations and local tradition. Although in nearly all, daily treatment is given on 5 days of the week, the number of fractions ranges from 15 to 35, the dose per fraction from 1.8 to 3.4 Gy, the total dose from 50 to 70 Gy and the overall duration from 3 to 7 weeks [23]. The most commonly employed regimen is 2 Gy per day, 5 days per week over 6-7 weeks to a total tumour dose of 60-66 Gy in 30-33 fractions

[56]. Split course schemes which insert a rest period halfway through treatment have been tried to allow time for recovery of normal tissues during treatment. Results for tumour control are poorer and the expected normal tissue benefits have not been realised [89].

Recent evidence from both clinic and laboratory has suggested that tumour cell repopulation may occur while a course of radiotherapy over 6-7 weeks is proceeding and that this may be an important cause for failure to cure [126,130].

Maciejewski et al showed in an analysis of 310 patients with T3/T4 squamous cell carcinomas of the larynx that local control rates decreased significantly from around 80% at overall treatment times of 32-35 days to only 16% at 56-63 days [74]. Similar clinical findings of the importance of overall treatment duration on probability of tumour control has been shown by others in head and neck cancer as well as at other tumour sites such as skin, bladder and cervix [29,147]. In studies of head and neck cancer it has been calculated that an extension of overall treatment time by 1 day needs to be balanced by on average an extra 0.6 Gy per day when treatment durations exceeded 28 days [29,125,147].

The cell kinetics of human tumours can now be determined following a single injection of bromodeoxyuridine (BUDR) and a biopsy performed 4-6 hours later. The tissue obtained is processed using a flow cytometer and the labelling index (LI) and

the duration of S phase (Ts) measured. From these two parameters the potential doubling time (Tpot) can be calculated [6]. Tpot is a measure of the proliferative activity of tumour cells taking into account the prescence of dividing and non-dividing cells but assuming the absence of cell loss [118]. In a study of the cell kinetics of human tumours it was shown that at least half of squamous cell carcinomas from head and neck sites had the potential to double their cell number in 5 days or less [144]. Even this may be an underestimate. If the tissue obtained is studied immuno-histochemically then the cells which have taken up BUDR can be seen clearly. Measurement of the LI using this method gives higher values for LI than that obtained by flow cytometry as the latter method underestimates the value of LI for diploid tumours being unable to distinguish between normal and tumour cells. Using the values of LI obtained immuno-histochemically as many as 84% of squamous cell cancers of the head and neck are found to have Tpot values of less than 5 days with two-thirds of tumours having Tpot values of less than 3 days [8].

In addition to this evidence concerning tumour control and the potential for tumour cell repopulation during treatment it has been shown that the giving of radiotherapy in many small doses leads to a sparing of late radiation damage [47,145]. This is illustrated in Figure 1 based upon work by Withers et al [147] and modified into the form shown by Drs M Joiner and S Dische. Varying the dose per fraction causes little alteration of effect in the early reacting normal tissues and of the response in tumour,

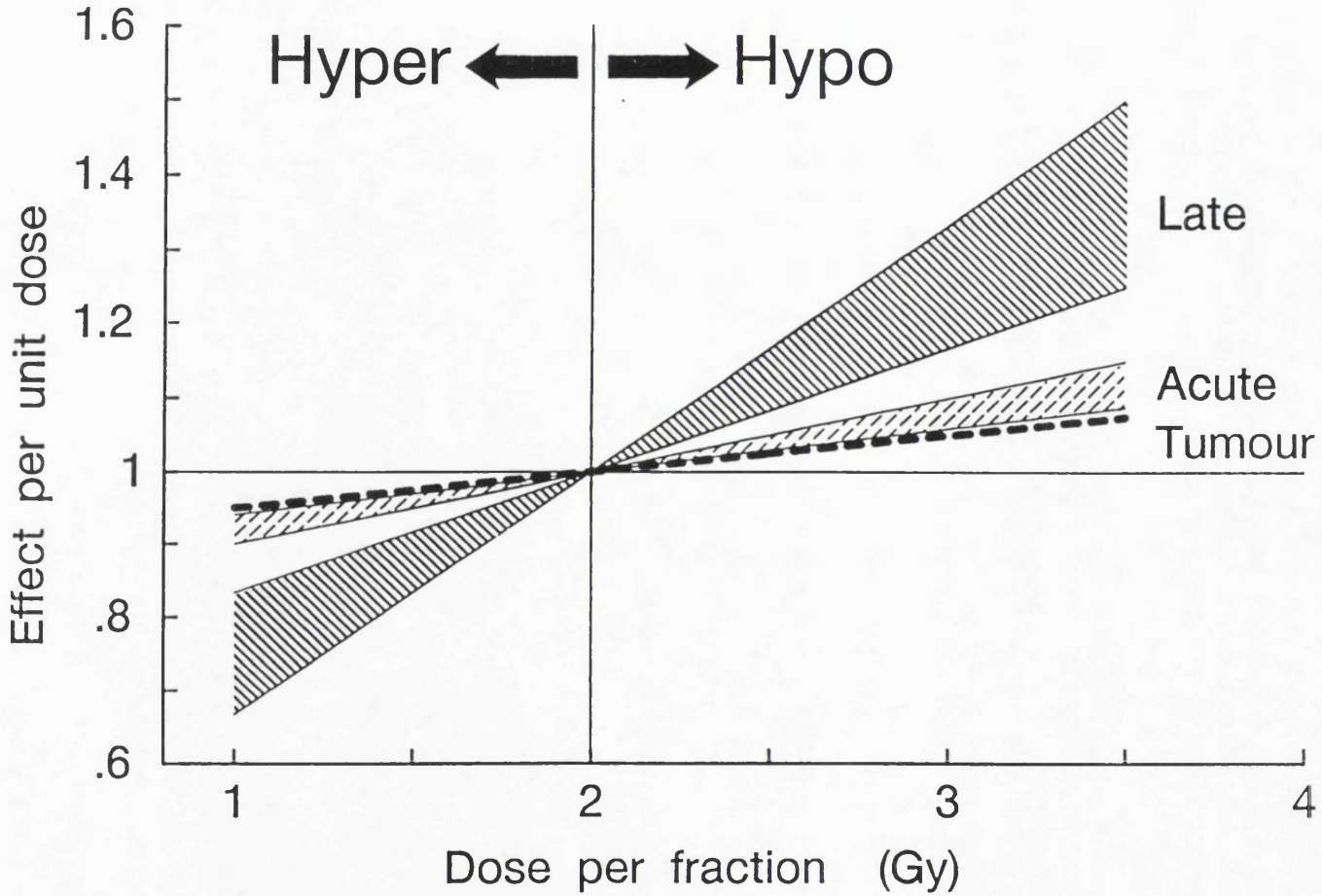


Figure 1. Relative radiation effect related to a change in the dose per fraction (produced by Drs M Joiner and S Dische)

but a great deal in the late effects in normal tissues. The most likely radiobiologic explanation for this is that the target cells for late effects are capable of repairing more sublethal injury than are the target cells in acutely responding tissues. It is also likely that some of the difference results from more "self-sensitisation" of acutely responding tissues, but not slowly responding tissues, through division cycle redistribution in regimens using a larger number of smaller dose fractions [146]. Expressed in terms of linear-quadratic theory; tumours and early reacting tissues respond to radiation predominantly with a linear relationship between dose and effect - the α or linear component. In the late reacting tissues a large part of the effect is related to the square of the individual dose given - the β or quadratic element [29,49,126]. Lowering the fraction size below the conventionally used 2 Gy should lead to a considerable sparing of late changes in normal tissues.

It follows that to limit the repopulation of tumours a reduction in the overall duration of treatment (acceleration) should reduce the opportunity for cellular proliferation by tumour. To minimise late damage to normal tissues, however, multiple small treatments must be given (hyperfractionation). To achieve both, more than one treatment must be given on each day [100]. A sufficient amount of time must be left between treatments given on each day to allow for maximum repair of sub-lethal injury in normal tissues, laboratory and clinical evidence suggest that an interval of at least 6 hours is necessary [48,98]. When an interval of less than 4 hours between fractions has been employed a considerable

and unnacceptable increase in early and late morbidity has resulted [29].

Several groups have devised regimens with these radiobiological principles in mind [26]. At Mount Vernon Hospital a scheme of continuous, hyperfractionated, accelerated radiotherapy (CHART) was devised with the objective of combining the greatest chance of eradicating all tumour with the minimal of late effects in normal tissues and the first patients were treated in January 1985. It was considered that there should be no interruption to permit repopulation to occur and once commenced treatment should continue every day until its conclusion. In the head and neck region all regimes of accelerated radiotherapy result in the appearance of marked mucosal reactions on the 13th or 14th days after initiation of radiotherapy and therefore it seemed best to complete treatment before this time. In this way there would be no problem of attempting to continue treatment through this period of reaction [29]. Radiotherapy is given three times a day for a continuous period of 12 days with a gap of 6 hours between treatments. An individual dose of 1.4 Gy was given to the first 11 patients so that a total tumour dose of 50.4 Gy was achieved in the 36 treatments. As tolerance appeared good the individual dose was increased to 1.5 Gy so that a total tumour dose of 54 Gy was achieved in subsequent patients [97,98].

Some increase in early radiation effects was predicted because of the reduction in overall treatment time [97]. Acute reactions have though proved tolerable with mucositis being most

troublesome and skin reactions being milder than expected [98]. Reducing the overall treatment time would not be expected to affect the severity of late reactions which are largely determined by the individual fraction size [126]. It was hoped that the lower dose per fraction employed with CHART would lead to a reduction of late normal tissue damage [46,97]. With the exception of spinal cord there is a suggestion that this may indeed be the case [24,67,98].

When a comparison was made with comparable patients treated previously by conventionally fractionated radiotherapy there were statistically significant improvements in local tumour control and survival [98]. To test these results in a prospective manner the CHART regime is currently being evaluated in a national, multi-centre randomised study. The trial commenced on the 1st of April 1990 and over 700 cases of squamous cell carcinoma of the head and neck have so far been entered. The study endpoints are local tumour control, survival and morbidity.

2) Physiology of saliva production:

The normal human salivary glands produce 600-700 mls of saliva per day [63,117]. Ninety percent of salivary secretion is produced by three pairs of major glands : the parotid, the submandibular and the sublingual glands with the remainder coming from numerous minor salivary glands distributed throughout the oral cavity [57]. The parotid and submandibular glands are the main

contributors to salivary secretion, the sublingual glands contribute only 2-5% of total salivary flow [69]. Under resting conditions the flow from the submandibular glands is as least as great as that from the parotids, however under conditions of stimulation (eg eating or drinking) the parotid glands become the main contributors [90,105]. The rate of salivary flow shows great variation between individuals although the output of any one individual is fairly consistent on different days [63,101]. There is evidence for a circadian rhythm with little saliva produced during sleep but relatively little change from 9am to 4pm [63,117]. A number of other factors can affect salivary flow rates, in particular degree of hydration, drugs, stress, food and smoking as well as gustatory and psychic stimuli [20,63].

The structure of salivary glands is similar to other exocrine glands, comprising a series of secretory units (acini) clustered around a central lumen. They are supported by myoepithelial cells and a basement membrane [69]. The parotid glands consist entirely of serous acini producing a clear watery product virtually devoid of mucin. The submandibular and sublingual glands contain mucous and serous acini whilst the minor salivary glands are predominantly mucous secretors [57,69,90]. From each acinus the secretions pass to a series of interconnected ducts before passing out through the major salivary duct into the oral cavity [57,63,69]. More than 99% of saliva is water and the specific gravity varies from 1.000 to 1.010 [57,69]. The total protein content of human saliva averages about 300 mg per 100 ml [63]. Approximately 30% of the protein found in saliva is

amylase [69] and other proteins found in significant amounts are immunoglobulins, lysozyme and glycoproteins [63]. Other organic constituents include glucose, at a similar concentration to blood, and small amounts of lipids and amino acids [63,69]. The ions found in all physiological fluids are also present in saliva, albeit at concentrations modified by salivary secretion [63,69,128,138].

The pH of saliva measured immediately after secretion into the oral cavity is acidic, in the range of pH 5.73-6.15 [63]. When saliva enters the oral cavity it loses dissolved carbon dioxide upon contact with air so that salivary pH rises to alkaline levels, in the range of pH 7.2-7.6. The bicarbonate concentration in saliva rises with increased salivary flow. The pH of saliva is thus extremely sensitive to the rate of flow and rises as flow increases, reaching values as high as 7.8 in stimulated parotid saliva [57,63].

3) Methods for the collection of saliva:

Whole saliva, the product of the major and minor salivary glands can be collected or saliva can be collected from individual glands. Saliva flow is termed unstimulated when no exogenous stimulus is used and is termed stimulated when secretion is promoted by mechanical, gustatory or pharmacological means [20]. A number of factors can influence salivary flow rates from an individual and care must be taken to standardise collection procedures [20, Chapter 1 section 2].

Collection of whole saliva :

Four methods have been employed [20,54].

a) Draining method - The subject bends the head forward and after an initial swallow allows saliva to drip off the lower lip into a graduated cylinder or preweighed container.

b) Spitting method - As with (a) except that the subject spits out every 60 seconds or as saliva accumulates.

c) Suction method - Saliva is sucked continuously from the floor of the mouth with a suction tube and allowed to accumulate in a collection vessel.

d) Swab method - Preweighed absorbent swabs are inserted in the mouth and removed for weighing at the end of the collection period.

Methods (c) and (d) introduce some degree of stimulation and in a comparison of all four procedures the spitting method proved the most reproducible [88].

Stimulated whole saliva can be collected using any of the above procedures after stimulation. Pharmacological stimulation with drugs such as pilocarpine is rarely used. Gustatory stimulation with citric acid or mechanical stimulation by the chewing of paraffin wax or rubber bands is usually employed [20].

Collection from individual glands :

Various methods have been developed for the collection of saliva from individual salivary glands. Unstimulated or stimulated saliva can be collected but it takes a considerable amount of time to collect without stimulation [20]. Parotid saliva can be collected by intra oral cannulation of the duct or more conveniently by the use of suction cups placed over the openings of Stensen's duct. Such a device was first described by Carlson and Crittenden in 1910 [13]. Numerous modifications of the original device have been made [102] and the device is now most commonly referred to as the Lashley cup after one of its modifiers [68].

Submandibular saliva is more difficult to obtain and cannulation of the ducts is difficult [20]. However a silicone rubber device that fits into the floor of mouth has been described to collect from both submandibular glands simultaneously [131]. This device can be used to collect sublingual saliva at the same time. Minor salivary gland secretions can be collected by pipette from the inner aspect of the lips or soft palate [19] or by absorption on filter paper of known weight [115].

4) The role of saliva in health:

Saliva has many functions and although not essential for the maintenance of life, it makes important and varied contributions to the efficient working and protection of the body [63].

The most effective way to gain an appreciation of the variety of roles played by saliva in humans and its importance to well-being is to sample the complaints of people with salivary dysfunction. They are miserable : "My mouth and throat are dry, rough and sticky. I'm hoarse; its so hard to talk. I can't wear my dentures, my mouth is always sore. I have to sip fluids frequently so my tongue won't stick to the sides or roof of my mouth. Eating is difficult and sometimes impossible. Food sticks to my mouth and teeth. I can't tell the position of food in my mouth. My mouth often feels numb. I have difficulty tasting and have to add more salt and sugar to my food. My fillings are falling out and my teeth are crumbling away" [78].

The list of complaints elicited from patients with salivary gland dysfunction reflects the two major functional roles of saliva : protective and digestive.

Glycoproteins are the main protein of saliva and give it a slimy/lubricant character. It forms a viscous coating on the epithelial surface of the oral cavity functioning both as a lubricant and as a barrier that protects against dessication. Its lubricant qualities help to protect the teeth by reducing wear,

facilitate food bolus formation and swallowing as well as aiding tongue movements and speech [63,69].

The main role of saliva in the digestive process is preparative with the formation of a food bolus which is readily chewed and moved to the back of the oral cavity where it is easily swallowed.

The high water content of the parotid secretions moistening the food and the salivary glycoproteins coating the food combine effectively to facilitate ingestion [78]. The contribution of saliva to the chemical digestion of food is small. Salivary amylase functions best at a pH of 6.8 and is largely inactivated by the acidic contents of the stomach, although after a large meal the pH of the food entering the stomach can remain nearly neutral for up to half an hour during which amylase activity may continue. The main action of salivary amylase is to digest starch molecules from food residues left in the mouth after a meal, rather than to contribute to digestion as a whole [57,63,69]. A lipase secreted by the lingual serous glands (von Ebner's) act in the acidic enviroment of the stomach initiating the digestion of fat [57,78].

The sensation of taste is produced only by substances in solution. Some foods such as fruits contain such a high proportion of water that the substances which give rise to taste are already in solution and their taste may be perceived as soon as they are released by mastication. Other foods without such a high water content require the saliva to dissolve out and deliver the substances giving rise to taste to the taste bud receptors

[63,140].

Saliva plays an important role in controlling the oral microbial population. There is a mechanical action whereby saliva removes bacteria from the mouth, conveying them to the stomach where they are controlled for the most part by the acidic enviroment. The buffering capacity of saliva maintains in health a slightly alkaline enviroment in the oral cavity which favours a microbial population that is non cariogenic [11]. In addition saliva contains a number of antibacterial substances, including lysozyme, peroxidases, lactoferrin and IgA [57,63,69,78].

Saliva is essential for the maintenance and protection of the teeth. As outlined above it acts as a mechanical protection and can aid the digestion and removal of food residue left in the mouth after a meal. It helps to maintain a normal non cariogenic oral microflora and salivary components such as calcium and phosphate promote surface mineralization of the dentition [3,63,69].

Besides its role in the oral cavity saliva helps to protect the oesophagus by the buffering of acidic contents refluxed from the stomach [55,57] and epidermal growth factor present in saliva and produced by the submandibular glands is involved in the repair and maintenance of the gastroduodenal mucosa [33,57].

5) Mechanism of salivary gland damage following radiotherapy:

Information on the acute morphological changes that occur in human salivary glands following irradiation is limited. The single largest study in humans examined salivary glands from surgical specimens removed 24 hours after exposure to single doses of radiotherapy of 10-20 Gy [65]. The glands exhibited acute inflammatory changes with infiltration by polymorphonuclear leucocytes, eosinophils and plasma cells. Marked degenerative changes in serous acinar cells were described along with pyknosis, cytoplasmic vacuolization and loss of zymogen granules. Mucous glands showed little change (Figure 2).

Although loss of serous acini occurs very quickly, early gross atrophy of the salivary glands may be concealed by swelling caused by the inflammatory reaction [123]. Once the acute inflammation subsides the salivary glands clinically exhibit atrophy or, in the case of the submandibular gland, firm to hard enlargement that may mimic neoplastic involvement [44,123]. Microscopically the principal features of the chronic changes are atrophy and loss of serous acini, fibrosis and chronic inflammation [94,123].

Morphological studies of rodent salivary glands after irradiation have shown similar findings to those seen in humans with serous cell degeneration and death occurring within hours of irradiation [14,36]. An acute inflammatory cell infiltration is not seen [121]

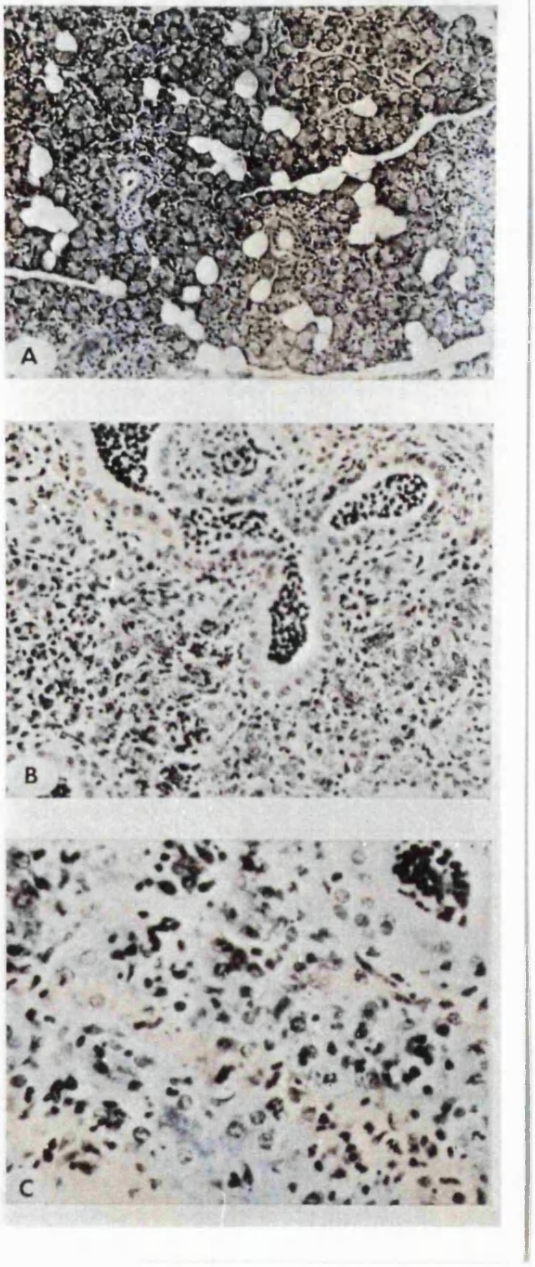


Figure 2. Histological appearances of human parotid gland tissue following irradiation (Kashima et al 1965).

a) Normal (X120)

b) 24 hours after 10Gy (X210)

c) 24 hours after 10Gy (X640)

and rodent salivary glands appear more resistant to radiation than those of humans [40,121].

These observations in rodents have been confirmed and further studied in rhesus monkeys whose salivary glands more closely resemble those of humans in terms of anatomy, physiology and radiosensitivity [120,123]. Stephens et al [120] studied the morphological alterations of parotid and submandibular salivary glands of rhesus monkeys 1-72 hours and 16-40 weeks post irradiation with single photon doses of 2.5-15 Gy or 10.2 Gy given in six fractions. Acute degeneration and necrosis of serous cells in both parotid and submandibular glands were clearly expressed by 24 hours after irradiation and this occurred in a dose dependent fashion. Mucous glands were far less sensitive. The predominant glandular alteration 16 or more weeks after irradiation was atrophy due to loss of serous acini. The effect of 10.2 Gy given in six 1.7 Gy fractions was approximately equivalent to that from 9.3 Gy as a single fraction suggesting that little sparing results from dose fractionation.

Salivary acini are composed of highly differentiated cells that display a low rate of mitotic activity, because most are in interphase. Studies in rat salivary glands have shown that the acinar cells turn over about every 40-65 days [17]. Based upon the rare occurrence of mitotic activity in human salivary glands, human salivary acinar cells have been assumed to have a life span of comparable length [120]. Studies of embryogenesis of normal salivary glands and histogenesis of salivary gland tumours in

vivo and in vitro have shown that acinar cells are derived from the terminal cells of the intercalated ducts rather than by division of the acinar cells themselves. Because of these characteristics, salivary acinar cells would not be expected to show the marked sensitivity to radiation that is observed [120,123].

From their studies in vivo on rhesus monkeys [120] and from in vitro work on organ cultures of rhesus monkey parotid glands [122] Stephens et al have concluded that the early cell death of serous salivary cells following irradiation is the result of interphase cell death occurring by the process of apoptosis. The cell membrane is suggested as the likely target of radiation injury [123].

6) Changes in serum and salivary amylase following the irradiation of salivary tissue:

Changes in the serum amylase following irradiation of the salivary glands in man was first described by Kashima et al in 1965 [65]. A rapid increase in the serum amylase within a few hours after irradiation and a return to normal levels in the subsequent days has been shown after a single dose and during fractionated irradiation [2,4,65,132]. The salivary origin of the amylase has been demonstrated by electrophoresis. Irradiation of other tissues rich in amylase such as the pancreas does not result in a rise in the serum amylase [65]. A correlation between the

amount of salivary tissue irradiated and the degree of amylase rise in the serum found in the serum has been reported [4,65,132]. It has been suggested that the parotid gland is the major scource of the radiation induced hyperamylasaemia [15] and indeed most salivary amylase activity is found in the parotid glands [69]. Becciolini et al studied the hyperamylasaemic response in patients receiving an accelerated split course of radiotherapy and found an earlier and more rapid peak of the serum amylase in these patients [5].

The appearance of amylase in the serum probably reflects the interphase death that the serous cells of the parotid gland undergo in response to radiotherapy [120]. It has been ascribed to the disruption of serous cells or changes in their cell membrane permeability resulting in the release of intracellular amylase [4,65,132].

Corresponding to the rise in serum amylase during irradiation, falls in the amylase content of stimulated whole saliva have been shown [76]. The secretion of amylase into parotid saliva has not though been shown to fall significantly during irradiation. The authors suggest that this may be the result of an inability to collect saliva from the majority of patients studied even after one week of treatment due to reduction in flow [85].

The rise in serum amylase that results from the irradiation of salivary tissue provides a unique early biochemical measure of the effect of radiation on a normal tissue. It has in fact been

suggested that measurement of serum amylase activity could provide a useful rapid indicator of tissue damage in the first 12-48 hours after accidental exposure to radiation [2,66].

7) Salivary gland function following radiotherapy:

Early effects :

Changes in salivary secretions quickly become apparent to the patient during a course of radiotherapy when a significant amount of salivary tissue is included in the treatment volume. Within the first week of treatment, many patients note reduced production of saliva and an increase in its viscosity [9]. The serous acini are considered to be the most sensitive to ionising radiation [90,108] and it is the loss of their watery product that results in the thick tenacious secretions observed during and after a course of radiotherapy [90].

Early and marked falls in the secretion of saliva occur when the major salivary glands are included in the treatment fields [31] (Table I). Shannon et al [104] collected unstimulated whole saliva by the drooling technique from 10 head and neck cancer patients treated by opposed fields that covered all of the major salivary glands. Treatment was given 4 days per week with daily fractions of 2.25 Gy to doses ranging from 22.5-54 Gy (mean 44 Gy). For treatment weeks 1 through 6 the salivary flow rate decreased to 40%, 29%, 24%, 19%, 9% and 5% respectively. In a similar study

TABLE I

EARLY CHANGES IN SALIVARY FLOW AFTER RADIOTHERAPY

(Tested salivary tissue fully included in treatment volume)

SUMMARY OF PUBLISHED DATA

SCOURCE	NUMBER OF PATIENTS	COLLECTION TECHNIQUE	RADIOTHERAPY	% OF PRETREATMENT SALIVARY FLOW
Shannon et al [104]	10	Unstimulated whole saliva (drooling)	9 Gy/wk 4 fractions/wk Total 22.5-54 Gy	40% after 1 wk 29% after 2 wks 24% after 3 wks 19% after 4 wks 9% after 5 wks 5% after 6 wks
Wescott et al [142]	13	Unstimulated whole saliva (drooling)	9 Gy/wk 4 fractions/wk Total 45-63 Gy	36% after 3 days
Dreizen et al [32]	42	Stimulated whole saliva (spitting)	10 Gy/wk 5 fractions/wk Total > 50 Gy	43% after 1 wk 24% after 6 wks
Shannon et al [105]	7	Unstimulated parotid saliva (Lashley cups)	9 Gy/wk 4 fractions/wk Total > 50 Gy	50% after 1 day 0% after 3 days
Eneroth et al [38]	4	Stimulated parotid saliva (Lashley cups)	10 Gy/wk 5 fractions/wk Total > 40 Gy	18% after 1 wk <2% after 4 wks
Mossman et al [85]	25	Stimulated parotid saliva (Lashley cups)	10 Gy/wk 5 fractions/wk Total 61 Gy (mean)	50% after 1 wk 0% after 6 wks

Wescott et al [142] found the flow of unstimulated whole saliva to be reduced by 36% after only three days of treatment. Dreizen et al [32] collected masticatory stimulated whole saliva from 42 patients treated by similar fields who received a minimum of 50 Gy given in 2 Gy fractions, 5 days per week. The mean flow rate decreased to 43% after the first week of treatment and 24% after six weeks.

Shannon et al [105] studied the resting flow from 7 parotid glands included in the treatment volumes of 7 patients receiving radiotherapy. A mean reduction in resting flow of 50% was found 24 hours after a single dose of 2.25 Gy. After two doses had been given, no measurable flow was obtainable from 6 of the glands and no flow was measurable from any of the glands after three doses. Eneroth et al [38] collected stimulated parotid saliva from 4 parotid glands included in the treatment volumes of 4 patients receiving radiotherapy. Treatment was given in 2 Gy fractions, 5 days per week to a dose of at least 40 Gy. The mean flow rate decreased to 18% after the first week of treatment and less than 2% after four weeks. Mossman et al [85] collected stimulated parotid saliva from 25 patients undergoing radiotherapy where the parotids were fully included in the treatment volume. Flow was decreased to 50% after the first week and was not produced in measurable quantities after six weeks of treatment.

Selective collection of saliva from submandibular or sublingual glands following radiotherapy in humans has not been reported.

Mossman [87] has constructed a dose response curve for the early changes in salivary gland function after radiotherapy by pooling the data from four studies measuring salivary flow after treatment. Salivary gland flow is seen to be extremely responsive to radiotherapy and overall was reduced to 50% after 10 Gy and to 20% after 60 Gy with radiotherapy given in daily fractions of 2-2.25 Gy on 4-5 occasions per week (Figure 3).

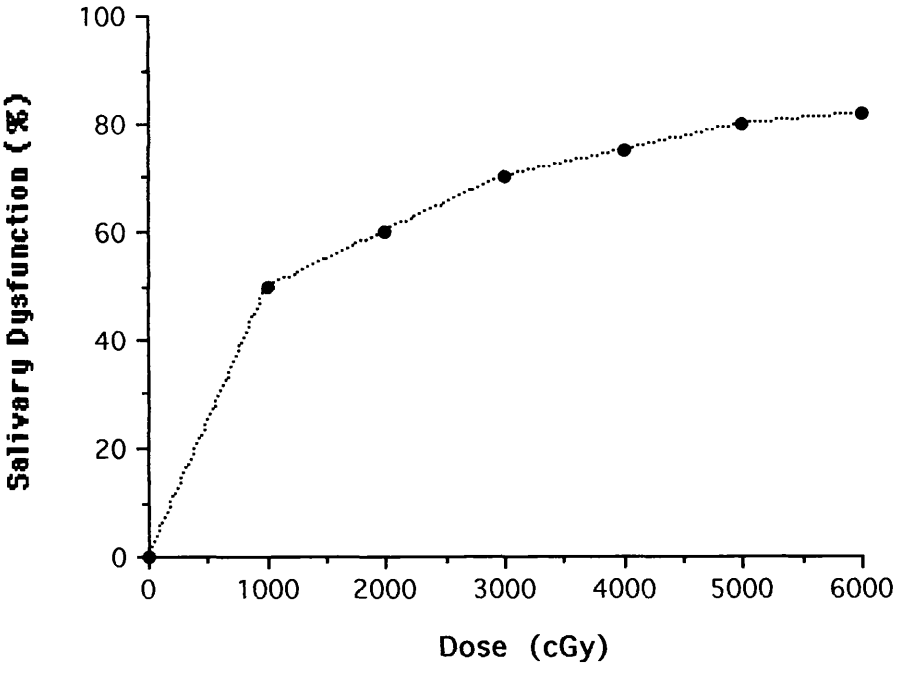


Figure 3 Dose response curve for salivary dysfunction during radiotherapy for head and neck cancer (Mossman 1983).

In addition to the early falls in salivary flow rate the pH of unstimulated [7] and stimulated whole [30,107] saliva falls by the end of a course of radiotherapy when all the major salivary glands are included in the radiotherapy fields. Changes in the electrolyte concentration of whole saliva have also been noted at the end of a course of treatment with concentrations of sodium, chloride, calcium, magnesium and protein all increased significantly whilst bicarbonate concentration falls [7,30]. The amylase content of stimulated whole saliva has been shown to fall during a course of irradiation [76]. Falls in protein secretion rate [85], pH and bicarbonate [1] have also been found in stimulated parotid saliva during a course of radiotherapy.

Late effects :

These marked early changes may persist in the period after irradiation and impaired salivary gland function following radiotherapy is a major cause of late morbidity following the treatment of head and neck cancer with radiotherapy [90]. Unstimulated and stimulated whole salivary flow can be demonstrated to be reduced even up to 25 years following irradiation [32,72,77]. Parotid glands included in the treatment volume have been shown to have markedly reduced stimulated flow rates for at least 9 years after treatment [16,20,37,38,86] (Table II).

The pH of unstimulated whole saliva is still reduced up to 6 years following radiotherapy [50]. For stimulated parotid saliva reduced

TABLE II

LATE CHANGES IN SALIVARY FLOW AFTER RADIOTHERAPY

(Tested salivary tissue fully included in treatment volume)

SUMMARY OF PUBLISHED DATA

SCOURCE	NUMBER OF PATIENTS	COLLECTION TECHNIQUE	RADIOTHERAPY	% OF PRETREATMENT SALIVARY FLOW
Liu et al [72]	19	Unstimulated and stimulated whole saliva (spitting)	40-75 Gy No other details given	unstim 22% * stim 19% (mean results) after 2-18 years
Dreizen et al [32]	26	Stimulated whole saliva (spitting)	10 Gy/wk 5 fractions/wk Total > 50 Gy	5% (mean) after 3 years
Makonnen et al [77]	8	Stimulated whole saliva (spitting)	10 Gy/wk 5 fractions/wk Total 62 Gy (median)	0-37% * after 3 years
Mossman et al [86]	12	Stimulated parotid saliva (Lashley cups)	10 Gy/wk 5 fractions/wk Total > 30 Gy	0-39% * after 1-7 years
Eneroth et al [38]	4	Stimulated parotid saliva (Lashley cups)	10 Gy/wk 5 fractions/wk Total > 40 Gy	0-46% after 8 months
Eneroth et al [37]	5	Stimulated parotid saliva (Lashley cups)	10 Gy/wk 5 fractions/wk Total 44-66 Gy	0-29% * after 1-9 years
Cheng et al [16]	4	Stimulated parotid saliva (Lashley cups)	9-1.25 Gy/wk 5 fractions/wk 50-65 Gy	0% after 4-8 months
Marks et al [79]	17	Stimulated parotid saliva (Lashley cups)	30-79 Gy No other details given	0-69% * after 5-47 months

* No pretreatment assessment or internal control used but comparison with control non irradiated glands from other patients or volunteers used.

pH and levels of IgA have been shown 5 months or more following radiotherapy [79] and protein secretion rate is reduced 1-7 years following irradiation as compared to normal controls [86].

Statements in the literature about the ability of salivary tissue to recover function after its impairment following radiotherapy are somewhat contradictory. Fletcher states that xerostomia is never a scource of trouble after one or two years [45]. Silverman suggests that recovery of function is seen in most patient several months after treatment although recovery of adequate saliva for oral comfort and function may take from 6 to 12 months and for some remains inadequate indefinitely [108]. In contrast Dreizen et al describe radiation induced xerostomia as irreversible and consider that whilst some patients report a subjective improvement in symptoms this is not matched by increased saliva production [31]. This view is also taken by Parsons although he suggests that recovery of function can occur in the 6 months following radiotherapy if the dose received by the salivary tissue is less than 30-35 Gy and if the patient is of young age [90].

These are largely clinical observations and there are only a few studies where saliva has been collected serially before, during and for extended periods of time after radiotherapy to determine objectively if recovery can occur. Wescott et al collected unstimulated whole saliva serially from 10 patients receiving between 45-63 Gy to volumes that included virtually all salivary tissue. Patients were followed for up to 17 months after radiotherapy. In no case was flow rate recovery observed despite

the subjective improvement of symptoms with time [142]. Dreizen et al found similar results after collecting stimulated whole saliva from 42 patients for up to 3 years after radiotherapy [32]. Frank et al report that after 2 to 6 months the unstimulated whole salivary flow increases in certain patients but details are not given [50]. Eneroth et al serially collected stimulated and unstimulated saliva from the parotid glands of 4 patients receiving radiotherapy by a unilateral technique that included one of the patients parotid glands in the treatment volume. Partial recovery of function was documented for one case treated to a dose of 40 Gy when tested at 8 months following radiotherapy [38]. In a separate study Eneroth et al reported a parotid gland functioning well 9 years after radiotherapy to a dose of 65 Gy [37]. This case was not though studied serially so it is unclear if the function observed represents recovery or simply residual function remaining following radiotherapy.

The literature is thus unclear on the question of the ability of salivary tissue to recover functionally after radiotherapy and the relationship of any such recovery to dose or the time scale at which it occurs is also obscure.

Factors influencing the effect of radiotherapy
on salivary gland function :

It is generally agreed that the degree of late impairment of salivary gland function seen after radiotherapy is related primarily to the volume of salivary tissue included in the

treatment volume and to the dose that the glands receive. Other factors such as the patients age and pre treatment salivary flow have also been reported to affect the degree of late impairment that results [90].

In the treatment of a carcinoma when all or most of the major salivary glands are included in the treatment volume dryness of mouth will inevitably result. If only the submandibular and sublingual glands are included and not both parotids, most patients note little or no difference in the quality and quantity of their saliva. If the parotids are irradiated and the submandibular glands spared, moisture may be preserved [83,90,105]. Unilateral treatments where only one side is treated rarely result in symptomatic dryness [22,79]. Mira et al showed that more than 50% of the parotid glands must be excluded from the treatment volume to prevent severe dryness, as measured by collection of unstimulated whole saliva when the rest of the major glands are included [83]. Cheng et al found that when 100% of the parotid gland was included in the treatment volume to a dose of 50 Gy or more no stimulated flow could be detected 4-8 months after treatment. If even a small portion, 10-20% of the gland could be excluded from the volume then flow, albeit reduced could be produced after stimulation [16].

Clinically patients treated to doses of 40 Gy as for lymphoma suffer less late salivary dysfunction than patients receiving doses of 60 Gy or more [9,72,79]. Marks et al studied the stimulated parotid flow from glands treated to a wide range of

radiotherapy doses from 5-47 months previously. A dose response effect was clearly demonstrated with parotid flow progressively decreased with increasing doses of radiation. Measurable flow was found in 50% of glands recieving 20-40 Gy, 20% receiving 40-60 Gy and in none receiving more than 60 Gy [79].

It has been shown for both unstimulated whole saliva [83] and stimulated parotid saliva [39] that a high pre treatment flow rate will result in a greater preservation of function following radiotherapy as compared to low pre treatment flow rates. The decrease in flow after irradiation follows an exponential decay curve. A certain dose reduces flow by approximately the same percentage, not by the same absolute amount in different patients [39,83,90].

Eneroth et al [37] have reported a patient whose parotid gland was functioning well 9 years after having received radiotherapy to a dose of 65 Gy in the treatment of a carcinoma of the tongue. The patient was only 26 years old at the time of treatment and this was felt to be a factor in the unexpectedly good preservation of salivary flow. Other authors have also suggested that young patient age at the time of treatment results in less late salivary gland dysfunction [90].

It has been suggested that split courses of radiotherapy might allow for greater recovery of salivary tissue after radiotherapy [83], others have failed to see any benefit from this approach [89,90]. To my knowledge there are no other data on the effect of

different fractionation schedules on salivary gland function.

8) Clinical sequelae of impaired salivary gland function following radiotherapy:

Irradiation of salivary glands during the treatment of head and neck cancer may lead to an alteration in the amount and quality of the saliva produced. As a consequence of this patients suffer dryness of mouth with oral discomfort, altered taste acuity, deterioration of dental hygiene and dental decay [90].

Impaired salivary gland function following radiotherapy is not only a cause of considerable discomfort to patients but is also a major cause of the dental problems that occur following radiotherapy. Decreased saliva production is accompanied by a fall in pH resulting in an altered microflora that is highly cariogenic [11]. In addition there is a decrease in the concentration of electrolytes [30] and immunoproteins [12] in saliva that normally help to protect against the development of caries. These factors are compounded by the tendency of patients to take frequent high carbohydrate meals as a result of dryness of mouth and alteration of taste perception [11,84].

To avoid dental problems where considerable amounts of salivary tissue are to be included in the treatment volume the traditional approach, still practised by many clinicians is to advise extensive dental extractions [21]. Others however advocate a

more conservative approach emphasising the importance of meticulous oral hygiene and the use of fluoride gel [9,31,62,90].

9) Prevention of radiation injury to salivary gland tissue:

Prevention of radiation injury to salivary gland tissue is currently limited to excluding the salivary glands, especially a portion of the parotid glands from the treatment fields by field configuration and shielding [124]. However this approach might compromise complete coverage of the tumour and or regional lymph nodes [123]. Interstitial techniques can allow the delivery of high doses of radiation to the treatment volume whilst sparing normal tissues such as salivary glands [9], external beam radiotherapy given with charged particles such as protons may also offer such an advantage over the use of standard high energy photons [109].

Although with limited indications, surgical transposition of the submandibular glands outside the treatment portals has been described as a successful method for the prevention of hyposalivation [10].

Radioprotection of the rat parotid gland with WR-2721 [110,111], isoprotenerol [112], chlorpromazine [36], cAMP [113] and acetylsalicylic acid [52] has been reported. Lidocaine has been shown to protect parotid serous cells from rhesus monkeys in

vitro [123] but this effect was not found in vivo [Peters LJ, personal communication]. Although most compounds are probably too toxic at radioprotective doses to be of clinical usefulness, WR-2721 is currently being tested in phase II/III trials [62].

In a small study of 6 patients salivary gland stimulation with pilocarpine during radiotherapy appeared to reduce subsequent impairment of parotid gland function [148].

10) Management of patients with impaired salivary gland function following radiotherapy:

The measures that can be employed in the treatment of impaired salivary gland function following radiotherapy are a) maintenance of oral hygiene, b) symptomatic relief of oral dryness, c) stimulation of residual salivary tissue [31,62].

a) Maintenance of oral hygiene

Continuous maintenance of effective oral hygiene and the use of an adequately protective topical fluoride are the most important methods for preventing the dental complications of impaired salivary gland function [62]. Before irradiation all patients should be instructed in oral hygiene measures including meticulous brushing techniques using a fluoride containing toothpaste and interdental techniques such as flossing as well as dietary instructions about non-cariogenic foods [32,59,73]. Topical

fluoride is applied daily, usually as 1% sodium fluoride gel. Oral hygiene must be maintained at a high level throughout treatment and thereafter requiring the close co-operation of patients [59,62].

b) Symptomatic relief of oral dryness

The simplest measure is frequent moistening of the mouth with water and many patients will carry with them a bottle of water for this purpose. The poor retention properties of water alone means frequent applications which is inconvenient [136]. For this reason more viscous glycerine containing mouthwashes which require less frequent application have been employed [92,143]. More complex saliva substitutes have been developed not only containing substances to impart viscosity such as mucin [135] or carboxymethylcellulose (CMC) [143] but also including inorganic substances such as calcium, phosphate and fluoride to aid remineralisation [106]. Often though patients object to the taste or inconvenience of using artificial saliva and return to the use of water [73,108]. Intraoral devices have been constructed which act as reservoirs providing slow and sustained release of artificial saliva [129,133,134].

c) Stimulation of residual salivary tissue

A large number of commonly prescribed medications can result in reduced saliva flow [102,116]. Wherever possible these should be avoided.

Salivary secretion can be stimulated by the use of gustatory, mechanical, pharmacological and electrical means. Acidic tasting substances such as lemon drops or pastilles are found helpful by some patients but preparations should be sugar free [62]. Sugarless chewing gum provides both mechanical and gustatory stimuli and is used to good effect by some patients [62,108].

The flow of saliva is mediated through the parasympathetic innervation of the salivary glands. Over a 100 years ago Hutchinson used Jaborondi, a dried leaf which is the scource of pilocarpine, to treat patients with dry mouths [61,117]. Pilocarpine alone [53] and in combination with anetholetrithione [41] has proved effective as a sialogogue in clinical trials. The usefulness of pilocarpine and other pharmacological sialogogues is though limited by their potential for side effects, particuarly gastrointestinal and cardiovascular [123].

Electrical stimulation of the oral and pharyngeal afferent nervous system with a specially constructed device is a further approach to increase saliva flow from residual salivary tissue [119,141].

11) Clinical observation of patients treated by CHART:

At Mount Vernon Hospital patients with advanced head and neck cancer have been treated with the CHART schedule since 1985. Some increase in early radiation effects was predicted because of the reduction in overall treatment time [97]. Acute reactions

have though proved tolerable with mucositis being most troublesome and skin reactions being milder than expected [98].

The clinicians involved in the follow up of these patients have consistently observed a reduction in late changes of the irradiated tissues such as skin and subcutaneous tissue although these differences are difficult to quantify. Reduced late changes after CHART was predicted on the basis of the low dose per fraction employed [46,97].

There is no internationally agreed way of measuring and recording radiation morbidity although the EORTC/RTOG system is widely used [28]. Dische has suggested a system for the recording of radiation change using a dictionary developed and used at Mount Vernon since 1985. It essentially abstracts all the separate elements on which morbidity scales are based eg telangiectasia, fibrosis etc and records them individually. It also places more emphasis on functional radiation effects than on morpholological changes as compared to other systems. Once the basic data has been obtained it can be sorted to produce the grades according to other systems of recording morbidity thus allowing comparisons to be made with different series of patients using a previously employed system [25]. In a study assessing acute buccal mucosal reactions during radiotherapy the Dische system was compared to the EORTC/RTOG system and the former was found to be the more sensitive [75].

The clinical impression of reduced late changes after CHART has been lent some support by a comparative study. The late radiation change observed in 15 patients treated for carcinoma of the oral cavity or oropharynx using CHART was compared to that seen in 15 similar patients treated with conventionally fractionated radiotherapy; the Dische system was employed. The late changes observed in skin and mucosa were similar in both groups but hair regrowth was observed in 6 out of 10 men treated with CHART compared with persistent partial or complete hair loss in all 9 men treated with the conventional scheme. In the CHART group only one patient complained of partial taste loss compared with 7 in the conventionally treated group. 11 of the CHART patients and 13 of the conventionally treated patients had some dryness of mouth. This was graded as severe in 3 of the conventionally treated patients but in none of the CHART patients [67].

In addition the morbidity of salvage surgery following CHART seems comparable to that encountered following conventionally fractionated radiotherapy. When operations were performed after CHART there was an impression that the longer the interval after treatment the easier was surgery to perform. This is in contrast to the usual experience after conventional radiotherapy when the optimum time for surgery appears to be between 6 and 8 weeks after treatment, and when late operations are associated with a risk of morbidity [96]. A reduction in late changes after CHART has not though been observed in spinal cord possibly due to particularly long repair half times [24,27].

CHAPTER 2
OBJECTIVES, METHODS AND MATERIALS

1) Objectives:

The objectives of this project are :

1) To document quantitatively the changes in salivary gland function that occur during and after a course of radical radiotherapy for head and neck cancer.

2) To compare the differences that result following treatment by CHART as compared to conventionally fractionated radiotherapy.

2) Methods and materials:

a) Techniques for saliva collection

Various methods of saliva collection (Chapter 1 section 3) were tested on both patients and healthy volunteers prior to commencement of the study. The spitting method for collection of resting whole saliva and Lashley cups to collect from the parotid glands following stimulation proved feasible and well tolerated. Repeated testing of the same subjects demonstrated that the tests were reproducible. These tests were thus used in the project.

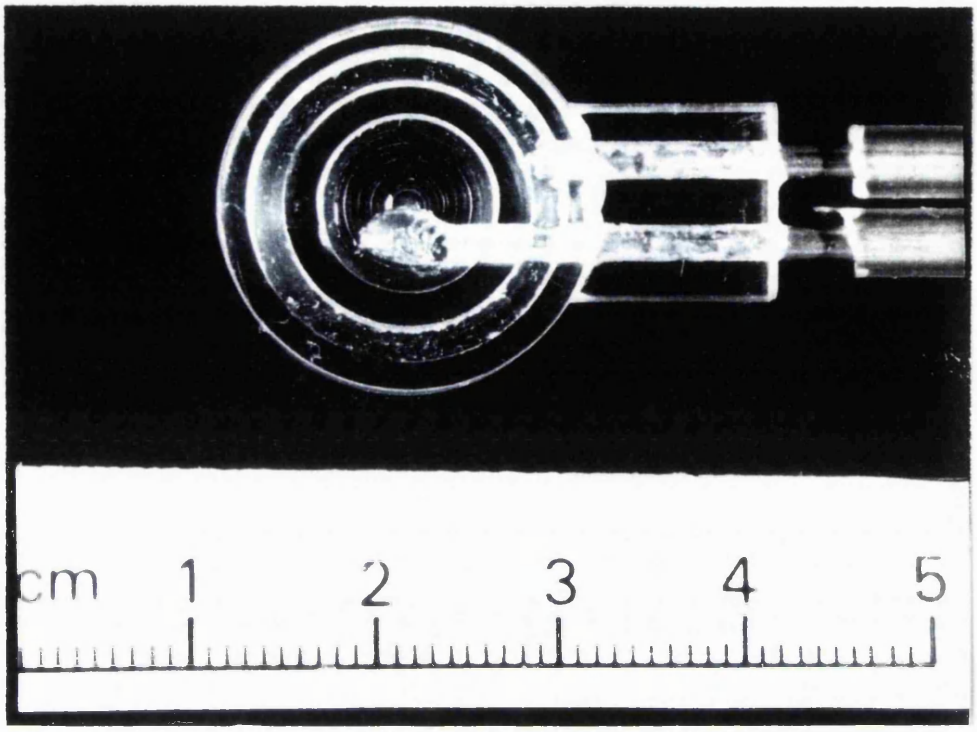
Collection of saliva was carried out under standard conditions to minimise the effect of outside influences (Chapter 1 section 2). All collections were carried out in a quiet room with patients having refrained from eating or smoking for at least 1 hour prior to testing. All samples were collected between 9am and 4pm.

Resting whole saliva :

The spitting method was employed. Patients expectorate saliva into a receiver at will for a period of 10 minutes. The volume of saliva collected is measured in graduated tubes and flow rate is expressed as mls/minute.

Stimulated parotid saliva :

Lashley cups [42,103] were used to collect saliva simultaneously from both parotid glands. These small round plastic cups (Fig 4a) have an inner and outer chamber, each being attached to fine bore plastic tubing. The inner chamber is placed over the opening of Stensen's duct (Fig 4b) and suction applied to the outer chamber from a 20ml syringe provides the vacuum to hold the device firmly on the buccal mucosa of the cheek whilst saliva is collected from the inner chamber (Fig 5). Stimulation of saliva flow was achieved by applying three drops of 2% citric acid to the dorsum of the tongue at intervals of 2 minutes and collection was carried out for 10 minutes. The volume of saliva collected is measured in graduated tubes and flow rate for each gland is expressed as mls/minute.



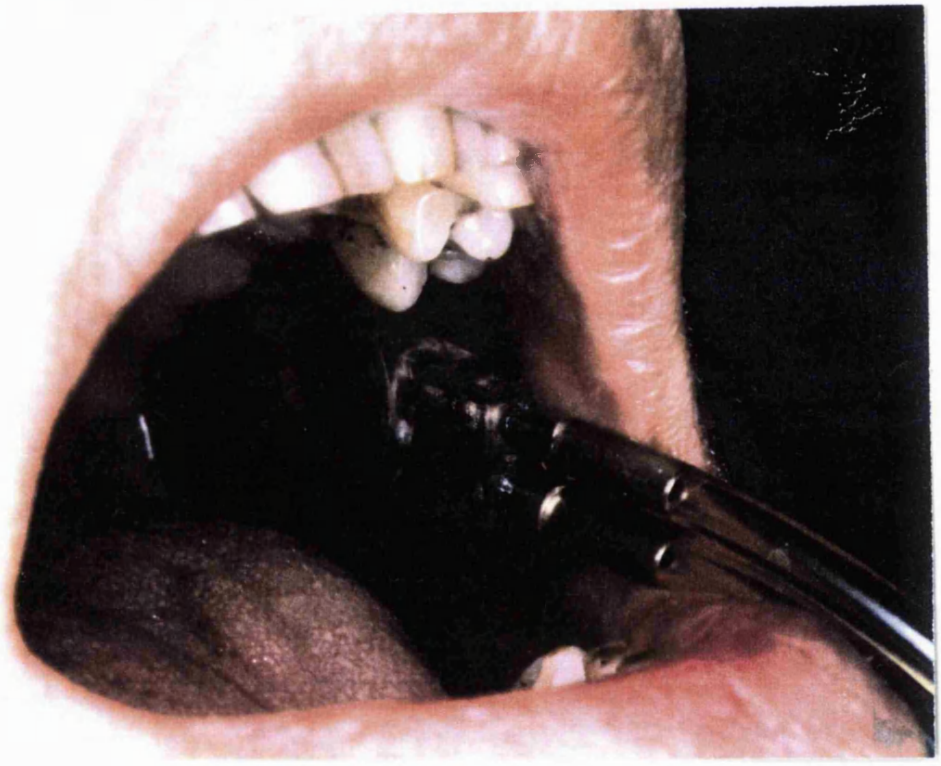


Figure 4. a) Lashley cup.

b) Lashley cup in place over Stensen's duct.

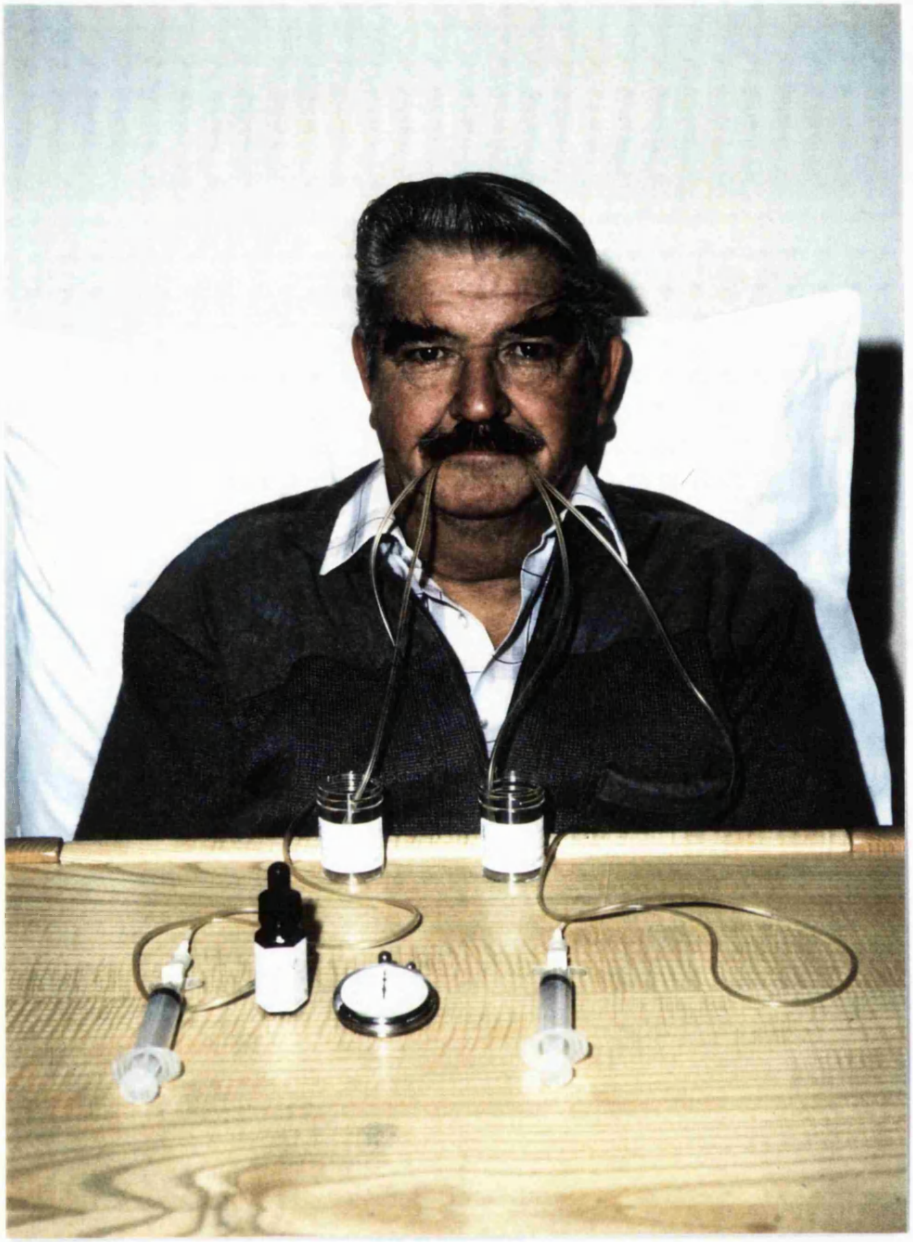


Figure 5. Simultaneous collection of saliva from both parotid glands.

b) Patients studied:

All patients were treated in perspex shells using a 5MV linear accelerator. All freely gave their informed consent for the study. No patient had undergone surgery to the major salivary glands or was receiving any medication known to interfere with salivary gland function. None showed any evidence for dissemination beyond the regional lymph nodes and all were considered suitable for treatment by radical radiotherapy.

Retrospective study :

The parotid gland function of 26 patients treated by radiotherapy was studied on one occasion nine months or more after its conclusion. All had received radiotherapy for a malignancy confined to one side of the head and neck region using a unilateral technique employing anterolateral and posterolateral fields. This resulted in the parotid gland on the ipsilateral side being encompassed in the treatment volume and a dose less than 25% of that prescribed being delivered to the contralateral gland. The contralateral gland thus acted as an internal control.

Three groups of patients were studied (Table III) :

Group I 12 treated with conventionally fractionated radiotherapy for a squamous cell carcinoma received between 60 and 66 Gy minimum tumour dose in 30-33 fractions treating daily over 6-7 weeks.

Group 2 8 treated with conventionally fractionated radiotherapy for lymphoma received between 35 and 40 Gy minimum tumour dose in 17-22 fractions treating daily over 3.5-4.5 weeks.

Group 3 6 treated with CHART for squamous cell carcinoma received 54 Gy minimum tumour dose in 36 fractions over twelve consecutive days treating 3 times a day with an interval between fractions of at least 6 hours.

For the 18 patients with squamous cell carcinomas the TNM stage (appendix 1) was T2, N0 in 10 and T1, N0 for the remaining 8. For all 8 lymphoma cases the disease stage was I or IE (Ann Arbor classification, appendix 2).

Prospective study :

47 patients receiving radical radiotherapy for squamous cell carcinoma of the head and neck have been studied (Table IV). 37 were treated with the CHART schedule and 10 received conventionally fractionated radiotherapy to a dose of 66 Gy given in 33 fractions treating daily over 6-7 weeks. The prescribed doses were target applied doses. A baseline assessment of salivary gland function is made on two occasions prior to the commencement of radiotherapy and all subsequent measurements are related to the mean of these two pre treatment measurements.

TABLE III DETAILS OF PATIENTS INCLUDED IN THE RETROSPECTIVE STUDY

No	Age	Sex	Primary site	Histology	Radiotherapy (Gy/fractions /days)	Time till testing (yrs)
01	54	M	Tonsil	SCC	66/33/49	1.8
02	68	M	Tonsil	SCC	60/30/45	2.3
03	67	M	Tonsil	SCC	64/32/46	2.2
04	72	M	Retromolar	SCC	60/30/43	1.6
05	75	F	Retromolar	SCC	64/32/51	3.8
06	63	M	Tonsil	SCC	64/32/49	1.0
07	78	M	Tonsil	SCC	64/32/48	2.8
08	79	M	Tonsil	SCC	64/32/49	3.5
09	74	M	Tonsil	SCC	64/32/49	6.4
10	55	F	Retromolar	SCC	65/32/57	7.5
11	67	F	Palate	SCC	60/30/43	5.0
12	63	F	Buccal	SCC	64/32/44	8.2
13	44	M	Tonsil	NHL	40/22/29	1.4
14	70	M	Tonsil	NHL	40/20/27	5.4
15	70	F	Tonsil	NHL	40/20/26	3.2
16	55	M	Parotid	HD	35/17/18	9.0
17	51	F	Tonsil	NHL	40/20/26	4.4
18	60	M	Tonsil	NHL	40/20/26	2.1
19	62	M	Tonsil	NHL	40/20/26	5.9
20	54	M	Tonsil	NHL	40/20/27	8.0
21	71	M	Tonsil	SCC	54/36/12	4.1
22	76	M	Tonsil	SCC	54/36/12	3.0
23	67	F	Palate	SCC	54/36/12	1.8
24	58	M	Retromolar	SCC	54/36/12	1.4
25	52	F	Retromolar	SCC	54/36/12	1.0
26	72	M	Alveolus	SCC	54/36/12	0.8

HD - Hodgkins lymphoma
NHL - Non Hodgkins lymphoma
SCC - Squamous cell carcinoma

TABLE IV DETAILS OF PATIENTS INCLUDED IN THE PROSPECTIVE STUDY

No	Age	Sex	Primary site	Stage	Radiotherapy
01	68	M	Tonsil	T2N0	
02	63	M	Tonsil	T2N1	
03	55	M	Post 1/3 tongue	T2N2	
04	64	M	Tonsil	T4N0	
05	63	M	Tongue	T4N0	
06	52	M	Tongue/Palate	T2N1	
07	55	M	Tonsil	T2N1	
08	50	M	Tongue	T4N1	
09	64	F	Post 1/3 tongue	T4N0	
10	59	M	Tonsil	T3N2	
11	47	F	Tongue	T2N2	
12	76	M	Piriform sinus	T2N3	
13	75	F	Post 1/3 tongue	T2N2	
14	60	M	Tonsil	T3N3	
15	51	F	Post 1/3 tongue	T2N2	
16	86	F	Alveolus	T4N0	
17	70	F	Alveolus	T4N0	
18	71	M	Alveolus	T4N0	CHART
19	81	M	Tonsil	T2N0	
20	48	M	Tongue	T2N0	
21	73	M	Tongue	T2N0	
22	78	M	Floor of mouth	T4N1	
23	47	M	Floor of mouth	T4N1	
24	55	M	Floor of mouth	T2N1	
25	63	M	Floor of mouth	T2N1	
26	58	M	Pharyngeal wall	T2N0	
27	52	M	Floor of mouth	T2N1	
28	70	M	Floor of mouth	T2N0	
29	70	F	Supraglottis	T4N0	
30	59	M	Supraglottis	T3N0	
31	59	M	Epiglottis	T2N0	
32	64	M	Supraglottis	T3N0	
33	68	F	Supraglottis	T4N0	
34	70	M	Columella	*	
35	85	M	Glottis	T2N0	
36	76	F	Columella	*	
37	79	M	Tongue	T2N0	
38	52	M	Post 1/3 tongue	T4N2	
39	70	M	Tonsil	T4N1	
40	63	M	Tonsil	T2N2	
41	64	M	Tonsil	T3N3	
42	73	F	Tongue	T4N1	CONVENTIONAL
43	75	M	Post 1/3 tongue	T3N1	
44	68	F	Post 1/3 tongue	T3N1	
45	53	F	Post 1/3 tongue	T2N2	
46	62	M	Tonsil	T2N2	
47	70	F	Retromolar trigone	T4N1	

* No TNM staging system is specified for columella

31 of the patients studied presented advanced tumours of the oral cavity, oropharynx or hypopharynx. A number of field arrangements were used to treat these patients but in all cases the upper border of the fields came up to above the level of the hard palate. 16 patients (patients 1-9 and 38-44, Table IV) were treated by opposed fields throughout treatment, a large volume being followed by a reduced volume coming off the spinal cord. Essentially all salivary tissue was included in the treatment volume throughout. Other cases (patients 10-15 and 45-46, Table IV) with nodes lying posteriorally in relation to the spinal cord were treated with opposed fields for the large volume and angled opposed fields for the reduced volume. This resulted in one parotid gland being included in the treatment volume throughout and the other being in the treatment volume for the large volume and then receiving 15-20% of the small volume tumour dose. Other less advanced tumours (patients 16-19 and 47, Table IV) were treated by a unilateral wedge pair technique throughout such that one parotid gland was in the treatment volume throughout, the other receiving less than 25% of the prescribed dose. 2 patients with carcinoma of the tongue (patients 20-21, Table IV) were treated by a unilateral wedge pair technique with "CHART" for 9 days only to a dose of 40.5 Gy given in 27 fractions, the second phase of treatment being given by an implant.

The remaining 16 cases, treated by CHART (patients 22-37, Table IV) consisted of tumours at a variety of head and neck sites with varying amounts of salivary tissue being included in the treatment volumes.

Patients receiving conventionally fractionated treatment are tested weekly during treatment and those receiving CHART on the 3rd, 6th and 10th day of treatment. Following the completion of radiotherapy patients are tested at 8 weeks and 12 weeks from the start of treatment and thereafter at 3 monthly intervals unless relapse occurs.

Amylase study :

41 patients receiving radical radiotherapy for squamous cell carcinoma in the head and neck region were studied (Table V). The mean age of the patients was 64 years (range 40-84), 32 patients were male and 9 female. Opposed fields were used for the first phase of treatment to the large volume with usually a second phase to a reduced volume given by smaller opposed fields or with a unilateral wedge pair technique. 29 were treated with CHART and the remaining 12 received conventionally fractionated radiotherapy to a dose of 66 Gy given in 33 fractions treating daily over 6-7 weeks. The prescribed doses were target applied doses.

Serum amylase was measured prior to treatment in 40 of the patients. For the first 8 patients studied serum amylase was measured hourly for eight hours after the first fraction of radiotherapy, and then at intervals of 24 hours after the start of treatment. For the subsequent patients serum amylase was measured at 24 hour intervals following the start of treatment. Samples were taken just before treatment and for the patients

TABLE V **DETAILS OF PATIENTS INCLUDED IN THE AMYLASE STUDY**

No	Site	Stage	Radiotherapy	Included in Phase I Treatment Volume	
				% parotid glands	% submandibular + sublingual glands
01	Tonsil	T3N2	CHART	100	100
02	Tonsil	T2N0	"	"	"
03	Tongue	T2N2	"	"	"
04	Post 1/3 tongue	T2N2	"	"	"
05	Tonsil	T4N0	"	"	"
06	Tonsil	T3N3	"	"	"
07	Post 1/3 tongue	T2N1	"	"	"
08	Tonsil	T2N1	"	"	"
09	Tongue	T4N1	"	"	"
10	Tonsil	T4N0	"	"	"
11	Post 1/3 tongue	T2N2	"	"	"
12	Post 1/3 tongue	T4N0	"	"	"
13	Alveolus	T4N2	"	"	"
14	Supraglottis	T4N1	"	"	"
15	Tonsil	T2N0	"	"	"
16	Tonsil	T4N1	CONVENTIONAL	"	"
17	Post 1/3 tongue	T4N2	"	"	"
18	Tonsil	T3N3	"	"	"
19	Epiglottis	T4N1	"	"	"
20	Supraglottis	T3N2	"	"	"
21	Tonsil	T2N1	"	"	"
22	Post 1/3 tongue	T3N1	"	"	"
23	Tonsil	T2N1	"	"	"
24	Soft palate	T2N1	"	"	"
25	Supraglottis	T3N3	"	"	"
26	Supraglottis	T4N2	"	"	"
27	Epiglottis	T2N2	"	"	"
28	Floor of mouth	T4N2	CHART	80	"
29	Floor of mouth	T4N1	"	"	"
30	Floor of mouth	T2N1	"	"	"
31	Glottis	T3N0	"	"	"
32	Floor of mouth	T2N1	"	50	"
33	Floor of mouth	T2N0	"	"	"
34	Glottis	T3N0	"	"	"
35	Glottis	T3N0	"	30	"
36	Glottis	T3N0	"	"	"
37	Tongue	T2N0	"	0	"
38	Columella	*	"	"	0
39	Glottis	T2N0	"	"	"
40	Glottis	T2N0	"	"	"
41	Columella	*	"	"	"

* No TNM staging system is specified for columella

receiving CHART before the 8 am treatment session. In 6 patients treated by CHART the concentration of amylase in the stimulated parotid saliva was also measured.

c) Clinical observation of patients:

At each assessment of salivary gland function dryness of mouth, loss of taste and saliva consistency are scored clinically (appendix 3). Dryness of mouth was graded using a score of 0 - 3 as shown below :

0	Assymptomatic
1	Mild dryness of mouth
2	Moderate dryness of mouth requiring the patient to carry water/artificial saliva
3	Severe dryness resulting in serious incapacitation

Taste loss was scored as no loss (0), partial loss (1) and complete loss (2). The consistency of resting whole saliva was scored as normal/watery (0), slightly thickened (1) and thick/sticky (2).

d) Localisation of major salivary glands:

All patients in the prospective and amylase studies had undergone

diagnostic magnetic resonance scanning (MRI) of the head and neck area prior to treatment. The proportions of the major salivary glands included in the treatment volumes were determined by review of the simulator films, treatment plans and MRI scans. The anatomical siting of the submandibular and sublingual glands is well defined [35] and their inclusion in the treatment volume of opposed fields was determined directly from the lateral simulator films. The parotid glands are visualised well with MRI (Figure 6) which provides images in multiple planes and without bone artifact [139]. Ericson and Hedin [42,43] have shown that the volume of the parotid gland is linearly correlated to the area of the parotid gland measured on the lateral view of a sialogram. We were thus able to determine the proportion of the parotid gland volume included in the treatment volumes for each patient. For patients in the retrospective study MRI scans had not been performed but review of simulator films and treatment plans confirmed that the entire parotid gland on the ipsilateral side was included in the treatment volume throughout.

e) Measurement of salivary pH:

pH measurements were carried out using a Russell model 3100 pH meter with a protein resistant electrode type TR/CMAWL/4/TB. Calibration was carried out at pH 4 and 7 with standard buffers (BDH Ltd) to an estimated precision of 0.02 pH units.

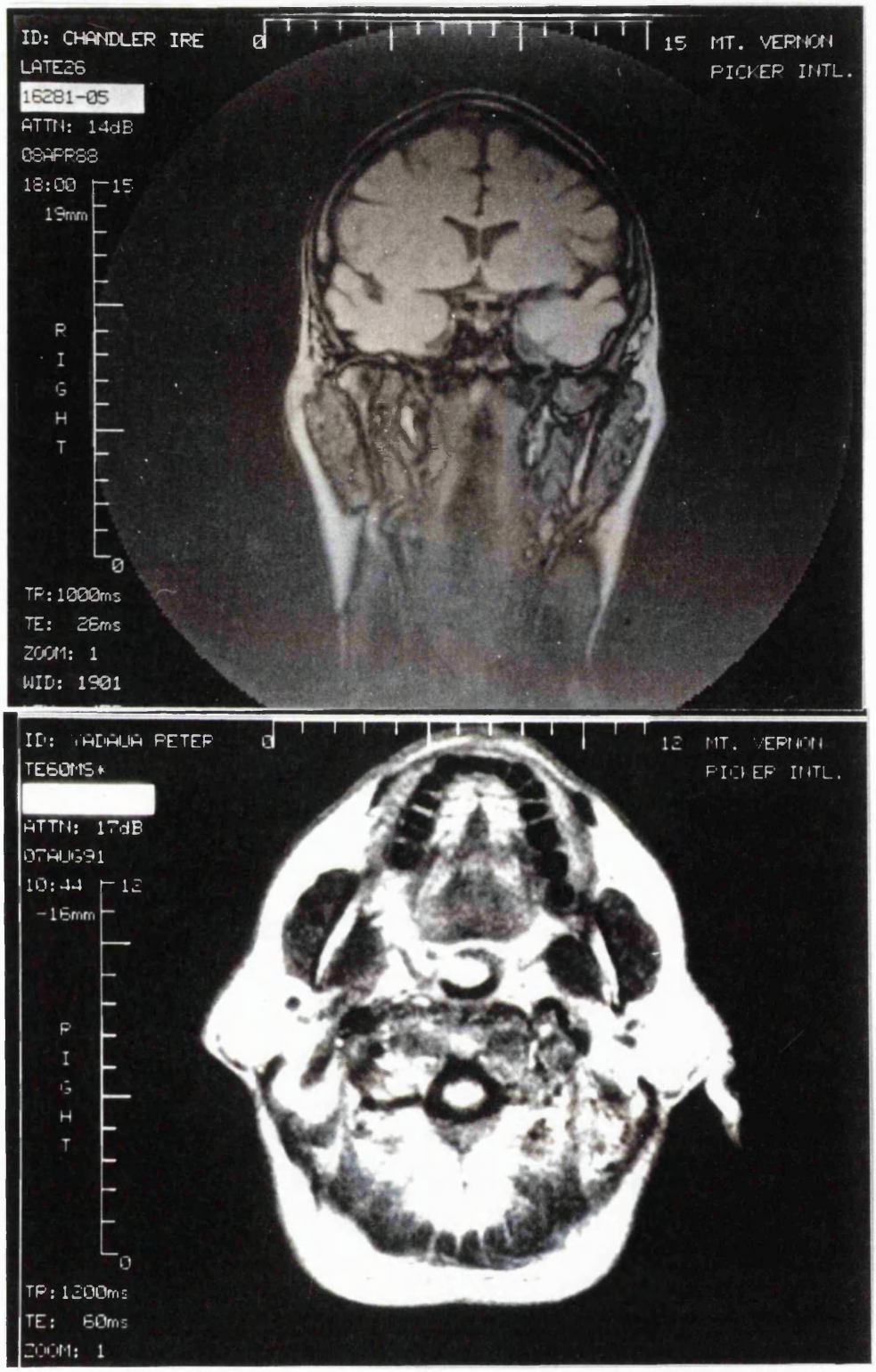


Figure 6. Parotid gland demonstrated by MRI.

a) Coronal section.

b) Transverse section.

f) Amylase measurements:

Amylase concentration in serum and saliva was determined using a liquid stable reagent set manufactured by Medical Analysis Systems, Inc. California, USA. The parotid saliva was diluted one thousand fold prior to testing. The test is based on the enzymatic action of amylase on the test substrate p-nitrophenyl maltoheptaoside which results in the production of p-nitrophenol [80]. The rate at which p-nitrophenol is formed is directly proportional to the amylase activity in the sample and this can be detected spectrophotometrically. The normal range was 150-300 International Units/ml (IU/ml).

g) Statistical methods:

All tests of statistical significance were carried out using the t test [58].

CHAPTER 3
RESULTS OF THE STUDY

The procedures for the collection of saliva proved relatively simple to perform and caused no discomfort or trauma to patients. In a few cases dislodgement of the Lashley cups during collection occurred but it was possible to successfully repeat the procedure. In most cases saliva collection was carried out at routine follow up visits.

1) Retrospective study of patients treated unilaterally:

a) Flow

The parotid flow rates from the contralateral ("untreated") glands of the three groups of patients studied are given in Figure 7a. Rates of flow from 0.30 to 0.68 ml/min were obtained. The pattern and range are similar to that obtained from 20 patients in the prospective study prior to treatment (Figure 8). Furthermore no differences can be seen between the patients of the three treatment groups. Figure 8 also demonstrates the wide variation in function of the normal population as described previously [101] and the correlation of flow rates between glands in the same individual.

In Figure 7b the flow rates from the ipsilateral ("treated")

parotid glands of the three groups are shown. These are reduced in all cases as compared with the corresponding contralateral glands with rates from 0.0 to 0.60 ml/min being obtained. In each case the flow rate of the ipsilateral gland was expressed as a percentage of the flow rate from the contralateral gland (Figure 7c). This removes the effect of the normal variation in flow between individuals and the contralateral gland acts as an internal control.

The greatest falls in flow were seen in glands treated by conventionally fractionated radiotherapy to a dose of 60-66 Gy. In 4 of the 12 ipsilateral glands there was no detectable function and in the other 8 the percentage flow of the ipsilateral gland as compared to the contralateral gland, did not exceed 41% and the mean percentage flow of the whole group was 20% (SE 4.7). A more modest impairment of function was seen in those glands treated either by conventionally fractionated radiotherapy to a dose of 35-40 Gy or by CHART. Percentage flow was greater than 50% for all cases treated to a dose of 35-40 Gy and for 5 of the 6 CHART cases. The mean percentage flows were 65% (SE 5.8) and 57% (SE 6.4) respectively (Figure 9).

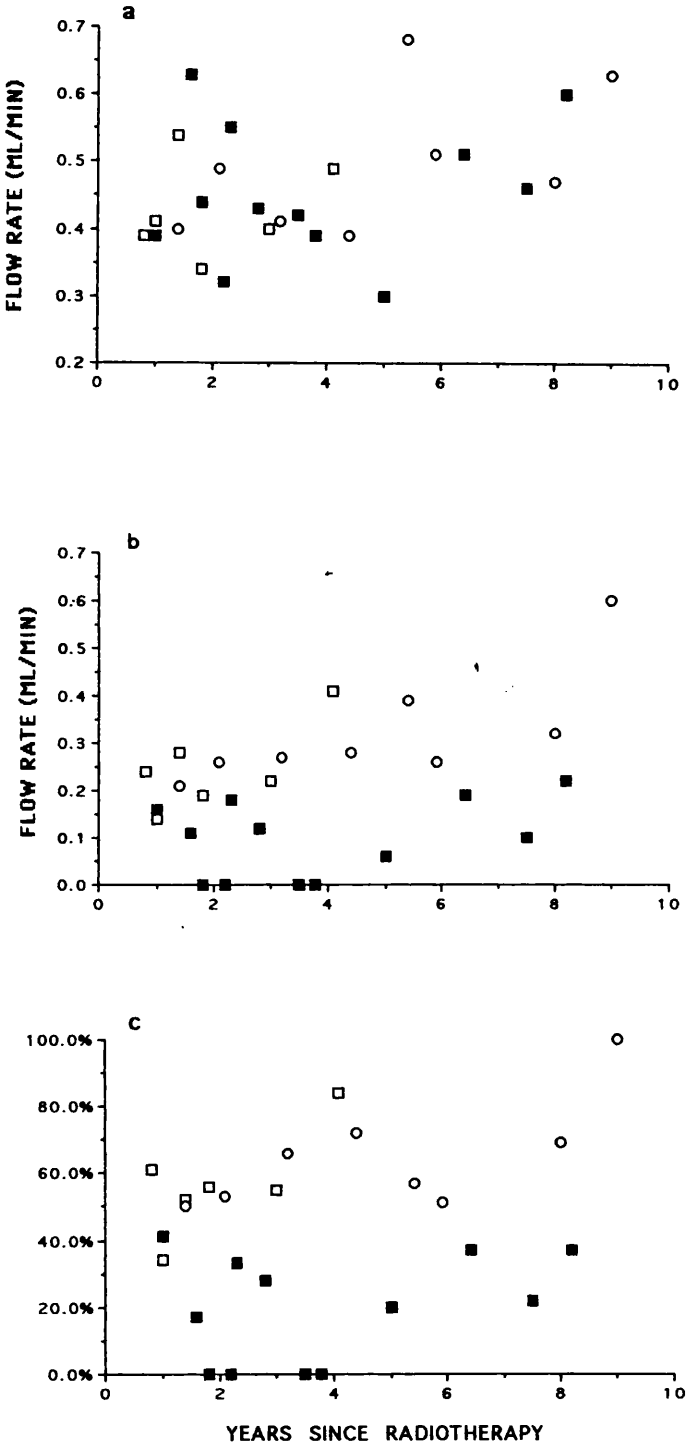


Figure 7.
Flow rates of parotid saliva from patients in the retrospective study

a) flow of contralateral glands
b) flow of ipsilateral glands
c) flow of ipsilateral glands expressed as % of flow from contralateral glands

60-66 Gy ■ 35-40 Gy ○ CHART □

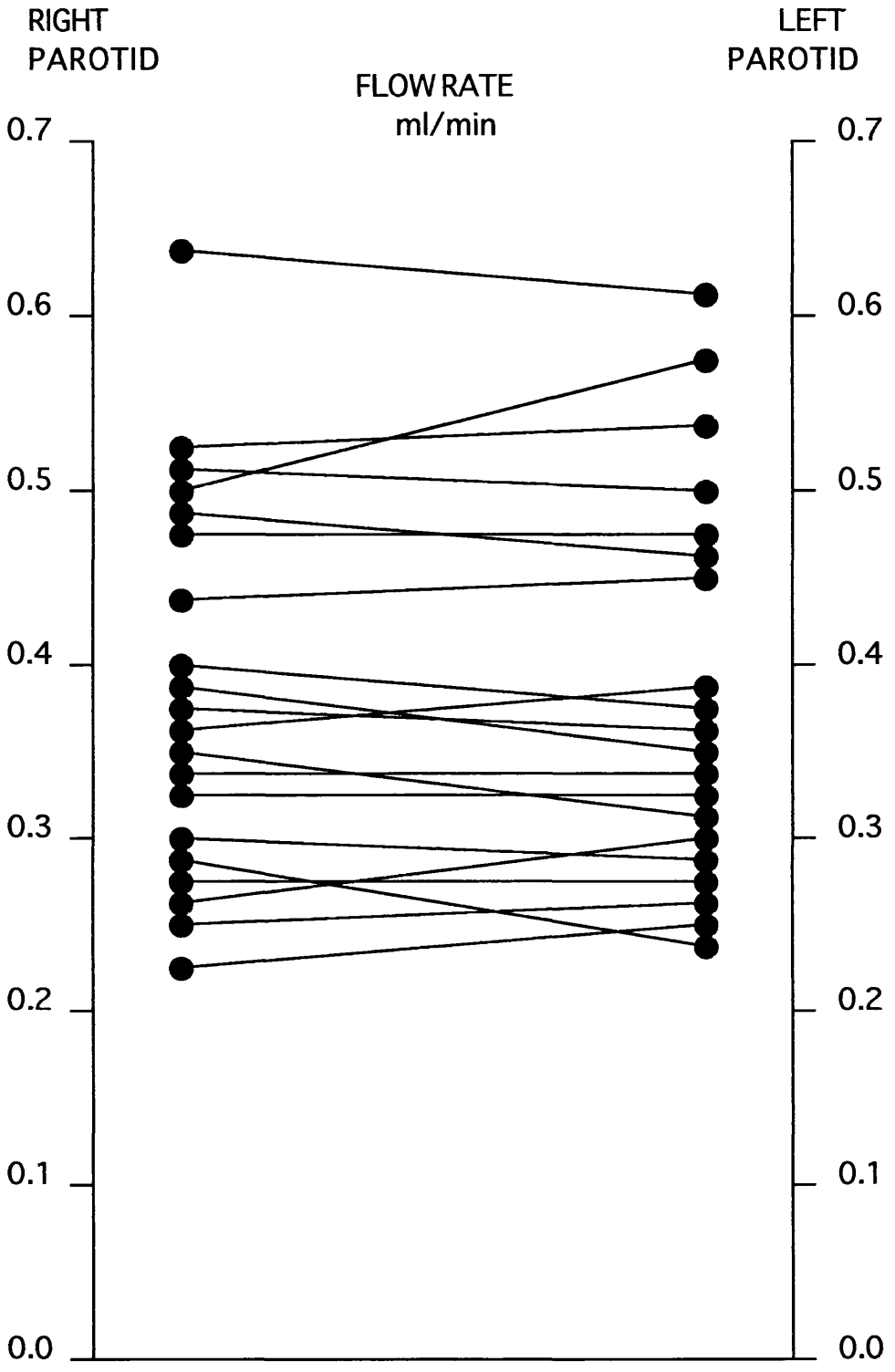


Figure 8. Flow rates of parotid saliva from twenty patients prior to radiotherapy.

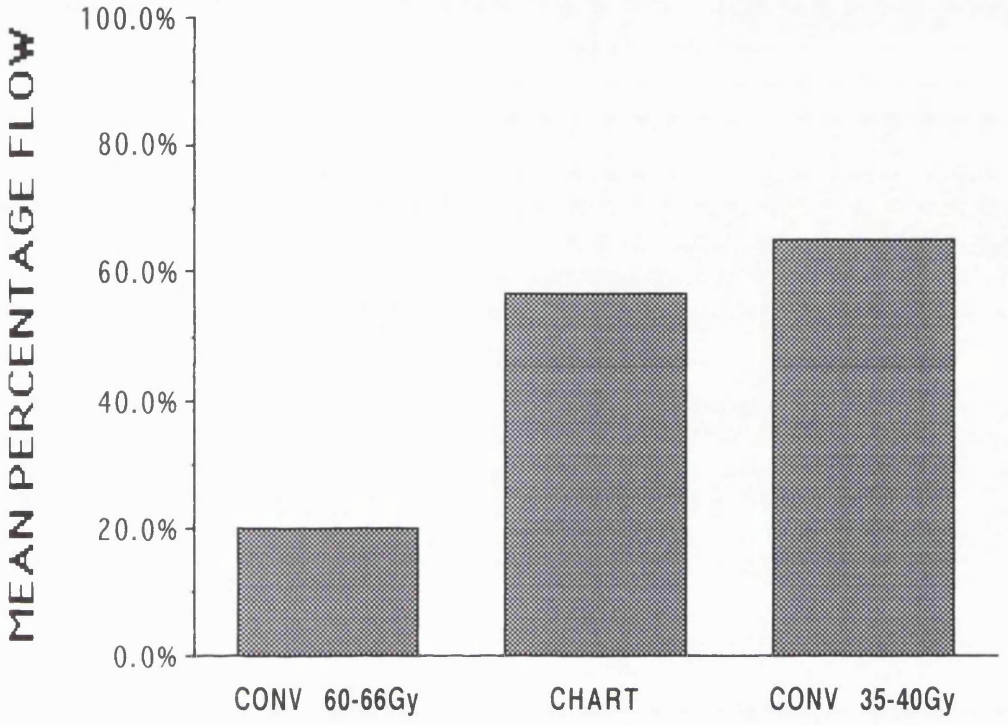


Figure 9. Mean percentage flow rates of parotid saliva from patients in the retrospective study.

b) pH

Results for the pH values of the parotid saliva are given in Table VI and are illustrated in Figure 10. Following irradiation by conventionally fractionated radiotherapy to a dose of 60-66 Gy there is a significant fall ($p<0.005$) in the mean pH of saliva from the ipsilateral parotid glands (7.23) as compared to that from the contralateral glands (7.42). In the cases irradiated to a dose of 35-40 Gy and in those treated by CHART, a small fall in mean pH was observed but in neither group did this reach statistical significance.

Table VI

pH of parotid saliva from patients in the retrospective study.

Treatment group	Conventional 60-66 Gy	Conventional 35-40 Gy	CHART 54 Gy
No of patients	8*	8	6
pH mean/SE (range)			
Ipsilateral	7.23/0.03 (7.19-7.31)	7.36/0.05 (7.28-7.47)	7.34/0.07 (7.29-7.44)
Contralateral	7.42/0.04 (7.36-7.50)	7.41/0.03 (7.29-7.47)	7.42/0.06 (7.31-7.49)
Difference	$p<0.005$	NS	NS

* Four cases had insufficient flow to measure pH.

c) Clinical symptoms

Only three patients (cases 3, 5 and 11, Table III) complained of any dryness of mouth. All were in the group treated by conventionally fractionated radiotherapy to a dose of 60-66 Gy and the flow rate from the ipsilateral gland was unrecordable in 2 and just detectable in the 3rd with a flow rate of 0.06 ml/min. The secretions from the contralateral glands in these cases were at the lower range of normal being 0.32, 0.39 and 0.30 ml/min respectively. No patients complained of loss of taste.

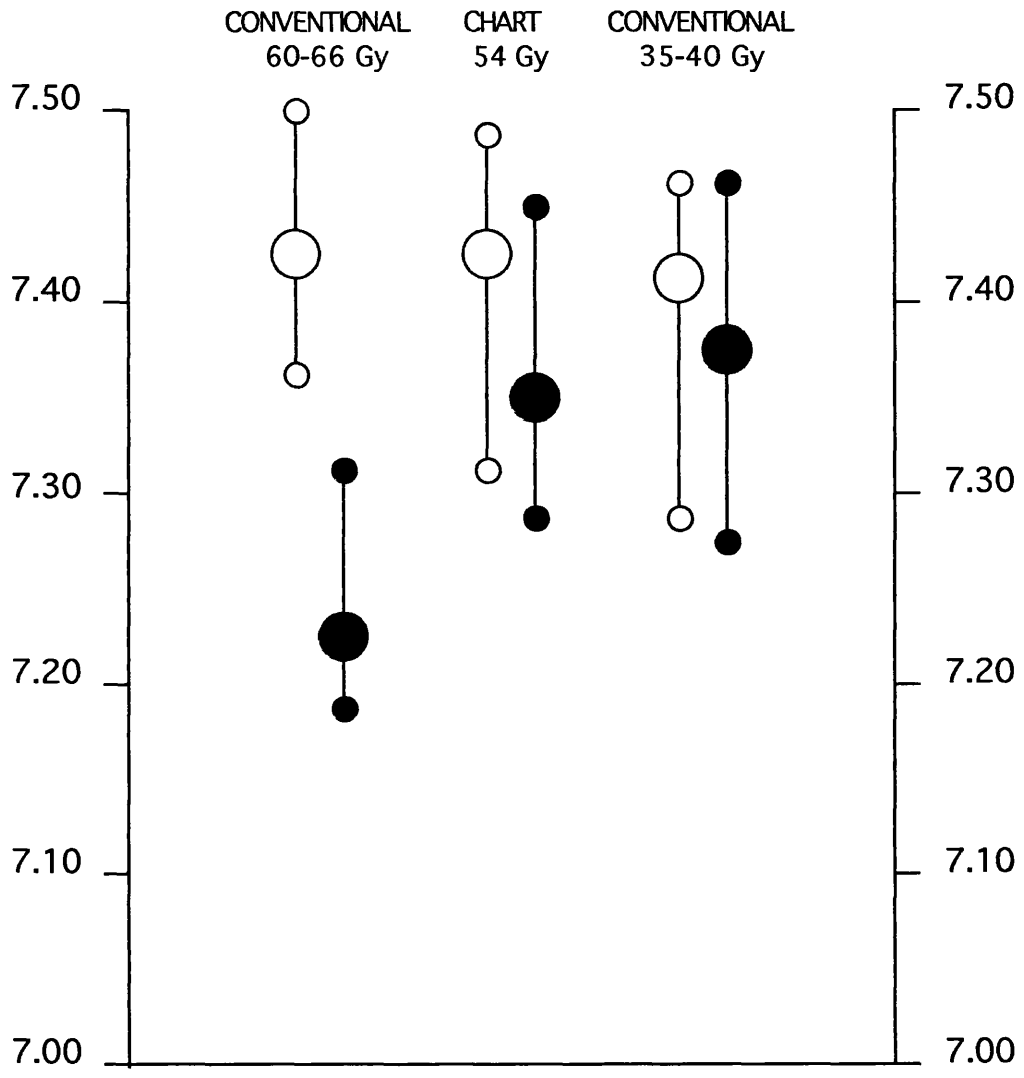


Figure 10. pH (mean and range) of parotid saliva from patients in the retrospective study.

Ipsilateral glands ●
Contralateral glands ○

2) Prospective Study:

Early changes (first 12 weeks from the start of radiotherapy)

a) Flow

16 patients with advanced oral cavity or oropharyngeal tumours were treated with similar opposed fields throughout treatment which included in the treatment volume essentially all salivary tissue. 9 received CHART (patients 1-9, Table IV) and 7 were treated with conventionally fractionated radiotherapy to a dose of 66 Gy (patients 38-44, Table IV). Marked falls in resting whole saliva (Figure 11) were seen during and early after the completion of treatment. Significantly earlier falls in flow rate were seen for the patients receiving CHART. By 8 weeks after the start of treatment the reduction in flow compared to pre treatment values was the same for both CHART and conventionally treated patients.The mean percentage flow at 12 weeks from the start of treatment for resting whole saliva was 9.4% for the CHART group and 7.1% for the conventionally treated group.

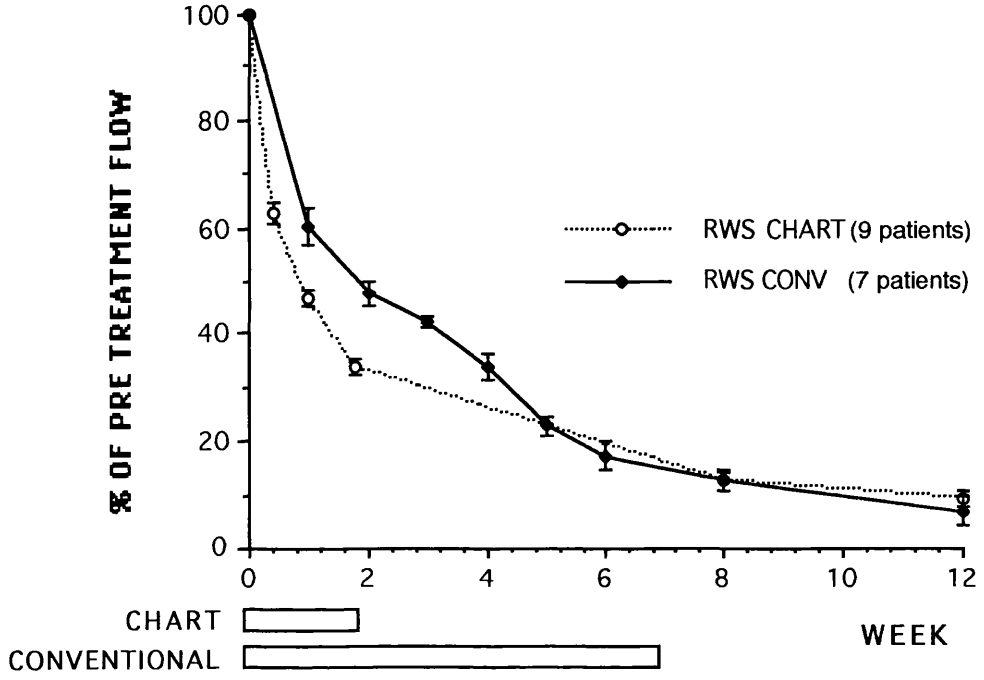


Figure 11. Early changes in resting whole saliva flow (mean and SEM). All salivary tissue included in the treatment volume.

In addition to the patients reported above a further 13 patients (patients 10-21 and 45-47, Table IV) had one parotid gland fully included in the treatment volume throughout. Thus a total of 45 parotid glands (28 CHART, 17 Conventional) receiving the full tumour dose and fully in the treatment volume have been studied. The changes in stimulated parotid flow for all these glands is shown in Figure 12. Marked falls are seen during and early after the completion of treatment. The rate of fall of stimulated parotid flow is greater than that seen for resting flow and as seen with resting flow significantly earlier falls in flow rate are seen for the patients receiving CHART. The mean percentage flow at 12 weeks from the start of treatment for stimulated parotid

flow was 2.6% for the CHART group and 3.0% for the conventionally treated group.

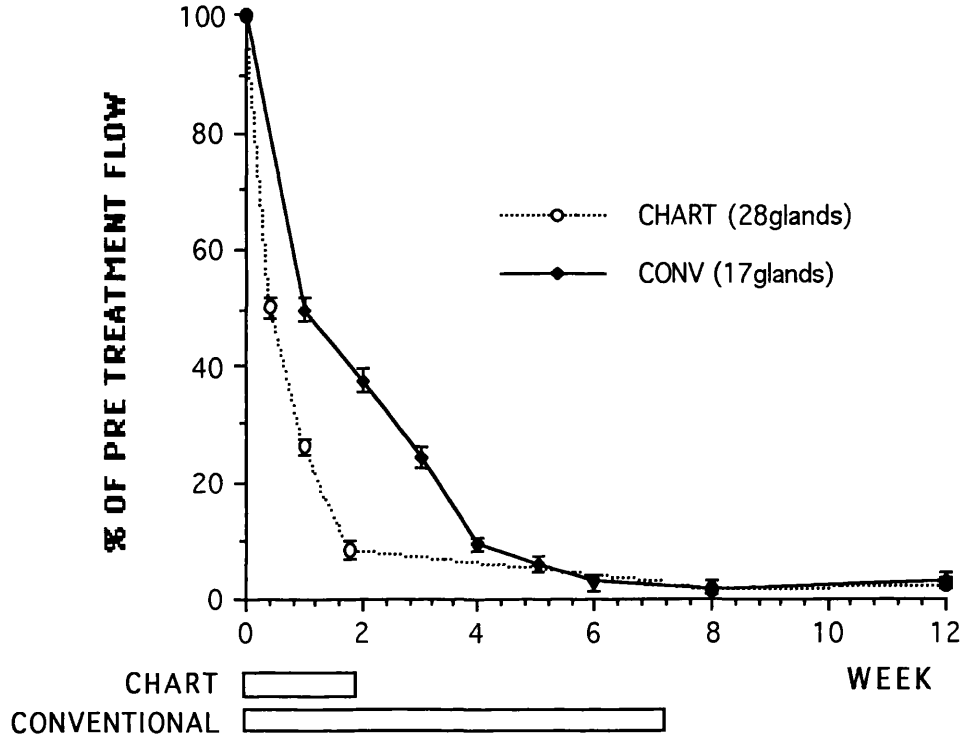


Figure 12. Early changes in stimulated parotid flow (mean and SEM). Parotid glands fully included in the treatment volume.

The effect of CHART on parotid flow at three dose levels when the parotid gland is fully included in the treatment volume is shown in Figure 13. There is a modest reduction in flow for glands receiving a total dose of 10-15 Gy. The reduction in flow for glands receiving 40-42 Gy is as marked as for those receiving the tumour dose of 54 Gy.

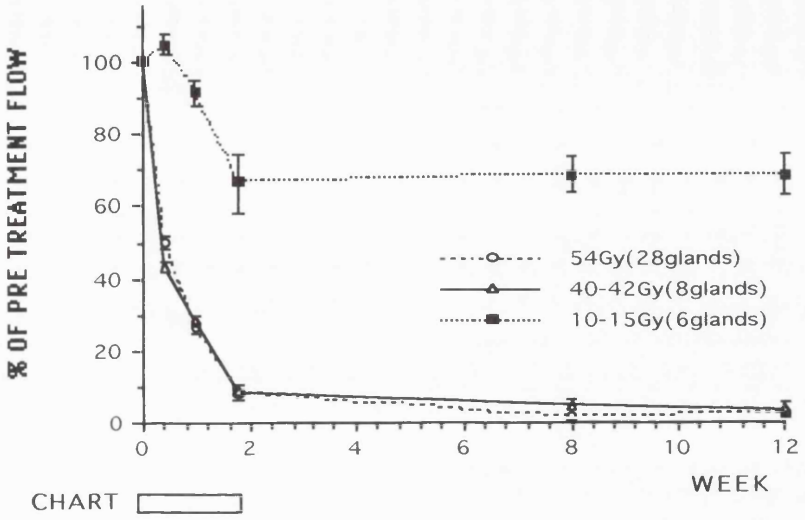


Figure 13. Early changes in stimulated parotid flow (mean and SEM). Glands receiving CHART at three dose levels.

A close relationship between the degree of reduced parotid flow after CHART and the proportion of the parotid gland included in the treatment volume is demonstrated (Figure 14).

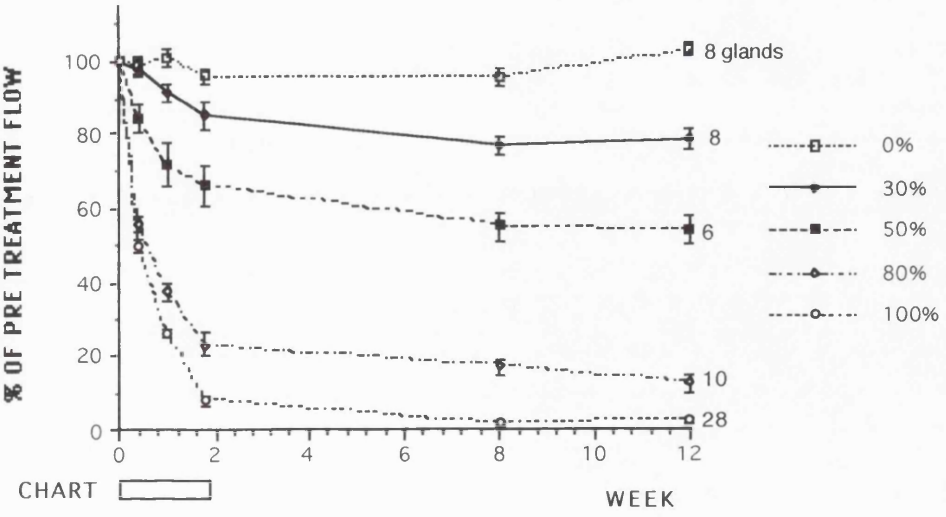


Figure 14. Early changes in stimulated parotid flow (mean and SEM). Variation with % of volume of gland irradiated.

b) pH

For the 16 patients treated by opposed fields throughout with essentially all salivary tissue incorporated in the treatment volume the mean pre treatment pH of the resting whole saliva was 6.58 (range 5.98-7.05, SE 0.09). Falls in pH were noted during and after radiotherapy in both treatment groups. Specimens of saliva were obtained from 11 of the 16 patients (2 produced no saliva, 3 were not tested) 12 weeks after the start of treatment. The mean pH for the CHART group was 6.37 (range 6.01-6.61, SE 0.09), and for the conventionally treated group 6.33 (range 6.07-6.64, SE 0.17). These falls in pH did not achieve statistical significance compared to pre radiotherapy values for either treatment group.

The mean pH of the stimulated flow from the 45 parotid glands receiving the full tumour dose and fully included in the treatment volume was 7.40 (range 7.20-7.62, SE 0.01) prior to radiotherapy. During and after radiotherapy the pH fell in both treatment groups. Specimens of saliva were obtained from 12 of the 45 glands (21 produced no saliva, 12 were not tested) 12 weeks after the start of treatment. The mean pH for the CHART group was 7.07 (range 6.90-7.16, SE 0.03), and for the conventionally treated group 6.98 (range 6.90-7.09, SE 0.04). These falls in pH were of statistical significance compared to pre radiotherapy values for both treatment groups, $P< 0.01$.

For the parotid glands which received CHART to a lower dose level specimens were obtained at 12 weeks from the start of

treatment from 3 of the 8 glands treated to a dose of 40-42 Gy (4 produced no saliva, 1 was not tested) and from all 6 glands receiving 10-15 Gy. The mean pH of the saliva from these glands was 7.10 (range 6.96-7.14, SE 0.14) and 7.45 (range 7.26-7.61, SE 0.09) respectively.

Figure 15 shows the pH of stimulated parotid saliva at 12 weeks after CHART according to the proportion of the parotid gland included in the treatment volume.

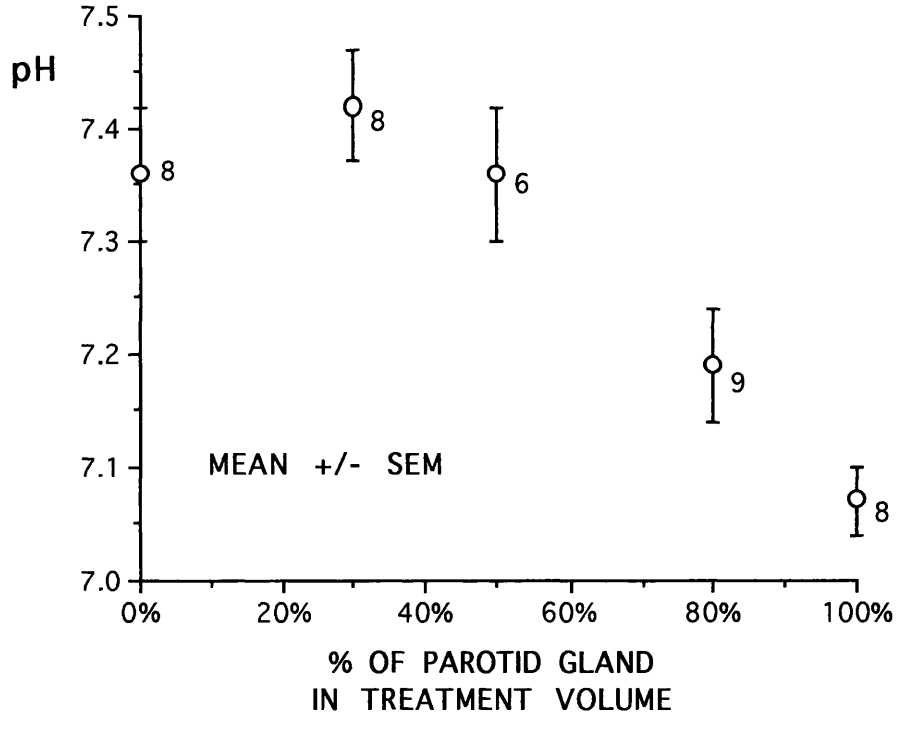


Figure 15. pH of stimulated parotid saliva at 12 weeks from the start of treatment (mean and SEM). Variation with % of volume of gland irradiated.

c) Clinical symptoms

The 16 patients treated by opposed fields throughout where essentially all salivary tissue was included in the treatment volume all reported increased stickiness of saliva secretions, dryness of mouth and partial loss of taste by the second week of treatment. 3 of the 9 CHART patients reported these symptoms by the fourth day of treatment. By 12 weeks from the start of treatment all complained of moderate dryness of mouth and in all saliva consistency was scored as thick/sticky. Complete loss of taste was complained of by 5 of the 7 treated by conventionally fractionated radiotherapy and by 4 of the 9 treated by CHART. For the remainder partial loss of taste was reported with usually only the preservation of taste for sweet being preserved.

In general the clinical symptoms experienced by patients reflected the amount of salivary tissue that had been included in the treatment volume. The extent of inclusion of the parotid glands in particular seemed to markedly affect the degree of symptoms. This is illustrated by a patient with a verrucous squamous cell carcinoma of the tongue treated with limited opposed fields to the anterior 2/3 of the tongue and floor of mouth with CHART (case 37, Table IV). The submandibular and sublingual glands were in the treatment volume but the parotid glands were entirely excluded. The patient had no complaint of dryness of mouth although partial loss of taste was complained of from 2 weeks after the start of treatment. The saliva consistency remained normal/watery throughout.

Later changes

a) Flow

The later changes in resting flow for the 16 patients treated by opposed fields throughout is shown in Figure 16. The number of patients decreases with time due to patients undergoing surgery (8), dying of disease (3) and lack of co-operation with follow up (1). The remaining 4 patients (2 CHART, 2 conventional) continue to be assessed in follow up.

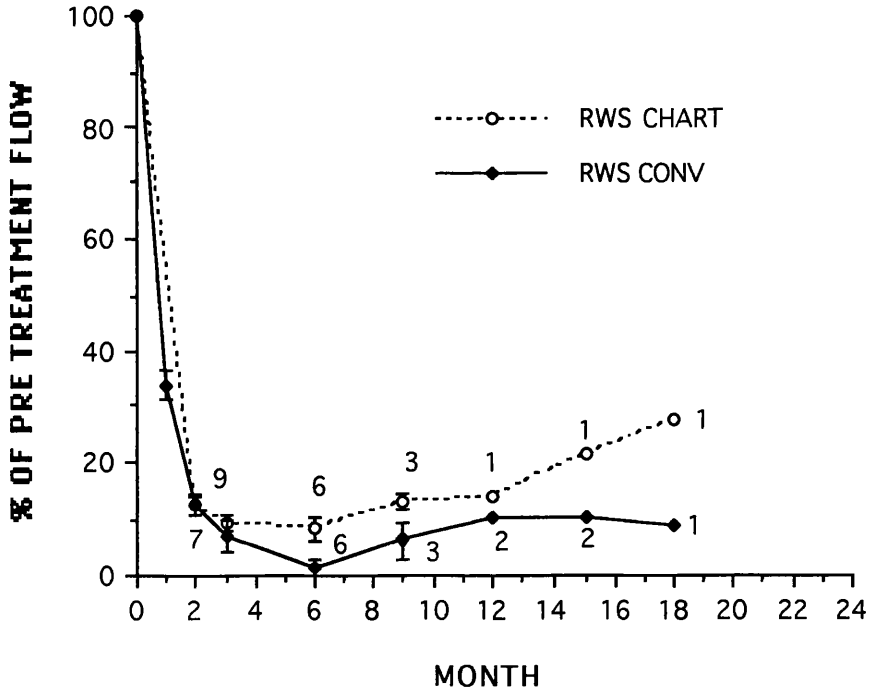


Figure 16. Changes in resting whole saliva flow (mean and SEM). All salivary tissue included in the treatment volume.

Results for the 45 parotid glands (29 patients) fully included in

the treatment volume is shown in Figure 17. Again the number of glands assessable decreases with time due to patients undergoing surgery (12), dying of disease (5) and lack of co-operation with follow up (1). The remaining 11 patients (15 glands - 9 CHART and 6 conventional) continue to be assessed in follow up.

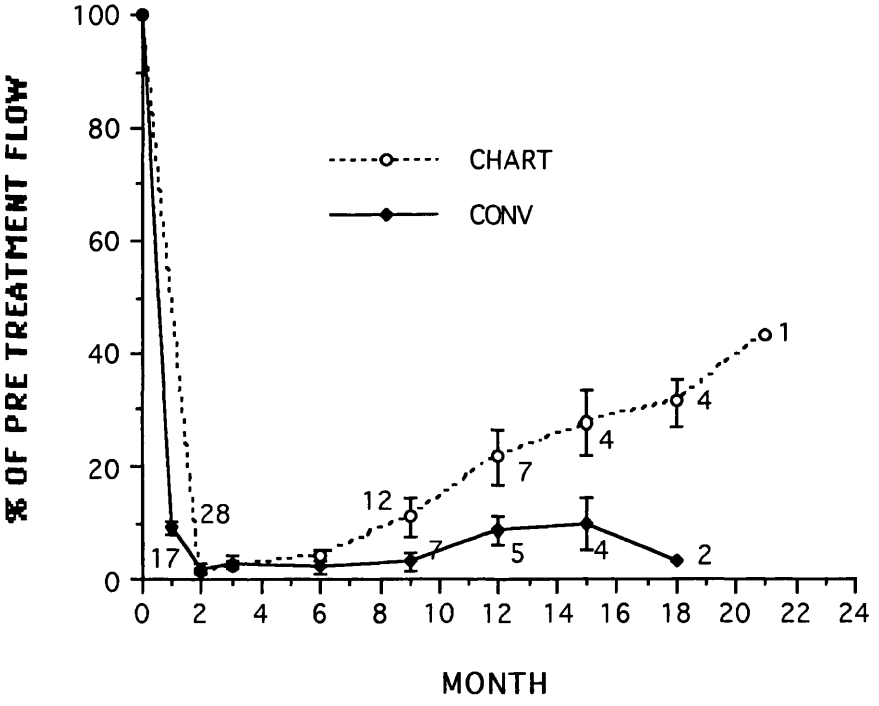


Figure 17. Changes in stimulated parotid flow (mean and SEM). Parotid glands fully included in the treatment volume.

There is a suggestion of a greater recovery of salivary flow, both for resting whole saliva (Figure 16) and stimulated parotid saliva (Figure 17) in the patients treated by CHART as compared to those receiving conventionally fractionated treatment. This recovery seems to commence at about 6 months after the start of treatment.

Recovery of flow in the glands treated by CHART to a dose less than 54 Gy is shown in Figure 18. Glands treated to a dose of 10-15 Gy show an early and relatively complete recovery of function. There also appears to be a greater recovery of function by glands receiving 40-42 Gy as compared with those receiving 54 Gy.

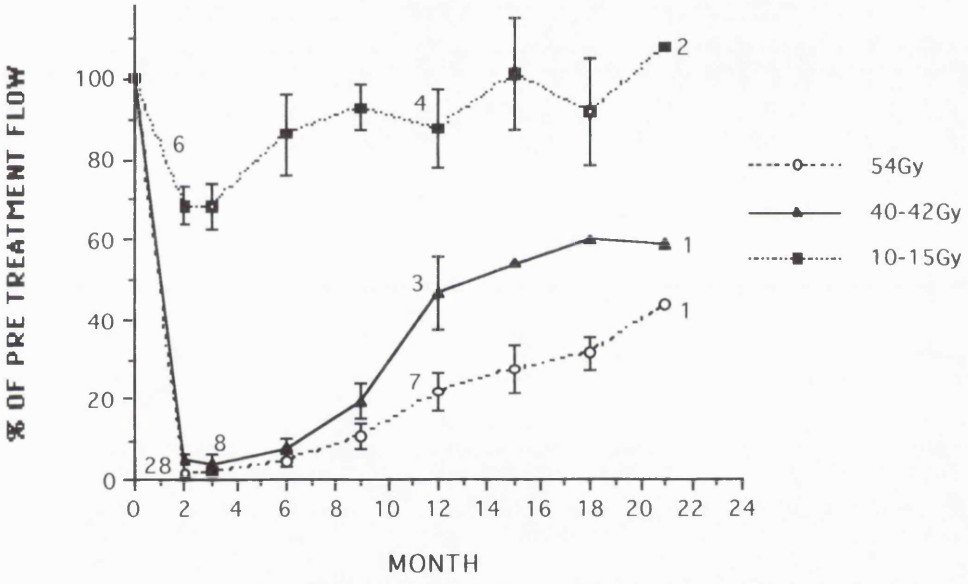


Figure 18. Changes in stimulated parotid flow (mean and SEM). Glands receiving CHART at three dose levels.

The recovery of flow from parotid glands not fully included in the treatment volume is shown in Figure 19.

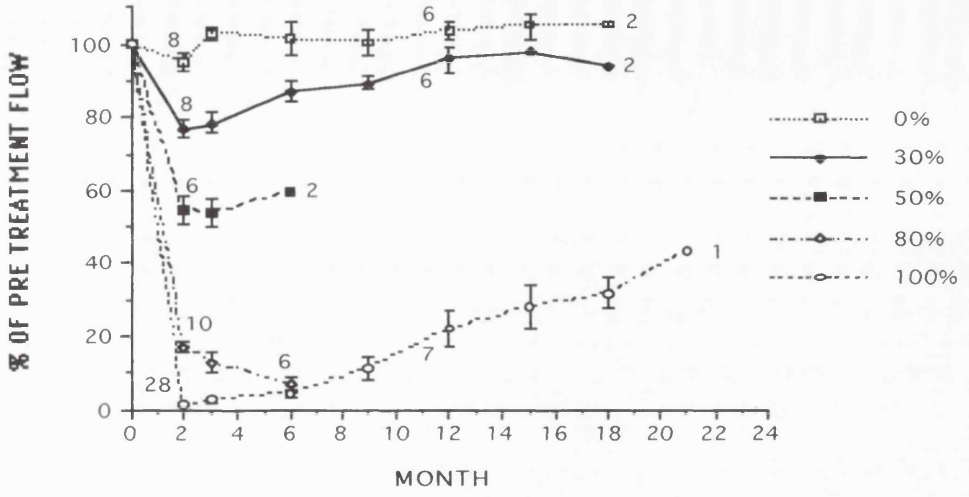


Figure 19. Changes in stimulated parotid flow (mean and SEM). Variation with % of volume of gland irradiated.

b) pH

Of the group of 16 patients treated by opposed fields throughout only 3 were available for collection of resting whole saliva twelve months after radiotherapy (Figure 16). For the 1 patient treated by CHART the pH was 6.50 and for the 2 treated by conventionally fractionated radiotherapy 6.41 and 6.49.

Stimulated parotid saliva was collected from 12 glands fully included in the treatment volume and treated twelve months previously. 7 had received CHART and 5 conventionally fractionated treatment (Figure 17). The mean pH and range for these two groups was 7.30 (7.26-7.40, SE 0.05) and 7.18 (7.10-7.34, SE 0.07) respectively. This difference was not of statistical significance. For 3 glands receiving 40-42 Gy the mean pH and range was 7.29 (7.26-7.33, SE 0.10) and for 4 glands receiving 10

-15 Gy, 7.38 (7.30-7.42, SE 0.06). For 6 glands where only 30% had been included in the treatment volume the mean pH and range was 7.40 (7.32-7.45, SE 0.04) and for 6 not included in the treatment volume at all it was 7.43 (7.36-7.49, SE 0.07). No data was available for glands where 50% or 80% had been included in the treatment volume (Figure 19).

c) Clinical symptoms

For the 16 patients treated by opposed fields throughout where essentially all salivary tissue was included in the treatment volume, dryness of mouth was most marked and persistent. 6 of these cases were assessable 9 months after the start of treatment. Of the three treated by conventionally fractionated radiotherapy all complained of moderate dryness of mouth and saliva consistency was for all three scored as thick/sticky. 2 of the 3 complained of persistent partial loss of taste. For the 3 treated by CHART, 1 complained of moderate dryness of mouth and 2 of slight dryness, saliva consistency was scored as thick/sticky for 2 and slightly thickened for 1. All 3 reported a complete recovery of taste.

As for the period during and shortly after treatment the degree of symptoms complained of later reflected the amount of salivary tissue, particuarly the parotids, included in the treatment volume. Case 37, Table IV where the submandibular and sublingual glands had been irradiated but not the parotids experienced no dryness of mouth and partial loss of taste was fully recovered by

6 months after the start of treatment with CHART. Saliva consistency remained normal/watery throughout.

3) Amylase study:

All but one patient had a serum amylase measured prior to the commencement of radiotherapy. For all 40 cases the value was within the normal range (150-300 IU/L). For the 8 patients in whom the serum amylase was measured hourly after the first treatment a rise in the serum amylase was detected after 4 hours.

For 27 of the patients (15 CHART, 12 conventionally fractionated treatment) with advanced tumours of oral cavity, oropharynx or supraglottis the fields for the first phase of treatment were similar being large opposed fields treating the whole neck and the superior border coming up to above the hard palate (patients 1-27, Table V). These fields incorporated all the major salivary glands within them. The serum amylase for the 15 days after the start of radiotherapy for these patients is shown in Figure 20. Marked rises in the serum amylase (8-21 fold) were seen in all these patients peaking 24-48 hours after the first fraction of treatment. The peak rise for the CHART patients was seen after 24 hours and for those receiving conventionally fractionated radiotherapy at 48 hours.

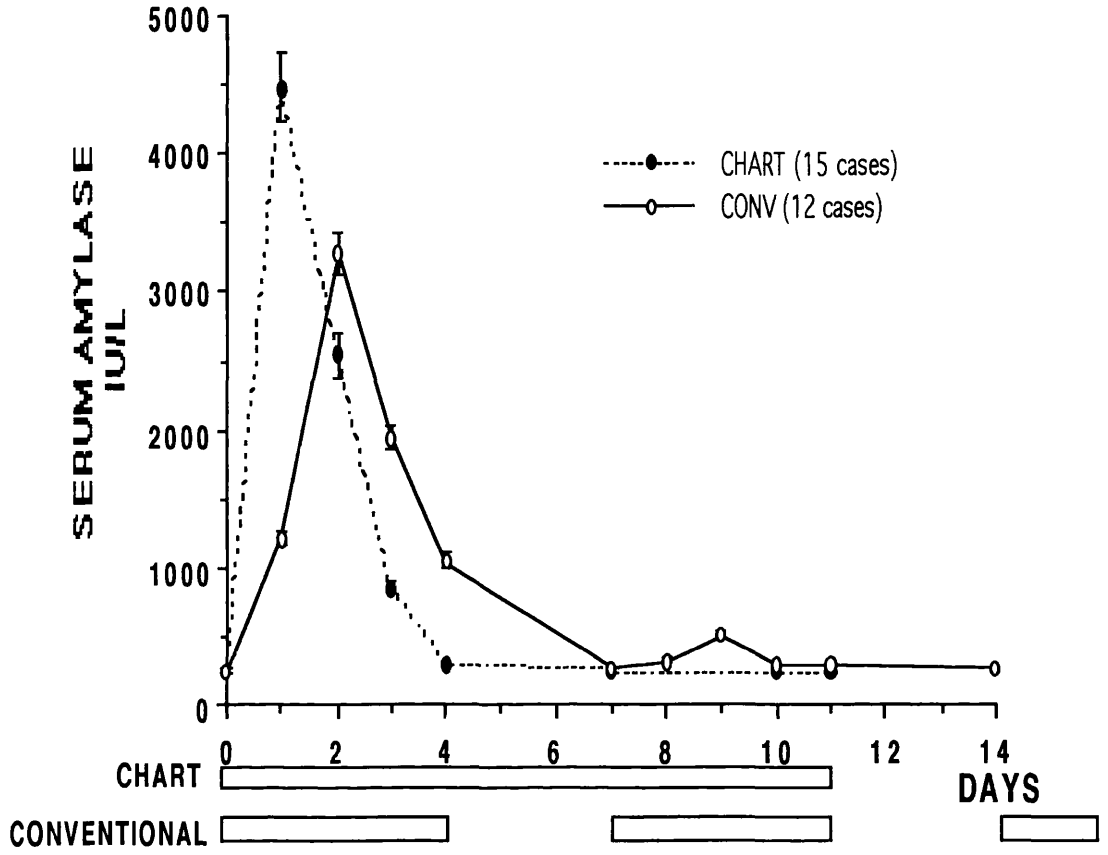


Figure 20. Changes in serum amylase (mean and SEM) during the first 2 weeks of a course of radiotherapy. All salivary tissue included in the treatment volume.

The mean peak rise for the CHART patients was 4477 IU/L and for the 12 treated by conventionally fractionated treatment 3275 IU/L. This difference is statistically significant ($P < 0.01$). Following this rise the serum amylase then falls rapidly reaching normal levels for the CHART patients by the 5th day after the start of treatment and for the conventionally treated patients by the 8th. No further rises were found in the patients treated by CHART but in those receiving conventionally fractionated treatment a small but significant rise was seen after the

weekend break and peaking on the 10th day after the start of treatment. Despite continued treatment out to 6-7 weeks no further rises in the serum amylase were seen in this group.

One patient (case 15, Table V) developed tender swelling of the submandibular and parotid glands on the afternoon of the first day of treatment with CHART when 2 fractions of 1.5 Gy had been given. This became marked over the next 24 hours (Figure 21) and then subsided over the following days. The peak serum amylase on the second day after the start of treatment for this case was 6446 IU/L; the highest value recorded in the series.

For the remaining 14 patients (cases 28-41, Table V) the treatment volume for the first phase did not include all the major salivary glands. These patients were all treated by CHART and smaller rises in the serum amylase were seen. The peak rise in serum amylase according to the proportion of the parotid glands included in the treatment volume is shown in Figure 22.

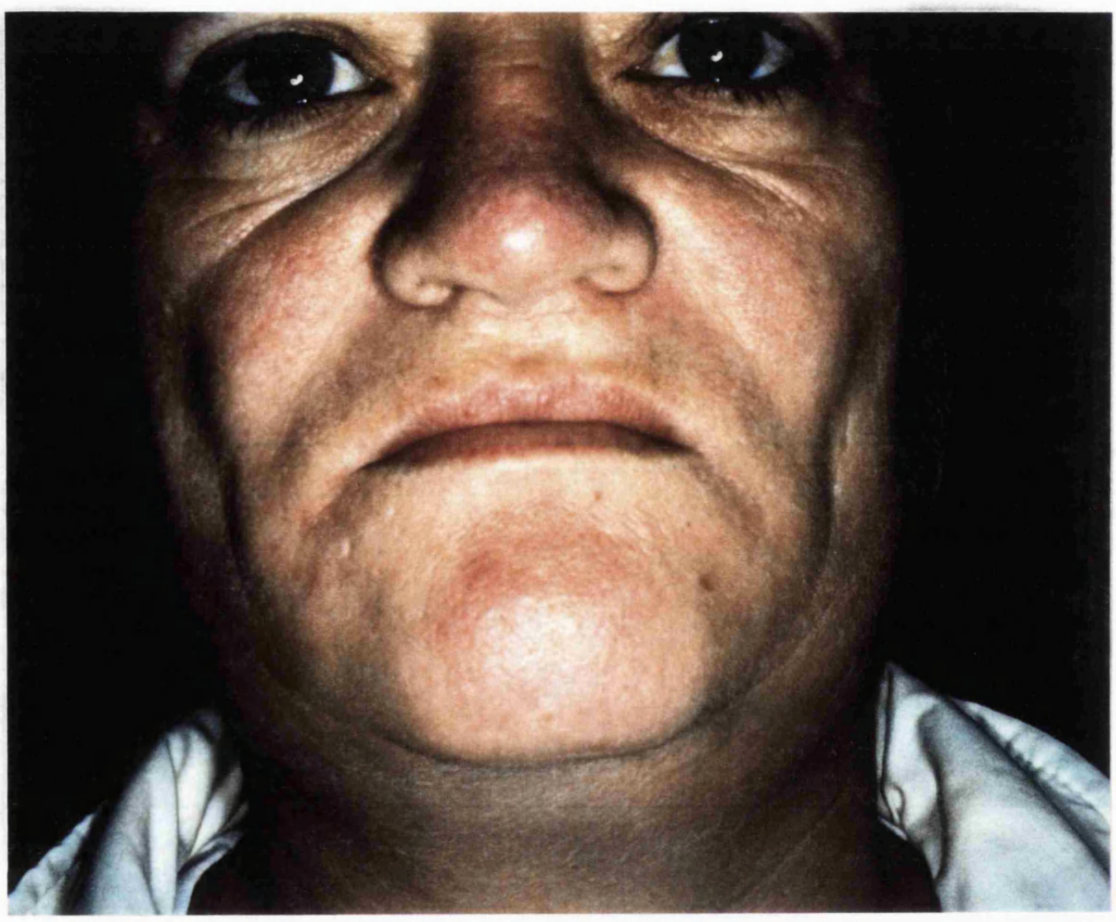


Figure 21. Tender swelling of submandibular and parotid glands following two fractions of 1.5 Gy given 6 hours apart.

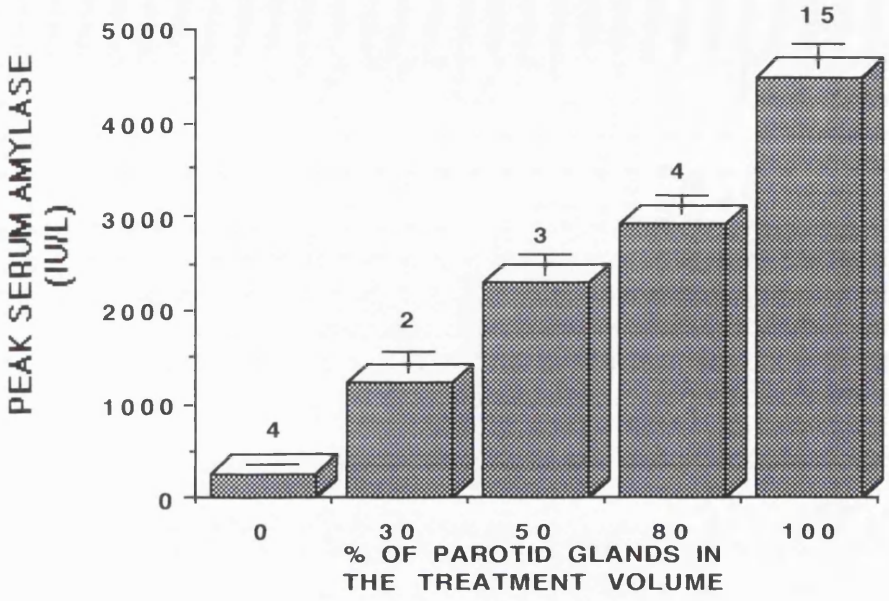


Figure 22. Peak (mean and SEM) serum amylase rise. Variation with % of volume of gland irradiated (28 cases treated by CHART).

One patient (case 37, Table V) with a verrucous squamous cell carcinoma of the tongue was treated with limited opposed fields to the anterior 2/3 of the tongue and floor of mouth. The submandibular and sublingual glands were in the treatment volume but the parotid glands were entirely excluded. For this patient only a small rise in the serum amylase was seen with a peak of 593 IU/L.

Saliva was selectively collected from 6 parotid glands of 6 patients (cases 2,3,4,9,11,12, Table V) during treatment with CHART and the concentration of amylase determined. These glands were fully included in the treatment volume throughout radiotherapy. The product of flow rate and concentration of

amylase in the saliva gives the total amount of amylase secreted by the gland/minute into the saliva. This declined rapidly to very low levels following the commencement of treatment (Figure 23).

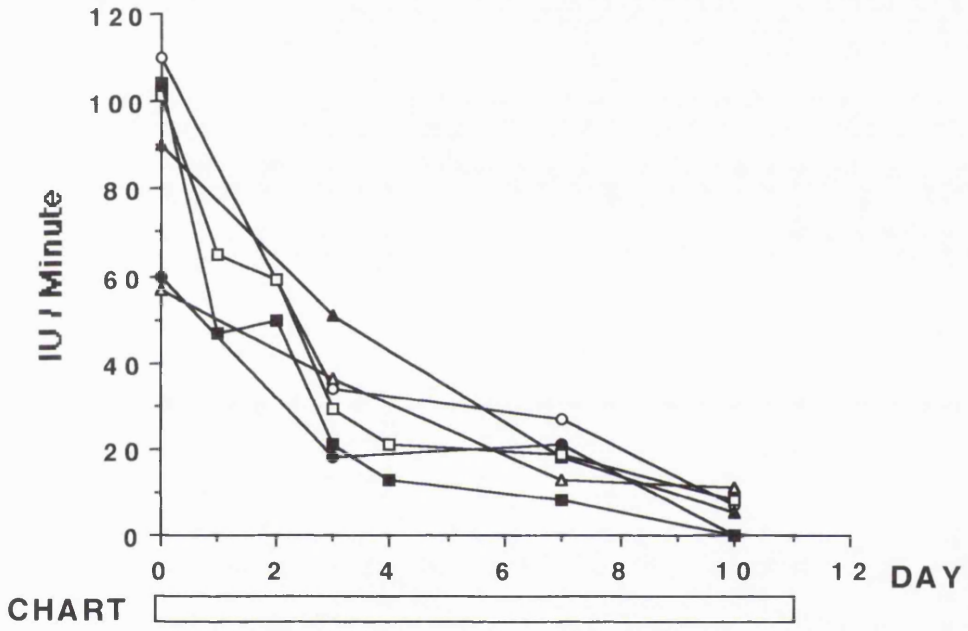


Figure 23. Total amount of amylase secreted by 6 parotid glands during treatment with CHART.

CHAPTER 4
DISCUSSION AND CONCLUSIONS

1) Retrospective study:

In this study the late effects of radiotherapy (nine months to nine years after treatment) on the function of the parotid gland were assessed by measuring salivary flow rate and alteration in pH of the saliva produced. The parotid gland not included in the treatment volume served as an internal control.

The results demonstrate a dose response effect with significant differences in salivary flow being seen between glands treated to a dose of 35-40 Gy compared to those receiving 60-66 Gy of conventionally fractionated radiotherapy. Marks et al [79] also reported a dose response effect finding measurable flow in 50% of glands receiving 20-40 Gy, 20% receiving 40-60 Gy and in none receiving more than 60 Gy. I was however able to demonstrate some function, albeit markedly reduced in 67% of the cases treated to a dose of more than 60 Gy. This difference may reflect the repeated stimulation with citric acid used in my collection procedure compared with the single application of "sour grape drops" which they used.

Less impairment of parotid flow after CHART was demonstrated as compared with conventionally fractionated radiotherapy to doses of 60-66 Gy; mean percentage flows being 57% and 20% respectively. The salivary flow from the parotid gland following

CHART is in fact more comparable to that seen when conventionally fractionated radiotherapy is given to the much lower dose of 35-40 Gy; mean percentage flow 65%. Similarly with respect to the fall in saliva pH following radiotherapy there is a significant fall in the pH of saliva from glands treated by conventionally fractionated radiotherapy to a dose of 60-66 Gy, but for glands receiving 35-40 Gy or CHART only a small fall in pH is seen which does not reach statistical significance.

Using the "two regimen" comparison of Thames et al [127] it would appear from the data showing similar parotid function after CHART and conventionally fractionated radiotherapy to a dose of 35-40 Gy that the α/β ratio for this endpoint is in the low range. Therefore the functional unit in question has a fractionation sensitivity akin to late responding tissues.

The three patients in this study who complained of dryness of mouth are of particular interest. All came from the group receiving conventionally fractionated radiotherapy to a dose of 60-66 Gy. In two there was no measurable function from the ipsilateral gland and in the third it was barely detectable. In addition, the flow from the contralateral glands was in all three cases at, or close to, the lower end of the normal range; a low pre -treatment salivary flow rate will result in a markedly reduced flow following radiotherapy [39,90]. This combination would seem to explain the symptom of dryness in these patients.

2) Prospective study:

The studies of early effects have demonstrated the marked sensitivity of human salivary glands, to irradiation. The parotid glands seem particuarly sensitive with falls in parotid flow seen earlier than for the resting whole saliva flow (largely the product of the submandibular glands) and this has been noted by other investigators [105]. This sensitivity is well seen when accelerated radiotherapy is given for the fall in salivary flow, for both resting whole flow and stimulated parotid flow, is seen earlier reflecting the more rapid delivery of treatment. There is no data in the literature dealing with the early effects of fractionated radiotherapy at different dose levels in humans. In this study the effect of 40-42 Gy on stimulated parotid flow is as marked as 54 Gy but a dose of 10-15 Gy produces a far more modest fall in flow. The dose required for maximal effect therefore lies in the range 15-40 Gy. I have also been able to demonstrate a close correlation between the percentage of the parotid gland included in the treatment volume and the early effects of radiotherapy in terms of reduced flow and fall in saliva pH.

As found by other investigators the flow of saliva from human salivary glands remains diminished for many years, if not permanently after their inclusion in the treatment volumes used for the radical treatment of head and neck cancer [31,72,86]. I have though clearly demonstrated some recovery of function for both resting whole saliva, largely the product of submandibular

glands and also of stimulated parotid saliva. Recovery is seen to commence at about 6 to 9 months from the start of treatment and continues out to at least 21 months : the patients continue to be studied in follow up. Parotid glands not fully included in the treatment volume or receiving less than the prescribed dose show a relatively greater ability to recover function. No effect of age was observed on the ability of salivary tissue to recover function. Of particular interest is the evidence for a greater recovery of function seen in patients treated by CHART as compared to those treated by conventionally fractionated radiotherapy.

3) Amylase study

The hyperamylasaemia seen following the irradiation of human salivary glands is of considerable radiobiological and radiotherapeutic interest. I have compared the hyperamylasaemic response in patients treated by CHART with that seen in patients treated by conventionally fractionated radiotherapy. The peak rise in serum amylase is seen earlier and is greater in the patients receiving CHART. This earlier and more rapid peak in the serum amylase following accelerated treatment has been described previously by Becciolini et al [4] who studied patients receiving an accelerated split course regime of radiotherapy. The area under the curves for both our groups of patients is though identical and the difference in time course probably reflects the more rapid delivery of treatment to the patients receiving CHART.

The small rise in serum amylase seen in the second week for the patients receiving conventionally fractionated treatment would suggest that after a dose of 10 Gy given in five daily fractions of 2 Gy there are still a small number of viable serous cells capable of responding to radiotherapy. A dose of conventionally fractionated radiotherapy of 20 Gy will however eliminate all such cells.

A clinical syndrome of tenderness and swelling of the salivary glands following the start of radiotherapy has been described [65,90]. It is rarely seen and it is noteworthy that in the one patient in the present series in whom it occurred the peak serum amylase was the highest value recorded.

Previous studies have shown some correlation between the amount of salivary tissue irradiated and the degree of amylase rise found in the serum [4,65,132]. It has been suggested that the parotid gland is the major scource of the radiation induced hyperamylasaemia [15]. With the aid of MRI scanning to localise the position of the parotid glands I have been able to show that the magnitude of the rise in serum amylase is very closely correlated with the amount of the parotid glands included in the treatment volume. If the parotid glands are outside the treatment volume the irradiation of the other salivary glands results in only a slight rise in the serum amylase. This can be explained as most salivary amylase activity is found in the parotid glands. The submandibular amylase activity is about 20% of that in the parotid and there is little amylase activity in the sublingual and

minor salivary glands [69].

Corresponding to the initial release of amylase into the serum I have found a rapid decline in the total amount of amylase secreted by the parotid gland into the saliva in the early days after the start of radiotherapy. This is the result of the elimination of serous salivary cells which are no longer capable of producing amylase.

4) Conclusions:

Marked falls in salivary flow and pH are seen shortly after the start of a fractionated course of radiotherapy when the major salivary glands are included in the treatment volume. There is some recovery of function commencing at about 6 to 9 months from the start of treatment although function remains considerably reduced compared to pre treatment levels. The early effects seen following CHART are as marked as those seen with conventionally fractionated treatment. There is though a greater recovery of function after CHART and this results in the reduced incidence of late effects observed clinically [67] and that can be measured objectively [70].

The interphase cell death of the serous salivary cells is manifested by a rapid rise in the blood amylase that can be detected following the first few fractions of a course of radiotherapy; the magnitude of the rise being closely related to

the volume of parotid tissue included in the treatment volume [71].

There would thus seem to be a dissociation between the acute effects of radiation on the function of the major salivary glands from the clinically more important late effects. Animal work would support this concept and studies on the salivary glands of rhesus monkeys [120] and rats [137] suggest that the early effects of radiotherapy result from interphase cell death (apoptosis) of salivary serous cells whilst later effects are determined by the ability of surviving stem cells to repopulate. Work published following the completion of this study has shown that this finding also applies to the lacrimal glands of rhesus monkeys. These are serous glands that are morphologically similar to parotid glands. The late atrophy of the rhesus lacrimal glands following irradiation has been shown to result not from the early loss of serous cells from apoptosis but to the failure of replenishment of the serous acini by surviving stem cells and to typical responses to radiation by the fibrovascular stroma [51]. Consequently, protection of serous cells from radiation apoptosis will not diminish serous gland atrophy. Thus lignocaine has been shown in vitro to inhibit apoptosis of serous cells from the parotid glands of rhesus monkeys [123] but it proved innefective in preventing glandular atrophy when studied in vivo [Peters LJ, personal communication].

The linear quadratic model predicts that by reducing the dose per fraction a sparing of the changes in late reacting tissues will

result but with little effect in early reacting normal tissues and of the effect in tumour [46,49,Chapter I section 1].

The sparing of late tissue injury with the use of small doses per fraction has been confirmed in three clinical trials of pure hyperfractionation in head and neck cancer [18,60,91]. In all three it proved possible to give considerably higher doses of radiation with no increase in late normal tissue effects when small doses of 1.15-1.2 Gy were given twice daily as compared to conventional treatment with 1.8-2 Gy fractions given daily. Similar findings have been reported in the treatment of carcinoma of the bladder [34]. The interval of time between fractions when treating more than once a day must though be sufficient to allow for repair of sub lethal damage in the normal tissues [18]. Laboratory and clinical evidence suggest that an interval of at least 6 hours is necessary [48,98].

The reduction in late changes after CHART was predicted on the basis of the low dose per fraction employed [46]. In addition the lower total dose of 54 Gy given when treating with CHART may be a factor in the greater functional recovery of salivary tissue that we have observed.

At Mount Vernon Hospital patients with advanced head and neck cancer have been treated with the CHART schedule since 1985. The clinicians involved in the follow up of these patients have consistently observed a reduction in late changes of the irradiated tissues such as skin and subcutaneous tissue. In a

comparative study of patients treated by either CHART or conventionally fractionated radiotherapy late changes in normal tissues was assessed using a clinical scoring system. Although no statistically significant differences in the changes in skin and mucosa were demonstrated there was evidence to suggest reduced late change for the patients treated by CHART in terms of less impairment of taste and dryness of mouth along with the observation of hair regrowth in 6 of the 10 patients treated by CHART [67, Chapter 1 section 11]. Patients being entered into the current randomised trials of CHART are being assessed for late changes in follow up using a clinical scoring system and hopefully more data will become available as this trial matures. This reduction in late changes after CHART has not though been observed in spinal cord possibly as a result of particuarly long repair half times [27]. The cohort of patients in this work being studied prospectively are under regular follow up and I will be continuing to study their salivary function to obtain further data on late effects and in particular on the observation of a greater functional recovery of salivary tissue following CHART as compared to conventionally fractionated treatment.

CHAPTER 5

IMPLICATIONS FOR THE RADIOTHERAPY OF HEAD AND NECK CANCER

The goal of cancer treatment is tumour control with a minimum of treatment complications. Currently many centres are exploring the use of altered fractionation schedules in the treatment of head and neck cancer with the dual aims of increased tumour control and decreased late normal tissue damage [26]. In pilot studies CHART appeared to give greater tumour control in head and neck and bronchial carcinoma when comparision was made with patients treated with conventional radiotherapy [98,99]. The CHART regime is now being compared with conventionally fractionated radiotherapy in the treatment of head and neck cancer in a multicentre randomised study funded by the Medical Research Council, the Cancer Research Campaign and the Department of Health. Accrual to this study has been excellent and over 700 cases have been entered so far (Dische S, personal communication). The study endpoints are survival, local tumour control and morbidity.

If it indeed proves possible by altered fractionation to improve tumour control and reduce late normal tissue damage then unconventional fractionation schedules such as CHART will become standard treatment. Many centres in the United States already regard radiotherapy treatment twice a day using fractions of 1.2 Gy as standard treatment for advanced head and neck cancer.

Impaired salivary gland function following radiotherapy in the treatment of head and neck cancer is an important cause of long term patient morbidity. The dental sequelae of impaired salivary gland function is the principle reason for the practice of extensive dental extractions prior to radiotherapy (Chapter I section 8). There is an increasing trend to a more conservative approach to dental extractions prior to irradiation and for greater conservation of teeth where possible. A reduction in long term salivary gland dysfunction as is seen following CHART adds further weight to this approach.

A greater preservation of salivary gland function following radiotherapy will lead to greater patient comfort and a less radical approach to dental extractions prior to treatment would result in a considerable improvement in the quality of life of these patients.

The quantitation of late radiotherapy change in humans presents problems, eg the difficulties in measuring changes such as skin fibrosis, telangiectasia etc. Measurement of late changes relies heavily on clinical scoring systems that have a limited sensitivity. If altered fractionation schedules are to become widely accepted it will be essential to demonstrate in addition to improved tumour control that there is no increase in late normal tissue effects. Salivary gland function has proved measurable and provides a valuable system for the study of human radiobiology. The patients studied prospectively continue to be examined by myself in follow up.

Other measurable systems are being investigated in this context and preliminary studies looking at electrical conduction across skin surfaces is showing promise in the investigation of skin eccrine gland function after radiotherapy (Dische S, personal communication).

The techniques of saliva collection described may also prove to have further application. I have recently been able to demonstrate in three patients with post radiotherapy xerostomia a measurable increase in salivary flow and relief of symptoms following the cessation of commonly prescribed medications given for co-existent medical problems (Clinical Oncology - in press). The methods described also provide a system for assessing attempts at reduction of late radiotherapy change in salivary tissue be it by altered fractionation as in this work or by other methods such as changes in planning technique, shielding of salivary tissue, implantation techniques and the use of particle therapy such as electrons in treatment. The assessment of pharmacological agents such as pilocarpine to treat post radiotherapy xerostomia would also be best made using measurement of salivary flow in addition to subjective scoring.

This study provides objective evidence that the CHART schedule with its lower dose per fraction and lower total dose gives the benefit of reduced late change in a clinically important normal tissue - the major salivary glands.

This study also gives direct support in humans for the concept of reduced late change resulting from a reduction in dose per fraction as predicted by the linear quadratic model [46,49]. This is an area of major importance to the current ongoing developments aimed at improving radiotherapy treatment by the determination of optimal treatment schedules and holds out the hope of improved tumour control combined with a reduction in late normal tissue effects.

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APPENDIX 1

TNM STAGING OF HEAD AND NECK CANCER
(UICC 1987)
SITES INCLUDED IN THE STUDY

ORAL CAVITY :

T1 Tumour 2cm or less in greatest dimension.

T2 Tumour more than 2cm but not more than 4cm in greatest dimension.

T3 Tumour more than 4cm in greatest dimension.

T4 Tumour invades adjacent structures, eg. through cortical bone, into deep (extrinsic) muscle of tongue, maxillary sinus, skin.

OROPHARYNX :

T1 Tumour 2cm or less in greatest dimension.

T2 Tumour more than 2cm but not more than 4cm in greatest dimension.

T3 Tumour more than 4cm in greatest dimension.

T4 Tumour invades adjacent structures, eg. through cortical bone, soft tissues of neck, deep (extrinsic) muscle of tongue.

HYPOPHARYNX :

T 1 Tumour limited to one subsite of hypopharynx.

T 2 Tumour invades more than one subsite of hypopharynx or an adjacent site, without fixation of hemilarynx.

T 3 Tumour invades more than one subsite of hypopharynx or an adjacent site, with fixation of hemilarynx.

T 4 Tumour invades adjacent structures, eg. cartilage or soft tissues of neck.

SUPRAGLOTTIS :

T 1 Tumour limited to one subsite of supraglottis with normal vocal cord mobility.

T 2 Tumour invades more than one subsite of supraglottis or glottis, with normal vocal cord mobility.

T 3 Tumour limited to larynx with vocal cord fixation and/or invades postcricoid area, medial wall of piriform sinus or pre-epiglottic tissues.

T 4 Tumour invades through thyroid cartilage and/or extends to other tissues beyond the larynx, eg. to oropharynx, soft tissues of neck.

GLOTTIS :

T1 Tumour limited to vocal cord(s) with normal mobility.
T2 Tumour extends to supraglottis and/or subglottis, and/or with impaired vocal cord mobility.
T3 Tumour limited to the larynx with vocal cord fixation.
T4 Tumour invades through thyroid cartilage and/or extends to other tissues beyond the larynx, eg. to oropharynx, soft tissues of neck.

REGIONAL LYMPH NODES :

N0 No regional lymph node metastasis.
N1 Metastasis in a single ipsilateral lymph node, 3cm or less in greatest dimension.
N2 Metastasis in a single ipsilateral lymph node, more than 3cm but not more than 6cm in greatest dimension, or in multiple ipsilateral lymph nodes, none more than 6cm in greatest dimension, or in bilateral or contralateral lymph nodes, none more than 6cm in greatest dimension.
N3 Metastasis in a lymph node more than 6cm in greatest dimension.

APPENDIX 2

ANN ARBOR STAGING CLASSIFICATION

FOR LYMPHOMAS

Stage I	Single node region.
Stage II	Two or more node regions same side of diaphragm.
Stage III	Node regions both sides of diaphragm.
Stage IV	Diffuse extranodal involvement.

The suffix E denotes localised extranodal involvement.

APPENDIX 3

PROFORMAS USED TO RECORD DATA

SALIVA STUDY - INITIAL ASSESSMENT

NAME HOSPITAL NUMBER

DATE OF BIRTH [ ][ ][ ][ ][ ][ ]

SEX [ ]

PRIMARY SITE

T STAGE [ ]

N STAGE [ ]

START DATE OF RADIOTHERAPY [ ][ ][ ][ ][ ][ ]

CHART OR CONVENTIONAL

DRUGS

PRE RADIOTHERAPY STATUS

DRYNESS OF MOUTH [ ] 0-nil,1-mild, 2-moderate, 3-severe.

TASTE IMPAIRMENT [ ] 0-none, 1-partial 2-complete.

SALIVA CONSISTENCY [ ] 0-normal/watery 1-slightly thickened 2-thick/sticky

	1	2	MEAN
RESTING WHOLE SALIVARY VOLUME ml/min			
pH MEASUREMENT			

SERUM AMYLASE

SALIVA STUDY - DURING TREATMENT CHART

NAME HOSPITAL NUMBER

START DATE OF RADIOTHERAPY

DAY	0	1	2	3	4	5	6	7	8	9	10	11
DATE												
DRYNESS OF MOUTH												
TASTE IMPAIRMENT												
SALIVA CONSISTENCY												
RESTING FLOW ml/min												
pH												
SERUM AMYLASE												

SALIVA STUDY - DURING TREATMENT CONVENTIONAL

NAME HOSPITAL NUMBER

START DATE OF RADIOTHERAPY

END OF WEEK	1	2	3	4	5	6	7	8	9	10	11	12
DATE												
DRYNESS OF MOUTH												
TASTE IMPAIRMENT												
SALIVA CONSISTENCY												
RESTING FLOW ml/min												
pH												
SERUM AMYLASE												

SALIVA STUDY - POST TREATMENT

NAME HOSPITAL NUMBER

START DATE OF RADIOTHERAPY

WEEKS POST RT												
DATE												
DRYNESS OF MOUTH												
TASTE IMPAIRMENT												
SALIVA CONSISTENCY												
RESTING FLOW ml/min												
pH												
SERUM AMYLASE												

SELECTIVE PAROTID STUDY CHART

NAME HOSPITAL NUMBER

PRE RT

PAROTID FLOW RATE
(stimulated ml/min)

	1	2	Mean
Right			
Left			

MEASUREMENT OF pH

	1	2	Mean
Right			
Left			

DURING RT

DAY		0	1	2	3	4	5	6	7	8	9	10	11
DATE													
Right	Flow												
	pH												
Left	Flow												
	pH												

POST RT

WEEKS												
DATE												
Right	Flow											
	pH											
Left	Flow											
	pH											

SELECTIVE PAROTID STUDY CONVENTIONAL

NAME HOSPITAL NUMBER

PRE RT

PAROTID FLOW RATE
(stimulated ml/min)

	1	2	Mean
Right			
Left			

MEASUREMENT OF pH

	1	2	Mean
Right			
Left			

DURING RT

WEEK		1	2	3	4	5	6	7	8	9	10	11	12
DATE													
Right	Flow												
	pH												
Left	Flow												
	pH												

POST RT

WEEKS													
DATE													
Right	Flow												
	pH												
Left	Flow												
	pH												

APPENDIX 4

ORIGINALITY OF THE STUDY

The methods of saliva collection used in this study were adapted and modified for use to enable the study of patients during and after radiotherapy.

This work has studied in a serial fashion the function of the salivary glands before, during and after radiotherapy in a much larger number of patients than have been examined previously. It adds considerably to the data on salivary gland function after radiotherapy and new data on dose and volume effects has been obtained. The use of MRI scans to precisely determine the volume of parotid tissue included in the treatment volumes has not been described before.

Measurement of salivary gland function from patients receiving the CHART schedule of radiotherapy has not been carried out before and indeed no studies have investigated salivary function after unconventional radiotherapy treatment schedules. Comparison of patients treated by CHART with those receiving conventionally fractionated radiotherapy is also a major feature of this work.

Finally I would like to add that the many hundreds of collections of saliva that form the basis of this work were all carried out by myself whilst a research fellow in The Marie Curie Research Wing at Mount Vernon Hospital.