MODEL STUDIES ON BREAST CANCER TREATMENT

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No man can hope to attain wisdom until he has come to see clearly how superficial ignorant and prejudiced most of his thinking really is.

Socrates 469-399B.C.

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PART I

REVIEWS OF THE LITERATURE

CHAPTER I

1. Introduction and rationale for the study

Cancer of the breast is the most frequent lethal malignant disease of women in western countries (Seidman, 1969). The Netherlands, having a mortality rate from this disease of 37.0 per 100,000 women (Centraal Bureau voor de Statistiek, 1974), and other northern countries of Western Europe together with the USA represent the so-called high rate areas. In 21 per cent of Dutch women who die of neoplasms, cancer of the breast is the cause (Ministerie voor Volksgezondheid en Milieuhygiëne, 1975). This represents one of the highest rates in the world (Clinical Oncology, 1973).

Several studies on the incidence of cancer of the breast from high (Salber et al., 1969; Lowe and MacMahon, 1970), intermediate (Ravnihar et al., 1971; Valaoras, 1969; Mirra et al., 1971) and low rate (Lin et al., 1971) areas show an increase in the incidence of this disease. The standardized overall mortality for cancer of the breast in The Netherlands shows an increase of 15 to 20 per cent during the last 25 years (Ministerie voor Volksgezondheid en Milieuhygiëne, 1975). A similar increase in mortality is found in the majority of countries in the world (Logan, 1975). This rise in mortality rates, however, appears to be largely due to the increase in the average length of life. Mijers (1973) reviewed data from 63,000 patients of breast cancer treated in the USA between 1940 and 1969. The survival rate after treatment had not improved in the past 20 years; indeed, the survival rate of the treated group relative to an age-matched population continued to show a downward slope up to 25 years after surgery, even for those women treated for localized disease. Mijers concluded that "apparently reduction of the excess risk of dying of breast cancer even for localized disease awaits some new therapeutic method". Is there anything wrong with the therapeutic approaches to breast cancer or is it essentially impossible to alter its course?

Many investigators and physicians are challenged to alter and improve the therapies of patients with breast cancer, particularly by attacking the initial phases of the disease.

2. Lowering the mortality rates can be achieved theoretically in three ways:

A. Primary prevention;
B. Secondary prevention;
C. Improvement of therapy.

A. Primary prevention

Ideally, we should aim at methods of primary prevention which are applicable to the entire population. Most of the control programs must be based on an individualistic approach to the "high risk" patient. The major predictors of high risk include sex, age, familial history of breast cancer, age of first pregnancy (Editorial, 1971), previous cancer in one breast (Welvaart, 1973) and the precancerous type of fibrocystic disease (Leis and Raciti, 1975; Ketcham and Sindelar, 1975). This means that we must identify the woman with unfavourable genetic or environmental factors so that we may manipulate her environment to avoid cancer development later. If we prevent the occurrence of the disease, we have achieved primary prevention. In breast cancer, as in most other diseases, this is our ultimate goal and should be the most cost-effective.

A drastic surgical prevention would be removal of the "trigger" organ, i.e., a prophylactic bilateral mastectomy for the person at very high risk. More convenient in this regard is a subcutaneous mastectomy with silicone implantation which leaves behind only a very small amount of breast tissue in the nipple area. Several authors have reported on the benefit of a surgical menopause in the reduction of breast cancer risk (Lilienfeld, 1956; Hiryama and Wynder 1962; Feinleib, 1968; Trichopoulos et al., 1972). These methods can only be justified, however, in selected cases with a family history involving numerous premenopausal breast cancers, especially if it is associated with no childbirth and fibrocystic disease, and it has to be evaluated on acceptability by the patient. These methods, however, do not provide a general solution for the problem.

B. Secondary prevention

Secondary prevention of breast cancer implies reduction in mortality or morbidity through early diagnosis and treatment, especially of

asymptomatic disease (Hutchison and Shapiro, 1968; Shapiro et al., 1971; Venet et al., 1971; Strax et al., 1973). Closely associated with early diagnosis is the question of screening. The efforts devoted to the "early" detection of mammary lesions certainly give the impression of an inverse relationship between survival and tumour size. Support for such a view arises from reports indicating that almost three-fourths of patients with tumours larger than 5 cm already have nodal metastases when first examined (Johnstone, 1972). The relationship between size of tumour, nodal metastases and prognosis have been for the most part confirmed by several studies (Fisher et al., 1969; Brightmore et al., 1970; Alderson et al., 1971). On the other hand, many studies have failed to show a close relationship between survival rates and the length of time the patient admits to being aware of the tumour (Bloom, 1965; Handley, 1972). Consequently, many reviews have concluded that it is the biologic features of the tumour and host rather than the time at which therapy is applied which are the major determinants of the clinical course (Devitt, 1967; Fisher et al., 1969). In further considering the usefulness of breast cancer screening, it is necessary to evaluate benefits against costs (Hutchison and Shapiro, 1968) not only economic but also in the hazard of tumour induction by the radiation needed to perform mammography (van Bekkum, 1977), selective screening of the high risk group against the whole population (Zippen and Petrakis, 1971) and the screening methods available (Furnival et al., 1970). Instruction in breast self-examination is vital as a first step in screening (Thiessen, 1971). The next steps include thermography (Nathan et al., 1972), mammography (Furnival et al., 1970; Dowdy et al., 1971; Egan, 1975; Pomerance et al., 1976; Lundgren and Jacobsson, 1976), breast examination by various health professionals (Gray, 1969) and combinations of these (Furnival et al., 1970; Shapiro et al., 1971; Venet et al., 1971; Strax, 1971).

Strax, Venet and Shapiro (Strax et al., 1973; Strax, 1976) have been conducting a prospective study to determine the value of screening and earlier treatment in reducing mortality from breast cancer. When studies such as this have been completed and confirmed by others, it will become clear whether, and to what extent, the time of treatment influences the mortality patterns of patients with breast cancer. It will also be clearer whether early treatment improves survival rates other then by merely starting the observation period that much earlier. It is however of significance that patients who

presented for surgery as a result of mass screening have smaller tumours and less nodal involvement (Urban, 1976; Zwaveling and Kleinveld 1976).

C. Improving the therapy

Optimal surgical treatment

The initial management of breast cancer has been characterized by interminable controversy during the past three decades. At this time, surgical removal of the tumour is the fundamental basis of all therapy but the type of surgical procedure continues to be a subject of great controversy.

For many years, surgeons have accepted the old maxim that the smaller the cancer the larger should be the operation. It has been stated that the chances of cure are best when a smaller cancer without apparent metastases is treated by wide removal of the primary tumour and the lymphatics and lymph nodes that drain it. The original aim of this kind of approach is to remove by surgery or to destroy by radiotherapy all of the cancer down to the last cell. It was believed that malignant disease is autonomous and spreads centrifugally through the surrounding tissues along lymphatic pathways to the local lymph nodes and, at a later stage, by the blood stream to form distant metastases.

Those who presently favour this kind of surgery, the standard radical mastectomy, such as Haagensen (1974) support the premise that breast cancer initially spreads to axillary nodes with a significant proportion of clinically negative nodes being positive pathologically. In this regard, Cutler and Mijers (1967) noted that 38 per cent of the axillas clinically regarded as negative exhibit metastases on histopathological examination; however, an essentially similar estimate of the converse has been made, that is, the finding of pathologically negative nodes in 35 per cent of patients in which the axillae were regarded clinically to be positive (Cutler and Mijers, 1967).

The basis for cancer surgery of this kind is purely anatomic and mechanistic. On the other hand, Crile (1964, 1967) has a different outlook on breast cancer surgery, based on systemic effects induced by treatment, instead of viewing the technique solely as a means of destroying the last cancer cell. He stated: "In small cancers of the breast without nodal metastases, the results of simple mastectomy

may be better than those that follow either radical mastectomy or simple mastectomy coupled with radiation therapy. There is a strong probability that early in the course of the disease, damage or destruction of regional nodes increases the tendency to systemic metastases" (Crile, 1967). This chance of concept is understandable when one considers that an operation designed to cure the patient (radical mastectomy) has a 5-year recurrence rate of 21 per cent in the absence of axillary nodal metastases, rising to 66 per cent in the presence of any nodal disease and to 81 per cent with four or more involved nodes (Fisher et al., 1969). The change of concept is also very important in another way: it is the recognition that surgery alone is not optimally effective in eradicating the last neoplastic cell; systemic factors should be evaluated in the treatment. It also includes the recognition that breast cancer is a systemic disease until proved otherwise. Currently, the operative options range from simple excision of the tumour or removal of the breast only, to the extreme of doing an internal mammary node dissection with resection of part of the chest wall. Since radiotherapy can be added to each of the varied surgical procedures, one has twice as many possibilities, even without considering oöphorectomy and adjuvant chemotherapy.

A general descriptive orientation to the surgical approaches to the breast is shown in Table I (Modified after Carter, 1976).

Table I. Surgical approaches in breast cancer treatment

			Pectoralis		Lymph nodes		
Type of mastectomy	Primary tumour	Breast tissue	major	minor	axillary	internal mammary	supraclavicular
lumpectomy	+	−	−	−	−	−	−
simple	+	+	−	−	−	−	−
modified radical	+	+	−	±	+	−	−
radical	+	+	+	+	+	−	−
extended radical	+	+	+	+	+	+	−
super-radical	+	+	+	+	+	+	+

What is the fundamental basis for these different kinds of treatment? What has happened in our understanding of the biology of breast cancer that has altered thinking relative to its management?

In challenging the classical concept of *en bloc* resection of the primary

tumour and regional lymphatics, Crile's choice of simple mastectomy in early stages of breast cancer was based on the notion that retention of regional lymph nodes, grossly uninvolved by tumour, is important in maintaining a degree of systemic immunity against further growth of metastatic microfoci which is dependent of these regional lymph nodes. He based this conclusion on studies performed in his laboratory using 3 transplantable murine tumours (Crile, 1964; 1965). Since then, many investigators have developed various experimental models for evaluating the role of the regional lymph nodes in cancer. Their importance is obvious, since these models provide an increase in the knowledge of tumour biology and can give information about the relative merits of certain therapies before clinical evaluation in carefully planned trials of different kinds of therapy. Duncan and Kerr recently stated that a long period of follow-up (to 25 years) is necessary to establish the presence of long-term survivors (Duncan and Kerr, 1976). Experimentally, during the past ten years, major emphasis has been placed in finding a model for surgical therapy sufficiently similar to early human breast cancer to aid in the selection of the best type of surgery.

Another important clinical and experimental feature relative to the importance of lymph nodes was the finding that lymph nodes in the area of a primary neoplasm are often enlarged and clinically palpable without containing tumour cells. For carcinoma of the breast, this was observed in 35 per cent of cases (Cutler and Mijers, 1967). These reactive changes in uninvolved lymph nodes were found to be due to enlargement of the paracortical and cortical areas of the lymph nodes. More specifically, these responses were tentatively identified as structural representations of immunological phenomena.

From experimental studies with various antigens, it was shown that the deep cortex, i.e., the paracortical area, is a thymus-dependent area populated mainly by T lymphocytes, whereas the cortical area containing follicles and germinal centres is a thymus-independent area (B cell) associated with the production of plasma cells and humoral responses. Subsequently, a routine reporting of lymph node histologic changes associated with variations in the immune response was proposed (Cottier et al., 1972). It was further pointed out that the immunological potential of lymph nodes draining a tumour removed at operation should provide further information on prognosis (Editorial, 1973). Indeed, several investigators noticed that palpable benign

nodes in the axilla implied a good prognosis (Cutler et al., 1970; Berg et al., 1973).

Again, experimental models are of great importance, since it is possible to make sequential and adequately controlled observations on the significance of the morphological changes and to investigate whether or not such a reaction commences in the regional lymph nodes and later extends to distant nodes and spleen. Proliferative changes in the regional lymph node(s) may, therefore precede the spread and subsequent growth of tumour emboli therein. Experimental findings could provide information on whether regional lymph nodes should be preserved rather than removed in the early stage of breast cancer. Only on such a fundamental basis can an optimal surgical approach to early stages of breast cancer with the hope of improving the cure rate of this feared tumour clinically be founded.

The use of chemotherapy as an adjunct to surgery

Despite all of the clinical and experimental efforts devoted to finding the best local treatment for breast cancer, 70 per cent of all patients with breast carcinoma will sooner or later present with metastatic disease (Shimkin, 1967) and require a general or systemic form of treatment. The above information suggests that the disease is disseminated at the time of diagnosis in the majority of cases and that systemic approaches will be a necessity if there is to be any hope of eradicating it. Until relatively recently, systemic treatment consisted primarily of ablative or additive hormone therapy. During the developmental stage of tumour chemotherapy, cytostatic agents were used almost exclusively in far advanced cases that had failed to respond to hormonal treatment. Animal model systems, however, have strongly supported the use of postoperative adjunctive systemic chemotherapy (Shapiro and Fugmann, 1957; Martin and Fugmann, 1960; Karrer et al., 1967; Schabel, 1976). These studies have also demonstrated that debulking of the tumour by local measures provides a setting in which a previously chemotherapeutically refractory disease can be cured by chemotherapy. In animals, chemotherapy is more effective against the small metastases as compared to gross visible advanced tumour (Skipper, 1971; Schabel, 1976). Thus, the combined approach of effective local and systemic therapies offers the best

chance for cure. This new concept for the treatment of breast cancer is known as the combined modality approach. A variety of single agents have been reported to cause tumour regressions in patients with breast cancer (Carter, 1974). Thirteen drugs have shown evidence of activity and response rates of 35 per cent are not uncommon for single agents (Wasserman et al., 1975).

In order to increase the effectiveness of these single agents, combination chemotherapy programs have been developed (Greenspan, 1963; Cooper, 1969; for reviews see Broder and Tormey, 1974; Carter, 1976). The basic principles of combination chemotherapy programs include the use of several agents which should ideally have nonoverlapping toxicities and mechanisms of action. This allows the utilization of drugs at almost full doses. Another important attribute of the combinations has been the use of these drugs in intermittent schedules to allow for recovery from host toxicity and of the immune response. The effectiveness of combinations has increased the proportion of responses as well as the duration of the responses.

As a consequence, prospective randomized clinical trials comparing no therapy with chemotherapy as an adjuvant treatment in operable breast cancer with histologically positive axillary nodes were designed and these began in the United States in September 1972 (Fisher et al., 1975) and in Milan, Italy in June 1973 (Bonadonna et al., 1976). The initial results of these studies are very promising: treatment failure differences were significant in both studies and these early results evoked much enthusiasm in the scientific world ("Major advance in breast cancer", Holland, 1976), as well as in the lay press (Time, March 1, 1976). Many critical comments were afterwards written in scientific papers (Burrington, 1975; Culliton, 1976; Editorials in Brit. Med. J., 1976; and New Eng. J. Med., 1976). Since long-term follow up – i.e., long-term survival – results are not yet available, these results should indeed be considered with caution. The implications that these studies should have was most forcibly stated by Dr. Bonadonna: "Whatever the conclusions of the combined local-systemic treatment are, they will, if they are convincing, be used in the redefinition of the usefulness of all available therapeutic tools. In the meantime, patients and physicians must be open to new findings and accept a period of therapeutic uncertainty" (Bonadonna, 1976).

One immediate consequence of these studies has been the universal recognition that there is justification for further trials, since the evident

delay in recurrence is in itself sufficient compensation for the toxic effects, even if no increased ultimate cure rate is observed. Many controlled trials of prolonged adjuvant therapy are now in progress throughout the world (Carter, 1976; Editioral 1977); such a trial has also been started in the Netherlands.

With this increasing emphasis on the use of combined therapy and the resultant logistical and ethical problems (control groups) of empirically determining "optimal combinations" clinically, there is a correspondingly greater need for animal model tumour systems that could predict the clinical success of combinations of new as well as of existing chemotherapeutic modalities.

3. *What is the place of the experimental work and clinical trials devoted to evaluating the best local surgical treatment in these new approaches to the treatment of breast cancer?*

It may justifiably be considered that, even with the favourable findings regarding adjuvant systemic chemotherapy, an evaluation of less extensive surgical procedures still relates to the null hypothesis, for there is no reason to believe that simple mastectomy can, in terms of producing a disease-free state, be better than radical mastectomy and chemotherapy. Such a less extensive procedure however, would certainly produce a positive gain in terms of cosmetics and morbidity. In the light of these comments, it might logically be conjectured that recently completed and present clinical trials evaluating simple versus radical mastectomy, and many experimental model studies evaluating these procedures, are outmoded and that the data are no longer meaningful to the future strategy of cancer treatment. That conjecture is not justified. The need for the information provided by such work is still as great as ever for several important reasons.

1. The most important aspect of the controversy on the extensiveness of surgery is concerned with clinical stage I disease in which adjuvant therapy has not yet been tested. In this stage of cancer of the breast, surgery alone might represent the optimal treatment.

The hypothesis forwarded by Crile, that removal of regional lymph nodes increases the tendency to systemic metastases, was based on suggestive evidence from clinical and experimental data. Experimentally, the detrimental effect of regional lymph node removal was found only early in the course of the disease. Clinically, these are the

patients with nonpalpable axillary lymph nodes (stage I). Especially in this already prognostic favourable group, simple mastectomy might be shown to be as good if not better in comparison to radical mastectomy. The effects of long-term chemoprophylaxis can be too heavy a burden in this group of patients, so that the remedy could be worse than the disease. This latter assumption is based on the following considerations:

A. Most cytotoxic agents are immune suppressive (Harris et al., 1976; Cleton, 1977). Both humoral and cell-mediated immunity may be depressed (depending on the drug, dose and schedule). This action could result in a shift of the potentially favourable immunological reaction of the host against growth of distant microfoci. It could even result in an enhancement of tumour development due to immunosuppression (Penn, 1974; Grundmann and Gross, 1975a).

B. Cytotoxic agents are clearly carcinogenic in animals (Weisburger, 1974; Grundmann and Gross, 1975b). When administering adjuvant chemotherapy for long periods, this could result in carcinogenesis in humans as well. Suggestive reports have appeared in the literature, e.g. increased occurrence of breast cancer after L-PAM treatment for multiple myeloma (Bell et al., 1976); Cyclophosphamide treatment has been implicated as the cause of bladder cancers (Wall and Clausen, 1975).

C. Cytotoxic drugs are myelosuppressive and often have additional organ toxicities (Chabner et al., 1975). In experienced hands, this is usually not a major problem, but, if not used very carefully, cytostatic drugs can kill human beings.

D. Even on its target, the malignant disease, chemotherapy can result in an inverse effect: Finney reported on a clinical trial in which patients in stage I and II carcinoma of the breast were treated with Cyclophosphamide pre- and postoperatively. After 3 years, results in the group receiving chemotherapy were poorer than those of the control group (Finney, 1971). A similar effect has been described experimentally (Sugarbaker et al., 1970). A very significant increase in induced pulmonary metastases was observed after Cyclophosphamide treatment which could not be explained by immune depression (van Putten et al., 1975).

The above considerations indicate that, since the extent of all these effects is not yet known, surgery alone could be as effective as a combined approach. In view of this, evaluation of the two procedures

i.e. comparison of simple versus radical mastectomy is essential. On the other hand these are clear arguments for extending the comparison of different types of surgery also to the situation where adjuvant chemotherapy is used; this leads us to the next point.

2. Results of clinical trials evaluating simple versus radical mastectomy need not necessarily be similar after adjuvant chemotherapy in combination with each of the procedures.

A. If, without adjuvant treatment, one of the two procedures is found to give a better chance of survival, the possibility remains that both of the procedures could produce equivalent results with effective systemic therapy. The possible favourable immunological reaction dependent on intact regional lymph nodes could easily be abrogated by the immune-suppressive action of chemotherapy. On the other hand if radical mastectomy alone would give better results, effective systemic therapy could equalize this difference by cytostatic drug effects on residual disease.

B. If results are equal, there is still a possibility of a difference after adjuvant chemotherapy: data from experimental models have shown that there is a direct relationship between the number of viable tumour cells present at initiation of chemotherapy and curability (Laster et al., 1969; Schabel, 1975). After simple mastectomy alone, there is an increased risk for the development of recurrences in the axilla, since regional lymph nodes itself represent a "soil" for the outgrowth of tumour. Even nonpalpable axillary lymph nodes may contain significant numbers of tumour cells. Since chemotherapy is more effective against microfoci than against palpable tumour masses, it might be less effective after simple mastectomy alone.

3. Experimental work may provide data on whether or not the regional lymph node is indeed of utmost importance in the initiation and maintenance of immunity against distant microfoci. In effect, it may provide an answer as to whether there really is a fundamental basis for less radical surgery producing better end results.

As a result of the mass screening methods, the number of patients detected in an early stage of cancer of the breast will increase. For this growing group of stage I patients, there remains the crucial question

of what kind of surgery is best: simple mastectomy or radical mastectomy?

Until more time has elapsed to permit the evaluation of the long-term effects of prolonged adjuvant chemotherapy, its use is best limited to histologically proven positive node patients. Consequently, the nodal status of every patient coming to operation must be known. This requires that axillary staging must be done in all cases. As soon as it can be determined that the systemic agents employed have no unacceptable delayed sequelae, they may be applied in negative node patients as well, since a significant number of these will suffer treatment failure within 10 years according to the *presently* available survival data. If all patients would receive a similarly intensive systemic therapy regardless of histologic nodal status, nodal staging would become unnecessary, since treatment would be independent of such findings. Indeed, as a result of the findings of the largest controlled clinical study so far undertaken to evaluate the results of simple mastectomy and radiotherapy versus simple mastectomy alone, some investigators have gone so far as to conclude: "Our findings would lend support to the view that a conservative form of primary treatment with subsequent adjuvant chemotherapy may be the treatment of choice in the future" (Editorial in Brit. Med. J., 1976). It seems much too early for a conclusion of this type; a modification of treatment in this sense would have to be preceeded by thorough clinical and experimental study.

The interaction of both the clinical disciplines and the basic disciplines has been and will be increasingly responsible for progress in cancer control. In order to give the best of patient care, these disciplines must appreciate each other's contributions and their initial and long-term management of the patient must be complementary. Therefore, there was and still is an increasing need for the development of appropriate animal models resembling the clinical tumour as closely as possible in its biologic characteristics for the evaluation of different surgical procedures and the determination of optimal combinations with chemotherapy. In this way, an optimal local and systemic treatment of breast cancer can be achieved.

4. Outline of the study

Although transplantable tumour systems have facilitated progress in breast cancer therapy, the search continues for animal models which resemble the clinical tumour in its characteristics as much as possible. The present study was designed in finding and defining such a model in which surgery in relation to the regional lymph nodes, lymph node morphology as well as adjuvant chemotherapy could be evaluated.

Part I contains the reviews of the literature:

Chapter II will review the varied surgical procedures in breast cancer surgery in view of existing knowledge of the tumour and its metastatic behaviour.

Chapter III deals with the experimental studies in which lymph node morphology and the effect of regional lymph node removal in experimental mammary carcinoma models will receive special attention.

Chapter IV will briefly summarize the experimental and clinical data on surgical adjuvant chemotherapy for breast cancer treatment.

Part II describes the experimental studies. Every chapter will give a description of the experimental procedures and techniques which have been used during the studies.

Model characteristics and some of the applications of the finally selected mammary carcinoma will be presented in chapter I.

A morphometric analysis of the immune response in lymph nodes draining an antigenic and a nonantigenic tumour is given in chapter II.

The influence of regional lymphadenectomy on metastasis and survival in three different rodent tumour models will be the subject of chapter III. Effects of adjuvant chemotherapy in the mammary carcinoma model will be described.

Results will be discussed separately in the three chapters. In the final chapter the information obtained will be integrated in the overall discussion like in the introduction.

CHAPTER II

BREAST CANCER SURGERY

1. Historical aspects of breast cancer surgery

Carcinoma of the breast has been recognized since the times of the ancient Egyptians. The renaissance saw a reawakening of interest in mastectomy for this disease as a result of the teachings of Vesalius and Fabricius. Since discovery of the lymphatics by Asselius (Asselius, cited by Rusnyák et al., 1960), professor of anatomy and surgery in Pavia in 1622 and observation of the thoracic duct and cysterna chyli by Pecquet in 1647 (Pecquet, cited by Crafoord, 1941), the role of lymph, lymph nodes and the lymph/vascular system in the biological phenomena of malignant disease has been the subject of much controversy. Le Dran (1685–1770) recognized the spread of cancer to the regional nodes, dissecting these out when enlarged and describing a poor prognosis associated with nodal involvement (Lewison, 1953). In the eighteenth century, numerous theories implicating lymph in the cause of cancer were in vogue: Descartes (Descartes, cited by Garrison, 1926) proposed that "sour lymph" was responsible; Monro (Monro, cited by Garrison, 1926) implicated lymphstasis and Hunter believed that cancer was a systemic disease arising wherever lymph coagulated. However, all who discussed dissemination of cancer, despite differences in concept, recognized the importance of the lymph vessels in the process.

In the Johns Hopkins Hospital Reports for 1890 and 1891, William Stewart Halsted first published a summary of 13 cases of breast cancer treated by his original technique of radical mastectomy: "About eight years ago (1882) I began not only to typically clean out the axilla in all cases of cancer of the breast but also to excise in almost every case the pectoralis major muscle, or at least a generous piece of it, and to give the tumor on all sides an exceedingly wide berth". Later he decided to remove minor as well as major pectoral muscles* (Halsted, 1924).

* Inspired by W. Meyer who advocated this principle (1894).

These surgical principles advocated by Halsted (originally adopted from C. Moore, surgeon to the Middlesex Hospital in England from 1848–1869), were splendidly conceived and entirely in keeping with the perspectives of cancer surgery of his time. The underlying philosophy upon which radical surgery was based was most forcibly stated by Handley in 1906 (Handley, 1906). He maintained that the spread of cancer was a continuous one along the lymphatic channels to the lymph nodes which acted as filter traps. When the lymph nodes were full of tumour, they acted as a further source of dissemination. En bloc resection was therefore considered mandatory to avoid spilling of tumour cells from the cut ends of lymphatics and radical operations were favoured in order to remove as many involved lymph nodes as possible.

For a time, William Handley's theory (Handley, 1922) of continuous permeation of the lymphatics by neoplastic cells influenced thinking about the manner in which neoplastic cells traversed lymphatics. He postulated that, if lymph nodes were involved but afferent lymphatics contained no neoplastic cells, cellular embolization was not responsible but that perilymphatic fibrosis had destroyed the continuous line of neoplastic cells so that they were no longer visible. In 1938, however, Gray showed that the mode of spread to lymph nodes was by cell emboli and that cancer cells do not ordinarily remain long within the lumen of lymphatic vessels (Gray, 1938).

In 1909, Bartels suggested that lymph from peripheral regions does not reach the blood without passing through at least one lymph node (Bartels, 1909). However, Engeset found that lymph can reach the venous circulation without passing through any lymph nodes (Engeset 1959). Many studies have shown that, besides the lymphatic venous communications at the venular angles in the neck, many communications between the vascular and lymphatic system exist (Pressman, et al., 1961; Bron et al., 1963; Malék et al., 1965). Fisher and Fisher (1966) found that tumour cells can readily pass from blood to lymphatic channels and back again, indicating that the two systems are probably inseparable in the pathogenesis of the disease. Of course, Halsted's early concept of cancer surgery could not conceivably take into account today's ever-increasing evidence of early blood-borne dissemination. Based on the early concept of the centrifugal spread through surrounding tissues along lymphatic pathways to local lymph

nodes and at a later stage formation of distant metastases (fig. 1), radical mastectomy, i.e. en bloc wide removal of tumour containing breast and both pectoral muscles with an axillary dissection, came to be regarded the routine treatment in most cases of cancer of the breast during the first half of the twentieth century.

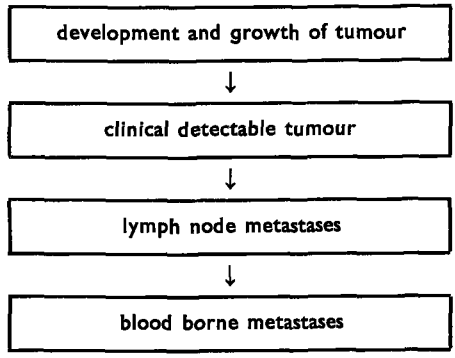


Figure 1. Early concept of the spread of cancer of the breast.

2. Discord*

Ten-year survival data after radical mastectomy in large series such as those from Harrington 1910–1946 (Harrington, 1952) of 35 per cent, Lewison et al., 1935–1940 (Lewison et al., 1953) of 29 per cent, Moore et al., 1935–1951 (Moore et al., 1958) of 33 per cent and Smith and Meyer 1924–1952 (Smith and Meyer, 1959) of 30 per cent in operable stages I and II show that an operation designed to cure the patient failed in about 70 per cent of cases. Despite the worldwide acceptance of the Halsted radical mastectomy, it was very disconcerting for many skilfull surgeons to realize that the breast cancer mortality rate had not

* An attempt to catalogue information concerning results achieved with all kinds of surgical procedures in carcinoma of the breast is an exercise in futility and frustration. The plethora of statistics and information gathered from all kinds of analyses of the experiences of individual surgeons or single institutions with different stage definitions makes comparison of results very difficult or even impossible, with the exception of prospective randomized clinical trials. Therefore, only a few data are included in this brief overview. Because the relevance of the regional lymph nodes in cancer is the subject of the experimental part of this thesis, special attention was given to clinical data evaluating simple mastectomy in comparison to other surgical procedures.

reflected improvement in surgical skill. The slight improvement found was possibly due to better staging of the disease and many patients on whom the operation was originally practiced would be considered inoperable today. Dissatisfaction with these results led to the employment of surgical alternatives as well as in continuation to be more precise in defining criteria of operability. Of course, elimination of unfavourable categories of patients from surgery, such as patients who have involvement of the toplevel of the axillary lymph nodes (determined by an infraclavicular biopsy) would have such an effect, however, such restrictions would be self-defeating and unrealistic. Therefore, another approach was explored: Breast cancer spreads to the internal mammary nodes as well as the axilla and these internal mammary nodes are more likely to be involved with centrally and medially placed tumours (Handley, 1964; Urban and Marjani, 1971b). It was therefore a logical extension, again on anatomic and mechanistic principles to employ a "more" radical surgical approach, i.e., an extended radical mastectomy and a super radical mastectomy (Table I).

3. The more radical approaches to operable breast cancer

It was hoped that super radical surgery, utilizing the same criteria of operability as employed for radical mastectomy, would result in a greater disease free survival rate and/or would permit the extension of criteria of operability so that patients considered unsuitable for conventional radical mastectomy would become candidates for cure by extended operation. Turner-Warwick in studying autoradiographs of surgical specimens found that both the axilla and internal mammary chain receive lymph from all quadrants of the breast (Turner-Warwick 1969). The ipsilateral axillary nodes commonly receive more than 75 per cent of the total lymph from the breast; the remainder drains into the ipsilateral internal mammary chain. Therefore, if the removal of the entire potential lymphatic drainage area is of utmost importance then the Halsted radical mastectomy should be considered inadequate.

Margottini first proposed the technique for extended radical mastectomy, which includes radical mastectomy plus the excision of the internal mammary nodes, in 1948 (Margottini et al., 1963). The percentage of patients alive and clinically free of recurrence at 5 years was 55 per cent versus 26 per cent following radical mastectomy. He subsequently stated that this was the operation that followed the

fundamental rules of cancer surgery. Urban found that 13 per cent of patients with no histologically demonstrable axillary involvement had internal mammary node metastases and the incidence of 5-year freedom of disease was 86 per cent after extended radical mastectomy (Urban, 1967). He believed that this procedure was particularly indicated for early stages of centrally and medially located breast cancers (Urban, 1967; Urban and Castro, 1971). Sugarbaker found differences in 5-year survival of 57 per cent for patients having conventional radical mastectomy, whereas, for those having extended radical mastectomies, the 5-year survival rate was 70 per cent (Sugarbaker, 1964).

However, Dahl-Iversen and Tobiassen who also performed a supraclavicular dissection (super radical mastectomy), concluded from their experience with this operation: "As far as we can see, the results following extended radical operation and classical mastectomy are practically identical" (Dahl-Iversen and Tobiassen, 1963). Also because of the inconclusive results of LaCour they abandoned extended operations for breast cancer (LaCour, 1967). In 1964, Gould found no difference in 5-year survival for patients treated by radical or extended radical mastectomy (66 per cent in the former, 65 per cent in the latter, Gould, 1964); local recurrences were identical (8 per cent). Many others (Cáceres, 1967; Donegan, 1968; LaCour et al., 1976) found no superior results with this kind of surgery when compared to the conventional radical mastectomy; the results of a clinical trial comparing extended radical mastectomy and simple mastectomy with irradiation, as advocated by McWhirter, showed no differences in 5 and 10-year survival (Kaae and Johansen, 1962; 1965).

The two-stage radical mastectomy combined with supraclavicular dissection and internal mammary and mediastinal lymph node dissection reported by Lewis (1953) carried such a high operative mortality (12 per cent in 50 patients) that this operation with its high morbidity and mortality rates could not be regarded as being justified.

The data presented show that some investigators (Margottini et al., 1963; Sugarbaker, 1964; Urban, 1967; Urban and Castro, 1971) obtained better results with a more aggressive operation than the conventional Halsted mastectomy. It must be pointed out, however, that this data gave no assurance that the case material compared was indeed equivalent as in the case of prospective randomized clinical trials. Moreover, the "superior" survival rates recorded after extended

operations were not really different from series from other hospitals where only the conventional mastectomy was performed (Fisher, 1970; Haagensen, 1972). However, one must realize that it is very hazardous to relate data from different clinics utilizing different methods of clinical staging. Many other series showed no differences in survival and recurrences, even those from a clinical trial (Kaae and Johansen, 1962; 1965) comparing a less extended operation than the Halsted mastectomy, showed no differences. This indicates that the theory of "some more lymph node dissections will cure more cancer" was not justified.

4. Less radical procedures for surgery of breast cancer: modified radical mastectomy

In 1938, Gray published the finding that "the deep fascia is a plane devoid of, or very poor in lymphatics and hence not an important potential plane of spread" (Gray, 1938). Patey and Dyson subsequently began to perform an operation that left the pectoralis major intact but removed the breast, pectoralis minor muscle and the axillary contents; their results were as good as those of the standard radical operation (Patey and Dyson, 1948). Handley pointed out that this modified radical mastectomy or "conservative" radical mastectomy, as he prefers to call it, secures the advantages which Halsted first pointed out, without the deformity which sacrifice of the pectoralis entails (Handley, 1965). This modified radical mastectomy is not a new operation. Moore described it as early as 1867 (An historical review of modified radical mastectomy is given in Madden et al., 1972).

A major objection to the modified radical mastectomy was made by Haagensen. He believed that a complete axillary dissection was less adequate than by the conventional procedure (Haagensen, 1973; 1974). Madden, however, in his well illustrated description of his technique (with preservation of both pectoral muscles), demonstrated that the axilla can equally be fully cleared by modified radical mastectomy (Madden, 1965). He later documented this ability to "clean the axilla" by lymphangiographic studies (Madden, 1972). Nemoto and Dao found that the number of axillary lymph nodes removed is equal whether a radical or a modified radical mastectomy is performed (Nemoto and Dao, 1975). During recent years many supporters of the modified radical mastectomy have reported their findings (Dunphy,

1971; Papatestas et al., 1975; Robinson et al., 1976). Their belief is that the modified radical mastectomy with the preservation of both pectoral muscles is equally as satisfactory as either the standard or extended radical operation in the treatment of cancer of the breast.

5. Simple mastectomy*

In 1948, McWhirter, professor of Radiotherapy at Edinburgh, reported on a series of patients with early breast cancer treated by simple mastectomy with radiotherapy to the axillary nodes (McWhirter, 1948). McWhirter's primary rationale for this "new" therapy was that the trauma of operation caused an increased tendency for tumour cells to disseminate to other sites and, should this occur before the application of radiotherapy, the use of this latter regimen would be ineffective in saving the life of the patient. It was his opinion that, when a simple mastectomy was performed in contrast to the situation following radical mastectomy, tumour cells would be trapped by the intact barrier of the axilla. Since wound healing was likely to take place more rapidly after simple mastectomy, radiotherapy could be applied with less delay. In this way, a reduction of the interval during which cells could be disseminated to distant sites could be achieved. This rationality is again in keeping with the original mechanistic and anatomic approach towards both tumour dissemination and eradication.

As a result of a retrospective analysis of 1,044 patients with breast cancer, Williams et al. concluded that, where efficient radiotherapy is available, radical mastectomy should be abandoned in favour of limited procedures (Williams et al., 1953). The results on survival were equal for both procedures and oedema of the arm occurred three times more often after radical mastectomy; however radical mastectomy reduced the incidence of local recurrence and this made radiotherapy desirable as an adjuvant to simple mastectomy. One of the best studies carried out to determine the worth of McWhirter's method is that of Kaae and Johansen: a clinical trial comparing simple mastec-

* The term "simple" mastectomy is used here because of its general use in the literature. Fisher, however, very properly observed that its precise meaning is ambiguous and could also mean partial mastectomies; therefore, he advises the use of the term "total mastectomy" instead of "simple mastectomy" (Fisher, 1970).

tomy followed by 4500 rads of X-rays in three weeks with extended radical mastectomy with dissection of supraclavicular and internal mammary lymph nodes. The results 5 and 10 years after treatment revealed no significant difference in survival and the incidence of local and regional recurrence was approximately the same regardless of the method of treatment.

As mentioned in the introduction, G. Crile Jr. of the Cleveland Clinic has a different outlook on breast cancer surgery based on systemic effects induced by the treatment. He advocated a simple mastectomy without postoperative irradiation for early operable breast cancer. This conviction was based on experimental findings regarding immunological resistance against metastases in which the regional lymph nodes play a major role: tumour-bearing mice showed an increase in metastasis formation when regional lymph nodes were removed between the fifth and seventh day after implantation. In another experiment, he noted that "simple" removal of the tumour induced a high resistance to subsequent reimplantation of the tumour on the contralateral feet, whereas removal of the regional node as well resulted in abolishment of antitumour immunity. Irradiation of the regional lymph nodes was as effective as surgical removal in preventing the development of systemic immunity to reimplantation (Crile, 1965; 1967). He therefore stated: "Surgeons should realize that prophylactic excision or irradiation of the regional nodes that drain small tumors not only increases morbidity but in some types or stages of cancer may increase the death rate from distant metastases" (Crile, A biological consideration of treatment of breast cancer, page 27, 1967).

In the same book, he reported his clinical results comparing survival rates after simple and radical mastectomies in the years 1955, 1956, and 1957. He found that patients with stage I ("On palpation of the axilla, there seemed to be no involvement of the lymph nodes") cancers treated by simple operations, usually without radiation, showed a 14 per cent higher survival rate than those treated by radical ones (54 per cent and 40 per cent, respectively, 8–10 years survival; page 62).

Again, this procedure was not "new": in his historical review of modified radical mastectomy, Madden (1972) reveals that Volkmann (1875) removed the axillary lymph nodes only when they were "diseased". Smith and Meyer in their publication in 1959 reported survival in a series of 448 patients treated between 1924 and 1952 either by simple or radical mastectomy; the 10-year survival rates were 32 and

30 per cent, respectively. They concluded that survival rates were not affected by the type of operation performed (Smith and Meyer, 1959). This conclusion was in agreement with Shimkin et al. who reanalysed the material more thoroughly in an attempt to exclude selection in the series (Shimkin et al., 1961).

Because there has been or is no more of a champion of simple mastectomy than Crile, his contribution deserves more detailed considerations, especially because his rationale is not based only on viewing the technique solely as a means of destroying the last cancer cell and he reported better results with a less extensive operation. His early clinical series are difficult to evaluate because of their irregularity: *about* half of the operations were radical or modified radical; the other half were simple mastectomies *or* local excisions without axillary dissections. Patients treated by simple operations *usually* received no postoperative irradiation (Crile, 1967, pp. 60–62). Besides the differences in survival, an important finding was also that the incidence of oedema of the arm in stages I and II (Manchester classification) had been 36 per cent after radical mastectomy alone, 21 per cent after modified radical mastectomy and irradiation, 7 per cent after modified mastectomy alone and 0 per cent after simple mastectomy with or without irradiation (Crile, 1964a, 1964b). In 1968, he reported that 5-year survival in patients whose axillae contained no palpable nodes was 13 per cent higher when the nodes were left in place and not irradiated than when they were removed with the breast (Crile, 1968). He then stated that "although both clinical and laboratory evidence indicates that uninvolved regional nodes contribute to the host's immunological resistance to systemic metastases, a large randomized study of patients with operative stage I breast cancers will have to be done before it can be stated with certainty that removal of uninvolved nodes promotes metastases". In 1975, Crile reported on survival data after 10 and 15 years of treatment by simple mastectomy (Crile, 1975). He compared his results with those of identically staged patients of the "National Cancer Registry" treated predominantly by radical mastectomy. His conclusions then were:

1. Delayed axillary dissection for occult cancer in nodes gave as good results as immediate dissection.
2. The incidence of local recurrence was no higher after conservative operations than after radical ones.

3. Late deaths after conservative operations were not associated with local recurrences.

He commented that, although the numbers were too small to be of statistical significance, the short-term survival seemed to be slightly shortened by radical treatment. The overall survival in 15 years in the two groups however, was the same (27 and 27.5 per cent for radical and simple mastectomy respectively).

Many "pro" and "contra" simple mastectomy reports have since appeared: Haagensen and Miller presented data that led them conclude that, after 10 years of follow up, the results of treatment of early breast cancer with the Halsted radical mastectomy were "overwhelmingly" better than those following simple mastectomy (Haagensen and Miller, 1967). Ten year survival data for radical and simple mastectomy were 70.2 per cent and 39 per cent, respectively (Columbia A classification). The patients compared were of two different institutions; the simple mastectomy patients also received postoperative irradiation of which Haagensen said that it was "so limited in amount that it is unlikely it could have influenced the end results – it never exceeded 1000 rads". In 1974 Haagensen again compared 10-year survival of his own series (radical mastectomy; survival Columbia A, 70 per cent; local recurrence 6.8 per cent) with those of Miller (simple mastectomy; survival Columbia A, 40 per cent; local recurrence 37 per cent). His conclusion then was that "the much higher local recurrence rate and the far lower survival rate after simple mastectomy should convince anyone that it is an inferior method of treatment" (Haagensen, 1974).

The data of Smith and Meyer were described by Anglem as "statistically worthless figures" (Anglem, 1974a). In the same paper, Anglem also pointed out that 10-year survival rates in Crile's series (which were "easily recognisible in favourable case selection for the simplified forms of treatment") and many other series employing simple procedures in comparison to those who employed radical procedures all showed a decreased 10-year survival for the former procedure. In his rebuttal to Anglem, Crile compared his data with those of the Cancer Registry and found no differences (Crile, 1974); moreover, he again pointed out that the five-year survival rate was 11 per cent higher when uninvolved nodes were *not* removed than when they were, suggesting the role of uninvolved lymph nodes in maintaining immunity clinically (Crile, 1969). Crile concludes that a controlled study is

crucial: "for it is tragic to continue what I consider to be the needless mutilation of the standard radical mastectomy". Anglem, however, had seen enough (Anglem 1974b). He even showed in an earlier paper (Anglem and Leber, 1973) that Crile's results with modified radical mastectomy were inferior to others employing the same regimen.

The results of these studies stimulated the controversy over what was to be the proper treatment for early breast cancer. There was confusion in many surgeons' minds as to what to advise for their patients. Some accepted the concept that limited surgery was at least sufficient, whereas others continued with the traditional radical mastectomy. This confusion is illustrated by surveys on what kind of surgical practice was used in the Netherlands (Zwaveling, 1966) and in Britain (Editorial, 1969). Of course, only properly controlled clinical trials can give surgeons the evidence they require to decide what their practice should be.

6. The clinical trials

Because of the *suggestive* evidence of the merits of simple mastectomy, provided by some authors, they called for randomized prospective clinical trials as early as 1961 (Shimkin et al., 1961; Crile, 1961).

Several randomized trials comparing simple mastectomy with radical mastectomy have since been designed and described (see Table II).

As shown from the data available, most studies conclude with no differences between the regimens used. Remarkable however, are the results of the largest controlled clinical study (2,268 patients), so far undertaken (Editorial, Brit. Med. J., 1976). A reduction from 15 per cent to 4.7 per cent in local recurrence percentage was found in the group treated by additional radiotherapy. These recurrences occurred mainly in the axilla; the prognosis of these patients was poor. However, despite the greater incidence of local recurrence in the simple mastectomy alone group, the 5-year survival rate in the two treatment groups was similar. Many trials have not yet produced definite data, especially the results of the "Fisher" trial are awaited with interest. Notwithstanding, these preliminary "hard" data, it may be concluded that the advantage of the more biological approach, i.e., by leaving the regional lymph nodes intact, based on the original suggestion of Crile that simple mastectomy for early stages of cancer

Table II. Clinical trials on surgical management

Trial	A	B	C	Stages	5-year survival	Investigators' conclusion
Copenhagen, 1951–1957 (Kaae and Johansen, 1962; 1965; 1969)	Extended Radical +supra-clavicular and internal mammary lymph nodes' dissection	Simple+RT		I, II, III	A = 77 per cent B = 75 per cent (Stage I)	Survival A = B Recurrence A = B (5 and 10 year)
Cambridge 1958–1965 (Brinkley and Haybittle, 1966; 1971)	Radical+RT	Simple*+RT		II	A = 58.3 per cent B = 62.8 per cent	Survival A = B Recurrence A = B Morbidity A > B (5 and 10 year)
S.E. Scotland 1964–1974 (Bruce, 1971; Hamilton et al., 1974)	Radical+ Oophorectomy	Simple+RT +Oophorectomy		I, II, III	A = 76 per cent B = 66 per cent (all stages)	Survival A = B (5 year)
Hammersmith, 1965 (Burn, 1974)	Radical+RT	Simple+RT		I, II	A = 70 per cent B = 65 per cent	A = B
Cardiff, 1967 (Forrest et al., 1970; Roberts et al., 1973)	Radical or Modified Radical	Modified Simple		I		"There is nothing in the preliminary data to suggest that a conservative approach is inferior to a radical one"
	Radical or Modified Radical+RT	Modified Simple+RT		II		
King's/Cambridge 1970 (Editorial, Lancet, 1969)	Simple+RT	Simple		I, II		not available
N.S.A.B.P., 1971 (Fisher, 1973; Fisher and Wolmark, 1975)	Radical vs.	Simple vs.	Simple+RT	I		September 1974: preliminary results analysis by lifetable and direct methods revealed no significant differences
	Radical	Simple+RT		II		
King's/London, 1971 (Edwards et al., 1972)	Simple+RT	Simple		I, II		not available
International Multicenter trial 1970–1975 (Brit. Med. J., 1976)	Simple+RT	Simple		I, II	A = 78.8 per cent B = 78.3 per cent	Survival A = B distant recurrence A = B local recurrence A < B

* Simple mastectomy involved removal of the breast and accessible axillary nodes; the latter at the surgeon's discretion, but done in 79 per cent of the patients (= modified simple mastectomy).

of the breast gives *better* survival results, could not be confirmed. This could also be interpreted as evidence that less extensive surgery is equally good. This resulted in even less aggressive surgical procedures for early mammary carcinoma as advocated by several authors: i.e., local excision of the lump.

7. Local excision

What is the rationale for this kind of surgical approach to breast cancer? Atkins described his rationale, based on his belief that the fate of circulating tumour cells, which are likely to be present in the early history of cancer of the breast, is determined by an immune mechanism as follows: "*This* in itself prompts the consideration of whether more is gained by removing the cancer tissue than is lost by a major operation which might disturb both the humoral and the cellular immune processes designed to supress them". (Atkins et al., 1972). The results he describes of a controlled clinical trial (Guys hospital-started 1961) designed to compare radical mastectomy with wide excision (extended tylectomy) both followed by radiotherapy are however not supporting this hypothesis. In patients with clinically uninvolved nodes, the survival rates at ten years are the same, although there was a significantly higher incidence of local recurrence after tylectomy. In patients with clinically involved nodes the wide excision regimen was a disaster: Both in survival and recurrences the rates were significantly better in those patients treated by radical mastectomy.

Were the results of others and the success of treatment by less extensive surgery already well enough established so that controlled clinical trials could be done without fear of doing an injustice to the patients receiving the less extensive treatment?

There indeed have appeared favourable reports: In 1954 Mustakallio published the results of 107 patients treated by local or wedge resection with subsequent irradiation. Five-year survival rate was 84 per cent and in a very small number of cases observed for ten years (13/18) 72 per cent (Mustakallio, 1954). In a later report he published similar results, however these rates were not corrected for control population death rates (Mustakallio, 1972). In 1964 Porritt contended that if one is not going to employ radical surgery, one should not mutilate at all (as by simple mastectomy). The results of his personal

series would give some confidence to this contention (50 per cent and 34 per cent 10-year survival after local excision and radical mastectomy respectively), however he acknowledged case selection (Porritt, 1964). Several others have reported reasonable results with local excision in personal series (Peters, 1954; Crile, 1965, 1971, 1972a, 1975; Taylor et al., 1971, Wise et al., 1971). Anglem however critically evaluated the series of Mustakallio, Porritt, Peters and Crile and clearly indicated favourable case selection in all series (Anglem, 1974a). Furthermore the suggestion (Crile, 1972b) that patients be encouraged to make a decision about the extent of surgery for their breast cancers is difficult to approve because of the obvious inability of a person not familiar with advantages and disadvantages of several types of surgery to make a wise selection. I would expect that many women already disturbed by tensions and emotions, which make them a priori unsuitable for such a decision, are even more disturbed by having to make such a decision. The preservation of feminine appearance is highly desirable but the most important consideration is the prolongation of active living, not disturbed by local or regional recurrences and the reduction of fatality from this disease. It presently is even uncritical to consider local excision for large numbers of women since the mentioned series were often small with poor statistical support. Moreover several publications indicated that great caution must be exercised for these types of surgery.

A. Whole breast subserial histological sections of mastectomy specimens have shown that not only localized residual infiltrating carcinoma but also multiple sites of non-infiltrating carcinoma are found in a large number of patients. Many studies (Qualheim and Gall, 1957; Gallager and Martin, 1969a, 1969b; Shah et al., 1973 and Fisher et al., 1975) have clearly demonstrated that carcinoma of the breast is not a focal process but a diffuse phenomenon involving the entire breast epithelium and in several areas of the breast one may find varying stages of development of invasive carcinoma of the breast. They also have shown that concurrent invasion at multiple sites from pre-existing carcinoma in situ is common. Thus, one can never be certain as to the likelihood of leaving behind viable residual or multicentric carcinoma of varying histologic types after local excision of the focally dominant mass.

B. Several warning communications about local excision showing that survival was significantly diminished when compared to radical

mastectomy should be reason enough not to recommend such a policy for a large number of women (Farrow et al., 1971; Atkins et al., 1972). C. For the patient not only survival is important but also the quality of life: Even if there are haematogenous metastases it is very important that local recurrences do not present. The Antoni van Leeuwenhoek Hospital performed a valid pilot experiment to evaluate less aggressive surgical treatment in selected cases with small tumours. On 28 patients a radical partial mastectomy was performed, followed by radiotherapy. This included a radical axillar dissection en bloc with the excision of about half of the breast and pectoral muscles. Positive nodes were found in the axilla in nine cases. Recurrence in this group up to now has occurred in 13 out of 28 patients (van Dongen, 1977) often very close to the previous surgical field. In some of these patients no secondary ablation was possible! (Hadisumarto et al., 1967; van Dongen et al., 1972; 1973).

These arguments clearly indicate that even very small lesions detected by mammography which show to be invasive cancers after excisional biopsies should be treated aggressively. In as much as there is no scientific method now available to determine the extent of the involvement of a breast by carcinoma prior to its surgical removal, there is no place for local excision as the definitive therapy in patients with potentially curable carcinoma of the breast.

8. Comments

The present dilemma that exists relative to the primary treatment of breast cancer is, at least in part, the consequence of the changed concepts that have resulted from new information concerning tumour biology. Our knowledge concerning tumour dissemination and metastasis formation for example, although still incomplete, is well beyond that of Halsted. As a result of this new information several surgeons abandoned the classical radical mastectomy to perform more or less extensive surgery. Just as a prime purpose of laboratory investigation is to provide guide lines and clues as to what may be expected to occur when such studies are applied to the human, so may the results of retrospective analyses supply hints for future definitive evaluation. The findings of Crile and others that have been described are of such nature. They have provided "suggestive evidence" that should be

accepted as such. The next step, as a result of this intimations, is the carrying out of properly conceived prospective clinical trials with randomization of similar patients so that one group receives the therapy to be evaluated and the other, which serves as the control group, is the recipient of the present "standard" therapy (Lévy, 1971; Staquet, 1972). Such trials endeavour to apply the scientific method for solution of clinical problems. Unfortunately however, the labeling of a study as "prospective" or "randomized" or "a clinical trial" does not necessarily ensure the worth of the data produced. Consequently, an uncritical and unqualified acceptance of results from such trials is no more justified than is the formation of firm conclusions on clinical management on the basis of experimental findings and retrospective clinical studies. Selection of patients can remain a problem even in carefully planned clinical trials comparing standard radical mastectomy versus simple mastectomy in clinical stage I cancer (with non-palpable axillary nodes). For instance a follower of the more aggressive standard treatment co-operating in such a trial probably will decide earlier in favour of a node dissection when he is in doubt on their palpability. It is very difficult to perform different surgical procedures in one hospital, without the patients knowing about it. Besides the "informed consent", this contributes to the selection, since the patient can refuse the more extensive procedure and the surgeon has no arguments to change her opinion. Add to this that most of these trials often do not produce exact data relative to incidence of local, regional or distant recurrences, nor do they point out the effectiveness of radiation in eliminating axillary metastases or of subsequent chemotherapy.

Fisher critically reviewed and commented results of many clinical trials such as the Copenhagen, Cambridge, S.E. Scotland, Cardiff, King's/Cambridge and Guy's trial, and pointed out many inadequacies in these trials (Fisher, 1972). Moreover it is becoming increasingly apparent that more meaningful data are obtained from subgroups of breast cancer patients having special characteristics. Reporting of results only for "all patients in the study" are much less meaningful than when data are produced according to stage, menopausal status, histologic characteristics, site of the tumour, etc. Consequently, large numbers of patients are necessary to provide minimal sample sizes in such a variety of subgroups. Most of the clinical trials mentioned in this review fail to fulfil the preceding considerations. Frequently the protocols

employed are "loose", implementation is questionable, sample size is suboptimal and above all, reporting is less than desirable.

The results presently available when evaluated collectively do suggest – *but do not prove* – that simple mastectomy may produce equivalent results on survival for stage I cancers. This in itself must stimulate the design of more and better trials and experimental research as well to obtain more insight in the process of invasion and metastasis formation and how it can be handled optimally. As mentioned earlier, in this evaluation in which survival is the major endpoint, the occurrence of regional recurrences and success of subsequent treatment should be carefully registered since regional recurrences can greatly affect the quality of life.

CHAPTER III

REVIEW OF THE EXPERIMENTAL STUDIES

1. Biologic considerations of the process of invasion and metastasis formation

Essential to understand the malignant nature of cancer, is the process of invasion and metastasis formation. With current therapeutic possibilities, it can reasonable be argued that in terms of *clinical importance*, in a large majority of cases, the greatest problem posed by cancer is represented by the second of these characteristics. In practical terms, the first stage is of most importance since without it the other stages cannot take place. The basic facts are straightforward in that we know that these stages occur, but we have very little knowledge of the mechanisms involved or of the relative importance of host and tumour factors, or of the frequency with which the various events take place. Each stage in the process is a result of an interaction of host and tumour factors.

The lymphatic system provides the most common pathway for the initial spread of many carcinomas. The pattern of lymph node involvement depends on the site of the primary tumour and its lymphatic drainage. Lymphatic spread may result in the infiltration of a draining regional lymph node by tumour cells, with metastases to distant lymph nodes occurring later. The outcome of the disease is however mainly determined by the occurrence of haematogenous metastases which lead to death. The intimate nature of these steps and the roles played by local and general mechanisms have not been elucidated and the state of knowledge is still in its infancy. The purpose of this review is not to be exhaustive but to discuss existing knowledge of mechanisms of neoplastic invasion and of the interaction between host and tumour with special reference to the lymphatic system especially in relation to clinical significance.

2. Mechanisms of neoplastic invasion

During transplantation passages most experimental tumours change their histological pattern towards cellular anaplasia (Stewart et al.,

1959) and this tumour progression has been reported to go together with an increased tendency to metastatic spread of spontaneous as well as of induced tumours (Rudenstam, 1968). Gershon-Cohen et al. in a clinical study of mammary carcinoma related the doubling time to the histology showing that the well differentiated tumour had the slowest growth rate (Gershon-Cohen et al., 1963). Kusuma showed for the same type of neoplasm that doubling time strongly correlated with survival time, the shorter doubling times being associated with the lowest survival rates (Kusuma et al., 1970). This finding was confirmed for many other types of neoplasms by Malaise et al. (1974). In general, undifferentiated tumours are more likely to metastasize than well differentiated tumours. However, several exceptions, e.g. basal cell tumours and gliomas rarely metastasize irrespective of their degree of differentiation. It is evident however that a continuous and progressive multiplication of tumour cells within a restricted space could bring about an increase in tissue pressure leading first to disruption in blood supply and ischaemia and later to pressure atrophy also obstructing local lymphatics resulting in local oedema. Young (1959) and Eaves (1973) supported the hypothesis that an increase in hydrostatic pressure in tumours may lead to an increase in metastasis formation. As early as 1913 Tyzzer found that external mechanical increase of pressure resulting from massage of the primary tumour resulted in a shortening of pre-metastatic period. Stoker found that limb exercise possibly by muscular massage of the regional lymph nodes enhanced the spread of Vx2 tumour (Stoker, 1975). However recent experimental data using plaster casts (Hammond and Rolley, 1970) or tumour massage and biopsy (Kaae, 1953; Peters, 1975) did not show a significant difference in frequency of lymph node and lung metastases. Saidel et al. (1976) found an increase of dislogement by a factor 10 but this did not affect the ultimate total number of metastases.

In general, clinical experience supports this view e.g., though Engell found no correlation between manipulation and number of circulating tumour cells (Engell, 1959) whereas Watne found a positive relationship (Watne, 1960) Griffiths and also Engell found no correlation between the presence of malignant cells in venous blood of colonic and rectal cancers and the subsequent development of metastases (Griffiths et al., 1972). It should be obvious however that a failure to identify tumour cells from the blood cannot be associated with the absence of the hazard of haematogenous spread and on the other hand

a massive haematogenous spread may be related to a relatively high level of cells in the blood. (A detailed analysis of factors influencing haematogenous metastases is given in van Dongen, 1961). Nevertheless, it is evident that the presence of tumour cells in the blood does not always imply metastatic tumour growth, since most circulating tumour cells die rapidly, only about 0.1 per cent survive to form secondary growths (Fidler, 1970).

In conclusion: Although mechanical forces may aid tumour cell invasion and dislodgement their influence on the outcome of the disease as a whole is probably limited. However, it remains preferable to avoid the liberation of circulating cancer cells if possible, and all efforts should be made towards this end (For a recent review on this subject see Salsbury, 1975). Many other mechanisms have been suggested; recently it has been demonstrated that many varieties of human and animal malignant tumours have a much higher content of collagenolytic enzymes than benign tumours or the corresponding normal tissues (Strauch, 1972; Yamanishi et al., 1973). Tumour cell motility is an important factor. Easty and Easty have recorded migration rates of 50–100 μm/day through normal tissues of a variety of tumour cells from the hamster, rat, mouse and man (Easty and Easty, 1974). Another important feature may be that neoplastic cells invade lymphatic vessels in a way similar to that in which white blood cells penetrate them: by the propulsion of fine cytoplasmic processes and passage – sometimes in clumps – through open cell junctions (Carr et al., 1975; 1976). Abnormalities in intercellular adhesion have also been stated to correlate with metastatic potential (Winkelhake and Nicolson, 1976) as well as lack of immunogenicity (Kim et al., 1975; Davey et al., 1976). In fact it is conceivable that *all* these processes could be involved in the invasion phenomenon. The relative weight of each of these factors is hard to evaluate and the importance of each could differ from one tumour system to another, making comparisons and generalized conclusions impossible.

3. Lymph nodes

In 1863 Virchow postulated the theory that the regional lymph node acted as an effective barrier to the spread of cancer. The structure of lymph nodes is significant to their barrier function (Rubin, 1970) and their anatomy converts flow of lymph into percolation. Many

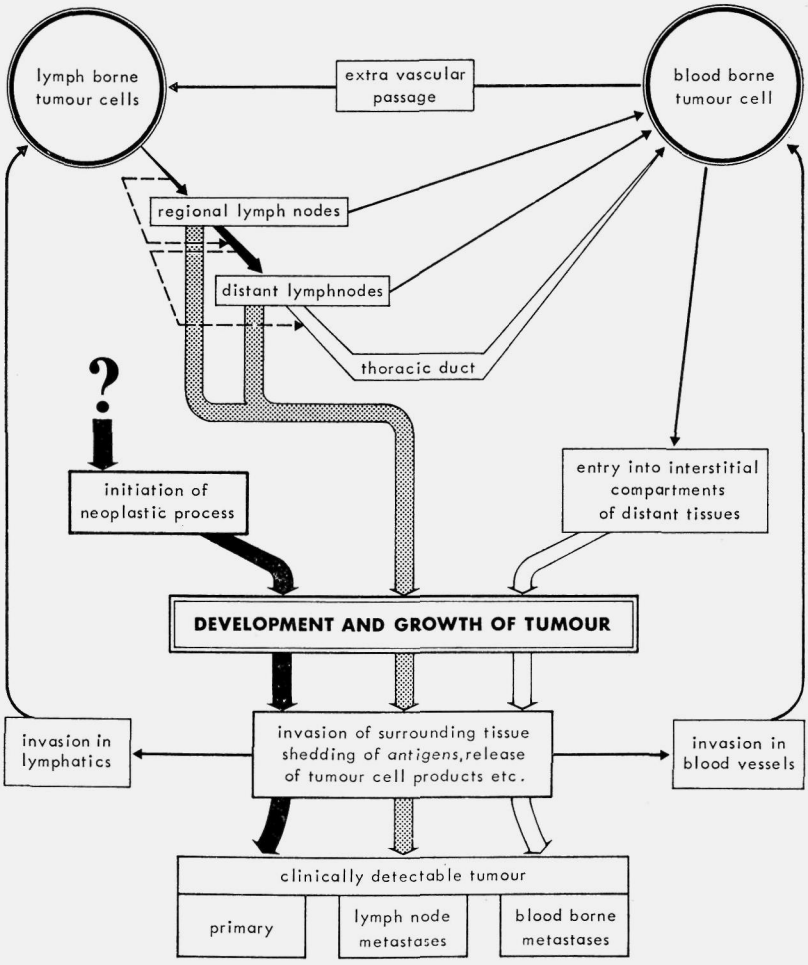


Figure 2. The spread and relationship of cancer in the lymphatic and vascular system.

authors described a temporarily effective barrier function of lymph nodes against cancer cells (Zeidman and Buss, 1954; Baker et al., 1969; see for review Sträuli, 1970). This concept has been challenged by many investigators either using injection of tumour cells into the afferent lymphatics (Fisher and Fisher, 1966; 1967; Madden and Gyere, 1968), or tumour cells naturally disseminated from a non-antigenic tumour (Hewitt and Blake, 1975), showing that tumour cells stream continually through lymph nodes with a low potential

for seeding. Fisher and Fisher (1966), found that just as neoplastic cells may traverse lymphatic pathways to become blood-borne (Confirmed by Hilgard et al., 1972), so may haematogenously disseminated cells find their way into lymphatics and hence back into the blood to complete a circuit. However different types of tumours e.g. carcinomas versus sarcomas have different preferences for the two modes of spread: It is common for carcinomas to metastasize to regional lymph nodes while most sarcomas do so infrequently.

The spread of cancer cannot anymore, in view of these findings be described as simply as in figure 1. Figure 2 shows the complex and intimate relationship that exists between the vascular and lymphatic systems in the spread of cancer. This scheme expresses that these systems are inseparable and can never be discussed alone.

4. The role of the lymphoreticular response to neoplasia

Immune reactivity to neoplasia is one host factor that may influence metastatic spread (Alexander et al., 1972). Any attempt to review immunologic considerations regarding all types of cancer would leave both the reviewer and the reader ensnared in a welter of conflicting and confusing data. Further one should not presume a priori that all host influences on the development and behaviour of cancer are immunologic in nature. (See for review of non immunological host defences, Apffel, 1976). Reactive changes in lymph nodes draining malignant tumours have been extensively studied in man (Black and Speer, 1959; Black, 1965; Anastassiades and Pryce, 1966; Alderson et al., 1971).

More specifically the lymphoreticular responses in human cancer patients were recognized as structural representations of immunological phenomena. Outside the field of oncology elaborate schemes have been proposed for describing reactive changes in lymph nodes according to their immunological function (Cottier et al., 1972). From experimental studies it has been shown that the deep cortex i.e. the paracortical area, is a thymus dependent area populated mainly by T lymphocytes (Parrott et al., 1966; Parrott, 1967; Parrott and de Sousa, 1971; Gutman and Weissman, 1972; Howard et al., 1972). This area is expanded and shows proliferation of large lymphoid cells early in the immune response against "thymus dependent antigens" (Scothorne and McGregor, 1955; Oort and Turk, 1965; Turk, 1967; Wiest and

Jung, 1970; Hellström et al., 1971; Meyer, 1971) and experimental tumours (Rosenau and Moon, 1966; Edwards, 1966; Alexander et al., 1969; Edwards et al., 1971; Fisher et al., 1973; Simar et al., 1975). Germinal centers have been shown to be thymus independent regions associated with the production of plasma cells and humoral immune responses (Turk, 1967; Bailif and Jones, 1969; Bilski and Jerusalem, 1969; Parrott and de Sousa, 1971; Gutman and Weissman, 1972; Howard et al., 1972).

The main finding of the reaction in draining lymph nodes to experimental tumours is an early hyperplasia of the paracortical area followed by an increase in the size and the number of cortical follicles (Rosenau and Moon, 1966; Edwards, 1966; Edwards et al., 1971; Fisher et al., 1973; Simar et al., 1975). Similar early and late changes have been found in human lymph nodes (Ahlqvist et al., 1974).

The prognostic significance of reactive changes in draining nodes in man has been stressed for carcinoma of the breast (Cutler et al., 1969; Berg et al., 1973; Black, 1974; Black et al., 1975; Tsakraklides et al., 1974; Hunter et al., 1975) as well as for carcinoma of head and neck (Berlinger et al., 1976) and bronchogenic squamous cell carcinoma (Kaufmann et al., 1977).

The clinical and experimental evidence just described indicate that at certain stages in the development of a tumour, the draining lymph nodes respond to it and that these early responses appear to be, at least temporarily, inimical to the tumour's progressive growth. Indeed experimental findings confirm that cellular (T cell) responses are relevant to the rejection of antigenic tumours (Barski and Young, 1969) and humoral (B cell) responses may allow enhancement (Kaliss, 1958). However, conflicting data exist showing that weakly antigenic tumours may lead to a cell-mediated response which stimulates rather than inhibits tumour growth (Prehn and Lappé, 1971; Medina and Heppner, 1973).

An important question in this regard is:

5. How antigenic is mammary carcinoma in man?

In experimental animals there is a profound immunological difference between "spontaneous" tumours and those induced by chemical, viral or other means. It appears that virtually without exception, those tumours that appear with low incidence and without known

cause have little or no capacity to effectively immunize animals syngeneic to the animal of origin. Mice immunized with such tumours usually grow a challenge inoculum of the same tumour as well as do the nonimmune controls (Hewitt et al., 1976). Whatever antigenicity they may exhibit, it is very weak. The situation with regard to induced tumours is quite different. If tumours are induced for instance by a powerful chemical oncogen, mice immunized with tumour cells are often highly resistant to the growth of a challenge inoculum. The immunity, in the case of chemical induction, is highly specific, being limited to the particular immunizing tumour and usually not cross-reactive with other tumours of the same etiology, histology, organ or animal of origin (Price and Baldwin, 1975; Prehn, 1976a, 1976b; Baldwin, 1976). For obvious ethical reasons evaluation of immunity in human cancer patients against transplantable cells is unacceptable. For the most part, the view that human tumours elicit immune reactions is based on results of *in vitro* studies and such findings must be considered critically until more substantial evidence of host rejection is available (Currie, 1974).

Is mammary carcinoma in man an induced or a "spontaneous" tumour?

Some human cancers occur in high frequency as a result of potent environmental oncogens, and these tumours may, like the analogous laboratory tumours, be influenced by an immune reaction e.g. ultraviolet induced skin cancers, bronchogenic squamous-cell tumours, and bladder cancers might fall in this category. For human mammary carcinoma such a factor has not yet been clearly identified. Non-immunologic factors are possibly of great importance. The low incidence of breast cancer in males as compared to females in man and mice and the decreased incidence rate of breast cancer among women who have been castrated before age 35 clearly demonstrate the importance of nonimmunologic factors in mammary carcinogenesis. Immunodeficient patients however, show an increased incidence of neoplasms. These tumours are however of a very restricted range e.g. in transplanted patients about 60 per cent of the tumours are of lymphoid origin and there is no increase of mammary carcinoma despite its common occurrence. Similarly Balner found that prolonged antilymphocyte serum treatment did not affect incidence and latency periods of malignancies other than lymphomas in mice (Balner, 1971). Simpson and Nehlsen chose the strain CBA because of its low frequency of spontaneous tumours and completely failed to increase tumour

frequency by antilymphocyte serum (Simpson and Nehlsen, 1971).

In vivo in man there is also little evidence for antigenicity. The use of immunotherapy in the treatment of human cancer has as its basic premise the assumption that human tumours are immunogenic and that the host is capable of reacting in a specific immunological manner to his or her own tumour. Unfortunately despite extensive clinical experimentation such attempts at immunotherapy have until very recently been far from successful (Currie, 1974b; Carter, 1976). Unequivocal success of immunotherapy would of course have vindicated any ideas about the existence of tumour specific immune responses in man. Several other "evidences" have been brought forward to substantiate antigenicity of human tumours e.g. *spontaneous regressions.* There are many convincing reports of the spontaneous regressions of established human tumours (Everson and Cole, 1966). The highest incidence of such regressions is seen in neuroblastoma, hypernephroma, choriocarcinoma and in malignant melanoma, but there is no systematic clearcut evidence from this sort of data that such regressions are always immunologically determined nor is there substantial evidence for common spontaneous regression of mammary carcinoma.

Prolonged survival of patients with tumours

After treatment for breast cancer, patients may survive for up to 20 years or more and then develop metastases with no evidence of further intervening primary lesions. Explanations have been given on an immunological basis, however, if one or a few cells have remained after treatment of the primary and do repopulate to a lethal number (Skipper, 1977) then such a long time is quite reasonable e.g. Kusuma observed doubling times of 500 days (Kusuma et al., 1972).

Histological evidence

Many types of primary malignant tumours are infiltrated by lymphocytes, macrophages and plasma cells. Furthermore occurrence and prognostic significance of local and lymph node morphological reactions to invasive breast cancer have been described extensively by Black (Black and Speer, 1960; Cutler and Black, 1969; Black, 1973). He related these morphological findings in draining lymph nodes to the antigenicity of the tumour in the breast (Black, 1975, 1976). Others have advanced the hypothesis that unstimulated lymph nodes may result from non or weakly antigenic tumours (Tsakraklides et al., 1974; Berlinger et al., 1976). It is remarkable that little or no attention has been paid experimentally to morphological changes in lymph

nodes draining tumours of known and different antigenicity. The mentioned clinical suggestions were however not generally confirmed e.g. Hartveit found no correlation between survival time and lymph node changes and suggested that the amount of tumour may be the major determinant (Hartveit, 1973). In fact, it is not quite clear what part of the reactivity of the lymph nodes must be ascribed to such nonspecific factors as cell necrosis and aspecific inflammation in the tumour. Moreover, a tumour is not a static target for the immune system; it is a dynamic, growing entity. The growth rate of the tumour, its metastatic potential, its anatomical location, its invasiveness, the cohesiveness of the tumour cells, the fibrous reaction surrounding the tumour and many other factors will influence the course of the disease and the interaction of the tumour with the immune system.

The wealth of available data, both experimentally and clinically and their incomparableness, invites speculation. Human breast cancer is antigenic in a way that it can induce morphological immune reactions in draining lymph nodes as well as immune reactions which can be measured by *in vitro* assays. That the immune reactions significantly influence tumour growth and tumour cell survival, comparable to that of chemically induced model-tumours, is not proven. So that the approximation of its antigenicity to be low or weak, as with spontaneous tumours in mice is more likely. Lack of antigenicity in spontaneous tumours in the sense of absence of modification of tumour growth by immune mechanisms does, however, not necessarily mean lack of antigens!

6. Experimental studies on the effect of regional lymphadenectomy

In challenging the classical concept of en bloc resection of the primary tumour along with its regional lymphatics for cancer of the breast, Crile's choice of simple mastectomy was based on his experimental findings that retention of regional lymph nodes early in the growth of a tumour could be important in maintaining a degree of systemic immunity against growth of metastatic microfoci (Crile, 1960, 1965, 1967, 1968, 1975). His first experimental studies were performed in an allogeneic tumour (Sarcoma 180) and a highly antigenic tumour (Sarcoma T241).

In both tumour models it was observed that when tumours were

Table III. Experiments showing a detrimental effect of regional lymph node removal

References	System		Time of lymphadenectomy in respect of tumour transplantation: effective	ineffective
Crile (1965, 1966, 1967, 1968, 1976)	Sarcoma 180 Swiss mice	▲	5–7 days after	4 days before and later than 11 days after
	Sarcoma T241 C57BL/6 mice	○	5–7–8 days after	10 days after
	Sarcoma 1 Strain A mice	○	—	10–14 days after
	Melanoma Hamster	▲	—	21–49 days after
Eltringham and Weissman (1970)	Fisher 344 rats immunity parameters		1–10 days after	—
Fisher and Fisher (1971, 1972)	spontaneous mammary carcinoma	□	7 and 14 days before	—
	C3HeB/FeJ mice		14–21–28 days after	—
	M.C.*-sarcoma C3HeB/FeJ mice	○	—	14 days after
Hall et al. (1972)	Vx-2 carcinoma Rabbit	▲	7 and 14 days after	21 and 28 days after
Perez et al. (1973, 1974, 1975) Stuwert et al. (1976)	Gardner lymphosarcoma C3H/AnF mice	○	4 days before until 7 days after	prior to 7 days before later than 8 days after
Pendergrast et al. (1976)	M.C.-sarcoma Babc/AnN mice	○	3 days after	7–21–14 days after

* 20-methylcholanthrene-induced
○ highly antigenic
□ non or weakly antigenic
▲ allogenic

growing progressively and the tumour-bearing feet were removed by simple amputation on the fifth to seventh day after implantation, the mice showed a high resistance to subsequent reimplantation of the tumour on the contralateral feet. But when the popliteal lymph nodes were removed at the time of the amputations, tumours reimplanted on the opposite feet grew almost as well as in unimmunized mice.

Crile's explanation for these findings was that early in the course of the growth of a tumour, the regional lymph nodes have not as yet become immunized, and they play no part in the systemic immunity of the host to either metastases or reimplantation of a tumour. Later, when the tumour is still small, there is a period during which systemic immunity to implantation of circulating tumour cells is dependent upon the integrity of the regional lymph nodes. Finally, there appears to be a time when the tumour becomes so large that its antigens "overwhelm" the immunity in the regional nodes and immunity becomes systemic (Crile, 1968).

As a result of his findings many investigators have developed various experimental models for evaluating the specific role of the regional lymph nodes in cancer. It is, however, surprising that little attention has been paid to the morphological reactivity in lymph nodes draining experimental tumours, whereas benign enlargement of regional lymph nodes clinically was used as an argument in favour of regional lymph node dependent immunity (such as observed by Berg et al., 1973). If regional lymph nodes, temporarily, have special properties in regard to their immunological function, one would expect a staging in the sense of a serial rather than a simultaneous response in the succeeding lymph nodes of the lympathic drainage system. This has however never been investigated on such a morphological basis. In fact most of the experimental models were explored to evaluate an effect of the removal of the regional lymph node. When reviewing the literature on these studies, differences are noted in tumour models explored, endpoints evaluated and final conclusions.

For the ease of survey, models and results are summarized in Tables III, IV, V and VI, since it is impossible to describe accurately all the variations in models and experimental designs.

Table III shows the models in which a detrimental effect of regional lymph node removal could be detected. Similarly as observed by Crile, time after tumour inoculation (or implantation) could be a major determinant as to whether such a detrimental effect is observed.

Table IV. Experiments showing a favourable effect of regional lymph node removal

References	System	Time of lymphadenectomy in respect of tumour transplantation	
		Effective	Ineffective
Sato (1964)	Ascites-hepatoma (C3H/HeN × dd)F_1 mice ?	5–15 days after	—

Indeed most of the models do show such a time dependency (Crile, Hall et al., Perez et al., Pendergrast et al., Table III). Each of these authors employed either allogenic tumours or highly antigenic (chemically induced) tumours. As a result of the previous discussion on antigenicity of human mammary carcinoma, it is reasonable to have some doubt about the relevance of this type of animal tumour systems as models for clinical cancer if they elicit forms of resistance which are clearly attributable to laboratory artefacts associated with their induction, or to genetic diversity such as in allogenic tumour systems. This is especially true when clinical significance is given by the authors to the results of their experiments.

Nevertheless, one experimental model, employing a spontaneous mammary carcinoma revealed a significant detrimental effect of regional lymph node removal in both the initiation (Fisher and Fisher, 1971) as well as in the maintenance of immunity (Fisher and Fisher, 1972). This mammary carcinoma, however, did not show such a time dependency with regard to regional lymph node removal. (Table III). In contrast to this, nodal excision did not impair the development of immunity in mice bearing a chemically induced sarcoma, so that the importance or regional lymph nodes was held by the authors to be a characteristic of weakly antigenic tumours (Fisher and Fisher, 1972). In both these studies secondary challenge of tumour was used as an endpoint for the existence of regional lymph node dependent immunity (Table VI). For the spontaneous mammary carcinoma a reduction in percentage tumours arising after secondary challenge was described in both experimental groups (with and without R.L.N. removal, 54 and 38 per cent respectively) compared to the first challenge in non-pretreated control animals (81 per cent). This clearly suggests antigenicity leading to an inhibition of tumour growth.

Table IV shows only one allogenic, or highly antigenic experimental model in which a favourable effect of regional lymph node removal

was observed on the number of pulmonary metastases (Table VI). This effect was supposed to be related to the effectiveness of the regional lymph node as a barrier to tumour cells, so that the amount of tumour removed was larger when including the regional lymph nodes, with the removal of the primary. While the barrier may be temporarily effective (Zeidmann and Buss, 1954; Sträuli, 1970) it is clear from other studies that the protection is at best only temporary since tumour cells may stream through lymph nodes with relatively little seeding (Fisher and Fisher, 1966; Hewitt and Blake, 1975).

Table V shows all experimental models in which no effect of regional lymph node removal was detected in either way. As shown this was observed independent of antigenicity of the primary tumour.

What is the significance of all these contradictory reports? Are these models appropriate for conclusions that there is, or is not a fundamental basis for simple mastectomy in a way that regional lymph node removal can affect ultimate survival clinically?

The presence or absence of some degree of immunological activity of the host against its tumour is a controversial condition, regarding its relevance to spontaneous cancer in man. As a result of the discussion on the antigenicity of human mammary carcinoma, I cannot regard tumours induced by chemicals or tumours not transplanted in syngeneic hosts, which embody artificial forms of antitumour immunity, as valid models for spontaneous mammary carcinoma in man.

In two spontaneous tumour models there was no effect of regional lymph node removal (Peters and McCredie et al., Table V). In these studies regional lymph node removal was however done at its earliest 10 days after tumour transplantation. It could have been that immunity had already become systemic at that time (see Table III). Apart from the criticism on these studies on account of the antigenicity of the tumours it is also relevant to assess the validity of the different endpoints used in the studies.

Is the spontaneous mammary carcinoma model of Fisher and Fisher, which is the only weakly antigenic tumour model in which a detrimental effect of regional lymph node removal was found, representative for human mammary carcinoma?

In their studies they employed secondary challenge as an endpoint, whereas in human studies both the occurrence of regional recurrences as well as survival, determined by haematogeneous spread, are the major endpoints. It is difficult to regard the take of second "primary"

Table V. Experiments showing no detrimental effect of regional lymph node removal

References	System		Time of lymphadenectomy in respect of tumour transplantation
Gardner and Rosen (1967)	Walker-256-sarcoma Wistar rats	▲	tumoursize (2 cm^3)
	M.C.*-sarcoma Wistar rats	○	before and after (time or size not given)
Bard et al. (1969)	M.C.-sarcoma C3H/HeN mice	○	tumour size (⌀ 6–10 mm)
Hammond and Rolley (1970)	M.C.-sarcoma C57BL/6N mice	○	8–12 days after (⌀ 1.5 cm)
Pilch et al. (1971)	M.C.-sarcoma C3H/HeN mice	○	1 week before tumour size (⌀ 6–10 mm)
Abe and Taneichi (1972)	Yoshida sarcoma Ascites hepatoma Donryu rats	▲	before
McCredie et al. (1973)	spontaneous mammary carcinoma	□	20 days after
	M.C.-sarcoma C3H/HeJ mice	○	
Sträuli and Lindemann (1974)	M.C.-sarcoma's non inbred golden hamsters	○	2–3 days before induction
Peters (1975)	spontaneous squamous carcinoma WHT/Ht mice	□	10–29 days after (90 mm^3)

* 20-methylcholanthrene-induced
○ highly antigenic
□ non or weakly antigenic
▲ allogenic

tumours, as a representative endpoint for extrapolation to clinical results. Survival was used in only three studies (Hall et al., Abe and Taneichi, Peters, Table VI) of which one showed an effect (Hall et al.), in an allogenic tumour system. In the model of Abe and Taneichi tumour cells were inoculated into the lymph nodes and survival was not affected by the occurrence of haematogenous metastases. A reasonable experimental model for human mammary carcinoma should have similar metastasizing characteristics. In fact, preferentially both lymphogenous and haematogenous spread should occur. In mice, however, few tumours show both lymph node and blood borne metastases. While blood borne metastases are frequently observed (Kaae, 1953; Anderson et al., 1974) lymph node metastases, if they occur are usually observed in a very low percentage (Hewitt, 1975; 1976). In these described experiments, some models (Hammond and Rolley, Peters, Sato, Sträuli and Lindemann; Tables IV and V) showed in a very low percentage lymph node metastases in addition to blood borne metastases in the lungs. Usually lymph node metastases occurred at a very late stage of the disease when pulmonary metastases were already established. In man, the earliest clinical evidence of metastases is usually recognized in the axillary lymph nodes. Most regional recurrences appear within two or three years after mastectomy (Spratt, 1967; Devitt, 1971). Therefore a more valid experimental approach would be the use of a syngeneic, non- or weakly antigenic mammary carcinoma which shows both metastasizing characteristics, in which the lymph node metastases tend to occur early in the course of the disease and the haematogenous metastases are the major determinants of survival. Lymph node removal should be done before and at different stages after tumour inoculation to evaluate if there is any effect and if so, if there is a time dependency. In this evaluation special attention should be given to the occurrence of regional lymph node metastases since in the human situation these recurrences can significantly affect quality of life even in the presence of haematogenous spread. Since the hypothesis was forwarded that antigenicity could be an important factor as to whether the regional lymph node possesses special properties in the lymphoreticular system, additional tumours of defined antigenicity should be evaluated in a similar way.

Only on the basis of such an approach valid information can be obtained. If it would be a general observation that regional lymph node removal tends to decrease survival in such an approach, then

Table VI. Type of experiments

References	Tumour challenge	Treatment of R.L.N.	Endpoint	Effect*
Crile (1965, 1966, 1967, 1968, 1976)	tumour plug S180	surgery	tumour incidence after 5.0×10^5 cells	↑
	tumour plug S180	irradiation	tumour incidence after 5.0×10^5 cells	↑
	tumour plug S180	surgery	incidence of pulmonary metastases	↑
	tumour plug S180	irradiation	incidence of pulmonary metastases	↑
	tumour plug T241	surgery	incidence of pulmonary metastases	↑
			tumour incidence after second challenge (number not given)	↑
	tumour plug S1	surgery	incidence of pulmonary metastases	=
	tumour plug hamster melanoma	surgery	incidence of pulmonary metastases	=
Eltringham and Weissman (1970)	antigenic stimulation	irradiation	delayed hypersensitivity reaction	↑
			Hemolysin titer	↑
			hemagglutinin titer	↑
Fisher and Fisher (1971, 1972)	tumour plug (1×0.5 mm) mammary carcinoma	surgery	tumour incidence after 10^4 and 1.5×10^4 cells	↑
	tumour plug (1×0.5 mm) M.C.-sarcoma	surgery	tumour incidence after 6×10^4 and 12×10^4 cells	= =
Hall et al. (1972)	tumour plug 2 mm^3	surgery	survival	↑
Perez et al. (1973, 1974, 1975) Stewart et al. (1976)	tumour plug or 2×10^6 tumour cells	surgery	tumour cure after irradiation	↑
		irradiation	tumour cure after irradiation	↑
Pendergrast et al. (1976)	10^5 tumour cells	surgery	tumour incidence after 10^4 cells	↑
Sato (1964)	tumour cell suspension (number not given)	surgery	number of pulmonary metastases	↓

Continued table VI

References	Tumour challenge	Treatment of R.L.N.	Endpoint	Effect*
Gardner and Rosen (1967)	5 mg M.C.	surgery	tumour incidence	=
	5 mg M.C. (chemical induction of tumours)	surgery	tumour incidence after 5–50 $\times 10^6$ cells	=
	tumour plug Walker 256	surgery	tumour incidence after 10–30 $\times 10^6$ cells	=
Bard et al. (1969)	10^3 tumour cells	surgery	tumour incidence after 750 cells	=
Hammond and Rolley (1970)	10^5 tumour cells	surgery	local recurrence	=
			pulmonary metastases	=
Pilch et al. (1971)	10^3 tumour cells	surgery	growth curve after 750 cells	=
			survival	=
Abe and Taneichi (1972)	10^5 tumour cells	surgery	survival	=
McCredie et al. (1973)	tumour plug mammary carcinoma	surgery	number of pulmonary metastases	=
		irradiation	and lung weights	=
	tumour plug M.C.-sarcoma	surgery	number of pulmonary metastases	=
		irradiation	and lung weights	=
Sträuli and Lindemann (1974)	1 mg M.C. (chemical induction of tumours)	surgery	tumour incidence	=
Peters (1975)	2×10^4 tumour cells	surgery	survival	=

* ↑ removal of R.L.N. has a detrimental effect
= there is no difference whether R.L.N. is removed or not
↓ removal of R.L.N. has a favourable effect

there would be a fundamental basis for less extensive surgery i.e. simple mastectomy, with the hope of better end results. If no such difference is found, the preference for simple versus radical mastectomy should be evaluated from the incidence of regional recurrences.

CHAPTER IV

POST SURGICAL ADJUVANT CHEMOTHERAPY FOR BREAST CANCER

Despite the uncertainty existing concerning the relative merits of a spectrum of operations, the surgeon must make a decision as to how he will surgically manage his patient. Presently there is another decision he has to make: Should his patient be treated by more than surgery alone?

Fisher et al. have clearly shown that, for patients with clinical stage I or II disease, the demonstration of tumour involvement of the nodes in the axilla is associated with a 75 per cent chance of treatment failure. Even when the nodes are histopathologically negative, 25 per cent of patients will be dead of breast cancer in 10 years (Fisher et al., 1975). There is firm evidence that a large number of patients with primary breast cancer who undergo surgery with intent to cure do in fact have occult disseminated disease at that time. Consequently, the removal of the primary tumour, by whatever operation, must be regarded as an incomplete form of treatment in a significant number of patients. Surgery and radiotherapy effectively eliminate the gross primary tumour masses. They are, however, local modalities that remove or kill tumour cells only where they are applied. Foci of disseminated microscopic disease are beyond their reach. The above information suggests that the disease is disseminated at the time of diagnosis in the majority of cases and, in addition to optimal surgical treatment, systemic approaches will be necessary if there is any hope of eradicating the disease.

1. The experimental basis for adjuvant chemotherapy

The experimental basis for adjuvant chemotherapy was already founded in 1957. Shapiro and Fugmann then showed in an experimental mammary carcinoma model that, after 15 days of growth, surgery alone or chemotherapy alone could not cure the animal. If, however, surgery and chemotherapy were employed together, more than half of the animals were cured (Shapiro and Fugmann, 1957).

Later, similar results have been reported in other experimental models (Karrer et al., 1967; Fugmann et al., 1970; Mayo et al., 1972; Skipper, 1974; Bogden et al., 1974; Martin et al., 1975; Griswold, 1975; Burchenal, 1976). Such quantitative experimental therapeutic trials and ancillary studies have given more insight into the use of chemotherapy in general and as an adjunct to surgery. Skipper and Schabel and co-workers have deserved well in this field (Skipper et al., 1964; Skipper, 1968; 1971; 1976; Schabel, 1975a, 1975b; Skipper and Schabel, 1977). The cardinal principles that have been established in these experimental studies are, briefly:

1. In all metastatic solid animal cancers studied, surgery followed by "optimal" chemotherapy has provided more cures than either modality used alone (see references listed above).
2. One or a few viable tumour cells can be lethal; thus, eradication of every viable tumour cell is required for cure by any therapeutic modality (Skipper et al., 1964; Schabel, 1976).
3. In all metastatic cancers, there is a direct relationship between the number of viable tumour cells present at initiation of chemotherapy and the probability of treatment failure (Skipper et al., 1964; Laster et al., 1969; Mayo et al., 1972; Schabel, 1975).
4. A given dose of a given drug will kill the same percentage, not the same number of tumour cells in populations of widely differing sizes so long as the growth fraction is constant and drug-resistant cells do not intervene (Skipper et al., 1964; Wilcox, 1966).
5. Combinations of drugs often are more effective than the single drugs used in the combinations and may eradicate up to 10 to 100 times as many tumour cells at equitoxic dosages (Goldin and Mantel et al., 1957; Shapiro et al., 1957; Martin et al., 1957; Goldin, 1973; Spreafico and Garattini, 1974; Schabel, 1975, 1976).
6. The most effective dose of most drugs alone or in combination is the maximum tolerated dose (Schabel, 1975b; Burchenal, 1976).

2. How can we apply these principles to human breast cancer?

One single cancer cell can establish fatal disease in several rodent tumour models. If this is true for human cancer, and Skipper thinks it is (Skipper, 1976; 1977), then this implies that total cell removal by surgery or kill by additional chemotherapy is required for cure.

One gram of tumour may contain as many as 10^9 tumour cells (Tabel VII). When cancer is first detected at this time, it has already gone through at least two-thirds of its growth, usually about 30 doublings. One billion cancer cells are already present and it is almost certain, since cell shedding increases with age and tumour mass, that tumour cells have shed into lymph nodes and/or peripheral blood by the time of diagnosis (Steel, 1968; De Vita, 1971). If metastases grow and the tumour is not treated, with 10 more doublings, sufficient tumour is present to kill the host (Table VII). If cure is no longer possible, then palliation can be achieved by reducing the number of tumour cells by fractionated drug treatment to prevent symptoms, even though there is no progressive eradication. One can achieve this by reducing the number of tumour cells at initiation of chemotherapy from 10^{10} to 10^8 cells. In most cases, symptoms disappear; further doses of drug treatment, however, often have to be reduced, since severe toxicity to gut and bone marrow occurs.

After surgical removal, when tumour is no longer clinically detectable, (statistically) in many cases late recurrent disease is found. Even with the apparent "complete" removal of tumour, up to 10^9 tumour cells can be scattered throughout the body without recognition. Clearly, as with bacteria, the host must be able to control the growth of small numbers of tumour cells in some cases, since surgery alone can be an effective treatment for apparently localized small tumours. Under the circumstances of a low tumour burden left after surgery, chemotherapy should result in cure for the patient. The prime candidates for use in the combined modality approach are those drugs having the highest degree of activity in advanced disease. A wide range of chemicals with different mechanisms of action can induce a shrinkage of measurable tumour by greater than 50 per cent. The degree of cell kill necessary to shrink a bulky solid tumour mass by greater than 50 per cent is quite large. If this degree of cell kill can be directed against the relatively small tumour burden remaining after surgical excision, perhaps eradication of the last neoplastic cell can be achieved.

Thirteen drugs have shown evidence of activity against human breast cancer and response rates of 35 per cent are not uncommon for single agents (Carter, 1972; 1974; 1976; Wasserman et al., 1975). As found in animal data, combination chemotherapy has an inducing ability superior to single agents in breast cancer.

Table VII. Seemingly reasonable estimates of the body burden of tumour cells at detection (Modified after Skipper, 1968; 1974)

Number of doublings	Approximate number of tumour cells		Weight	If in a single spherical[1] clone or mass: Diameter (mm)		Palpable (depending on site)
0	2^0	1	0.001 μg	0.012	<	no
		10^1	0.01 μg	0.025	<	no
		10^2	0.1 μg	0.06	<	no
10	2^{10}	10^3	1.0 μg	0.12	<	no
		10^4	10 μg	0.25	<	no
		10^5	100 μg	0.6	•	no
20	2^{20}	10^6	1 mg	1.2		no
		10^7	10 mg	2.5		no } (palpable in mouse)[2]
		10^8	100 mg	6		? }
30	2^{30}	10^9	1 gr	12	>	yes } (palpable in man;
		10^{10}	10 gr	25	>	yes } lethal to mouse)
		10^{11}	100 gr	60	>	yes
40	2^{40}	10^{12}	1 kg	120	>	yes (about lethal to man)

[1] the volume of a sphere = $0.5236\ d^3$

[2] subcutaneous solid animal tumours are usually first palpable and measurable in this range if one knows where they were implanted, has experience and is looking for recurrences

Greenspan was one of the first investigators to successfully exploit the potential of combination chemotherapy (Greenspan, 1963; 1966). Shortly afterwards, Cooper presented very impressive response rates in patients with far advanced breast cancer to a five-drug combination (Cooper, 1969). Many others have reported superior results with a combination of different drugs in advanced disease (Ahmann et al., 1975; Brunner et al., 1975; Creech et al., 1975; De Lena et al., 1975; Rubens et al., 1975; Shnider et al., 1975; Smalley et al., 1976; For reviews, see Carter 1972; 1974; 1976; Broder and Tormey, 1974; De Vita et al., 1975). Of course, many problems remain because of the large number of possible drugs and schedule variations. It becomes very difficult to determine which schedule for the individual agents would be optimal in combination (van Putten, 1974; Broder and Carbone, 1976). Also, very little is known of the relative contribution of each agent in combination. This is even more important in those combinations which utilize agents which have overlapping toxicities. One must often reduce dose to avoid additive toxicity, often compromising the therapeutic effect.

Much work still remains to be done in elucidating the optimum drug combination and sequences of drug administration. At this point, no individual combination can be recommended as optimal and the definite value of combinations over sequential use of single agents for palliating advanced disease remains to be established. Despite this, the cell kill potential of combinations as evidenced by remission induction figures appears to be higher and makes this approach highly attractive for use in combined modality regimens.

3. Combined modality therapy

The experimental concepts were being developed when the initial trials in breast cancer were started in the 1960's. The first trials involved short-term perioperative chemotherapy and were based on the notion that surgical radiotherapeutic treatment failures might be due to tumour cells being dislodged into the circulation during operative manipulation of the lesion (Watne et al., 1960). The results of these early trials were not conclusive and have been reviewed by Tormey (1975). We now know that these studies were based on an erroneous concept of tumour cell growth. The kinetics of breast cancer growth indicate that metastatic foci are already present in a

number of patients at the time of diagnosis and surgical removal of the tumour. Destruction of these metastatic tumour cells will require intensive or repetitive courses of treatment for a prolonged time, as may be logically deduced from the low growth fractions and long cell cycle times of breast cancer cells. Despite the incorrect rationale, there was a significant decrease in recurrences and an improvement in survival at 5 and 10 years in patients who were premenopausal and had 4 or more nodes histopathologically positive in the thio-TEPA trial (Fisher, 1972; Fisher et al., 1975). At present, there are two studies that illustrate how adjuvant chemotherapy, when applied for longer periods of time, has significantly delayed recurrences. In these studies, not only the concept of adjuvant therapy was being tested but also the opportunity was presented to answer the question of what activity in advanced disease patients is necessary in the more favourable adjuvant situation. This new generation of prospective randomized adjuvant studies began in the United States in 1972 (Fisher et al., 1975) and in Italy in 1973 (Bonadonna et al., 1976a; 1976b) in histopathologically proven N^+ patients. The trials were identical in terms of patients selection criteria (so that information can be exchanged) but they differed as far as chemotherapy was concerned. L-PAM was selected by the American group because it could be administered orally by a simple intermittent schedule for two consecutive years and produced only moderate toxicity. The Italian group employed the combination of Cyclophosphamide, Methotrexate and 5-Fluorouracil, a more toxic regimen, that was given in 12 cycles after surgery (about 12 months). When comparing the initial results of the two trials, the available data indicate that multiple drug treatment is superior to the single agent in decreasing the total failure rate after radical mastectomy (Fisher et al., 1976; Bonadonna et al., 1976c). In both studies, adjuvant treatment was found to be advantageous, particularly for premenopausal women. Although the absolute recurrence rate is presently unknown, the difference in the relapse rate was particularly evident among patients with four or more positive nodes. Loco-regional recurrences were reduced from 13.3 per cent in controls to 3.3 per cent in CMF treated patients (at about 20 months; Bonadonna et al., 1976c).

Although the initial results of these two trials suggest that intermittent combination chemotherapy given over long periods is potentially of major benefit, we are still ignorant of potential interactions

that could intervene (see the introduction). The results of these and many other adjuvant chemotherapy trials already designed should help define future directions with adjunctive therapy in breast cancer. This may hopefully result in optimal adjuvant chemotherapy. Survival time is generally accepted as the principal measure of the effectiveness of treatment of patients with cancer and five-year survival represents the major yardstick. However, survival for five years after treatment is not equivalent to cure and death from recurrent disease occurs as many as 20 years after diagnosis. Theoretically, to determine the cure rate, the fate of each member of a defined patient population should be followed to his death. In practice, one cannot wait that long. Therefore, in establishing future directions, experimental research will play an important role and may guide the clinician.

If this tendency will be confirmed, it is likely that, after the Golden Age of the radical mastectomy as the optimal treatment for breast cancer, the next years may well go down in the history of Medicine as the Golden Age of adjuvant therapy.

PART II

EXPERIMENTAL STUDIES

CHAPTER I

A NEW METASTASIZING MAMMARY CARCINOMA MODEL IN MICE: MODEL CHARACTERISTICS AND APPLICATIONS

1. Introduction

In the experimental study of the formation of tumour metastases and the influence of surgery, radio- and chemotherapy, it would be desirable to use an animal model which resembles the clinical tumour as closely as possible (*Hilf, 1971*; *Bogden et al., 1974*; Martin et al., 1975). Many of the reported breast cancer models consist of induced tumours which usually display marked antigenicity, in contrast with tumours of spontaneous origin (Prehn and Main, 1957; McCredie et al., 1973; Hewitt et al., 1976). Moreover, tumours which metastasize in a similar way as in man are rarely used.

The main and primary route of spread of mammary carcinoma in man is by way of the regional lymph nodes and the main cause of death is from haematogenous metastases. In mice few tumours show both lymph node and blood borne metastases. While blood borne metastases are frequently observed (Kaae, 1953; Anderson et al., 1974), lymph node metastases, if they occur, are seen only in a very low percentage (Hammond and Rolley, 1970; Hewitt and Blake, 1975; Hewitt et al., 1976). Only a few model systems are known in mice which give lymphatic dissemination in a reproducible pattern and these only rarely produce parenchymatous secondaries. These systems use intratibial (Franchi et al., 1968), intratesticular (van Putten et al., 1975), tail (Sato, 1961), thigh and foot pad inoculation (Tsukagoshi et al., 1973).

A suitable animal model for breast cancer should fulfil a number of criteria:

1. It should be transplantable and suitable for use in a syngeneic tumour host system and reproducible in this sytem.
2. The tumour should arise in the same organ for which it serves as a model and the antigenicity should be known and low.
3. There should be similarities in metastasizing pattern i.e. lymphogenous and haematogenous spread should occur consistently.

Table VIII. Tumour-host systems initially studied

Tumour	Histology	Origin	Mouse strain	Lymph nodes	Lungs
18/76	adenocarcinoma	spontaneous	C57BLxC3H/f	—	+
3520/75	adenocarcinoma	spontaneous	C57BL/Rij	—	+
3641/75	adenocarcinoma	spontaneous	CBA/Rij	(10 per cent)*	+
191/74	adenocarcinoma	spontaneous	C3H/f	—	+
2661/61	adenocarcinoma	spontaneous	CBA/Rij	+	+
30L/57	lymphoma	radiation-induced	C57BL/RijxCBA/Rij	+	—
C22LR/58	osteosarcoma	radiation-induced	C57BL/RijxCBA/Rij	—	+
3LL/51	sarcoma	spontaneous	C57BL/Ka	(10 per cent)*	+
51CoL/76	carcinoma	chemically-induced	BALB/cxDBA/2	+	± (40 per cent)

10 mice per trial

* regional lymph nodes only

4. There should be possible a certain natural staging with regard to tumour spread.
5. The tumour should respond to the same therapeutic modalities as does its counterpart in man.

This chapter describes the characteristics of a selected mammary carcinoma which fulfils these criteria.

2. Materials and methods

Tumours

Several cell suspensions from tumour lines of different histological types were injected subcutaneously into the foot pad of mice. For each trial, 2×10^5 viable tumour cells were inoculated in a volume of 0.02 ml with a Hamilton microsyringe. After local growth to a critical volume, the tumours were removed surgically in order to prevent death from the primary growth. After death from residual disease, all mice were autopsied to record the extent of metastatic disease. Table VIII indicates those tumour-host systems initially studied. Only one of the tumours tested, mammary adenocarcinoma 2661, which originated spontaneously in a CBA/Rij mouse in 1961, gave metastatic growth in popliteal, inguinal, paraaortic and renal lymph nodes as well as lethal haematogenous metastases in the lungs. Therefore, it was selected for further studies. Since a large number of cells of passage 61 had been stored in liquid nitrogen, the majority of the studies could be performed with passages 61–65. Tumour cell suspensions were prepared by the trypsinization method described by Reinhold (1965).

Mice

All mice (inbred strains indicated in Table VIII) were bred by brother-sister mating in the Radiobiological Institute's colonies. For the experiments, nine to ten-week-old CBA/Rij females weighing 20 ± 2 grams were used as the recipients for tumour transplantation. They were fed standard laboratory chow and tap water ad libitum.

Antigenicity studies

Two methods were employed to test the antigenicity of the selected tumour.

A. End-point dilution titration was carried out by a modification

(Kallman et al., 1967) of the method of Hewitt (1953). One further modification was effected; the four injections per mouse were administered subcutaneously on the back of the animal, in the neck, the flanks and over the sacrum, rather than in the axillary and inguinal areas. This was done in order to facilitate differentiation between tumour growth at the inoculation sites and possible lymph node metastases. In order to determine the suitability of the mammary carcinoma, tests of the effect of prior immunization of the recipients were performed. Experiments performed by Woodruff and Dunbar indicated that the proportion of inoculation sites in which tumour develops is not altered whether 10 or one single site are inoculated in the same mouse, so that the 4 sites were evaluated as single observations (Woodruff and Dunbar, 1974).

B. Boone (Boone et al., 1973) provided a rapid intravenous assay technique of tumour immunity in mice as a substitute for the conventional procedure of Hewitt. Many studies have shown that most pulmonary tumour metastases in mice are found near or on the surface of the lungs (Polissor and Shimkin, 1954; Wood et al., 1954; Ketcham et al., 1961; Wexler, 1966). Since the contrast between tumours and lung is adequate for counting after adding microspheres* to the inoculate and after fixation of the lungs in Bouin's fluid, this method was employed instead of microscopically counting of tumour cell colonies from flattened lobes.

Tests for the presence of an oncogenic virus (mammary tumour virus) were done with the quantitative sepharose bead immunofluorescence assay using a polyvalent and a monospecific antiserum and purified mammary tumour virus antigens.

Surgery

Tumour removal was done under general anaesthesia by administering 60 milligrams of sodium pentobarbital per kilogram of body weight intraperitoneally, the line of amputation being between the tumour and the popliteal lymph node. The wound was closed with metal clips, which were removed ten days later.

Adjuvant chemotherapy studies

A combination of Cyclophosphamide, 5-Fluorouracil and Metho-

* Carbonized microspheres from the 3M Company, 15 ± 5 μm in diameter (Hill and Bush, 1969; Steel and Adams 1975).

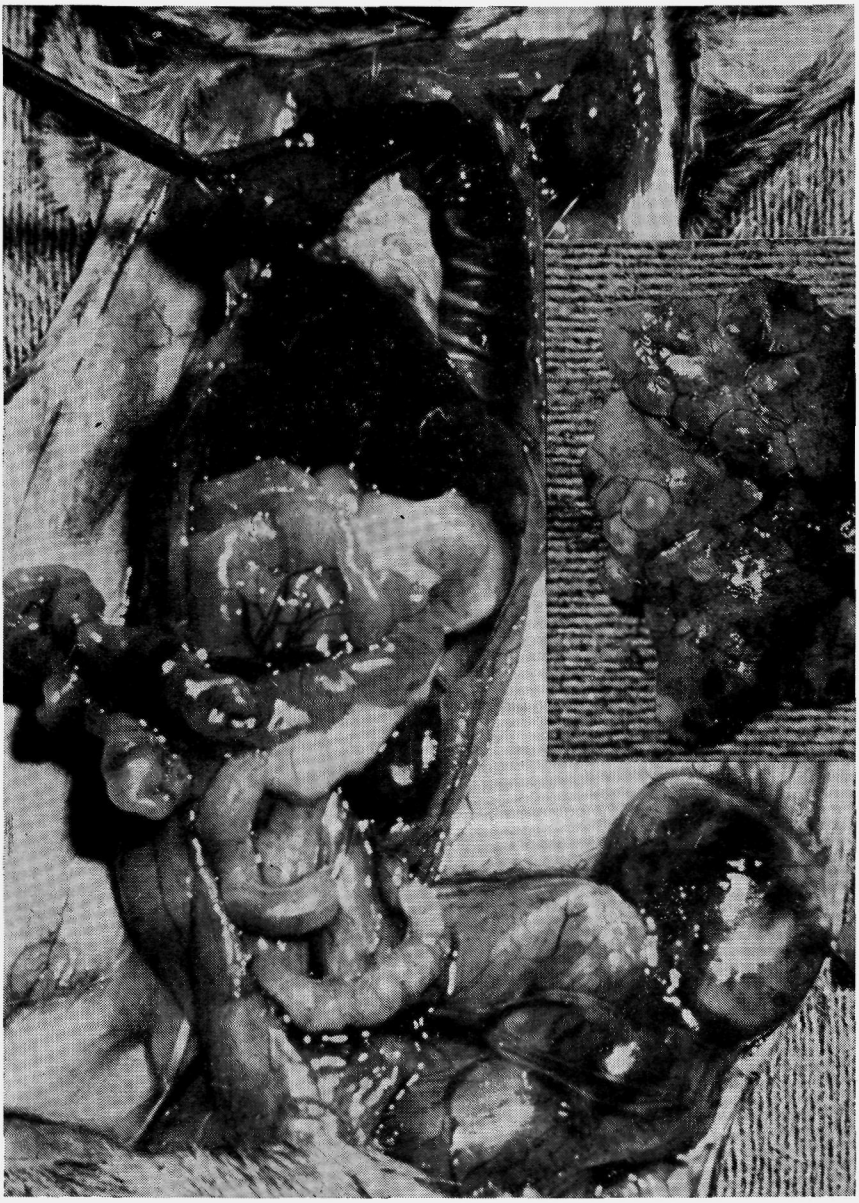


Figure 3. Animal killed 40 days after removal of the primary tumour. Metastases in inguinal and axillary lymph nodes (popliteal and paraaortic lymph node metastases not exposed); as well as in the lungs (see insert).

trexate was used in a dosage used clinically by Bonadonna et al (1976). This schedule was adapted from man to mouse on the basis of equal dose per m^2 body surface per 4 weeks (Freireich et al., 1966). The treatment was divided into four weekly cycles, so that each week the mice received:

Cyclophosphamide	100 mg/m^2 day 1, 2, 3	i.p.
Methotrexate	20 mg/m^2 day 1	s.c.
5-Fluorouracil	300 mg/m^2 day 1	i.v.

Results

All of the tumours tested gave rise to metastatic spread causing death after removal of the primary growth. All, with the exception of one line, gave lung metastases (Table VIII). One mammary adenocarcinoma (3641/75) showed a low percentage of lymph node metastases in addition to lung metastases. Mammary adenocarcinoma 2661 was the only one which gave early lymph node and later lung metastases in 100 per cent of recipients. Both lymph node and lung metastases can be easily detected at autopsy (fig. 3). The typical morphology of the tumour cells allows recognition even at early stages of metastasis formation (figs. 4, 5 and 6).

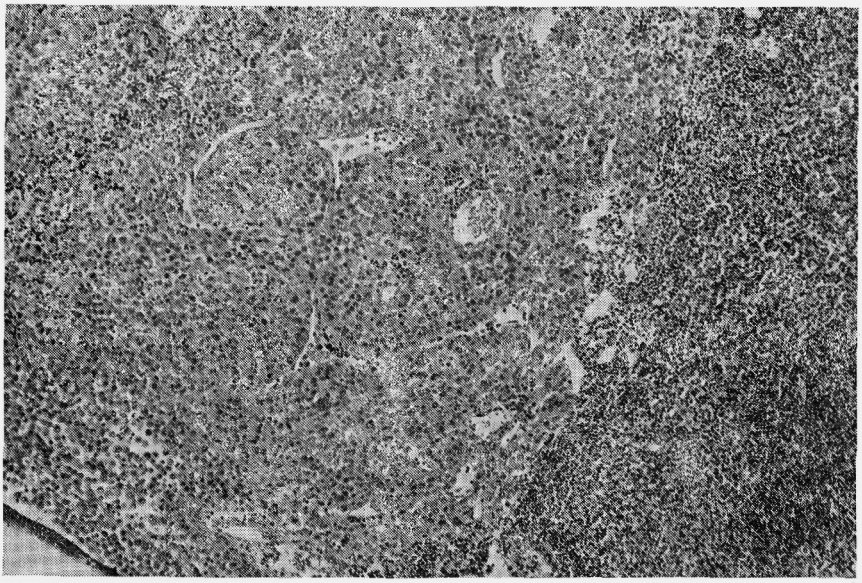


Figure 4. Large metastase in lymph node. Methyl-methacrylate embedding, P.A.S. staining.

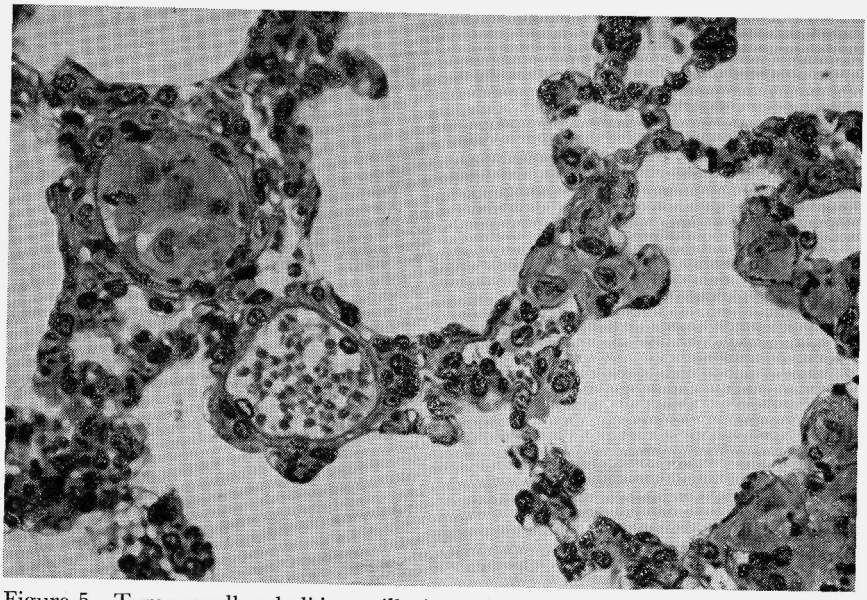


Figure 5. Tumour cell emboli in capillaries and larger vessels. Methyl-methacrylate embedding, methenamine-silver staining.

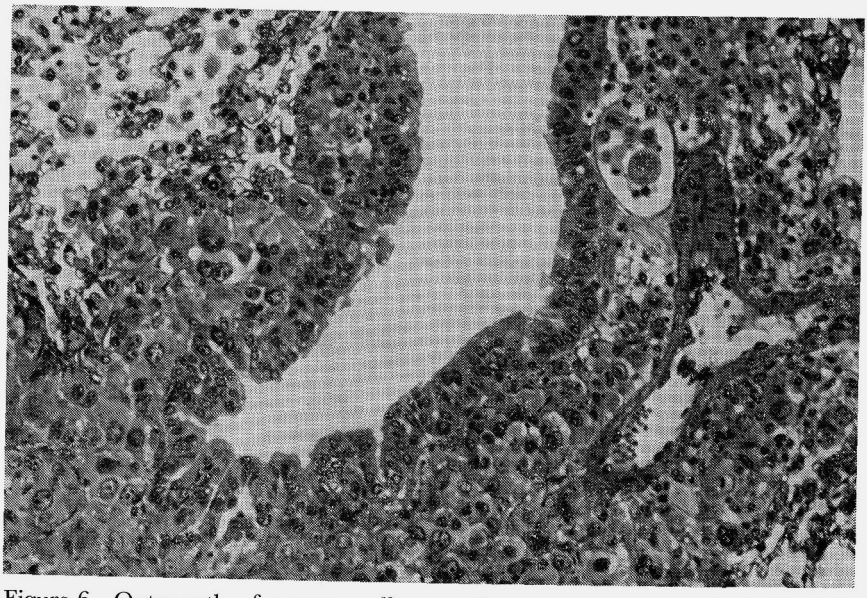


Figure 6. Outgrowth of tumour cells around a bronchus. Methyl-methacrylate embedding, methamine-silver staining.

3. Antigenicity studies of mammary adenocarcinoma 2661

A. *Subcutaneous challenge*

It is well known that antigenic differences between tumour and host may interfere with tumour growth and tumour cell survival. To test the effect of prior immunization, two subcutaneous injections of 10^6 tumour cells which had been irradiated *in vitro* with 10,000 rad of X-rays were given at two-week intervals before injection of graded numbers of viable tumour cells. Titrations were then performed simultaneously in both female and male immunized and nonimmunized control mice. The results are presented in Table IX. According to the probit method (Finney, 1962), two curves were fitted to the two TD_{50} values per sex; the difference was not found to be significant ($p > 0.10$). An analogous analysis by the Spearman-Kärber method gave similar information (Kärber, 1931).

Table IX. Effect of immunization on the TD50*

Controls	Immunized
♀122	67
♂194	239

* Estimated number of cells necessary to obtain a tumour take in 50 per cent of inoculation sites.

B. *Intravenous challenge*

By intravenous injection of different numbers of tumour cells together with 10^6 microspheres into the tail vein, the number of cells giving a significant and countable yield of metastases after 15 days was determined. This is shown in Table X.

Table X. Relationship between the number of intravenously injected tumour cells and number of pulmonary metastases after 15 days

Number of cells*	Average number of lung colonies ±S.E.M.
10^5	21 ± 4.9
2.5×10^5	41.6± 6.42
5×10^5	82.4±19.0
7.5×10^5	innumerable
10^6	innumerable

* 10^6 microspheres were added to each inoculum

Subsequently, 2.5×10^5 tumour cells were used for tumour immunity studies. Two groups of mice were inoculated intravenously at the same time. The first group consisted of mice from which a 10-day-old tumour (10^6 cells in the flank) had been removed three days prior to challenge, following the method of Boone et al. (1973). The average tumour volume was about 300 mm³. The second group consisted of normal mice from which a skin flap, designed to mimic the trauma of tumour excision, had been removed three days prior to tumour challenge. Table XI shows that there is no significant difference in the numbers of lung colonies developing in the two groups.

The sepharose bead immunofluorescence assay showed no antigens of the mouse mammary tumour virus (Brinkhof and Bentvelzen, 1976).

Table XI. Number of lung colonies 15 days after i.v. inoculation of 2.5×10^5 tumour cells into control and immunized mice

	Average number of pulmonary metastases ±S.E.M.
Control (15)	35.5±3.3
Immunized (15)	43.3±4.2

($p > 0.10$) Student's two-sample test

4. Surgery and metastasis formation

Complete cure can be obtained in an observation period of 150 days when amputation is performed before the 10th day, whereas animals die from metastatic disease when amputation is performed after 15 days. In the experiments, the median survival time of mice which die after removal of the primary tumour is about 45 days. However, the spread in survival times in different experiments is great, with several mice surviving over 60 days. This range of survival in a homogeneous system where standard tests did not reveal host resistance indicates the presence of random factors influencing the growth of metastases. Within the range of 10–15 days postoperatively, there is no significant correlation between the volume of the primary growth and the risk of metastases. None the less, mice that had tumours removed when less than 100 mm³, as measured by 2 diameters at right angles and the thickness of the tumour, did not exhibit metastases, whereas mice that had tumours of more than 500 mm³ removed, all died of metastatic disease (fig. 7). It appears therefore that, as with human breast cancer (Mühlbock, 1958; Fisher et al., 1969; Alderson

et al., 1971), the size of the primary growth is a *crude* index of metastatic risk.

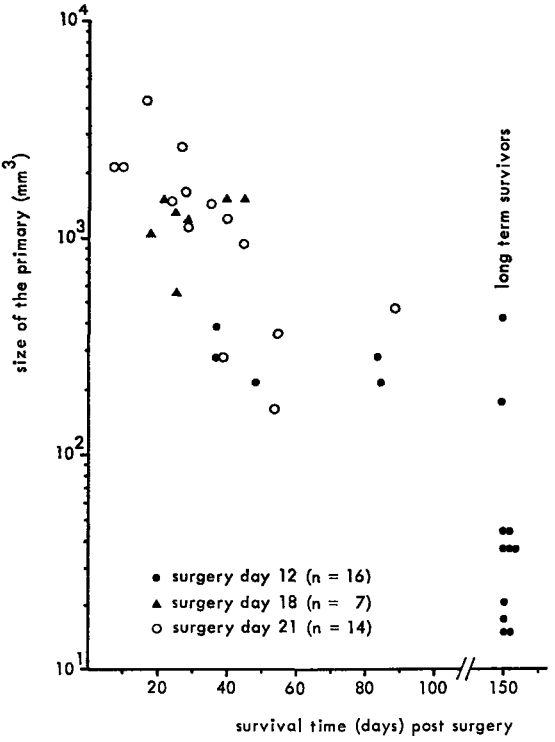


Figure 7. Survival time post surgery as a function of the size of the primary growth.

5. Lymph node metastases

The possibility of direct intralymphatic injection, as often used in lymph node metastases models, was unlikely in view of the absence of tumour spread if amputation was performed before day 10. In addition, it was excluded histologically. Histological sections of the lymph nodes were made immediately after, 1 day after and every other day until 17 days after injection of the tumour cell suspension. Five female mice were used for each group examined; weights of lymph nodes were measured with a Mettler type B5-balance. The weight of the lymph nodes had already increased on the first day after inoculation and

Table XII. Weight in mg (±S.D.) of lymph nodes draining mammary carcinoma 2661

Day	Popliteal	Inguinal	Paraaortic
0 (controls)	1.06±0.09	2.80±0.77	0.52±0.18
1	1.82±0.34	3.04±0.26	0.76±0.11
3	2.84±0.44	3.24±0.80	1.00±0.21
5	3.46±0.46	3.94±0.35	1.46±0.44
7	6.68±1.57	4.64±0.99	2.00±0.90
9	8.04±1.40	4.52±1.05	1.68±0.40
11	13.10±2.15	6.36±1.92	2.62±1.14
13	10.82±1.07	5.38±0.43	3.20±1.31
15	19.96±0.93	9.92±4.75	9.54±3.45
17	25.48±4.03	10.40±0.95	9.76±3.70

continued until tumour cells appeared in the sinus* (Table XII). The first tumour cells appeared in the sinus of the popliteal lymph node at day 13; at day 15, all popliteal lymph nodes contained tumour cells infiltrating and destroying the lymph node; other nonregional lymph nodes also showed the presence of tumour cells at this time. As shown in table XII, the inguinal lymph node plays an active role, as shown by enlargement and subsequent metastasis formation. This finding is surprising, in view of the normal lymph drainage from the foot pad. When Patent Blue Violet (2.5 per cent solution for lymphography) is injected into the foot pad in a manner similar to tumour cell inoculation, the first node to stain is the popliteal (regional) lymph node. Afterwards the paraaortic and renal lymph nodes are stained. Even when the liver is blue, indicating the presence of P.B.V. in the circulation, the inguinal lymph node remains unstained, suggesting that metastasis formation itself, possibly by lymphatic obstruction (as observed by Crile and Schofield, 1969), produces changes in the lymphatic drainage pathways. Accordingly figure 8 gives a schematic representation of the lymphatic drainage system of the foot pad. Figure 9 shows an animal killed 14 days after tumour cell inoculation. Popliteal, inguinal and paraaortic lymph nodes are enlarged.

The nodal status of tumour bearing animals appears to be of interest: all animals have palpable popliteal nodes on day 12. This is mainly due to paracortical hyperplasia and increased germinal center activity (Chapter II). After surgical removal of the tumours on day 12, some

* (see Chapter II).

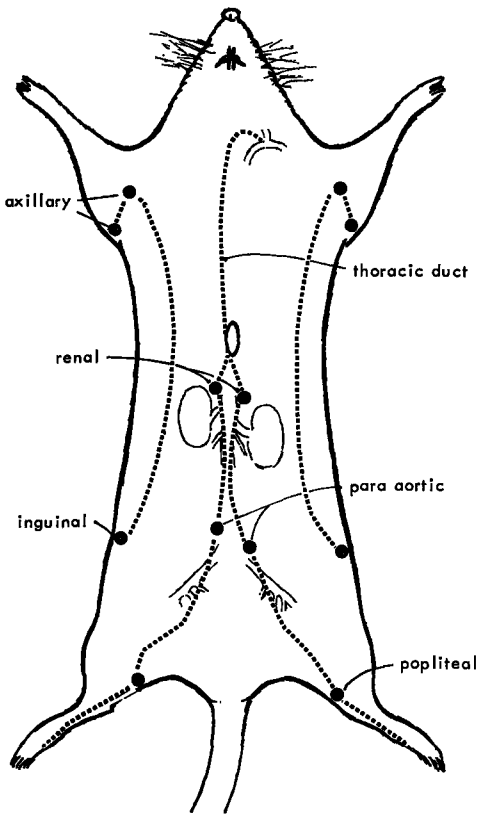


Figure 8. Schematic representation of the lymphatic drainage pathways of the foot pad.

of the nodes regress in volume; others show an increase in volume due to metastatic growth. This phenomenon has also been observed clinically by Edwards et al., after simple mastectomy (Edwards et al., 1972). To investigate the prognostic value of the lymph node status in conjunction with the surgical removal of the tumour, animals were grafted with tumour cells into the foot pad. On day 12 after inoculation, the tumours were removed and, on day 20, all lymph nodes were palpated and the animals were divided into 3 groups as follows:

1. popliteal and inguinal nodes positive
2. popliteal node positive and inguinal node negative
3. popliteal and inguinal nodes negative

(Positive and negative as judged by enlargement on palpation).

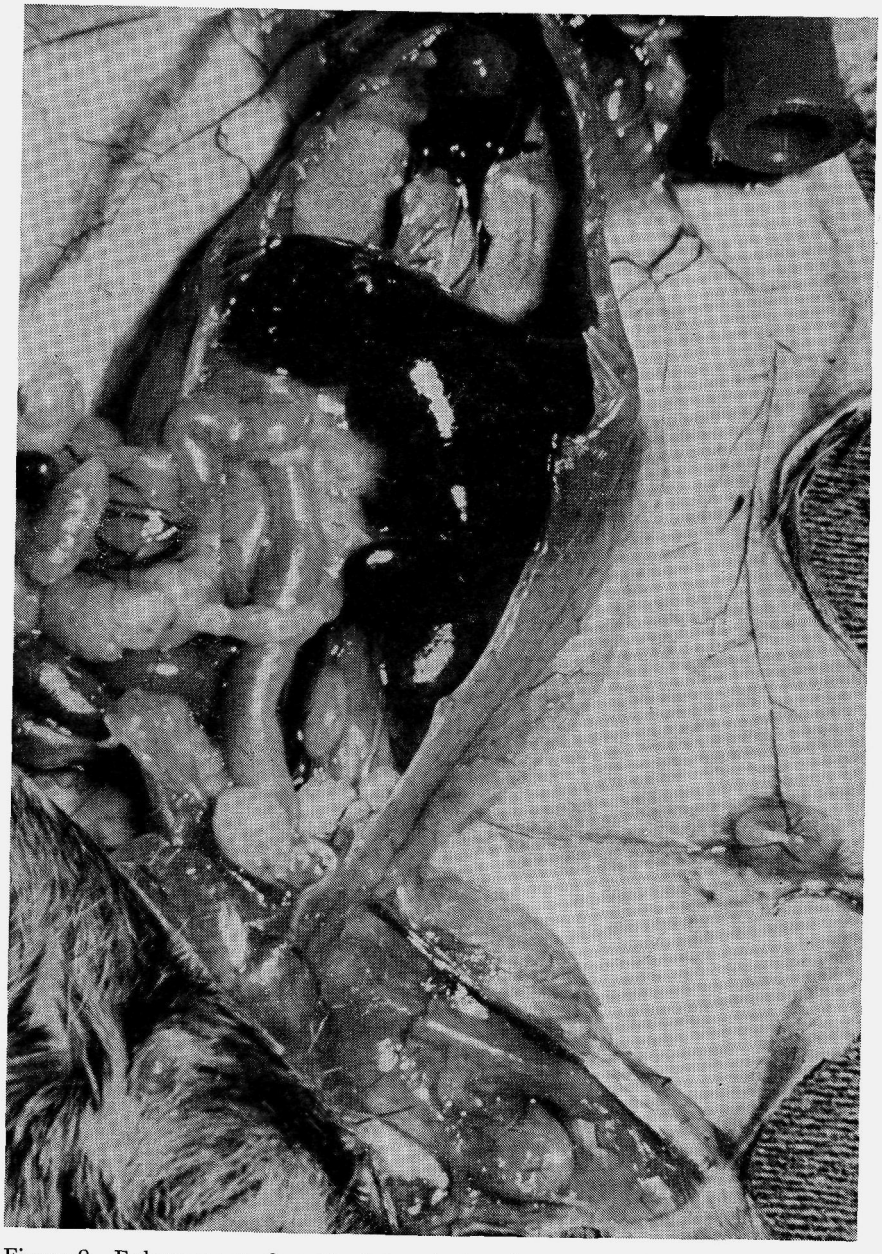


Figure 9. Enlargement of popliteal, inguinal and paraaortic lymph nodes 14 days after tumour cell inoculation.

Figure 10 shows the survival curves of these animals, together with untreated ones, indicating that the nodal status is a reasonable prognostic indicator for survival.

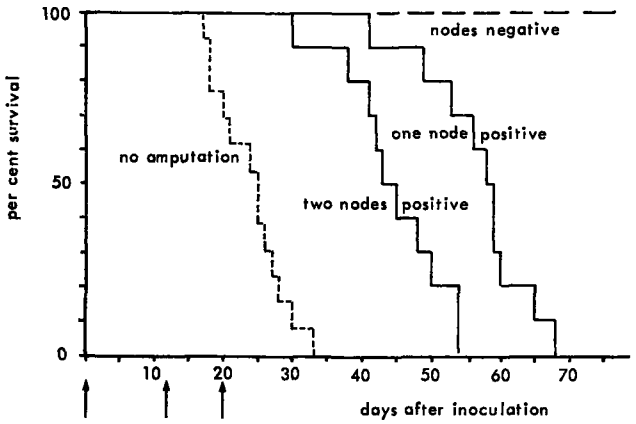


Figure 10. Survival of mice dependent on node status and treatment. Positive and negative as judged by enlargement on palpation.

6. Chemotherapy

Because of recent interest in the Bonadonna trial (Bonadonna et al., 1976), it was decided to test the drug combination used for both established tumours and in the postoperative adjuvant situation. For the former, this was done by utilizing objective response criteria (shrinkage of measurable tumour by greater than 50 per cent; standard definition of objective regression). Flank tumours were used for practical reasons and therapy was started when tumours were well established in the early log phase of measurable growth and was continued for four weeks. The results as shown in figure 11 indicate that this schedule is effective when given as a combination but, if treatment is stopped, all tumours resume growth; no toxic deaths occurred. Only when the dose was doubled mice very soon developed diarrhoea and their stools contained Giardia and Hexamita; after two weeks mice died of severe bone marrow depression.

As adjuvant therapy, this schedule was started 3 days following surgery. Surgical removal took place at 18 days after tumour injection into the foot pad, the average tumour volume being 1280 mm^3. In

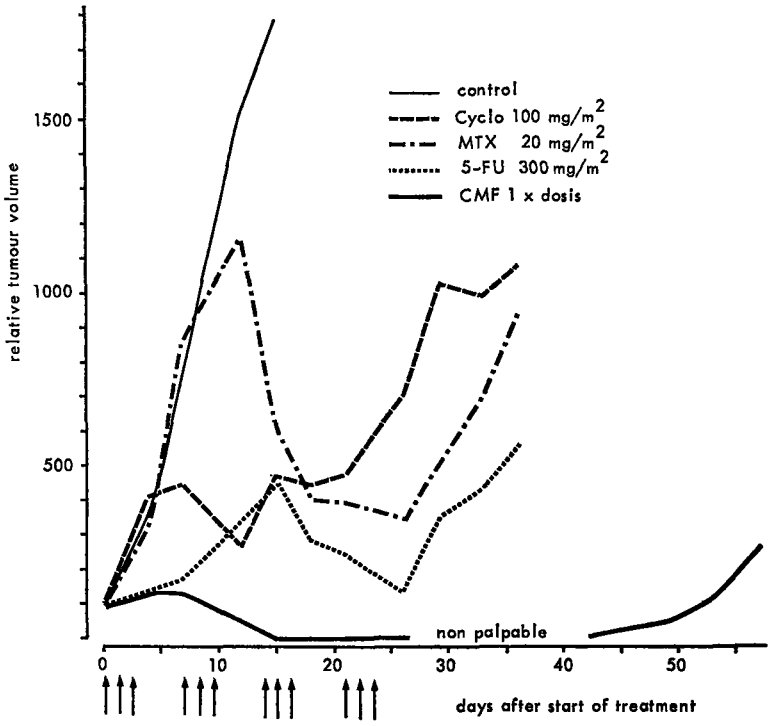


Figure 11. Growth delay of flank tumour by chemotherapy, given as single drugs and in combination.

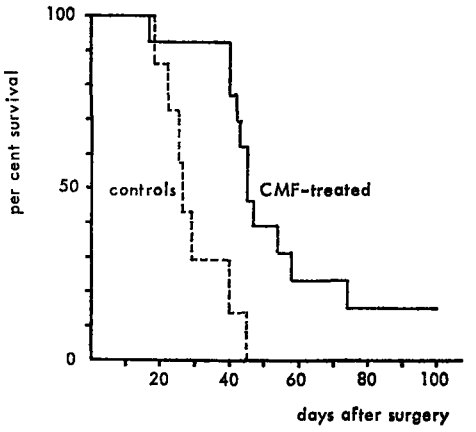


Figure 12. Survival of mice after removal of the primary growth, given adjuvant chemotherapy.

Table XIII. Reduction in lymph node metastases after adjuvant chemotherapy

	per cent positive nodes*	
treated	22.7	n = 11
untreated	100	n = 6

$P < 0.001$, Cochran's test (Cochran, 1954)

* Four nodal areas were examined. The value presented is the per cent total number of nodes positive/total nodes. The lymph nodes examined were the popliteal, inguinal, paraaortic and axillary on the ipsilateral side with respect to the primary tumour.

this stage, some animals had already died of their primaries and lymph node metastases were already established. Figure 12 shows the survival of treated and untreated animals and demonstrates a significant prolongation of survival in the treated group ($p < 0.05$). A striking difference was found in the site of metastases in the treated group. Whereas all untreated mice showed large lymph node metastases in addition to lung metastases at autopsy, a significant reduction in lymph node metastases was seen in the treated group (Table XIII), in contrast to their massive lung metastases. At first I had the impression that the lungs of animals in the treated group showed more heavy metastases than did those from untreated mice dying at the same time. As a result of reports indicating that Cyclophosphamide can give under certain circumstances, an increase in metastases both experimentally

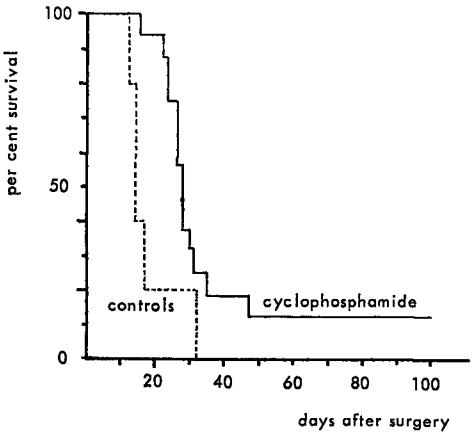


Figure 13. Survival of mice after removal of the primary growth, given cyclophosphamide alone.

(Sugarbaker et al., 1970; van Putten et al., 1975) and clinically (Finney, 1971; Brunner, 1971) it was thought that this drug was responsible for this subjective observation. Cyclophosphamide given alone as adjuvant treatment however also produced in a different experiment, a delay in survival as well as a reduction in percentage of lymph node metastases (fig. 13) to 31 per cent (per 4 nodes).

7. Discussion

The limited current insight into the mechanisms of metastasis formation may be due in part to limitations in experimental models used to approach this complex problem. With better models, a rational basis on which to found attempts at influencing metastasis formation may be investigated. This is especially true for cancers showing two modes of spread. Mammary carcinoma in man is one example: lymph node and subsequent evidence of haematogenous spread of tumour generally occurs. The main finding which emerges from this work is that there is now an experimental model which shows these two modes of spread in 100 per cent of cases. One way in which the model can be used is as a useful tool in testing the effectiveness of different types of therapy in different stages on metastasis formation. However, I would stress that no animal tumour can represent all of the variations in growth potential and metastasizing characteristics that are encountered in humans. However, it is of significance in this system that the two modes of spread are both 100 per cent phenomena, which may be unrealistic but very convenient to evaluate therapy differences. These special properties of metastasis formation have not been met in other useful breast cancer models (Hilf, 1971; Bogden et al., 1974; Martin et al., 1975). The validity of a therapeutic model should, of course, always be confirmed by findings of parallelism in response between the clinical tumour and the experimental model.

Special attention was given to experiments testing whether the tumour is antigenic or not in its host of origin. Several investigators (Vaage, 1973, 1976; Alexander and Eccles, 1975) found that anti-tumour immunity has a higher level of effectiveness in the lungs than in subcutaneous tissue. It is likely that the passage of the former type of inoculum in the blood stream exposes the tumour cells more readily to the hazard of elimination by antibody; in the latter case, cellular immune responses are more likely to be effective. For that reason, an

additional intravenous test according to Boone et al. (1973) was added to the conventional one using subcutaneous challenge (Hewitt, 1953).

Both tests (see Tables IX and XI) showed that no antigenicity could be detected, as one would expect for a syngeneically transplanted tumour of spontaneous origin in a low cancer strain. This is in contrast to mammary tumour mouse strains such as C3H/+ where a vertically transmitted exogenous virus induces the tumour. Spontaneous can be used in the true sense of the word here: no identifiable oncogenic agent which could entail artifactual immunity was involved.

The foot pad location was chosen intentionally: subcutaneous transplants of the mammary carcinoma in the flank of mice can grow to a very large size; yet they metastasize with a low frequency. Inoculation into the foot pad leads to a tumour which metastasizes much earlier. Intramuscular inoculation gives lung metastases and a low percentage of lymphogenous metastases. This can possibly be explained on pure mechanical and anatomical bases. There is an increased pressure in the foot pad rather than in the flank when the tumour starts growing, promoted possibly by movements of the foot (Stoker, 1969). Muscle itself does not contain lymphatics. Lymphatics are present, however, in the fascial planes enclosing and dividing muscles.

The reason why so few rodent tumours metastasize via the lymph nodes is unknown. It is possible that intrinsic properties of the tumour cells themselves help to determine whether they enter local lymphatic vessels or are retained in the draining nodes. Similarly in man, it is unknown why it is common for carcinomas to metastasize to regional lymph nodes while most sarcomas do so infrequently; but differences in fine surface structure of the tumour cells may perhaps be implicated. There is no evidence that sarcoma cells are inherently less capable of invading lymphatic structures.

Whether there is any relationship between a lack of immunogenicity and metastasizing capacity of tumours as stated by Davey et al. (1976) and Kim et al. (Kim, 1970; Kim et al., 1975) is uncertain. This is especially true because metastasizing tumour models are known with chemically induced antigenic tumours (Bogden et al., 1974) from which the model of Carr et al. (Carr and McGinty, 1974; Carr et al., 1974) also utilises foot pad inoculation. Mammary carcinoma 2661 in its present state is an undifferentiated carcinoma. During transplantation passages, most tumours change their histological pattern towards anaplasia; this tumour progression has been reported to

coincide with an increased tendency to form metastases of spontaneous as well as induced tumours (Rudenstam, 1968; Woodruff and Symes, 1969). It is not known if this is the case here, since the test for metastases was done only in its 61th passage; however, another mammary carcinoma (3641/75, Table VIII) was tested in its first transplantation generation and also gave lung metastases in 100 per cent of cases.

The ultimate reason why this particular murine tumour gives rise to lymph node and lung metastases when inoculated via the foot pad remains unknown, as intralymphatic injection was ruled out, but this property makes this model a unique and valuable one. Time, tumour size and lymph node palpation were shown to be reasonable parameters for staging the disease. A late stage of the disease was used in chemotherapy experiments to determine whether cures could be obtained with chemotherapy against the primary tumour. Combinations of drugs have been shown to be more effective than single drugs (e.g. Goldin, 1973; Broder and Tormey, 1974; Rubens et al., 1975; Brunner et al., 1975; see also part I, Chapter IV); for that reason, the Bonadonna schedule was applied (Bonadonna et al., 1976). There were no cures in any experimental group (fig. 11). When flank tumours were used, resumed growth occurred for all tumours after an objective regression, when given as a combination. However, they did not show an increased doubling time in the regrowth phase, in contrast to the findings of Shewell (1976). When the same treatment schedule was used in a late stage adjuvant situation, a striking difference between the sensitivity of lymph node and lung metastases, not seen in other models (Sato, 1961; 1964; Rosso et al., 1970; Tsukagoshi et al., 1973; Bogden et al., 1974), was found. This could possibly be evaluated more fully in experiments using single drugs and other combinations. Unfortunately all experiments devoted to evaluate this have failed due to an infection in the breeding colony, so that these experiments could not be included in this publication. Nevertheless an extensive program has been developed for this model which will be carried out in the coming years.

It is evident that this model, which fulfils the criteria mentioned in the introduction, has essential metastasizing and therapy response characteristics which permit evaluation of therapy combinations and therapy sequences. Although no animal model can completely mimic mammary carcinoma in man, this model seems to be a very attractive experimental one in mice.

CHAPTER II

LYMPH NODES IN THE IMMUNE RESPONSE TO AN ANTIGENIC AND A "NONANTIGENIC" TUMOUR: A MORPHOMETRIC ANALYSIS

* The following abbreviations are used in this chapter: P.C.A. stands for Paracortical area and C.A. stands for Cortical area. TD50 stands for take dose 50 i.e. estimated number of cells necessary to give a tumour take in 50 per cent of inoculation sites.

1. Introduction

Morphologic changes in lymph nodes draining tumours in experimental animals have been studied in considerable detail (Albert et al., 1954; Carter and Gershon, 1966; Rosenau and Moon, 1966; Alexander et al., 1967; Edwards et al., 1971; Fisher et al., 1973; Simar et al., 1975; Nelson and Kearney, 1976). Experimental models are attractive since they permit the making of sequential and adequately controlled observations. Turk and Heather have pointed out that the immune response as induced by the majority of antigens encompasses both a humoral and a specific cellular reaction (Turk and Heather, 1965). The deep cortex of the lymph node, which has been called the paracortical area (Oort and Turk, 1965), has been shown to be a thymus dependent area populated mainly by T lymphocytes (Parrott et al., 1966). This area expands and shows proliferation of large lymphoid cells in the immune response against thymus dependent antigens (Oort and Turk, 1965; Parrott et al., 1969; Parrott and de Sousa, 1969; Weissman et al., 1974). Germinal centers have been shown to be thymus independent regions associated with the production of plasma cells and humoral immune responses (Parrott and de Sousa, 1966; 1971). This localization of cellular and humoral immunological mechanisms within the lymph node allows the lymph nodes' response to a tumour to be more clearly interpreted. A standardized system to correlate immunological function with lymph node morphology has been proposed (Cottier et al., 1972). It was further pointed out that the immunological potential of lymph nodes draining a tumour, removed at operation should provide further information on prognosis

(Editorial, 1973). Whether regional lymph nodes draining a tumour possess properties which make them uniquely different from the rest of the lymphoreticular system is controversial in the literature, especially in so far as preservation or removal of the regional node at an early stage of tumour growth is concerned. Literature on this possible specific role of the regional lymph nodes was discussed in part I, Chapter III.

Morphological responses in regional and nonregional lymph nodes draining experimental tumours of known and different antigenicity have never been investigated quantitatively. An accurate quantification of response can be obtained, however, from histological sections by morphometric analysis of the paracortical and cortical areas as major parts of the active lymph nodes. The purpose of the present study was to analyse morphometrically the structural modifications occurring in the regional and nonregional nodes of mice grafted with syngeneic tumours. Attention was focussed on two main points:

1. Is there a difference in the morphological aspects of the immune response evoked by an antigenic and a nonantigenic syngeneic tumour?
2. Is there a "staging" in the sense of a serial rather than a simultaneous response in the succeeding nodes of the lymphatic drainage system?

2. Materials and methods

Tumours and mice

Mammary adenocarcinoma 2661 originated spontaneously in a CBA/Rij mouse in 1961 and has since been transplanted serially in the flanks of inbred mice of this strain and stored in liquid nitrogen. After inoculation into the foot pad it gives rise to metastatic growth in popliteal, inguinal, paraaortic and renal lymph nodes as well as in the lungs. For a detailed description of the tumour and its applications see Chapter I.

The Lewis Lung carcinoma arose spontaneously in the lung of a C57BL mouse in 1951 (Sugiura and Stock, 1955) and was obtained from Prof. S. Garattini of the "Mario Negri" Institute, Milan, Italy in 1973. It was transplanted into C57BL/Ka mice of the Radiobiological Institute's colony. After foot pad inoculation, it always metastasizes to the lungs but only rarely to lymph nodes.

Tumour cell suspensions were prepared by a modification of the method described by Reinhold (1965): fetal calf serum was not added to the inoculate. For both lymph node morphometry experiments utilizing the mammary carcinoma and the Lewis Lung carcinoma, 2×10^5 viable tumour cells were inoculated into the foot pad in a volume of 0.02 ml with a Hamilton microsyringe. Ten week-old CBA/Rij and C57BL/Ka female mice were the recipients of the two tumour lines described.

Antigenicity tests

An intravenous assay technique according to a modification (see Chapter I) of the method of Boone et al. (1973) was applied to both tumour systems.

Lymph nodes

Popliteal, inguinal and paraaortic lymph nodes were removed from

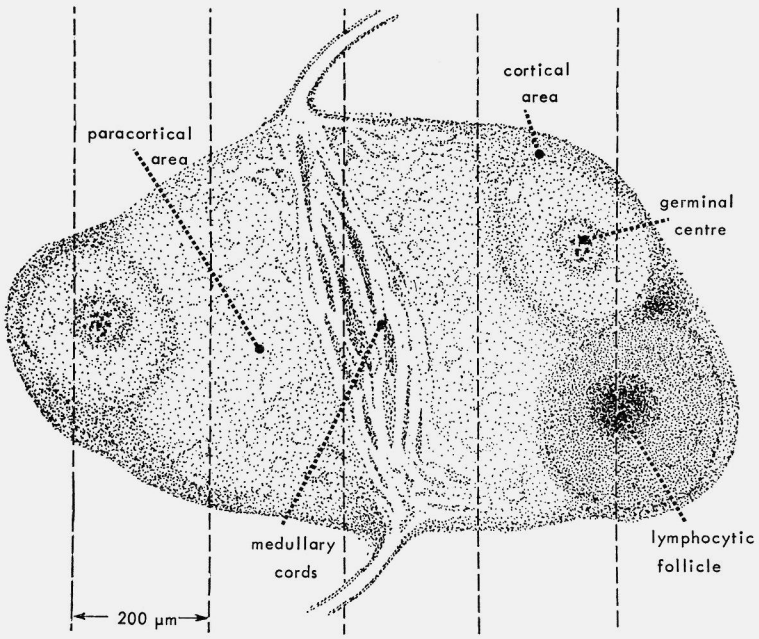


Figure 14. The principle of the estimation of the relationship between the volume of the paracortical area; the cortical area containing the lymph follicles, and the volume of the whole lymph node by measurement of their areas in a systematic series of semiserial sections.

5 animals before, 1 day after and on every other day until 17 days after injection of the tumour cell suspension. The lymph nodes were fixed in buffered 4 per cent formol and after removal of fatty tissues weights of lymph nodes were determined on a Mettler type B5 balance.

The lymph nodes were then embedded in paraffin and cut into sections at a thickness of 4 μm. Every fiftieth section was retained to obtain semiserial morphology at distances of 200 μm. Hematoxylin-Eosin (HE) staining was used for morphometric analysis.

The principle of the estimation of the relation between the volume of the paracortical area (P.C.A.), the cortical area (C.A.) i.e. the lymph follicles and the volume of the whole lymph nodes by measuring their areas cut in semiseries (fig. 14) was carried out according to the following formulas:
Volume P.C.A.: volume C.A.: volume whole lymph node = summated surface areas P.C.A.: summated surface areas C.A.: summated surface areas whole lymph nodes.

When different lymph nodes have different sizes during the immune response, the distance between the sections remains the same (= 200 μm) only the number of serial sections increases. So for P.C.A.'s: Volume P.C.A. lymph node 1: volume P.C.A. lymph node 2 = summated surface areas 1: summated surface areas 2.

In summary:

$$\frac{\text{summated surface areas P.C.A.'s}}{\text{summated surface areas whole lymph nodes}} =$$

$$\frac{\text{volume P.C.A.}}{\text{volume lymph node}} \times 100 = \text{per cent P.C.A.}$$

A similar principle was used for the estimation of the cortical area.

Morphometric analysis

Morphometric determination of the cross sectional area of entire lymph nodes, lymph node follicles and paracortical areas was carried out with a computerized semi-automatic system for direct morphometry on microscopic images, similar to that described by Cowan and Wann (1974). This system consists of a microscope with drawing tubus and a graphic tablet interfaced to a small laboratory computer (PDP12, Digital Equipment Corporation, Maynard, Mass., USA). The graphic tablet is used for digitizing contour coordinates of figures

drawn on the tablet with a capacitive probe. In order to facilitate the direct tracing of contours in microscopic images the probe is used to trace the microscopic image projected via the drawing tubus. This is facilitated by mounting a light emitting diode in the center of the sensor which is visible as a small red spot against a dark background. In this way, contours of objects in the microscopic image can be easily traced manually under eye control. The digitized contours are fed into the computer which calculates the area and perimeter. The size of the contour on the tablet can be adjusted to a convenient format by using appropriate projection optics in the drawing tubus. The magnification is calibrated with a stage micrometer and used as a correction factor in the computer program. The graphic tablet has a resolution of 0.1 mm. A more detailed description of the system and its potentialities is in preparation (Cornelisse et al.).

Statistics

All information on the lymph nodes was fed into the computer. For each day of observation and each of the three lymph nodes locations (popliteal, inguinal and paraaortic), mean and standard deviation were calculated of the following parameters: weight, surface area, surface area of paracortical and of cortical area and percentage surface of P.C.A. and C.A. The course in time for each parameter with its standard deviation was then plotted graphically by the computer. Statistical analysis was used to determine the correlation of weights and surface areas. A variance analysis was done to evaluate differences between the three lymph nodes per mouse for each tumour system. The analysis of variance was carried out in the manner described by Scheffé (1964). The experiment was regarded as a three factor experiment, with the factors "mice" as random factor and "lymph nodes" and "time" as fixed factors. (Factor mice nested within factor time, factor node crossed with mice and time). This was mainly done in order to evaluate the significance or insignificance of interaction between "time" and "lymph nodes".

3. Results: Antigenicity tests

In the previous chapter (I), the antigenicity of mammary carcinoma 2661 was determined following a subcutaneous and an intravenous assay technique. Neither of these systems showed an effect of prior

immunization of the recipients for this mammary tumour virus negative tumour. The latter method was used for determining the antigenicity of the Lewis Lung carcinoma in C57BL/Ka mice. The technique of this method has been described in detail previously (Chapter I). Table XIV shows the numbers of lung colonies in immunized and control animals. The difference is highly significant ($p < 0.001$). A similar effect of immunization was found utilizing subcutaneous challenge on the TD_{50} indicating that this tumour is antigenic in its host (Mulder, 1976).

Table XIV. Number of lung colonies 15 days after i.v. inoculation of 5×10^5 Lewis Lung tumour cells into control and immunized mice

	Average number of pulmonary metastases $\pm$S.E.M.
Control (10)	44.4 ± 4.77
Immunized (15)	8.7 ± 1.70

($P < 0.001$) Student's two-sample test.

Immunization was accomplished by inoculating 10^6 tumour cells into the flank and removal of a 10-day-old tumour three days prior to challenge.

4. Results of the morphometric analysis

A. *Correlation between weights and summated surface areas of whole lymph nodes in both systems*

Measuring the weights of the lymph nodes showed that, in both tumour systems, the weight of all three lymph nodes, popliteal, inguinal and paraaortic, respectively, already was raised on the first day after inoculation. This increase continued until day 17. For the 2661 mammary carcinoma system this is shown in figure 15. For the Lewis Lung system this is shown in figure 16.

The validity and reproducibility of the measured results on absolute and relative surface areas in the lymph nodes could be evaluated only from the degree of correlation existing between weight and summated surface areas of the lymph node cut in semi series. Figure 17 shows the visual correlation of these two parameters during the development of the response for the mammary carcinoma. As shown there is a good correlation until day 15 and day 17. At that time, lymph node weight increases, whereas total surface area decreases. This is due to ingrowth and destruction of all three lymph nodes by metastatic tumour.

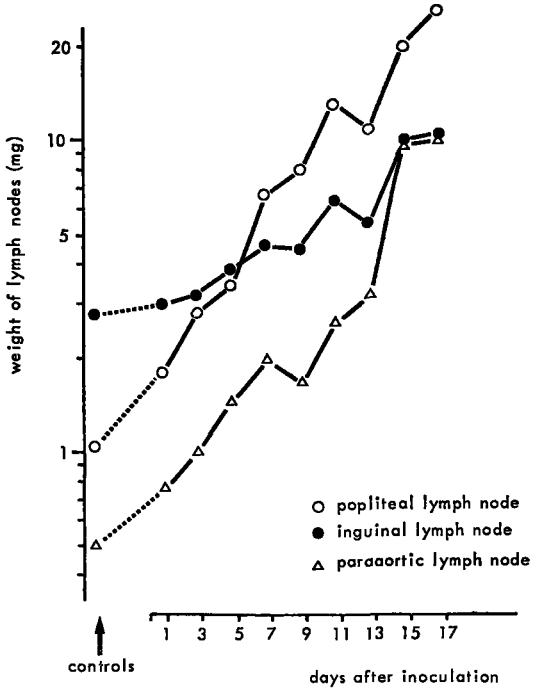


Figure 15. Weights of lymph nodes during the growth of the 2661 mammary carcinoma.

Tumour area was never included in the morphometric analysis and for this reason massive tumour growth in the nodes is expressed by weight increase, not associated with surface increase. Since all lymph nodes obviously had to be weighed before histological analysis, there is no longer a good correlation between lymphatic tissue surface area and the weight of the lymph node after its capsule is infiltrated by growing tumour. Consequently for the results of the morphometric analysis, days 15 and 17 were omitted for the mammary carcinoma system. Correlation coefficients were calculated for the data obtained up to day 13. Table XV shows the results for both the antigenic and the nonantigenic tumour. For both systems, a strong correlation exists between summated surface areas and lymph node weights, suggesting validity of the method.

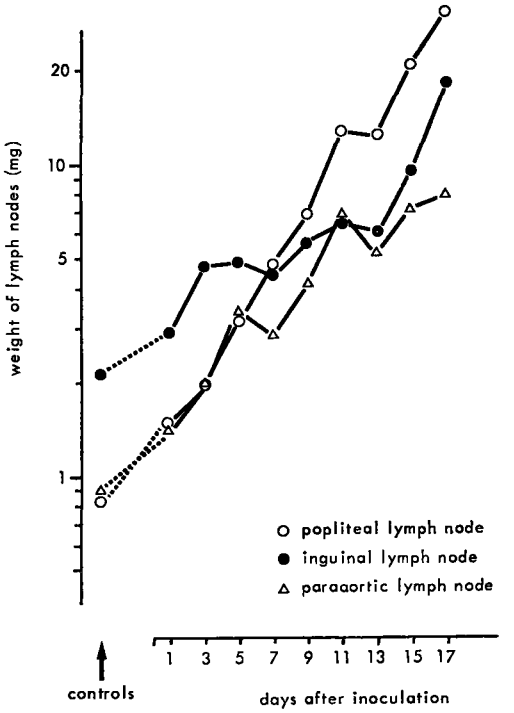


Figure 16. Weights of lymph nodes during the growth of the Lewis Lung carcinoma.

B. *Morphometric analysis of the paracortical areas of the lymph nodes draining the mammary adenocarcinoma and the Lewis Lung carcinoma*

Figure 18 shows the mean percentages P.C.A. of the popliteal, inguinal and paraaortic lymph nodes in the mammary carcinoma 2661 system. There is an initial increase in the percentages P.C.A. in all three lymph nodes reaching a maximum by day 3 and followed by a decrease with time. This phenomenon is observed in all three subsequent lymph nodes with only minor differences in percentages. However, during the relative decrease, the absolute surface area of the P.C.A. still increases, especially in the regional popliteal lymph node as shown in figure 19. Remarkable is the finding that even when the percentage of the paracortical area has returned to control values, the absolute size is still increasing. This absolute increase in paracortical surface area was also found in the antigenic tumour. The relative increase and subsequent decrease in percentages of para-

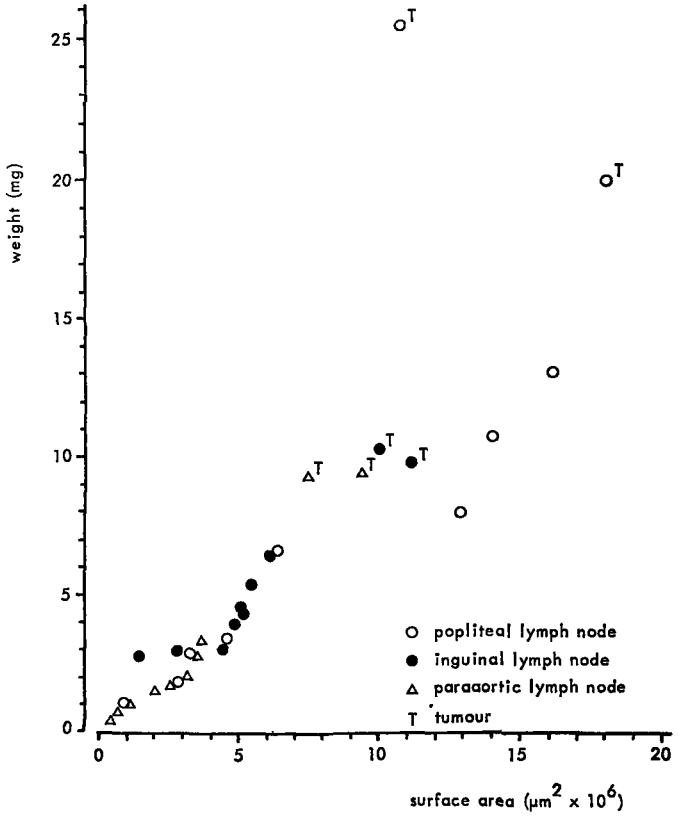


Figure 17. 2661 Mammary carcinoma: visual correlation between mean weight and mean surface area of lymph nodes per day. T near a symbol indicate lymph nodes containing tumour on days 15 and 17 after inoculation.

Table XV. Correlation coefficients between mean weight and mean summated surface areas of lymph nodes draining the antigenic and the non-antigenic tumour

	Tumour	
Lymph node	Lewis Lung	Mammary 2661
popliteal	0.999	0.935
inguinal	0.978	0.839
paraaortic	0.955	0.995

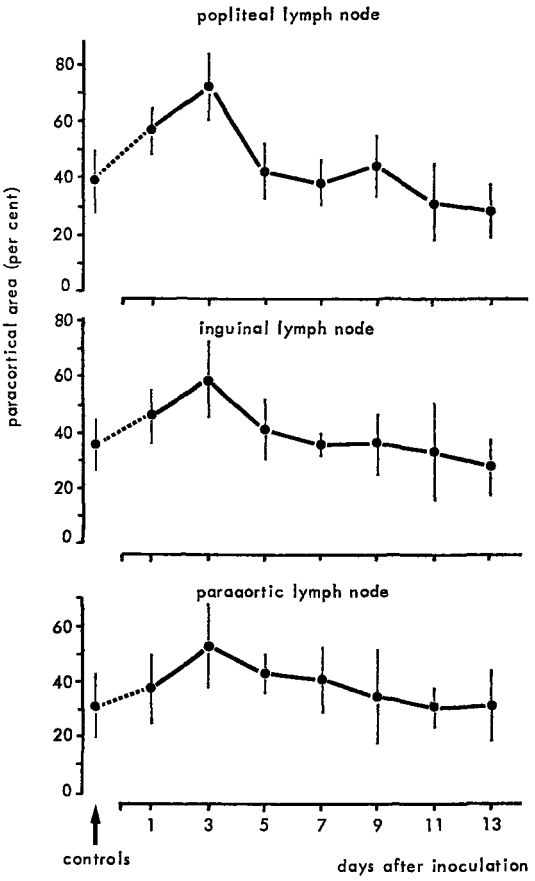


Figure 18. Calculated mean percentages of paracortical areas during the growth of the 2661 mammary carcinoma.

cortical areas was, however, not as clear as found in the nonantigenic tumour (fig. 20). Both systems (figs. 19 and 21), show, however, that the immune response of the P.C.A.'s in absolute numbers is the strongest in the regional (= popliteal) lymph node; they differ significantly between the two tumour systems. Percentages of P.C.A.'s of the whole lymph nodes are not essentially different from one node to another, nor was it observed that there was a staging between the subsequent nodes in the response developing.

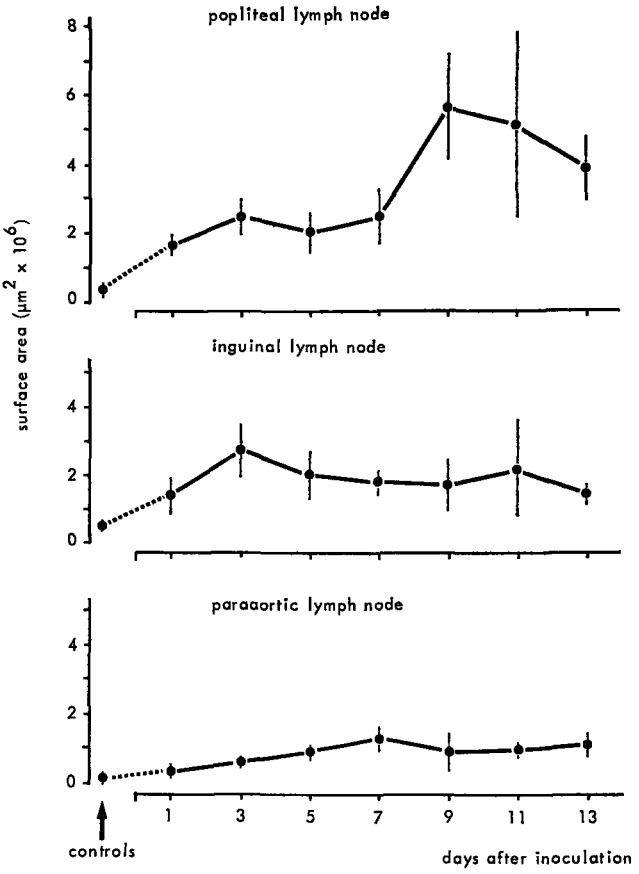


Figure 19. Mean of summated surface areas of paracortical areas during the growth of the 2661 mammary carcinoma in absolute figures.

C. *Morphometric analysis of the cortical areas of the lymph nodes draining the mammary adenocarcinoma and the Lewis Lung carcinoma*

Figure 22 shows the mean percentages of cortical areas of the popliteal, inguinal and paraaortic lymph nodes in the mammary carcinoma system. The maximum response is also found simultaneously in all three lymph nodes at the time when the percentage P.C.A. has decreased to normal or subnormal values (fig. 18). This maximum response coincides with the maximum number of germinal centres and follicles per section as shown in figure 23. The latter values were counted visually from every section. In the antigenic tumour, the

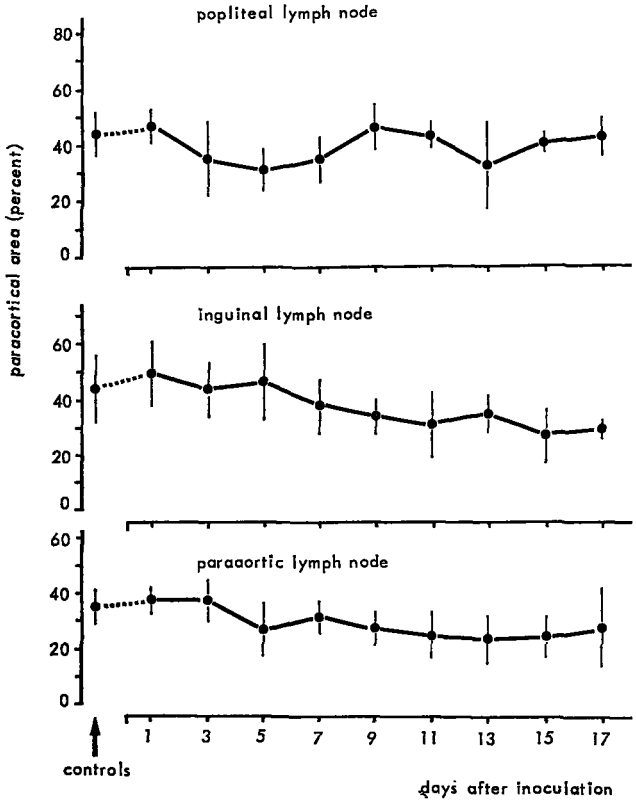


Figure 20. Calculated mean percentages of paracortical areas during the growth of the Lewis Lung carcinoma.

increase in percentage of cortical area was proceeding more slowly until day 13 (figs. 24 and 25). Again, no staging in percentages of cortical response in the nodes of either system was observed; in absolute numbers the response was the strongest in the regional lymph nodes (figs. 26 and 27).

D. *Results of the analysis of variance*

The analysis of variance was performed for each of the following variables: weight of lymph node, surface area of lymph node, surface area of P.C.A., surface area of C.A., P.C.A. as percentage of total surface area and percentage C.A. For each variable the following interpretation was applied: A significant interaction of time and lymph

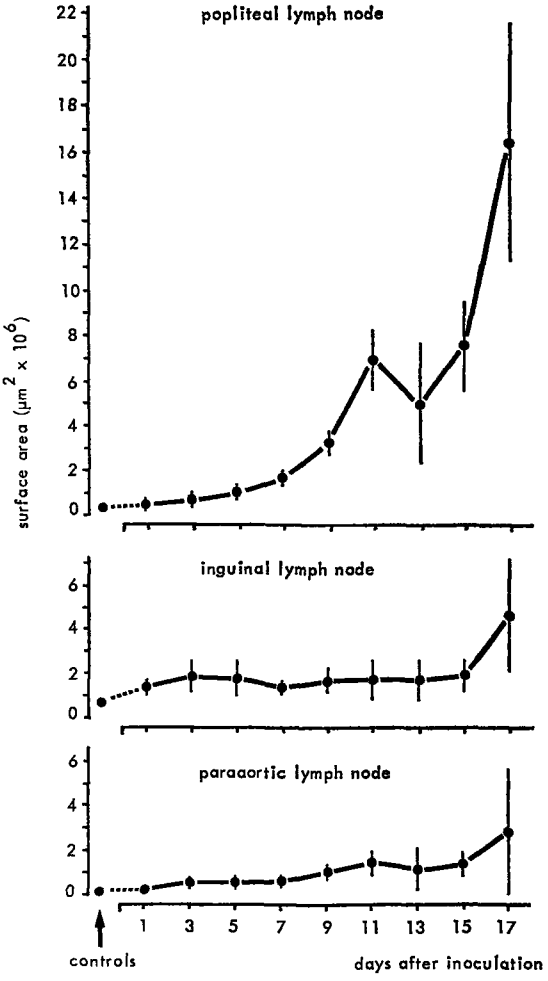


Figure 21. Mean of summated surface areas of paracortical areas during the growth of the Lewis Lung carcinoma in absolute figures.

nodes indicates that the course in time is different for the three subsequent lymph nodes, i.e.: popliteal, inguinal and paraaortic lymph node. A significant value for lymph node indicates differences in mean over time for the three subsequent lymph nodes. A significant value for time indicates that the mean of the three lymph nodes changes with time.

Table XVI shows the results of this analysis. As shown for the mam-

Table XVI. Results of the analysis of variance

Mammary carcinoma	F-values		
Variable	Time[1]	lymph node	time × lymph node
lymph node weight	45.13**	136.17**	15.64**
surface area whole lymph node	57.27**	80.39**	16.78**
surface area P.C.A.	28.30**	61.63**	11.67**
surface area C.A.	20.56**	26.64**	5.48**
percentage P.C.A.	9.16**	5.35**	1.05 ·
percentage C.A.	10.68**	8.44**	0.81 ·
Lewis Lung carcinoma			
lymph node weight	70.96**	53.48**	8.74**
surface area whole lymph node	55.17**	28.80**	6.88**
surface area P.C.A.	34.24**	34.43**	9.06**
surface area C.A.	18.69**	21.08**	2.06 ·
percentage P.C.A.	3.57*	8.18**	1.59 ·
percentage C.A.	9.32**	7.53**	2.22*

[1] up to day 13
* significant at 5 per cent level
** significant at 1 per cent level
· not significant at 5 per cent level

mary carcinoma, there is no significant interaction of time and lymph nodes in percentages P.C.A. and C.A., whereas time and lymph node values all are significant. This indicates that both the absolute and relative areas of P.C.A. and C.A. have their own identity for node, point in time and tumour system. However, the course in time of the changes in percentages P.C.A. and C.A. is similar in all three lymph nodes. It appears, in spite of the slight interaction in percentage C.A. for the Lewis Lung system (5 per cent), that the same is true in this essentially different system. This suggests a generalized response of thymus-dependent and independent immune systems as a reaction to the growing tumours.

5. Discussion

Two different tumour systems of spontaneous origin were used in the present study to evaluate differences in the response in the lymph nodes. Following subcutaneous and intravenous immunity challenge tests, the mammary carcinoma was found to be non-antigenic and

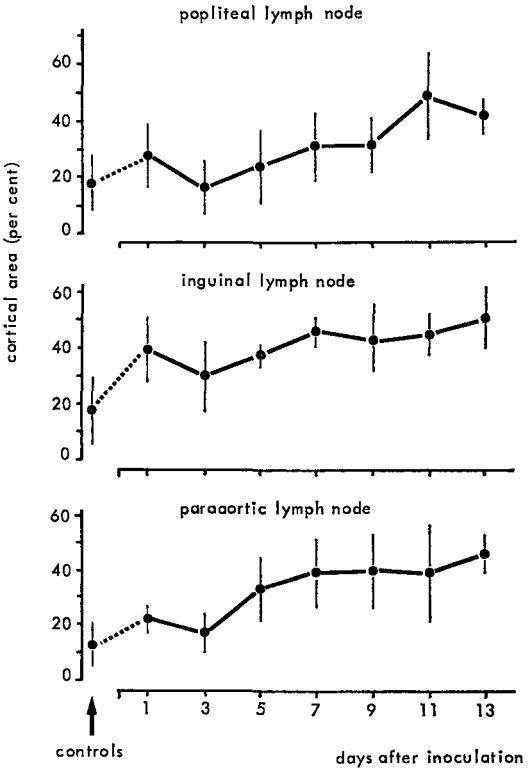


Figure 22. Calculated mean percentages of cortical areas during the growth of the 2661 mammary carcinoma.

the Lewis Lung carcinoma was found to be antigenic. Spontaneous regressions in early transplantation generations of this latter tumour are described in the literature (Sugiura and Stock, 1955) and the identification of the tumour as antigenic is in agreement with immunization results of Carnaud et al. (1974). Others, however, (Karrer et al., 1967; Steel and Adams, 1975; Treves et al., 1976) did not find this particular tumour to be antigenic, possibly due to differences in sublines of the tumour, differences in the animals or different immunity challenge tests. The characteristics of the two tumours are different in another way as well: The mammary carcinoma metastasizes through the lymph nodes and lungs, whereas the Lewis Lung carcinoma always metastasizes to the lungs but rarely to lymph nodes. When inoculated into the foot pad both tumours evoked res-

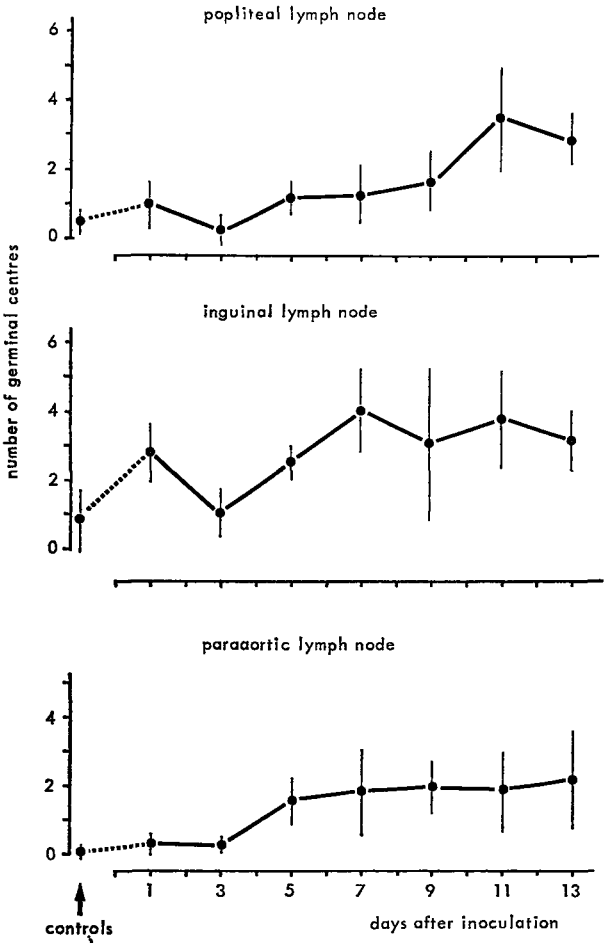


Figure 23. Mean number of germinal centres per section of the lymph nodes in the mammary carcinoma model.

ponses in regional and nonregional lymph nodes so that the weights of the lymph nodes were already increased on the first day after inoculation continuing until the end of the observation period. The weights of the lymph nodes correlated visually and statistically with the calculated summated surface of the lymph node sections; this allowed us to make calculations of percentages of P.C.A. and C.A. within the lymph node. The increase in accuracy of this method of morphometric quantification over visual estimation of the sizes of

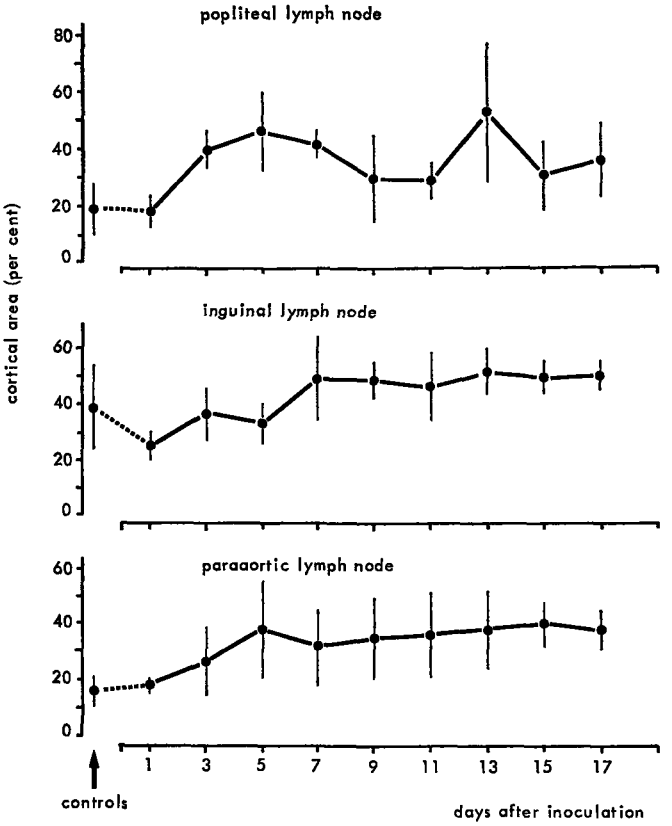


Figure 24. Calculated mean percentages of cortical areas during the growth of the Lewis Lung carcinoma.

different parts within the lymph nodes is evident and this opens many opportunities for the application of statistics. The reaction patterns of the percentages P.C.A. in the nonantigenic mammary carcinoma system showed an early increase and subsequent decrease in time. This percentual decrease in P.C.A. coincided with an increase in percentage C.A. and in numbers of germinal centres. A similar pattern was observed for the antigenic Lewis Lung carcinoma. These similar patterns indicate that antigenicity of the tumours is not an essential factor in producing different morphological reactions in draining lymph nodes and despite the quantification of the morphological findings these results are in agreement with lymph node reaction patterns described for various experimental tumours in the literature

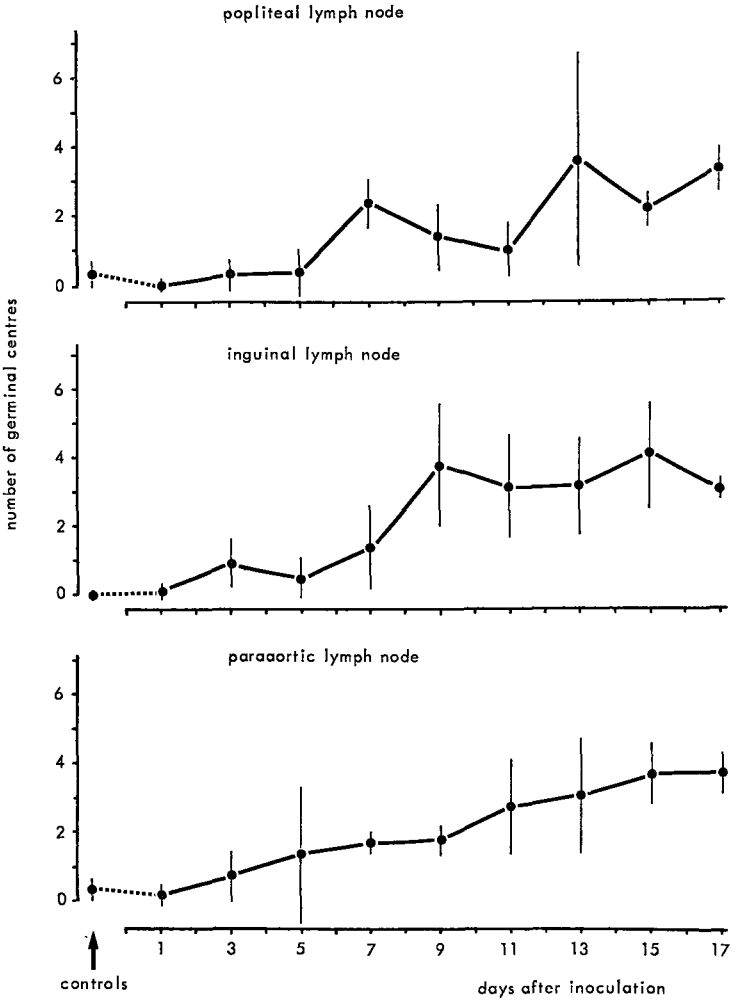


Figure 25. Mean number of germinal centres per section of the lymph nodes in the Lewis Lung model.

(Albert et al., 1954; Carter and Gershon, 1966; Rosenau and Moon, 1966; Alexander et al., 1967; Edwards et al., 1971; Fisher et al., 1973; Simar et al., 1975; Nelson and Kearney, 1976) in which sizes of different parts in the lymph node were estimated visually and compared with those of normal lymph nodes.

In clinical studies evaluating the prognostic value of reactive changes

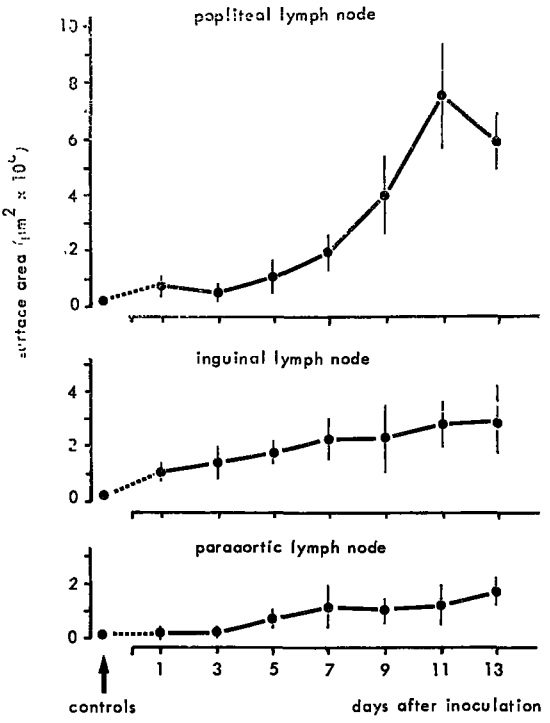


Figure 26. Mean of summated surface areas of cortical areas during the growth of the 2661 mammary carcinoma in absolute figures.

in lymph nodes of mammary carcinoma (Tsakraklides et al., 1974) and carcinoma of head and neck (Berlinger et al., 1976), the hypothesis was advanced that unstimulated lymph nodes may result from nonantigenic or weakly antigenic tumours. In the present study, the reaction patterns of the two tumours differing in antigenicity were essentially similar, suggesting that this conclusion is not justified. In the clinical study on the morphology of axillary lymph nodes in cancer of the breast, "lymphocyte predominance" with an increase in the P.C.A. without germinal centres was usually associated with a good prognosis, whereas the prognosis of "germinal centre predominance" was found to be poorer (Tsakraklides et al., 1974). This latter phenomenon was in agreement with findings of others (Hunter et al., 1975). In the present study, an "early" response consisted mainly of an increase in percentage P.C.A., whereas a "late" response was associated with an increase in C.A. This "early" response of P.C.A.

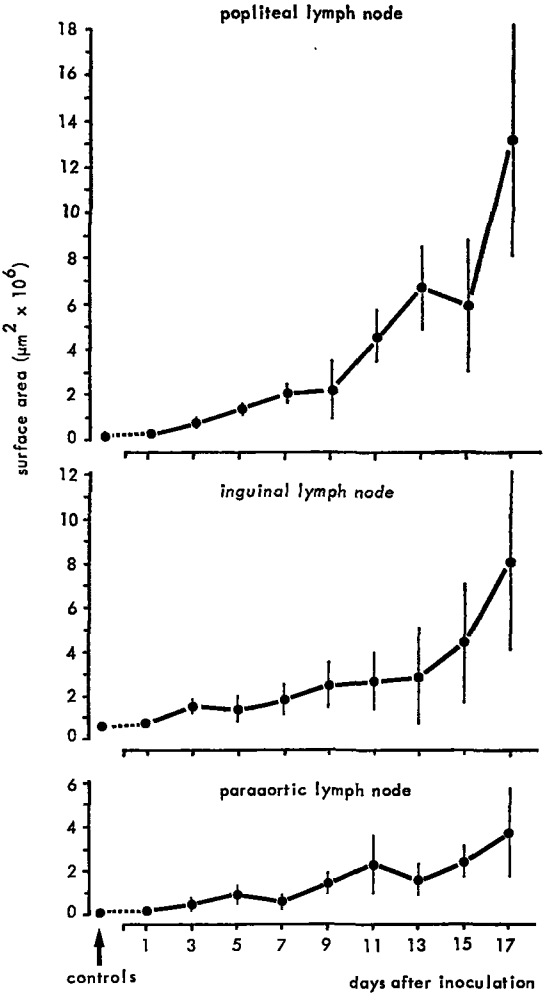


Figure 27. Mean of summated surface areas of cortical areas during the growth of the Lewis Lung carcinoma in absolute figures.

had already waned when tumours became visible or palpable (unpublished observations). Similar early and late changes have been found in human lymph nodes by Ahlqvist et al. (1974), who also calculated percentages of P.C.A. and C.A. using a point sampling technique. An oversimplified explanation would be that cellular (T cell) responses are relevant to the rejection of a tumour (Barski and Youn, 1969) and humoral (B cell) responses may result in enhance-

ment (Kaliss, 1958). However, there are conflicting data showing that weakly antigenic tumours can lead to a cell-mediated response which stimulates rather than inhibits tumour growth (Medina and Heppner, 1973). Clinically, the correlation of poor prognosis with lymphocyte and germinal centre predominance was not confirmed in a study on the regional lymph node histology in relation to survival in head and neck carcinoma; both patterns represented a favourable prognostic indicator (Berlinger et al., 1976). These results are in agreement with the findings of Kaufmann et al. (in the press) on immunomorphological lymph node changes in patients with operable bronchogenic squamous cell carcinoma.

The absolute numbers of sizes of P.C.A. and C.A. suggest a phase dependency in intensity in the three subsequent lymph nodes evaluated: The popliteal = regional lymph node always shows the highest absolute response. This would be in agreement with the findings of Flannery et al. on microcytotoxicity assays (Flannery et al., 1973). As shown in figure 15 the slope of increase in weight is less for the inguinal than for the popliteal and paraaortic lymph nodes, suggesting as taging as well. This was also found in the antigenic tumour system (fig. 16). This observation can possibly be explained by findings described in the previous chapter (Chapter I, Part II): It was found that after injection of Patent Blue Violet into the foot pad in a way similar to tumour cell inoculation, the first node to stain is the popliteal = regional lymph node. Afterwards, the paraaortic lymph node is stained but the inguinal lymph node remains unstained suggesting no direct communication of the lymphatic drainage of the foot pad with the inguinal lymph node.

When considering the relative numbers, i.e., the percentages P.C.A. and C.A. of the three lymph nodes, a different pattern is observed: The course in time is similar. This visual correlation of courses in time was confirmed by an analysis of variance in percentages P.C.A. and C.A. in interaction of "time" and "lymph nodes". This general response gives rise to some doubt about the question as to whether the regional lymph node possesses properties that makes it essentially different from the rest of the lymphoreticular system and whether it should be preserved rather than removed with a primary tumour.

Experimental data on the effect of lymph node removal are conflicting. Some find a detrimental effect of early removal (Crile, 1968; Fisher and Fisher, 1971; 1972; Hall et al., 1972; Perez et al., 1973;

1975; Pendergrast et al., 1976), whereas others did not find such an effect (Gardner and Rosen, 1967; Bard et al., 1969; Hammond and Rolley, 1970; Pilch et al., 1971; Sträuli and Lindemann, 1974; Peters, 1975). Fisher (Fisher and Fisher, 1972) suggested that the regional lymph node may play a more significant role in influencing tumour growth in tumours of "low" antigenicity. Since both an antigenic tumour and a nonantigenic tumour were used here, this could not be confirmed on a morphological basis in the present study. The special properties found for the regional lymph node were quantitatively but not qualitatively different from "distant" lymph nodes evaluated. Treves et al. (1976) observed an increase in spleen weight as early as two days after implantation of the Lewis Lung carcinoma into the foot pad. This suggests that the response by that time is already beyond the lymph nodes. In this regard present findings are in agreement with the findings of Edwards et al. (1971) and Simar et al. (1975) who studied the morphological alterations of contralateral lymph nodes in an experimental study. In contrast, however, are the studies of Alexander et al. (1967), Carter and Gershon (1966) and Fisher et al. (1973) who found that only regional lymph nodes have a reaction pattern similar to that described earlier.

In view of all the controversy existing in the literature, I feel that a quantitative approach is essential in analysing the morphological response. Antigenicity, defined as evoking an immune response which induces significant effects in tumour cell take and tumour cell growth, was found to be of no relevance in evoking a morphological response; for that reason, the nonantigenic tumour was placed between quotation marks in the title of this chapter. The absolute value of total node area and weight reaches a peak response which is greater in the Lewis Lung than in the 2661 mammary carcinoma system, but this cannot be clearly interpreted as a consequence of stronger antigenicity alone, since the area in control hosts is also different in the same direction. In addition, comparison of mice in which the 2661 mammary tumour developed at different growth rates have suggested that the growth rate may also be a factor determining surface area response (unpublished observations). In view of these findings, it may be concluded that perhaps in both man and the mouse, typical morphological changes in the lymph nodes cannot with certainty be interpreted as evidence for immunological reactions interpretable as influencing tumour cell growth. It is not even quite clear what part of the

reactivity of the lymph nodes must be attributed to such nonspecific factors as cell necrosis and aspecific inflammation in the tumour. A quantitative approach such as a morphometric analysis with the possibilities of applying statistics may represent an improvement in evaluating responses in lymph node studies.

CHAPTER III

EFFECTS OF REGIONAL LYMPHADENECTOMY AND ADJUVANT CHEMOTHERAPY ON METASTASIS AND SURVIVAL IN RODENT TUMOUR MODELS

* The following abbreviation is used in this chapter: R.L.N. stands for Regional Lymph Node.

1. Introduction

The controversy existing regarding the best local treatment for breast cancer is, at least in part, the consequence of the changed concepts which resulted from new information relative to tumour biology. In the field of cancer dissemination, animal experimentation aims at a better understanding of the course of the disease in the human patient. At the present time, this has a particular bearing on the role of the regional lymph nodes draining a tumour. Some experimental studies (Crile, 1965; 1967; 1968; Fisher and Fisher, 1971; 1972; Hall et al., 1972; Perez et al., 1973; 1975; Pendergrast et al., 1976; Table III) have shown that, at certain stages in the development of a tumour, the regional lymph nodes respond to it and that these early responses appear to be, at least temporarily, inimical to the tumour's progressive growth or resulting metastases. The implications of these observations may be important for the management of uninvolved regional lymph nodes in patients with breast cancer and discussion continues on the merits of retaining or ablating the draining nodes at certain stages in the development of cancer of the breast (Futrell and Hoopes, 1976). In this controversy, one essential condition for valid experimental research is the use of a model which shows similar essential characteristics to the human cancer to which it refers. Even if there is no perfect animal tumour model for any human cancer, certain principles do carry over. The essential characteristics needed for this study include: staging, metastasizing pattern, i.e., lymphogenous and haematogenous spread, similarities in response to therapy and low antigenicity, as expected for spontaneous tumours (Hewitt et al., 1976). For the experiments a rodent mammary carcinoma (2661) which shows these characteristics was selected (Chapter I). Although

this model was shown to have essential metastasizing and therapy response characteristics, a single animal tumour model can only serve as a model for a certain class of tumours found within a disease. Therefore, two additional tumours were evaluated in a similar way. Since most of the tumour models explored to evaluate an effect of removal of regional lymph nodes were antigenic tumours (Sato, 1964; Crile, 1965; 1967; 1968; Gardner and Rosen, 1967; Bard et al., 1969; Hammond and Rolley, 1970; Pilch et al., 1971; Abe and Taneichi, 1972; Fisher and Fisher, 1972; Hall et al., 1972; McCredie et al., 1973; Perez et al., 1973; Sträuli and Lindemann, 1974; Perez et al., 1975; Pendergrast et al., 1976; see Tables III, IV and V, Chapter III, Part I) one moderately and one highly antigenic tumour were additionally used.

In the experimental design, I wanted to evaluate whether there was at any stage before or after inoculation of the tumours a favourable or unfavourable effect of regional lymphadenectomy on survival and tumour growth in the area of the regional lymph node. Tumours were removed at a stage when early metastasis had taken place. This meant leaving a low tumour burden which could be influenced by the host's immunological system. The implications of variations in extent of surgery for breast cancer treatment cannot be judged without taking into account the effect of adjuvant chemotherapy. This implied to study also the effect of combinations of adjuvant chemotherapy with more or less extensive surgery in the most appropriate model, i.e. the 2661 mammary carcinoma model.

2. Materials and methods

Tumour-host systems

Three different tumour-host systems were used in the present study:
1. Mammary adenocarcinoma 2661 was selected from a trial testing tumours on lymph node and lung metastases. Only this particular tumour, which had originated spontaneously in a CBA/Rij mouse, consistently showed metastatic growth in popliteal, inguinal, paraaortic, renal and axillary lymph nodes as well as lethal haematogenous metastases in the lungs, if inoculated via the foot pad. Following subcutaneous and intravenous immunity challenge tests it was found to be nonantigenic (Chapter I). For each of the experiments 2×10^5

tumour cells were inoculated into the foot pad of female CBA/Rij mice weighing approximately 20 grams.

2. Lewis Lung carcinoma, a widely used experimental tumour, arose spontaneously in the lung of a C57BL mouse in 1951 (Sugiura and Stock, 1955). After foot pad inoculation, it always metastasizes to the lungs but only rarely to lymph nodes. Following similar immunity challenge tests as those described for the mammary carcinoma system it was shown that this tumour is antigenic in its host (Chapter II). For each of the experiments 2.5×10^5 tumour cells were inoculated into the foot pad of female C57BL/Ka mice weighing approximately 20 grams.

3. The Rd/3 tumour was obtained from Dr. Carr* in 1975, together with the inbred strain of albino rats, in which it was originally induced by injection of dibenzanthracene. This tumour model has been described as a valid experimental model for lymphatic metastases after foot pad inoculation (Carr and McGinty, 1974; Carr et al., 1974; Wood and Carr, 1974). According to results of the migration inhibition test and growth curves in immunized and nonimmunized hosts, this tumour was found to be antigenic (Carr et al., 1974). Immunization with 10×10^6 viable tumour cells in the flanks and subsequent removal of tumours resulted in the regression of all growing tumours (after 5×10^6 tumour cells) whereas non immunized sham operated control animals showed exponential growth of the tumour (fig. 28). Accordingly, this tumour may be described as a highly antigenic neoplasm. For the experiments with this tumour, 5×10^6 tumour cells were inoculated into the foot pad of rats weighing approximately 200 grams.

Tumour cell suspensions were prepared by the trypsinization method described by Reinhold (1965).

Technique of regional lymphadenectomy and tumour removal

In a previous chapter it was described that following Patent Blue Violet injection in a manner similar to tumour cell inoculation, the mouse foot pad's lymphatic drainage is to the popliteal lymph node (= R.L.N.) (Chapter I). In rats a similar observation was made. Removal of the regional, popliteal lymph node was carried out under

* For putting this tumour at my disposal, thanks are due to Dr. I. Carr, Department of Pathology, University and Weston Park Hospital, Sheffield, England.

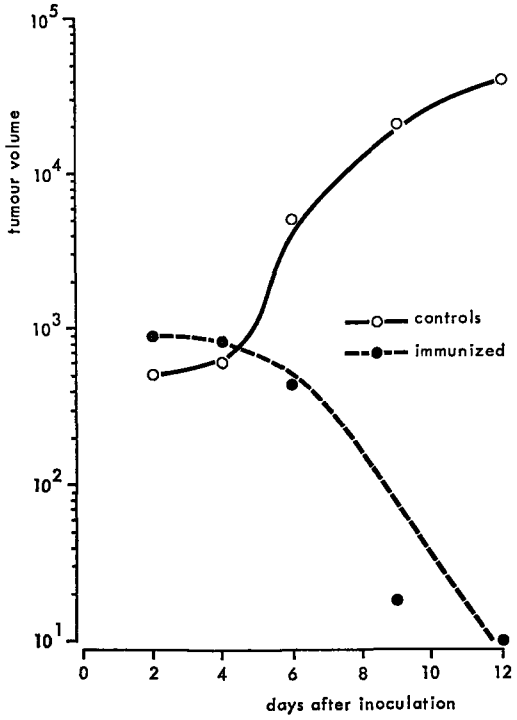


Figure 28. Growth curve of the Rd/3 tumour in immunized and control rats, after 10×10^6 tumour cells in the flank.

pentobarbital (60 mg/kg i.p.) anaesthesia after shaving and cleaning the popliteal skin area with alcohol. Control groups always underwent a sham lymphadenectomy under general anaesthesia, designed to mimic the trauma of lymph node excision. This procedure consisted of a similar skin incision in the popliteal area without exposure of the node. Incisions were closed with metal clips, which were removed ten days later.

3. Experimental design

Mammary carcinoma 2661

Except where otherwise indicated tumours were removed by amputation 14 days after inoculation. This interval was chosen intentionally, since it provided a number of deaths from recurrent disease as well as long-term tumour free survivors. The time of regional lymph node

removal or sham lymphadenectomy was used as a variable in order to investigate whether presence or absence of R.L.N.'s before and at any stage after tumour cell inoculation had any discernible effect. In different studies R.L.N. removal was performed 4 days before, 4 or 8 days after tumour inoculation; simultaneously with tumour removal at day 14, or 6 days later as a secondary procedure. The major endpoint for evaluation was survival, plotted as the percentage of mice surviving. At autopsy the extent of metastatic spread was recorded and special attention was given to tumour growth in the popliteal area.

Lewis Lung carcinoma

Tumour removal was performed 18 days after inoculation. Times of R.L.N. removal or sham lymphadenectomy were, respectively, 4 days before and 4 and 8 days after tumour inoculation and finally simultaneously with amputation. Secondary removal of R.L.N.'s was not performed for several reasons: 1. In the other models R.L.N.'s were already infiltrated by tumour at that time and the main purpose was to evaluate if extent of tumour removal showed an effect on survival at that stage. 2. For the role of the R.L.N. this time in the Lewis Lung system would be of no relevance: systemic immunity would be present at that time as haematogenous spread had already taken place.

Rd/3 tumour

Time of removal of tumours and treatment of R.L.N.'s was somewhat different in this system. Amputation was performed 5 days after inoculation and R.L.N. treatment was done 3 days before, 1 day after and 3 days after inoculation. Secondary R.L.N. removal was done 8 days after inoculation. These times were chosen earlier than in the other systems because R.L.N.'s were almost completely replaced by tumour at day 5. This is similar to data described by Carr and McGinty (1974).

Chemotherapy as an adjunct to surgery

This study was carried out only in CBA/Rij mice. A similar combination of drugs as described in chapter I was used both on established flank tumours and in the adjuvant situation. When given as full dosage the mice received each week:

100 mg/m²	Cyclophosphamide	day 1, 2, 3	i.p.
20 mg/m²	Methotrexate	day 1	s.c.
300 mg/m²	5-Fluorouracil	day 1	i.v.

This schedule was adapted from the clinically used dosages of Bonadonna et al. (Bonadonna et al., 1976; Bonadonna 1976; Brambilla et al., 1976) on the basis of equal dose per 4 weeks per m² body surface (Freireich et al., 1966) and was continued for 4 weeks. Variations in drug doses were used in order to find a level of drug treatment which had an effect on flank tumours comparable to that of C.M.F. in established recurrences in human mammary carcinoma. Subsequently, the full and the half dose schedules were employed in the adjuvant situation to determine the effect of these levels of chemotherapy, with and without surgical removal of R.L.N.'s at the time of amputation.

4. Results: Effects of regional lymphadenectomy

All mice that died after treatment in the mammary carcinoma model showed almost total replacement of the lungs by metastatic tumour and in addition lymph node metastases in various sites. Other sites of metastatic spread were observed only very rarely (1 ovarian metastasis, 1 brain metastasis and 2 adrenal gland metastases). Evaluation of experiments was done after a long observation period, since late deaths due to recurrent disease were to be expected. Long-term survivors never showed macroscopic or microscopic evidence of tumour, nor was any benign regrowth of removed lymph nodes observed.

Figure 29 shows the survival curves of animals which had their R.L.N.'s removed or underwent a sham procedure prior to inoculation of the nonantigenic mammary carcinoma. There is no significant difference in percentage surviving animals ($P = 0.89$). It appears however, that animals which had their R.L.N.'s removed have a somewhat longer delay before death. This difference is not significant and can possibly be explained from autopsy records: all sham lymphadenectomized animals always showed large masses of tumour in the popliteal area (sometimes even over 1 gram) which could have affected the ultimate date of death. A similar difference is found in most of the groups to be presented. In a separate experiment estimation of lung weights and ^{125}I-(5)-Iodo-(2')-Deoxyuridine (0.5 μCi/g body weight i.p.)-activity in the lungs of similarly treated animals, simultaneously

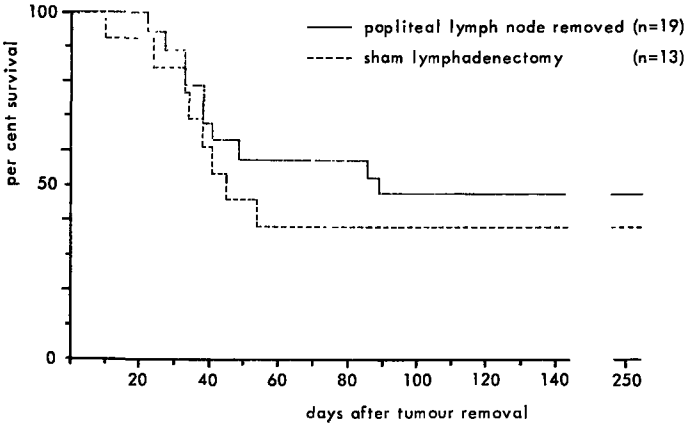


Figure 29. *2661 mammary carcinoma.* Survival of mice after removal of the primary growth: Popliteal lymph node removed or sham lymphadenectomy before inoculation.

killed, revealed no differences between lymphadenectomy and sham operated animals, although these parameters seem to be valid indications of the degree of metastatic tumour growth in the lung, similar to that described by Bonmassar et al. (1975), as shown by higher lung weights and ^{125}IUdR activity (de Ruiter and Cramer, 1976). If R.L.N.'s are important in the initiation of immunity it could have been that this specific function, after removal of the R.L.N. prior to inoculation was taken over by other lymph nodes nonregional to the foot pad.

At days 4 and 8 after inoculation R.L.N.'s are never infiltrated by metastatic tumour. They are however enlarged due to a reaction to the growing tumour, which has been morphologically interpreted as a sequential combination of a cellular and a humoral immune response (Chapter II).

It was shown that an "early" response consisted mainly of an increase in thymus dependent areas in the lymph node associated with the cellular response. A "late" response was associated with an increase of the thymus independent humoral response areas. If in the R.L.N., thymus dependent cellular responses are relevant to the rejection of tumour (Barski and Youn, 1969) and humoral responses may result in enhancement (Kaliss, 1958), there should be a difference in "early" (day 4) or "late" (day 8) R.L.N. removal. Figures 30 and 31 show the data of these experiments. Evaluation of the experiments

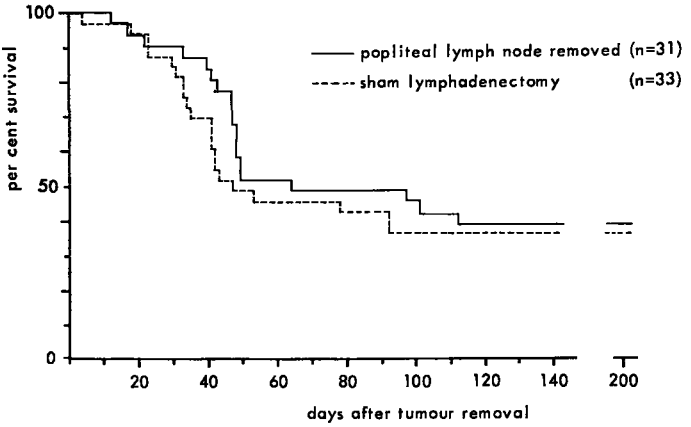


Figure 30. *2661 mammary carcinoma.* Survival of mice after removal of the primary growth: Popliteal lymph node removed or sham lymphadenectomy at day 4 after inoculation.

showed that survival was similar whether treatment of R.L.N.'s took place at 4 or at 8 days (P = 1.0 and P = 0.74, respectively).

Removal of R.L.N.'s at the time of tumour removal could theoretically influence tumour spread, since R.L.N.'s in most cases contain tumour at this time (14 days after inoculation). Figure 32 shows the survival curves which indicate no difference (P = 1) dependent on

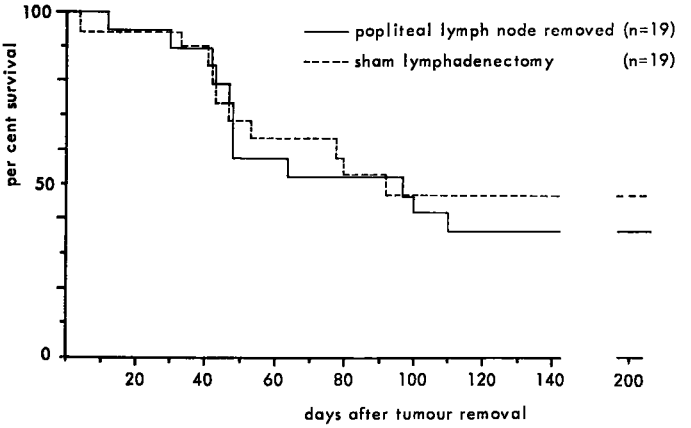


Figure 31. *2661 mammary carcinoma.* Survival of mice after removal of the primary growth: Popliteal lymph node removed or sham lymphadenectomy at day 8 after inoculation.

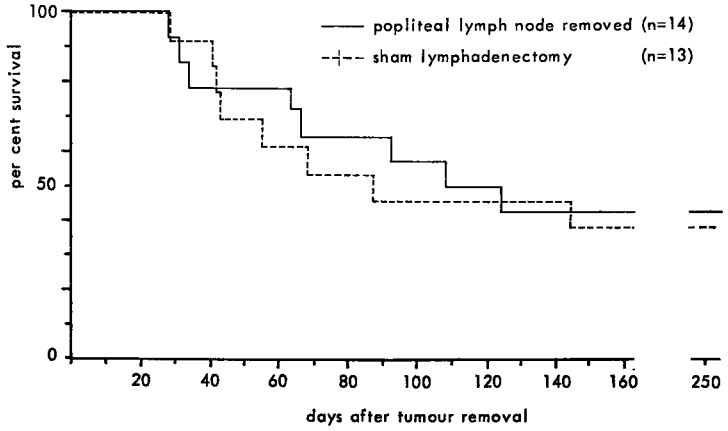


Figure 32. *2661 mammary carcinoma.* Survival of mice after removal of the primary growth with or without the regional lymph node.

treatment. It appears that the extent of surgery has no effect on development of blood borne metastases. This finding is similar to that of secondary (tumour containing) lymph node removal at day 20 (fig. 33). Removal of metastatic lymph nodes independent of whether one (popliteal) or two (also inguinal) lymph nodes were grossly metastatic on palpation at that time after tumour removal had no

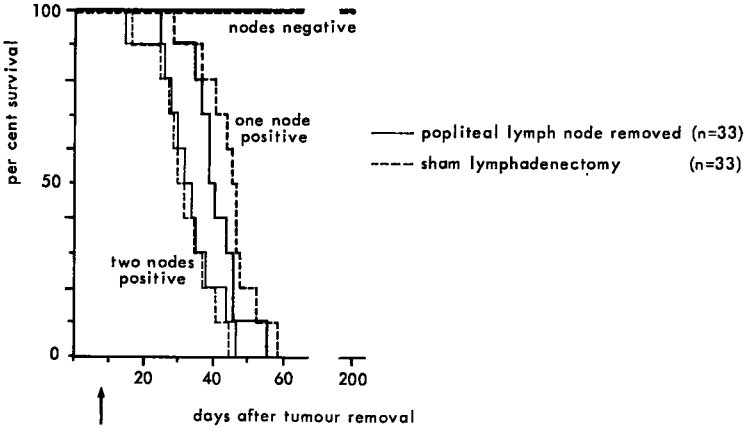


Figure 33. *2661 mammary carcinoma.* Survival of mice dependent on node status and treatment: secondary lymph node removal.

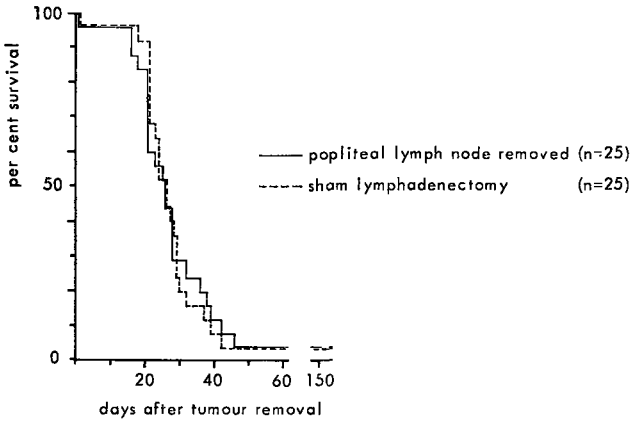


Figure 34. *Lewis Lung carcinoma.* Pooled data of experiments comparing removal of the popliteal lymph node with sham lymphadenectomy on survival.

discernible effect on survival. Nevertheless, the number of palpable metastatic nodes at that time appeared to be a crude index for survival.

As mammary carcinoma 2661 was found to be nonantigenic it could be that findings with tumours which clearly demonstrate antigenicity by influencing tumour cell survival, would differ.

The Lewis Lung carcinoma spreads primarily via the blood stream so that this model is less adequate for evaluating recurrence of tumours in lymph nodes. Figure 34 shows the pooled data of experiments similar to those performed in the mammary carcinoma model.

Evaluation of experimental groups in which R.L.N.'s were treated at different times showed no difference; for that reason results were pooled. The surviving fraction of mice is very small in these studies, as a consequence of an earlier haematogenous spread to the lungs. Survival curves run parallel for both groups evaluated.

The Rd/3 tumour is a highly antigenic tumour which metastasizes mainly through R.L.N.'s. In contrast to the findings of Carr and McGinty (1974) our rats always died as a result of extensive lymph node metastases in the abdomen. Paraaortic lymph nodes almost completely replaced the abdomen in the absence of any macroscopic or microscopic evidence of tumour cells in the lungs. The reason for this different finding is not clear. The time of R.L.N. treatment differed from that used in the other two models since very early lymph node replacement by tumour was observed, similar to findings of Carr

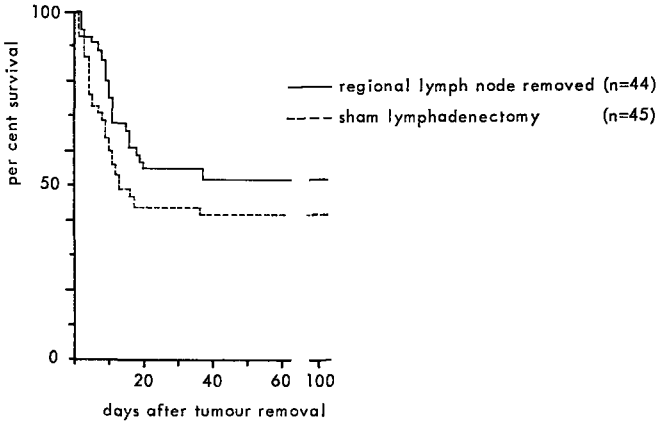


Figure 35. *Rd/3 tumour.* Pooled data of experiments comparing removal of the popliteal lymph node with sham lymphadenectomy on survival.

and McGinty (1974). Treatment of R.L.N.'s was performed before, and 1 and 3 days after tumour inoculation of 5×10^6 tumour cells. A smaller number of 10^6 cells usually produced temporary enlargement of the foot pad which regressed afterwards. For similar reasons as for the Lewis Lung carcinoma data were pooled and figure 35 shows the results. The difference in surviving fraction is not significant ($P = 0.46$). A similar phenomenon as found for the mouse mammary carcinoma is observed in the survival curves: i.e. sham operated animals have a

Table XVII. Absence of effect of lymphadenectomy on survival*

	Per cent mortality from tumour		
Time of treatment of R.L.N.	Lymphadenectomy	P-values	Sham
2661 mammary carcinoma			
before inoculation	**52.6**	0.89	**61.5**
4 days after inoculation	**61.3**	1	**63.6**
8 days after inoculation	**63.2**	0.74	**52.6**
at amputation	**57.1**	1	**61.5**
secondary	**60.6**	1	**60.6**
pooled data	**59.5**	1	**60.4**
Lewis Lung pooled data	**96.0**	1	**96.0**
Rd/3 pooled data	**47.7**	0.46	**57.7**

* Since staging was not always similar in these experiments, valid comparison is possible only with horizontal groups but not between lines.

somewhat shorter survival probably due to large tumour masses in the popliteal area.

As shown in each of the three models evaluated, at any time of treatment of R.L.N.'s there was no effect in either way. A summary of the surviving fractions in different experiments is presented in Table XVII.

However, for the two models which always showed lymph node metastases when left untreated significant differences were recorded for the incidence of recurrent disease in the popliteal area as shown in Table XVIII. Recurrent disease was also seen, but in a lower frequency, in animals which had their R.L.N.'s removed, possibly as a result of spread by lymphatics cut at surgery.

5. Adjuvant chemotherapy and R.L.N. treatment

Figure 36 shows the effects of different dosages of chemotherapy on established flank tumours. The standard dose refers to the schedule as

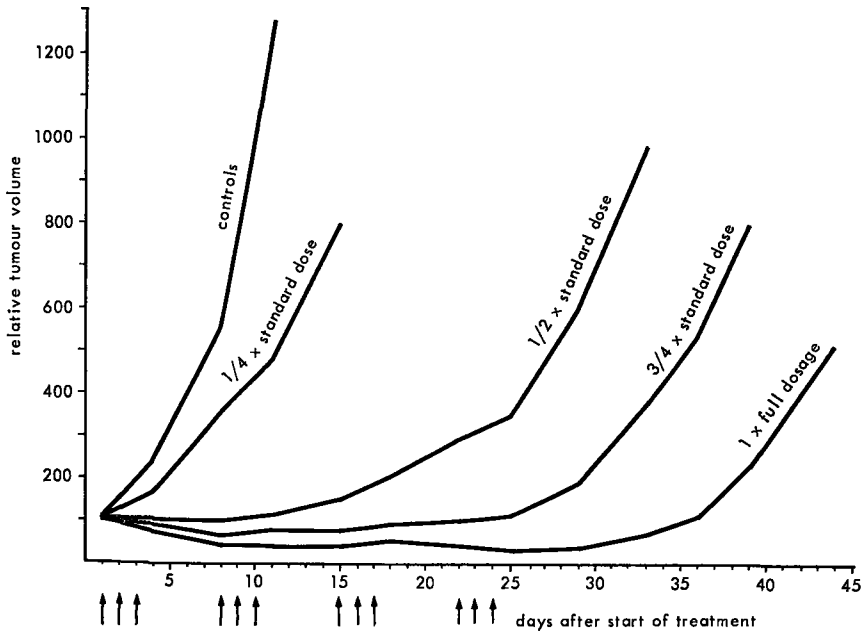


Figure 36. *2661 mammary carcinoma.* Growth delay of flank tumours dependent upon chemotherapy dose; 5–8 animals per group, each carrying two tumours.

Table XVIII. Tumour growth in popliteal area after lymph node removal or sham lymphadenectomy*

Time of treatment of R.L.N.'s 2661 mammary carcinoma	Per cent popliteal tumour growth observed in dead animals			Per cent popliteal tumour growth observed in all animals (including survivors)		
	lymph-adenectomy	P-values	Sham	lymph-adenectomy	P-values	Sham
1. before inoculation	**20.0**	<0.001°	**100**	**10.5**	=0.007°	**61.5**
2. 4 days after inoculation	**57.9**	=0.020°	**100**	**35.5**	=0.045°	**63.6**
2. 8 days after inoculation	**83.3**	=0.57	**100**	**52.6**	=1	**52.6**
3. at amputation	**20.0**	=0.014°	**100**	**9.0**	=0.023°	**61.5**
4. secondary two nodes+	**40.0**	=0.011°	**100**	**23.5**	=0.089°	**58.8**
one node +	**30.0**	=0.003°	**100**	**18.8**	=0.029°	**62.5**
pooled data	**42.4**	<0.001°	**100**	**27.4**	<0.001°	**60.4**
5. Lewis Lung; pooled data	**20.0**	>0.10	**12.0**	—	—	—
6. Rd/3; pooled data	**66.7**	=0.021°	**96.2**	**31.8**	=0.040°	**55.5**

* Absolute number of animals in each group are given in the relevant figures

P-values counted according to Fisher's exact test for two probabilities (Fisher, 1963)
° = significant values

extrapolated from the clinically applied dosages per m^2 body surface area. As shown, only the full dosage is adequate in producing an objective regression, whereas reduction of dosages resulted in resumption of tumour growth during therapy.

Since the size of the primary growth was shown to be a crude index for survival (Chapter I) a large tumour was chosen for the adjuvant treatment to determine whether cures could be achieved. Chemotherapy was given at the full and at the half dose level. Surgical removal of the primary tumour with or without simultaneous lymph node ablation took place at 18 days after tumour inoculation; lymph node metastases were already established at this stage. Figures 37 and 38 show the survival curves for treated and untreated animals. Animals treated by adjuvant chemotherapy consisted of 4 groups in order to evaluate whether variation in the extent of the removal of tumour mass (i.e. with and without regional nodes) and degree of adjuvant chemotherapy would result in differences in survival. As shown, the full dose schedule results in a significant prolongation of survival ($P < 0.05$; fig. 37). This was not significantly dependent on the extent of surgery. Reduction of the chemotherapy schedule to half dose abolished the effect: survival was similar to controls (fig. 38). The extent of surgery also failed to affect survival significantly in this group. A similar difference in the sites of metastases as found earlier (Chapter I) was found in the groups treated with adjuvant chemo-

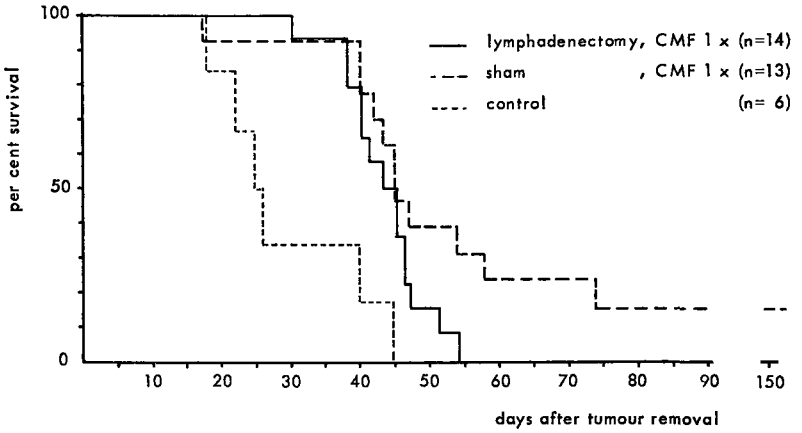


Figure 37. *2661 mammary carcinoma.* Survival of mice after removal of the primary growth dependent upon lymphadenectomy and chemotherapy, given as full dose.

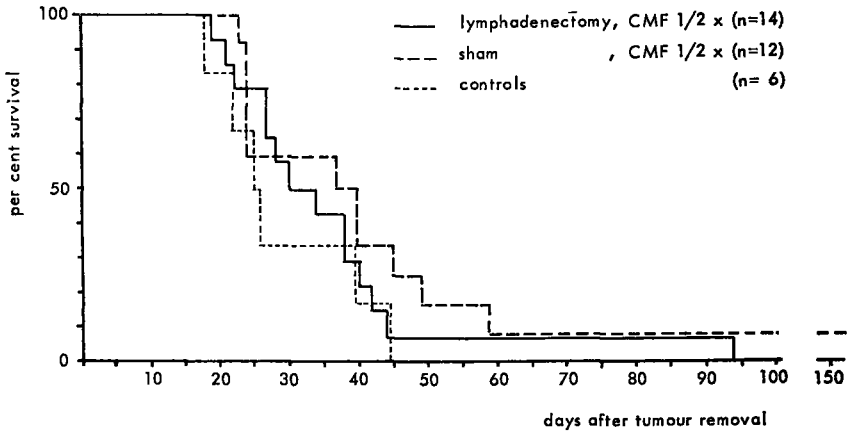
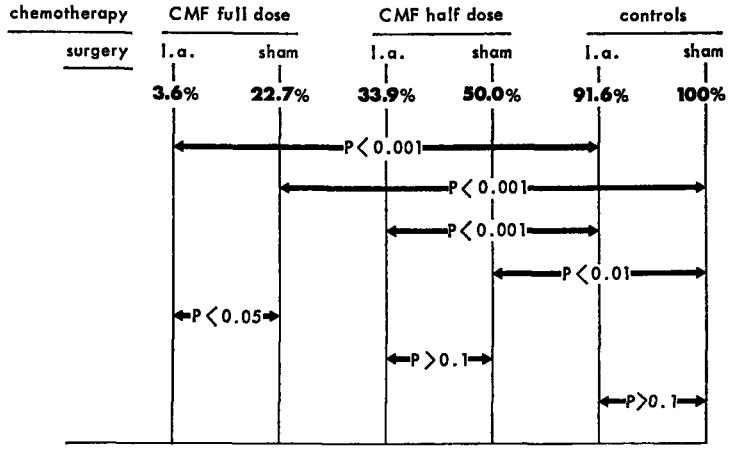


Figure 38. *2661 mammary carcinoma.* Survival of mice after removal of the primary growth dependent upon lymphadenectomy and chemotherapy, given as half dose.

Table XIX. Per cent positive nodes* dependent upon regional lymphadenectomy and chemotherapy



* 4 nodal areas were examined. The value presented is the total number of nodes positive/total nodes. The lymph nodes examined were the popliteal, inguinal, paraaortic and axillary on the ipsilateral side with respect to the primary tumour.

therapy (Table XIX). All animals treated by surgery alone, showed extensive lymph node metastases in addition to lung metastases at autopsy. A significant reduction in numbers of lymph node metastases occurred in the treated groups, in contrast to the much less noticeable effect on the massive lung metastases from which the animals apparent-

ly had died. Even though there was no prolongation of survival in the animals receiving only half the dosage, the difference in numbers of lymph node metastases was highly significant. Only in the full dose regimen, a significant difference in the number of lymph node metastases was found between mice treated with lymphadenectomy or not. It must be pointed out, however, that data presented here are expressed as percentages of 4 nodal areas.

6. Discussion

In challenging the classical concept of en bloc resection of the primary tumour along with its regional lymphatics for cancer of the breast, Crile's choice of simple mastectomy (i.e. with preservation of R.L.N.'s) was based on his experimental findings that retention of R.L.N.'s uninvolved by tumour could be important in maintaining a degree of systemic immunity against growth of metastatic microfoci. Results of his early clinical survival studies seemed to confirm this hypothesis (Crile, 1965; 1966; 1974a; 1974b; 1975b).

Since then, in addition to the clinical studies, devoted to this controversy, many investigators have developed various experimental models for evaluating the role of R.L.N.'s in cancer. When reviewing the literature on these studies, differences are noted in tumour models explored, endpoints evaluated and final conclusions (Part I, Chapter III). In the present studies, one model, the 2661 mammary carcinoma model, is more fully described, since this is the first model in which an effect on the two modes of spread, i.e., lymphogenous and haematogenous spread, could be evaluated. On the basis of the findings described, in both this nonantigenic tumour model and in two additional antigenic tumour models, I must express serious doubts of the unique role ascribed to R.L.N.'s in antitumour immunity at any stage of tumour growth. On the other hand, relative to the possible barrier function of R.L.N.'s to tumour cells (Zeidman and Buss, 1954; Baker et al., 1969; Sträuli, 1970) no favourable effect of the removal of R.L.N.'s which contain tumour was observed in contrast with findings of Sato (1964). An important finding in two models was a significant reduction of tumour growth in the popliteal area. This reduction was observed after R.L.N. removal at any stage. These results indicate that the lymph node itself may present a privileged site in the outgrowth of metastases. Some experimental data even show that the

number of cells necessary to give a "take" in the lymph node is much smaller than for successful inoculation at other sites (Hewitt and Blake, 1976). In my studies the large tumour masses in the popliteal area may have influenced survival to a small extent; similar recurrences in the clinical situation would probably have been treated by radiotherapy and/or chemotherapy, so that this influence would be minimal.

What is the value of these findings among the large list of contradictory reports of experimental studies? Are these models appropriate for conclusions that R.L.N. removal cannot affect ultimate survival?

The presence or absence of some degree of immunological reactivity of the host against its tumour is a controversial condition, regarding the relevance to spontaneous cancer in man of a transplanted animal tumour system. When using quantitative morphometric analyses of draining lymph nodes of the nonantigenic mammary carcinoma and the antigenic Lewis Lung carcinoma I could not find a clear difference in response (Chapter II). It is, however, reasonable to have some doubt about animal tumour systems as models for clinical cancer if they elicit forms of resistance which are clearly attributable to laboratory artefacts associated with their induction. Most of the tumour models previously used for study of the effect of R.L.N. removal involved either highly antigenic, chemically induced, or allogenic tumours. Results from these studies are contradictory: some described a detrimental effect of R.L.N. removal (Crile, 1965; 1967; 1968; Fisher and Fisher, 1971; 1972; Hall et al., 1972; Perez et al., 1973; 1975; Pendergrast, 1976), whereas others did not find such an effect (Gardner and Rosen, 1967; Bard et al., 1969; Hammond and Rolley, 1970; Pilch et al., 1971; Abe and Taneichi, 1972; McCredie et al., 1973; Sträuli and Lindemann, 1974; Peters, 1975) independent of the endpoints evaluated (Table VI).

Tumours of spontaneous origin were used in only three of the studies: two of them revealed no effect (McCredie et al., 1973; Peters, 1975), but one showed a detrimental effect of R.L.N. removal in both the initiation (Fisher and Fisher, 1971) as well as in the maintenance of immunity (Fisher and Fisher, 1972). In these latter studies secondary challenge of tumour was used as an endpoint for the existence of R.L.N. dependent immunity. A reduction in percentage tumours arising at secondary challenge was described in both experimental groups (with and without R.L.N. removal) compared to the first

challenge in nonpretreated control animals. This clearly suggests tumour growth affecting antigenicity.

Nevertheless in the present studies antigenicity of the tumour was shown to be of no relevance for the effect of R.L.N. removal. In these studies however, I used survival as an endpoint. Many survival curves in the various experiments show "breaks" before reaching the horizontal level. Skipper (1977) observed these breaks in many animal tumour models as well as in human survival curves after surgical treatment. He related these breaks in the slopes of survival curves to the presence of a tumour load of a few or even one tumour cell. For the present experiments this is illustrated in figure 39 which shows the pooled data of an experiment comparing removal of the popliteal lymph node with sham lymphadenectomy in the mammary carcinoma model. In this separate experiment R.L.N.'s were treated 4 days before and 4 and 8 days after tumour transplantation, i.e., before R.L.N.'s were infiltrated by tumour. Similarly like in the other experiments tumour removal was done at day 14 after tumour transplantation. One animal which died 188 days after tumour removal showed no metastatic foci at autopsy, however, a large new primary mammary carcinoma had developed which had caused death. Figure 40 shows the growth curve of the 2661 mammary carcinoma. The calculated doubling time upto 20 days after inoculation of 2×10^5 tumour cells is 2.33 ± 0.33 days (according to the method of Lloyd, 1975). When assuming that about 30 doublings are necessary to kill the mouse by metastatic tumour (Table VII) 70 days would be necessary for a repopulation of one single tumour cell, left after tumour removal. This is clearly within the range of the breaks observed in both survival curves. If this relation is justified R.L.N. dependent immunity would certainly be expected to have influenced the outcome of an experiment dependent on the survival of such small tumour cell numbers.

From present studies I can qualify R.L.N.'s only in their properties as part of the reticuloendothelial system's response to tumours. A morphometric analysis of the immune response supports this assumption since it was shown that the reaction of R.L.N.'s was quantitatively but not qualitatively different from "distant" lymph nodes evaluated. As a result I cannot support the hypothesis of Crile based on his experimental findings.

The clinical trials comparing simple with radical mastectomy do not confirm Crile's clinical studies. Most of the preliminary reports

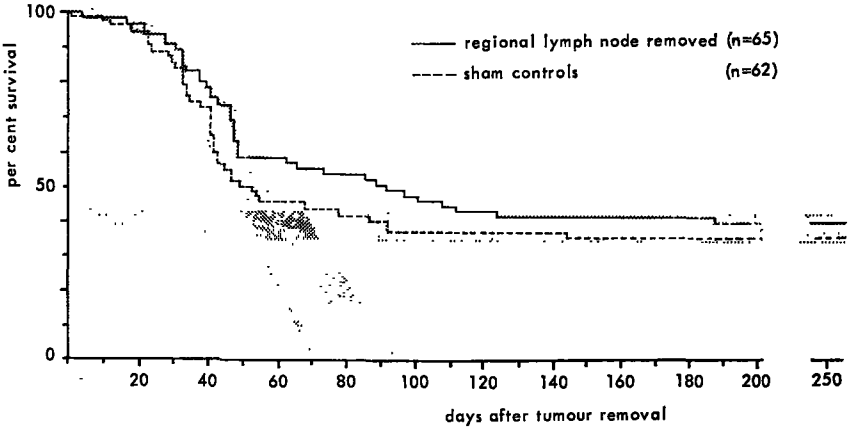


Figure 39. *2661 mammary carcinoma.* Pooled data of experiments comparing removal of the popliteal lymph node with sham lymphadenectomy on survival; "Breaks" in both curves.

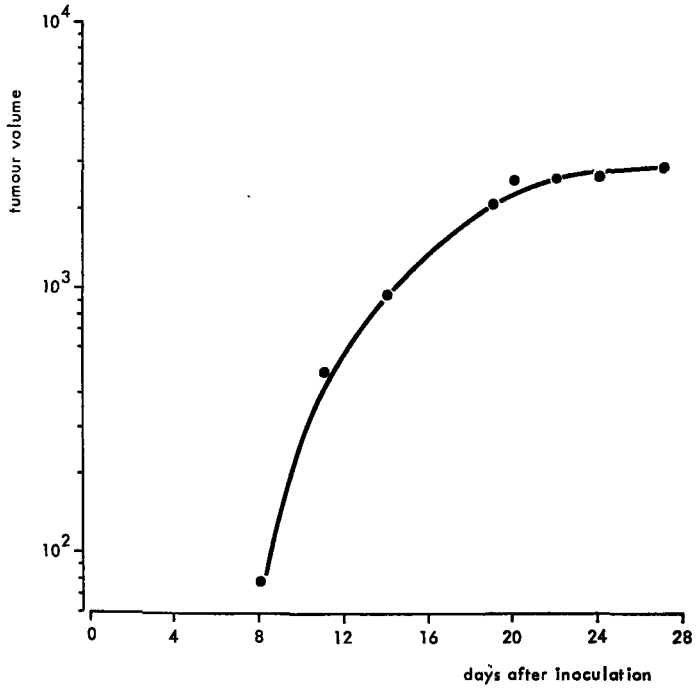


Figure 40. Growth curve of the 2661 mammary carcinoma.

show no difference in survival (Roberts et al., 1973; Forrest et al., 1974; Fisher and Wolmark, 1975). This could also be interpreted as evidence that less extensive surgery is equally good. From an emotional and aesthetic point of view, this would be a major improvement. Great caution however must be exercised in this regard: preliminary reports of an international multicentre trial comparing simple mastectomy with radiotherapy to simple mastectomy alone, did show, independent of survival, a significant increase in axillary nodal metastases when left untreated (Editorial, 1976). The ten years report of a clinical trial comparing radical mastectomy with wide excision showed a similar difference in patients with uninvolved axillary nodes; when axillary nodes were involved, local recurrence and survival were both significantly worse in the latter regimen (Atkins et al., 1972). Bonadonna noted that local recurrences were decreased by adjuvant chemotherapy (Bonadonna, 1976). Therefore, I applied this combination of drugs at different levels. Since many experimental studies have shown that there is an inverse relationship between the number of viable tumour cells present at initiation of chemotherapy and curability, (Laster et al., 1969; Schabel, 1969) the comparison of more or less extensive surgery was included. The findings showed however that there was no relation between extensiveness of surgery and survival. This could be due to the fact that the stage at which amputation was performed was too late. There was apparently a large tumour cell load. A significant reduction in lymph node metastases in contrast to haematogenous metastases was observed after chemotherapy. This was even the case at a level of drug treatment which was ineffective in prolonging survival. Van Putten et al. observed a similar specific decrease of lymph node metastases after cytotoxic drug treatment, associated with an increase in the number of lung metastases (van Putten et al., 1975). It is presently not clear whether this finding is due to a difference in cell kill in metastases existing at the time of treatment or to a different localization of regrowth and spread of recurring tumours. The possibility that the lymph node bed is a poorer area for regrowth after damage of the lymphatic system by drug treatment cannot be excluded.

Before the general application of less extensive surgery in breast cancer patients is justified by an effect of adjuvant chemotherapy, the presently running trials on adjuvant treatment must be evaluated more fully. Similarly its effectiveness in combination with different surgical approaches should be tested in view of the present findings.

Experimental models may share mechanisms and responses to treatment with certain aspects of clinical cancer. In this way employment of suitable models may have a predictive mode and guide the clinician. If experimental results are translatable to man, even if only broadly, it would appear that using such tumour models, the experimentalist is in a position to indicate possibly useful approaches to surgery, adjuvant chemotherapy and the combination of these. Of course such approaches will have to be subjected to the final test of usefulness, the controlled clinical trial. In the evaluation of the results in which survival is the major endpoint, the quality of life closely associated with the incidence of regional recurrences must not be forgotten.

CHAPTER IV

GENERAL DISCUSSION AND CONCLUSIONS

A decade or two ago, radical mastectomy was generally accepted as the standard therapy for breast cancer; however, there is great controversy today concerning the proper primary treatment for clinically curable cases of this disease. Dissatisfaction with the mortality rates led many surgeons to challenge the radical procedure with the hope of better results. Originally, tumours were considered to be autonomous of their hosts; lymph nodes were considered to be effective filters for trapping tumour cells and cancer was equated as "early" or "late" depending the size of the tumour. It is understandable that, on this basis, the hope was justified that more lymph node dissections would, by removing the last tumour cell, cure more patients. In general, survival data after more extensive procedures proved not to be superior to those after the classical approach; this indicates that this hope was not confirmed. We know presently that tumour cells may have spread into the circulation very early in the course of the disease. There they are beyond the reach of surgery or radiotherapy. To eradicate these cells more extensively, when present in the circulation or as distant microfoci, Crile proposed that an optimal use of systemic antitumour factors, i.e. antitumour immune reactions, would result in eradication of the remaining systemic tumour cells. This antitumour reaction of the host was assumed to be dependent on the integrity of the regional, tumour draining, lymph nodes. Clinically, this resulted in the employment of simple mastectomy for early operable breast cancer. Experimentally, the proposed specific and unique role of the regional lymph nodes in the lymphoreticular system was more fully explored. Some experimental models indeed showed that the initiation and maintenance of immunity was dependent on intact regional lymph nodes, whereas others did not show such an effect. The inadequacies of all of these experimental systems, as models for clinical breast cancer, have been discussed in detail earlier (Part I, Chapter III). Therefore, a more valid experimental model for breast cancer was developed and characterized as to its therapy response characteristics. It was shown that both surgery (dependent on the time after

tumour inoculation) and chemotherapy (dependent on the dose) could influence ultimate survival. In this model, after tumour removal, no effect on survival was detected whether regional lymph nodes were removed or not. This finding was independent of the stage of tumour growth and metastatic spread in which regional lymph nodes were treated (either by removal or by a sham procedure). Regional recurrences were, however, greatly reduced by radical surgery, i.e., by removal of regional lymph nodes. In the experiments, regional recurrences, when registered at death, were always associated with lethal haematogenous metastases in the lungs. It appears therefore that, though the two systems of spread of cancer (see fig. 2, Chapter III) are inseparable, the presence or absence of regional recurrences after tumour removal has little influence on the already present haematogenous metastases. Obviously, if regional recurrences would be the only site of tumour growth, then the recurrences could act as a further source of spread. In the experiments, they can be regarded at best as inconvenient, expressed by a somewhat (insignificant) shorter delay before death. This inconvenience would be of more significance in man. It is impossible to determine by palpation or multiple biopsies, even during surgery whether regional lymph nodes are or are not involved by tumour. Therefore, if tumour is left untreated in regional lymph nodes, it is likely that regional recurrences will develop in a significant number of patients, who will require further treatment. Secondary treatment of involved regional lymph nodes by dissection or radiotherapy usually carries a high morbidity (e.g. oedema of the arm) and survival in general is poor. Devitt and Spratt observed a 5-years survival of 4 per cent and 9 per cent, respectively, after the appearance of regional recurrences (Devitt, 1967; Spratt, 1969). But even if haematogenous metastases are present and the prognosis is poor, I sincerely believe that it is important that the patient is not distressed by regional recurrences. As a result of these clinical considerations and the experimental findings in the mammary carcinoma model, these can only be arguments to condemn less than radical surgery as treatment for operable breast cancer at any stage.

Clinical breast cancer involves many different forms of neoplasms with individual differences in tumour growth, invasiveness, histopathology and responsiveness to therapy. It is obvious therefore that no one animal tumour model can completely represent and be predictive for clinical breast cancer. Nevertheless, since animal models

can be found to mimic certain responses to therapy in a reproducible manner, the results with the present model can at best be regarded as predictive for a certain class of tumours found within the clinical disease. In this class, mastectomy with en bloc resection of regional lymph nodes would represent the optimal surgical treatment.

Clinically, it was proposed on the basis of morphological analyses of axillary, tumour draining lymph nodes that tumours might differ in antigenicity, as expressed by differences in morphological response. Experimentally, we know that one of the most important factors in influencing tumour cell growth and tumour cell survival is antigenicity (see Part II, Chapters I, II and III). The tests used for determining antigenicity* are, obviously, only applicable to experimental model systems. Could this variation in antigenicity not be the major characteristic of the primary tumour in influencing tumour cell growth clinically, dependent on the integrity of regional lymph nodes?
Experimental evidence is firmly against this contention:

1. A morphometric analysis of the response evoked by a defined antigenic and a nonantigenic tumour showed that reaction patterns were comparable. These findings suggest that great caution must be exercised when interpreting morphological lymph node changes as evidence for immunological reactions interpretable as influencing tumour cell growth. Moreover, the morphological reactions of "distant" lymph nodes were quantitatively but not qualitatively different when compared to regional lymph nodes.
2. In antigenic tumour models, there was no detrimental effect of regional lymph node removal at any stage of tumour growth. In fact, in one model in which the tumour metastasized via the lymph nodes, a difference in favour of radical surgery was observed in numbers of regional recurrences.

Even if these models are complete nonrepresentative for human breast cancer, (I think that the two antigenic tumour systems are) and regional lymph nodes are important in the initiation of anti-tumour immunity, as found in some models, then this was only observed for a very short period after tumour cell inoculation. When clinical breast cancer is detected at an early stage, e.g. 1 cm in diameter, it has already gone through 30 doublings (see Part I, Chapter IV). Since doubling times for human breast cancer may vary from 20 to over 200

* (in the sense described earlier, i.e., as affecting prognosis).

days, it can be calculated that the time required for a tumour to reach such a size may vary from 23 months to 17 years! Consequently, when a patient with breast cancer is presented for surgery, the tumour has probably been present for at least a year, during which any immunological response against the tumour should certainly have become fully mounted and systemic. How, at this "late" stage of tumour development, can local surgical ablation of regional lymph nodes or irradiation depress an immune response to a degree which proves harmful to the host? I think that those studies, which did show a detrimental effect (with their inadequacies), do not have a clinical counterpart. Some clinical investigators have even gone so far, on the basis of their findings, to state that regional axillary lymph nodes are immunologically incompetent (Humphrey et al., 1971).

Clinical trials, so far, do not show a difference in survival whether regional lymph nodes were removed or irradiated versus no treatment. This could of course also be interpreted as evidence that less extensive surgery is equally good.

A reduction in the extensiveness of surgery, such as local excision of the tumour, would naturally be an improvement for the patient with regard to her feminine appearance. My criticism against local excision has already been discussed in Chapter II (Part I). I think that, besides the arguments put forward then, there is no justification for local excision without fear of doing an injustice to the patient, before it has been proved that simple mastectomy is equally as good as radical mastectomy for clinical stage I cancers. In fact, the clinical trials comparing simple versus radical mastectomy should have produced data which show that this is indeed the case. A quite properly conducted trial in this regard is the N.S.A.B.P. trial which was started in 1971 and which ended in 1974 (Chapter II, Part I). The very preliminary results reveal no significant difference in survival and distant recurrences. It is of significance however, that, in this short follow-up period, 16 per cent of patients treated by simple mastectomy have already required a delayed axillary dissection (Fisher, 1976). Besides that this procedure usually carries a high morbidity, we do not know yet if these recurrences are really the top of an iceberg. Further follow-up will show what the ultimate percentage of patients is who require such a secondary procedure and will show whether in this subgroup of patients prognosis is adversely affected compared to that of clinically proven N^+ node patients after radical

mastectomy. But, even if this is not the case, I believe that the hazards and inconveniences of regional recurrences are too heavy a burden against the slight cosmetic improvement. This should especially convince the original followers of simple mastectomy, who believed that, by leaving the regional lymph nodes intact, the balance of cure dependent on a few tumour cells would be tipped positively. Since there are no methods available as yet to detect small metastatic microfoci in regional lymph nodes by means other than careful histopathological analysis of removed axillary nodes, radical mastectomy should also be the treatment of choice for patients detected as a result of mass screening with very small invasive breast cancers.

In the introduction to this thesis, it was stated that one way of reducing mortality rates would be optimal therapy. Optimal therapy consisted of two procedures: optimal surgical therapy and optimal adjuvant chemotherapy. I think that the first item has already been established in the radical mastectomy, the modified version of which has been shown to be equally good with the advantage of the preservation of the pectoral contour. Optimal then not only means the best chance of cure; it also means the best chance of avoiding the risk of regional recurrence, which can greatly affect the quality of life. (On the basis of the present studies described, I can not give an opinion on the relative merits of replacing part of the surgery by radiotherapy).

The other item was concerned with additional therapy. Present trials on the value of adjuvant chemotherapy have produced valid information that regional recurrences can be delayed, for which combination chemotherapy offers the best change. An immediate consequence of these studies has been the universal recognition that there is justification for further trials, despite the fact that long-term survival data and long-term toxic effects are not yet known. Therefore, adjuvant chemotherapy was only given to patients who were known to have a reasonable chance of lethal recurrence, i.e., patients with histopathologically proven positive axillary nodes.

Present experimental studies have indicated that adjuvant chemotherapy when given at the maximum tolerated dose could result in a very striking reduction in already established regional recurrences as well as in a prolongation of survival.

As a result of the clinical and experimental experiences, could the disadvantage of simple mastectomy not be overcome by adjuvant chemotherapy?

There are several conjectures against this hypothesis, some of which

have already been discussed in the introduction (Chapter I, Part I). This discussion led to the question of whether, in a group of patients already having a favourable prognosis (Stage I), long-term chemoprophylaxis could not be too heavy a burden. Delayed sequelae are not yet known and optimal combinations of chemotherapy have not yet been found. These are reasons for future research and further clinical trials in N^+ patients. When more information is available and if delayed sequelae are minimal or acceptable, this could then result in a reevaluation of the surgical procedures based on an individual approach.

Personally, I am quite sceptical about this reevaluation of surgical procedures. The best method presently available to obtain some idea of the prognosis of the patient is the axillary status. This implies removal of the axillary contents in all patients. For patients with involvement of the top-level of the axillary lymph nodes a more toxic schedule will be justified than for those who have only involvement of the basis of the axillary nodes. Experimentally, we have learned that there is a direct relationship between the number of viable tumour cells present at initiation of chemotherapy and curability (Chapter IV, Part I). Even with small cancers, we do not know and cannot detect whether tumour cells are present in the draining lymph nodes, so that a mild regimen could not be optimally effective. The future of the treatment of breast cancer therefore must primarily be concerned with the optimalization of adjuvant chemotherapy. The results of the Bonadonna and Fisher trials on adjuvant chemotherapy have already shown a difference in response whether patients were pre- or postmenopausal, in favour of the former group.

This implies that hormonal factors should be integrated in a search for optimalization of adjuvant chemotherapy. It is likely that the future improvements will, in time, further define and extend the optimal design of breast cancer therapy.

SUMMARY

Based on the early concept of centrifugal spread of tumour through surrounding tissues along lymphatic pathways to regional lymph nodes and formation of distant metastases at a later stage, radical mastectomy has been the routine treatment for most patients with cancer of the breast during the first half of the twentieth century. This procedure consists of an *en bloc* wide removal of tumour-containing breast, and both pectoral muscles with an axillary dissection. Dissatisfaction with the survival percentages led many surgeons to challenge this procedure with the hope of better results. Many of the challenging procedures were the consequence of the changed concepts that had resulted from new information concerning tumour biology. The performance of more lymph node dissections with the hope of removing the last cancer cell gave no better results. We know presently that tumour may have spread into the circulation very early in the course of the disease. It was then proposed that, since breast cancer should be regarded as a systemic disease in most patients, perhaps systemic antitumour factors should be exploited more fully. In fact, Crile challenged the classical concept of *en bloc* resection of the primary tumour and regional lymph nodes by propagating simple mastectomy for early operable breast cancer. Crile's choice for this procedure was based on the notion that retention of regional lymph nodes, grossly uninvolved by tumour, is important in maintaining a degree of systemic immunity against further growth of metastatic microfoci. It is the management of these regional lymph nodes that has created one of the most controversial problems in surgical oncology today and this has stimulated the research presented in this thesis.

The first part of the thesis is concerned with reviews of the literature on both the clinical and experimental management of breast cancer. Clinical series of different surgeons on the value of simple mastectomy for early operable breast cancer are controversial as well as are the experimental studies devoted to evaluating the specific unique role of the regional lymph nodes more fully. Crile's favour for simple mastectomy was based on experimental studies in which it was shown that, early after tumour transplantation, removal of the regional nodes had a detrimental effect on the antitumour reaction of the host. Another experimental and clinical argument relative to the importance

of regional lymph nodes was the finding that these structures are often enlarged and clinically palpable without containing tumour cells. More specifically, these responses in lymph nodes were tentatively identified as structural representations of immunological phenomena. These findings stimulated the investigation of the role of the regional lymph nodes on a morphological basis in this thesis.

For technical and ethical reasons, the design of therapeutic trials in cancer bearing animals is often simpler and interpretation is easier than in the clinical situation. There is no perfect animal model for any specific human cancer; however, it is important to recognize and exploit therapeutic principles whenever possible, since important therapeutic results often do carry across species bearing somewhat similar neoplasms. But, before giving any clinical significance to experimental data without fear of doing an injustice to patients with an experimentally found "better" therapy, one must critically consider the suitability of experimental models as models for clinical cancer. Because previous experimental models on the value of simple mastectomy had too many shortcomings in this regard, research was undertaken to find a reasonable experimental model for breast cancer which fulfilled a number of criteria. The finally selected mammary carcinoma model fulfilled these criteria and had essential metastasizing and therapy response characteristics which could be explored in evaluating different kinds of therapy (Chapter I, Part II).

From the experimental studies described in the literature, it was proposed that there are two major determinants as to whether regional lymph node removal has a detrimental effect, which are both difficult to evaluate in the clinical situation. One is the time after tumour transplantation in which regional lymph nodes are treated; the other is the antigenicity (as affecting prognosis) of the tumour.

Therefore, a study was made in order to determine whether the regional lymph node plays a major role in the morphological response to tumours. A defined antigenic and a nonantigenic tumour were used in a study in which lymph node morphology was quantitatively measured. Lymph nodes were cut in semi series and a morphometric analysis was done on the paracortical and cortical areas as major parts of the active lymph nodes. These latter parameters could be expressed in both absolute and relative numbers with confidence, since a strong correlation existed between the weights of lymph nodes and the summated surface areas. In order to determine whether the regional lymph

nodes play a major role in this response, two nonregional lymph nodes were analyzed in a similar way. It was observed that antigenicity defined as evoking an immune response which significantly influences tumour cell take and tumour cell growth, was of no relevance in evoking a morphological response. In absolute numbers, the response evoked was the strongest in the regional lymph nodes in both systems. The percentages paracortical and cortical areas reacting, however, were not essentially different from one node to another during the growth of both tumours. This observation, though determined *in vitro* already gives rise to some doubt about the question as to whether the regional lymph nodes are uniquely different from the rest of the lymphoreticular system. The findings also suggest that great caution must be exercised when interpreting morphological lymph node changes as evidence for immunological reactions interpretable as influencing tumour cell growth (Chapter II, Part II).

To evaluate the controversy *in vivo*, it was investigated whether regional lymph node removal at any stage of tumour growth had a favourable or unfavourable effect on survival after removal of primary tumours. Removal of tumours was performed at a stage when early metastases had taken place; this implied leaving a low tumour burden which could possibly be influenced by the host's immunological system. In three models which differed in antigenicity, there was no significant effect of regional lymph node removal on survival. In two tumour models which consistently showed lymph node metastases if left untreated, a significant reduction in regional recurrences was noted when regional lymph nodes were removed.

Since implications of variations in the extent of surgery cannot any longer be judged without taking into account the effect of adjuvant chemotherapy, a combination of drugs was applied in the most appropriate model, i.e., the nonantigenic mammary carcinoma model. A very pronounced reduction in lymph node metastases was observed when adjuvant combination chemotherapy was given after tumour removal. This was even the case at a level of drug treatment which was ineffective in prolonging survival. In this combined approach, more or less extensive surgery had no discernible effect on survival (Chapter III, Part II).

In view of these findings, regional lymph nodes can be regarded only as being quantitatively but not qualitatively different from the rest of the lymphoreticular system; they represent, however, favourable

sites for outgrowth of tumours as evidenced by the occurrence of regional recurrences.

In the overall discussion, it was attempted to integrate experimental results with clinical data into a "best" policy for early operable breast cancer. So far, clinical trials do not show a difference in survival whether regional lymph nodes are removed or irradiated versus no treatment. There are, however, preliminary data indicating that a significant number of patients treated by simple mastectomy required a delayed axillary dissection, since regional recurrences occurred. The risk of these recurrences with the simplified approaches to operable breast cancer should receive more critical attention when more data are obtained, since these recurrences can, even in the presence of haematogenous spread, greatly affect the quality of life. On the basis of the studies described no opinion can be given on the merits of replacing part of the surgery by radiotherapy. Therefore it was concluded that, for the moment, (modified) radical mastectomy represents the optimal surgical treatment for operable breast cancer.

Present trials on the value of chemotherapy as an adjunct to surgery have produced valid information that regional reccurrences can be reduced by drug treatment. Before application of less extensive surgery in breast cancer is justified by an effect of adjuvant chemotherapy, the presently running trials both on the value of simple mastectomy as well as on adjuvant treatment must be evaluated more fully. Similarly its effectiveness in combination with different surgical approaches should be tested in view of the present findings.

SAMENVATTING

Gedurende de eerste helft van de twintigste eeuw is de radicale mastectomie, bestaande uit en bloc verwijdering van mamma, beide pectoralis-spieren en de oksellymfklieren, de onbetwiste behandeling geweest voor patiënten met een operabel mammacarcinoom. Deze chirurgische behandeling was gebaseerd op de oude opvatting van tumor-verspreiding, nl. via locale doorgroei naar de regionale lymfklieren en, in een later stadium, optreden van haematogene metastasen. Daar de overlevingspercentages na deze behandeling teleurstellend waren, begonnen vele chirurgen andere technieken te ontwikkelen in de hoop betere resultaten te kunnen bereiken. Deze veranderde technieken waren mede het gevolg van veranderde opvattingen betreffende het proces van tumor-metastasering.

Het verwijderen van meer lymfklieren, met de hoop daarmee de laatste tumorcel te kunnen verwijderen, leverde geen betere resultaten op. We weten tegenwoordig dat er in een zeer vroeg stadium van mammacarcinoom reeds haematogene uitzaaiing kan plaatsvinden, zodat we carcinoma mammae als een systeemziekte moeten beschouwen. Dientengevolge werd voorgesteld systemische antitumor-faktoren beter te benutten. Crile propageerde ablatio mammae (zonder verwijdering van de axillaire lymfklieren) als behandeling voor carcinoma mammae in vroege stadia. De voorkeur van Crile om de axillaire lymfklieren niet te verwijderen was gebaseerd op het idee, dat regionale lymfklieren belangrijk zijn om de immunologische afweer tegen micrometastasen in stand te houden. Momenteel is het wel of niet verwijderen van deze regionale lymfklieren één van de grote controversiële problemen in de oncologische chirurgie. Deze controverse heeft het experimentele onderzoek, beschreven in dit proefschrift, gestimuleerd.

Het eerste deel van het boek geeft een overzicht van de literatuur, zowel voor wat betreft de klinische behandeling van mammacarcinoom als een aantal experimentele vraagstukken die hier nauw verband mee houden. De klinische resultaten van diverse chirurgen, verkregen bij verschillende modificaties van de radicale mastectomie evenals de experimentele gegevens over de waarde van het al of niet intact laten van de regionale lymfklieren, spreken elkaar tegen. Crile vond in experimentele studies dat verwijdering van regionale lymf-

klieren een nadelige invloed had op de anti-tumor-reactie van de gastheer.

Een ander argument ten aanzien van de specifieke functie van de regionale lymfklieren is de bevinding dat deze vaak vergroot zijn zonder tumorcellen te bevatten. De morfologisch herkenbare veranderingen in de lymfklieren, verantwoordelijk voor deze vergroting, werden tentatief geassocieerd met immunologische processen in de lymfklieren. Deze bevinding stimuleerde het onderzoek van lymfklieren op een morfologische basis.

Het is belangrijk klinisch-therapeutische vraagstukken te herkennen en uit te diepen in het experiment, omdat belangrijke experimenteel-therapeutische resultaten ook voor de mens kunnen gelden. Het ontwerpen van therapeutische experimenten met proefdieren met mammacarcinoom is uit technisch en ethisch oogpunt eenvoudiger en de interpretatie van de verkregen resultaten is gemakkelijker dan de uitvoering en interpretatie hiervan in de kliniek. Voordat aan de experimentele gegevens klinische relevantie gegeven kan worden, dient men kritisch te beschouwen of de resultaten in de experimentele modellen geschikt zijn voor klinische extrapolatie. De in de literatuur beschreven modellen over de waarde van het wel of niet verwijderen van regionale lymfklieren hadden in dit opzicht veel tekortkomingen; daarom werd voor het in dit proefschrift beschreven onderzoek getracht een beter experimenteel mammacarcinoom model te vinden. Het uiteindelijk geselecteerde model bleek aan een aantal, voor dit doel opgestelde, criteria te voldoen; in het bijzonder vertoonde de tumor met het menselijk mammacarcinoom vergelijkbare essentiële eigenschappen ten aanzien van metastasering en beïnvloedbaarheid door chirurgie en chemotherapie (Hoofdstuk I, deel II).

Uit de experimentele studies, beschreven in de literatuur, bleek dat er twee factoren kunnen zijn die bepalen of verwijdering van regionale lymfklieren een nadelig effect heeft. Beide factoren zijn moeilijk te evalueren in de klinische situatie, nl. tijd na tumortransplantatie waarin de lymfklieren worden verwijderd en antigeniciteit in de zin van beïnvloeding van tumorgroei.

In hoofdstuk II (deel II) werd in een experimentele studie nagegaan of regionale lymfklieren een belangrijke rol spelen in de morfologische respons, opgewekt door tumoren. Een antigene en een niet-antigene tumor werden hiervoor gebruikt. De antigeniciteit van beide tumoren werd door verschillende testen bepaald. Lymfklieren werden vervol-

gens in semi-serie-coupes gesneden en een morfometrische analyse werd op de paracorticale en corticale gebieden, als belangrijkste reactiecentra in de morfologische respons in lymfklieren, uitgevoerd. Deze laatste parameters konden zowel in absolute als in relatieve getallen uitgedrukt worden, daar een sterke positieve correlatie bestond tussen de gewichten en de gesommeerde oppervlakten van de lymfklieren. Om na te gaan of regionale lymfklieren een speciale rol spelen in deze respons werden twee niet-regionale lymfklieren op dezelfde wijze geanalyseerd. Waargenomen werd dat antigeniciteit, als eerder gedefinieerd, niet essentieel was om een morfologische reactie op te wekken. Absoluut gezien was de respons het sterkst in de regionale lymfklieren in beide tumorsystemen. De berekende percentages paracorticale en corticale gebieden in opeenvolgende lymfklieren waren echter, gedurende de groei van beide tumoren, niet essentieel verschillend. Deze waarneming, hoewel *in vitro* verkregen, wekt reeds enige twijfel op ten aanzien van de vraag of regionale lymfklieren unieke eigenschappen hebben in het lymforeticulaire systeem. De bevindingen suggereren ook dat men voorzichtig moet zijn morfologische veranderingen in lymfklieren te interpreteren als immunologische reacties die de groei van tumorcellen kunnen beïnvloeden.

Om de controverse *in vivo* te evalueren, werd nagegaan of verwijdering van regionale lymfklieren, in welk stadium van tumorgroei dan ook, een voor- of nadelige invloed had op overleving, nadat de primaire tumoren waren verwijderd. De verwijdering van de tumoren werd uitgevoerd op een tijdstip waarop in de meeste dieren metastasering had plaatsgevonden; dit hield in dat de resterende hoeveelheid tumorcellen mogelijk door het immunologisch-afweerapparaat van de gastheer kon worden beïnvloed. In drie proefdiermodellen, die verschilden in antigeniciteit van de tumoren, werd geen significant effect op overleving gevonden van verwijdering van regionale lymfklieren. In twee modellen, waarin altijd lymfkliermetastasen optraden, werd een sterke reductie in regionale recidieven waargenomen, indien regionale lymfklieren werden verwijderd.

Daar resultaten van variaties in de uitgebreidheid van chirurgie niet langer volledig beoordeeld kunnen worden zonder daarbij het effect van toegevoegde chemotherapie te betrekken, werd een combinatie van cytostatische middelen gegeven in het niet-antigene mammacarcinoom model, dat het meest geschikt bleek te zijn. Een sterke reductie in het aantal lymfkliermetastasen werd gevonden indien aan-

sluitend aan tumorverwijdering combinatiechemotherapie werd gegeven. Deze reductie trad zelfs op wanneer een dosering werd gegeven, die niet resulteerde in uitstel van sterfte. In deze gecombineerde benadering had meer of minder uitgebreide chirurgie geen duidelijk effect op overleving (Hoofdstuk III, deel II).

Op grond van deze bevindingen werd geconcludeerd dat regionale lymfklieren zich uitsluitend kwantitatief doch niet kwalitatief onderscheiden van de rest van het lymforeticulaire systeem. Zij vertegenwoordigen echter een goede voedingsbodem voor de uitgroei van tumorcellen, gezien het frequent optreden van lymfkliermetastasen.

In de algemene discussie werd gepoogd deze experimentele resultaten te integreren met de klinische gegevens om tot een gedragslijn voor de behandeling van het operabele carcinoma mammae te komen. Tot nu toe vertonen de resultaten van klinische trials, die radicale mastectomie met ablatio mammae vergelijken, geen verschil in overleving. Sommige voorlopige gegevens tonen echter aan dat bij een significant aantal patiënten, uitsluitend behandeld met ablatio mammae, een heroperatie noodzakelijk was wegens het optreden van een recidief in de axillaire lymfklieren. Het risico van deze recidieven bij de minder uitgebreide operatieve behandeling van mammacarcinoom dient kritisch te worden beschouwd, daar deze recidieven, zelfs in de aanwezigheid van haematogene metastasen, de kwaliteit van het leven nadelig kunnen beïnvloeden. Op grond van de beschreven studies kan geen oordeel gegeven worden over de voordelen van gedeeltelijk vervangen van chirurgie door radiotherapie. Aan de hand van de huidige gegevens werd geconcludeerd dat de (gemodificeerde) radicale mastectomie de optimale chirurgische behandeling voor mammacarcinoom is.

De huidige klinische trials over de waarde van langdurige chemotherapie na radicale mastectomie duiden op een vermindering van het aantal regionale recidieven dankzij de chemotherapie. Echter, voordat minder uitgebreide chirurgie als behandeling van mammacarcinoom gerechtvaardigd is vanwege het effect van chemotherapie, dienen de huidige trials, zowel ten aanzien van de waarde van ablatio mammae als ten aanzien van langdurige chemotherapie, uitgebreid geëvalueerd te worden. Evenzeer moet de effectiviteit van de chemotherapie in combinatie met verschillende chirurgische benaderingen getest worden, gezien de bevindingen in dit proefschrift.

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