THE NEED FOR PRAGMATISM IN EPIDEMIOLOGY, ILLUSTRATED IN RESEARCH ON THE DETERMINANTS OF ESTROGEN RECEPTOR STATUS IN BREAST CANCER

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ABSTRACT

A survey was planned to explore how estrogen receptor (ER) status of breast cancer was related to dietary and reproductive factors in postmenopausal patients from Toronto. Unforeseeable circumstances created major delays and, even after enhancements of design, the number of subjects who could be included was seriously reduced. As statistical power had thus become undesirably low, emphasis is placed on the realities of epidemiologic research of this nature, i.e. how inevitable difficulties arise, have to be identified and, at the very least, mitigated. Despite small numbers, the odds of positive ER status were found to be low for patients with many pregnancies and high for those with one or two pregnancies, but intermediate for cases who had never been pregnant. This very strong association underlines the weaknesses of those with measured dietary intakes, which appear unlikely to be of major relative importance.

RESUME

Nous avons planifié un sondage auprès de patientes postménopausiques de Toronto afin d'étudier comment l'état de récepteur d'oestrogène dans les cas de cancer du sein était relié à l'alimentation et à la reproduction. Des circonstances imprévisibles ont causé d'importants délais et malgré des améliorations à la conception du projet, le nombre de sujets potentiels a été grandement réduit. La fiabilitié statistique d'un tel échantillon étant très basse, nous avons mis l'accent sur les difficultés de recherches épidémiologiques de cette nature, c'est-à-dire comment d'inévitables problèmes se posent, comment ils doivent être identifiés et, autant que possible, leur impact atténué. Malgré le petit nombre de sujets, les chances d'un état positif des récepteurs d'oestrogène nous sont apparues faibles chez les patientes ayant eu de nombreuses grossesses et élevées chez celles n'ayant eu qu'une ou deux grossesses, tout en restant moyennes chez les sujets n'ayant jamais été enceintes. Cette très forte association souligne les faiblesses de celle avec un régime alimentaire, qui ne semble pas être d'une importance majeure relativement à l'autre.

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PREFACE

My first enrollment into the PhD program was in September 1977; because I held a master's degree from the Department of Epidemiology and Health, I was entered into the second year (i.e. PhD 2). This thesis is, then, being completed at the end of PhD 11. However, the time available for the relevant research has been less than five years. A full explanation was given in the thesis originally submitted, but has been removed at the request of the Oral Examination Committee.

In January 1980, Dr. G. E. Eyssen, with whom I had worked as a teaching assistant at McGill University before we both moved to Toronto, offered to act as my thesis supervisor. Although the understanding was that my research would have to be in cancer epidemiology, this offer was gratefully accepted. At that stage, I abandoned earlier work in different areas, and embarked on my present thesis research project.

Progress was slow because none of us (neither myself, nor Dr. Eyssen, nor any faculty member I consulted at McGill) had expert knowledge in the field of estrogen receptors in breast cancer. It took many months to delineate the issues, and for me to gain some understanding of the underlying endocrinological mechanisms. In addition, two preliminary investigations were found to be necessary (see sections 1.3 and 3.3 of this thesis).

Throughout most of the two years from June 1981, I was ill, and progress was negligible.

In July 1984, Dr. W. O. Spitzer, the recently appointed chairman of the renamed Department of Epidemiology and Biostatistics, agreed to let me finish my degree, subject to certain conditions, including that my supervisor would have to be a member of the McGill Department. Dr. F. D. K. Liddell volunteered to act in this capacity, and this offer was gratefully accepted. He and I had already discussed and agreed the principles of the necessary re-design of the project.

In March 1986, I became too ill to work, and could not re-start until four months later.

In the less than five years effectively spent on this research, in addition to designing and carrying out the survey (into which I incorporated two methods of analysis), and to writing up the thesis, I did the following:

- conducted a feasibility project related to medical chart information;
- carried out an examination of the age-specific incidence of ER- and ER+ breast cancer for Ontario in 1981;
- expanded - and corrected - the food frequency questionnaire; and
- designed, pre-tested and further refined a questionnaire related to reproductive and medical histories.

[The remainder of this Preface is as originally submitted, with the same pagination.]

Claims for Originality

When this survey was designed, there had been few investigations of the relationships between ER status and breast cancer risk factors. Several other studies have been reported in the last five years; nevertheless, the present research is the first to include direct measures of dietary intakes.

Two questionnaires were required for this project; one had to be designed and tested, the other had to be modified (and corrected). A study of the feasibility of collecting information from patients' hospital records, and of their comprehensiveness, was an important preliminary investigation. As I had carried out the examination of the age-specific incidence of ER- and ER+ breast cancer in Ontario in 1981, I had been invited to be senior author of the article, but had to decline because of my commitment to the present researeh.

To help resolve controversy as to whether discriminant analysis or logistic regression should be used on data such as those I had collected, both forms of discriminatory analysis were performed. The agreement of the findings from the two analytical methods was close; this is an empiric finding of practical importance for biostatisticians.

Pitfalls and problems in conducting an epidemiologic survey are, of course, far from unusual, but they have seldom been documented. Indeed, they have often been glossed over, leaving the impression that the protocol has been followed more closely than has been the case. This thesis, however, describes difficulties that arose in the survey, and explains how they were handled. This aspect is of undoubted importance, and will be of major interest to future researchers in this, and in many other, areas of epidemiologic survey.

Organization of the thesis

This thesis is divided into eleven chapters. The first sets the stage for the development of the research question, by reviewing the present knowledge on breast cancer, estrogen receptors and their inter-relationship. It has been improved by the careful criticism of Dr. R. J. B. King (Head, Hormone Biochemistry Department, Imperial Cancer Research Fund Laboratories, London), who endorsed the main lines of argument, particularly in his field of expert knowledge.

Chapter 2 describes the development of the methodological approach to this project - early plans, their shortcomings (particularly in the light of restrictions imposed in 1984), and how they were salvaged. The next chapter gives details of the measurement instruments used and how they were developed.

Chapters 4 and 5 describe, respectively, the findings and the discriminatory analyses of breast cancer patients with ER negative and ER positive tumours. Methodological issues are discussed in Chapters 6 through 9: those pertaining to fieldwork in Chapter 6; considerations of statistical power and related matters in Chapter 7; problems associated with measurement in Chapter 8; and comparison of the analytical methods in Chapter 9.

Despite the shortcomings imposed on it, the survey did yield findings of considerable epidemiologic interest; these are discussed in Chapter 10. The final chapter provides conclusions, with suggestions for further research.

Chapter 1

BREAST CANCER AND ESTROGEN RECEPTORS: EPIDEMIOLOGY AND ETIOLOGY

In this chapter, the knowledge about breast cancer and estrogen receptors is reviewed.

Breast cancer has been the subject of much epidemiologic research, in Canada, in North America, and indeed throughout the world. However, the present intention is not to summarize all this material but rather, after a short comment on the burden of breast cancer in North America, to review briefly epidemiologic investigations in which distinction was made between premenopausal breast cancer and postmenopausal disease (section 1.1).

Section 1.2, which includes the appropriate definitions, provides some background information on estrogen receptors, an account of the development of an assay, and its use as a prognostic indicator of response to treatment of breast cancer. The section continues with a review of how estrogen receptor (ER) status of breast cancer is related to other factors.

The next section (1.3) presents a detailed examination of the relationship of ER status with age, through a description of work which the author herself carried out on estrogen receptor status in breast cancer in the Province of Ontario.

Section 1.4 reviews the knowledge (up to July 1984) on the relationship between breast cancer risk factors and ER status; some inferences drawn from this literature are presented in section 1.5. An additional section (1.6) describes work published more recently, and so too late to be taken into account in preparing the proposal. Most publications after December 1986 have necessarily been excluded.

1.1 The burden of breast cancer in North America

The burden of breast cancer on the lives of North American women is heavy. Kelsey (1979) has estimated that, in the United States, 100,000 cases of the disease were diagnosed each year between 1973 and 1976, in a population of about 110 million females; in this period, the annual death toll was over 30,000, roughly 5% of total female mortality. In Canada, a woman's lifetime expectation of developing breast cancer has recently been quoted as 9.3 % [for the year 1981] (Canadian Cancer Society, 1987). In the Province of Ontario, with a female population of about 4.4 million, 1435 deaths of women in 1982 were attributed to breast cancer, accounting for 20% of all (7070) cancer deaths, or 5% of the total mortality (29,254) in females (OCTRF, 1983). Within the age group 35-54 years, malignant neoplasms were responsible for half of all deaths; 32% of these neoplasms were breast cancers (OCTRF, 1983).

Despite the rising incidence of lung cancer among women, the breast remains the most common single site of cancer morbidity and death in the North American female population (Canadian Cancer Society, 1987). Among Ontarian women, in 1982, and women in the United States, in 1978, there were more deaths, i.e. 1,435 and 28,299 respectively, from breast cancer [rubric

174 in the 9th revision of the International Classification of Diseases] than the 953 and 19,894 deaths from cancer of the trachea, bronchus or lung [rubric 162] or even than the 1,340 and 24,517 deaths from cancer of the stomach, colon, or rectum [rubrics 151-154] - although the numbers of deaths from all gastro-intestinal malignancies [rubrics 150-159] were higher (OCTRF, 1983; Doll and Peto, 1981). However, as can be seen in Table 1.1, in women who died at the age of 65 years or more, stomach and colo-rectal cancers [rubrics 151-154] accounted for substantially more deaths than did cancer of the breast, or cancer of trachea, bronchus or lung.

Table 1.2 shows the numbers of surgical procedures for breast cancer among women aged 50 to 79 years, in Ontario, over the four years 1981-1985. In this period, numbers increased by over 20% throughout the province; this trend was a reflection of similar increases in the six hospitals at which the most breast cancer surgery is performed in Toronto, in the other 21 Toronto hospitals, and in hospitals outside the capital. The reasons for such increase are not understood, because - at least in Canada as a whole - age-standardized incidence rates for female breast cancer had remained almost unchanged (between 65 and 71 cases per 100,000 population) from 1972 to 1982, and mortality increased only slightly from 1981 to 1985 (when age-standardized rates per 100,000 population were 23 and 24, respectively) (Canadian Cancer Society, 1987).

There are marked international differences in the rates of incidence and mortality of breast cancer in females. Incidence in the 1970's [standardized for age to world population], presented in the (World Health Organization) International Agency for Research on Cancer's publication Cancer in Five Continents, Fourth Edition (Waterhouse et al, 1982) ranged

TABLE 1.1: Number of deaths due to malignant neoplasms in females, by site and age, Ontario, 1982

Site of malignancy*	Ontario, 1982		
	Age at death		
	< 65	65 +	All ages
Breast [174]	732	703	1,435
Trachea, bronchus lung [162]	466	487	953
Stomach, colon rectum [151-154]	375	965	1,340
Other sites	1,239	2,103	3,342
Total	2,812	4,258	7,070

Source: OCTRF (1983)

* Figures in square brackets are rubrics in the International Classification of Diseases (9th revision).

TABLE 1.2: Surgical procedures for the treatment of breast cancer in Ontario for women aged 50 to 79 years

	Years, April to March			
	1981-82	1982-83	1983-84	1984-85
Hospitals (27) in Toronto				
The six performing the most breast surgery:	380	426	449	453
Others (21):	375	360	418	444
Total:	755	786	867	897
Hospitals outside Toronto:	1500	1639	1716	1839
Total:	2255	2425	2583	2736

Source: Ontario Ministry of Health

from a rate of 12 per 100,000 population, in Dakar (Senegal), to rates roughly seven times higher - in Californian whites and in both Caucasian and Hawaiian residents of Hawaii. Patterns are difficult to discern: at least geography and race appear to be determinants. Even within Canada, the 1973-77 incidence rates were highly variable; in Alberta, British Columbia, the Maritime Provinces, Quebec, and Saskatchewan, they were between 60 and 73 per 100,000 population, but only 50 for Newfoundland. The only figure for Ontario in Waterhouse et al (1982) was 65 per 100,000 population - for the years 1969-71.

An inference commonly drawn from studies of migrants is that international variation in cancer incidence rates cannot be due entirely to genetic factors: there have been well-documented instances in which the breast cancer rates of immigrants were much closer to those of their adopted country than to those of their country of birth (Staszewski and Haenszel, 1965; Buell, 1973; Kelsey, 1979).

The importance of the disease is unquestioned. In all countries, breast cancer is a condition of prime concern.

1.1.1 Premenopausal and postmenopausal breast cancer

There has been much speculation that breast cancer manifest before the menopause is a different disease entity from postmenopausal breast cancer. Evidence for separate etiologies comes from three types of investigation: (a) international differences in the shapes of age-specific incidence curves of breast cancer; (b) studies of correlation between national mortality rates of disease and national averages of possible etiological factors; and (c) analytical investigations, which have examined many variables as potential risk factors for breast cancer.

(a) National incidence curves

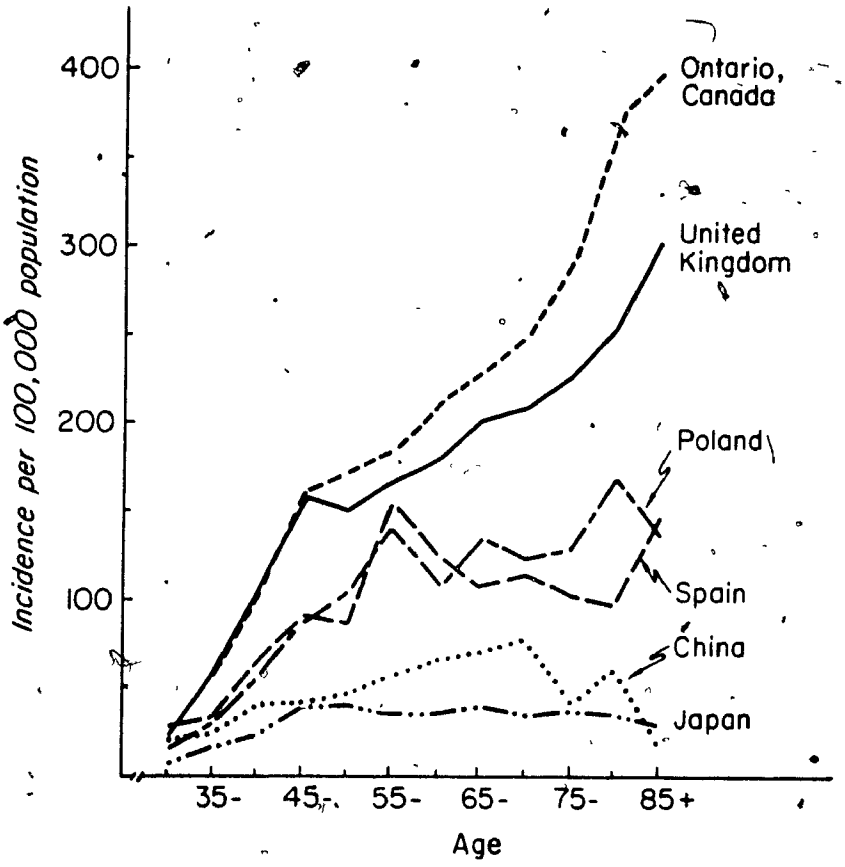
Figure 1.1 shows the age-specific incidence curves (as frequency polygons) for six countries (adapted from Waterhouse et al, 1982), illustrating those where the risks for breast cancer are relatively high (Ontario and the United Kingdom), intermediate (Poland and Spain) and low (China and Japan). It must be noted that a frequency polygon appears to imply a change in rate between two adjacent ages when the rate plotted at each point is, in fact, the average for the age range; care in interpretation is, therefore, required.

In all six of the selected countries, incidence was quite low under 30 years of age. However, at ages 40-44, rates ranged from 25 per hundred thousand (Japan), through 68 (Spain), to 106 (United Kingdom). From Waterhouse et al (1982), it can be seen that in some countries (especially in North America and northern Europe), breast cancer rates at older ages (from about age 45-54 years) were substantially higher the older the group; consequently, the overall risk for breast cancer was high. In certain other countries (such as several in southern Europe and South America), rates for all postmenopausal ages were broadly similar; in such countries, overall rates were intermediate. In most Asian and African countries, the incidence rates were lower in older women (de Waard, 1979; Kelsey, 1979); in these countries, the overall risk was low.

(b) Correlation studies

Correlations between national breast cancer mortality rates and corresponding environmental factors have been examined for definitely postmenopausal women [aged 65-69 years] and also for mainly premenopausal women [aged 40-44 years] (Hems, 1970). The rates for 22 countries were

FIGURE 1.1: Age-specific incidence rates of breast cancer



Source: Rates from Waterhouse et al (1982)

positively correlated with population estimates of mean daily percapita intake of total calories, fat, meat protein, total carbohydrate and sugar; the correlation coefficients were all higher (except those with carbohydrates) for the older women than for the younger. The coefficients of multiple correlation between (A) age-standardized breast cancer rates and (B) intakes of sugar and of fat were .86 in the older women and .68 in the younger group. However, the exclusion from consideration of other factors that may well also have been closely associated with diet suggests that these may possibly be "nonsense correlations" (Sokal and Rohlf, 1969). The usage of intakes per capita (males and females) is a further complication. Certainly, it is unlikely that such high proportions of the international variation in breast cancer mortality rates as might be inferred from the correlation coefficients, could be fully explained by these dietary factors.

(c) Analytical investigations

(i) Several reproductive variables have been examined as risk factors, treating premenopausal and postmenopausal women separately.

Table 1.3 summarizes findings concerned with a late age at menarche. Stavraky and Emmons (1974), Paffenbarger et al (1980), Lubin et al (1982), Pike et al (1981b) and Choi et al (1978), all found risk of breast cancer decreased in those whose menstruation had been of late onset. In the first three investigations mentioned, the association was reported only in premenopausal women (although older women had also been studied); the fourth investigation was only of young premenopausal women. However, Choi et al (1978) found the association only for postmenopausal women, although the premenopausal had also been studied. These findings had been obtained with rather different methodologies, in particular, in the sources of patients

TABLE 1.3: Relationship between late age at menarche and breast cancer risk by menopausal status

Investigation	Risk of breast cancer	
	premenopausal	postmenopausal
Stavraky and Emmons (1974)	↓	..
Paffenbarger et al (1980)	↓	..
Lubin et al (1982)	↓	..
Pike et al (1981b)	↓	n.a.
Choi et al (1978)	..	↓

Legend:

↓ = decreased risk of disease

↑ = increased risk of disease

.. = association not demonstrated

n.a. = not applicable

and of referents. It remains evident, however, that the disagreement between the results of Choi et al (1978) and those from the other four investigations cannot be attributed to obvious differences in the methodology.

A summary of results in relation to age at birth of first child is presented in Table 1.4. The risk of breast cancer was positively associated with a late age at the birth of first child (sometimes after the age of 25, sometimes at ages greater than 30), according to Brinton et al (1979) and Paffenbarger et al (1980) - in both premenopausal and postmenopausal breast cancer patients - according to Stavraky and Emmons (1974) and Lubin et al (1982) - in postmenopausal subjects only - and according to Craig et al (1974) - only in younger patients. On the other hand, no such association was reported by Pike et al (1981b) in their project on premenopausal breast cancer patients. Meanwhile, Choi et al (1978) had failed to demonstrate association whether or not cases were stratified by menopausal status; they themselves suggested they might have over-matched on some factor related to age at first birth. Indeed, the important inconsistencies in the results presented in Table 1.4 may have been due to differences in selection of cases and referents: as instances, Craig et al (1974) included only prevalent cases of breast cancer surviving at least five years, while Stavraky and Emmons (1974) used other cancer patients as "controls". Another possible reason for discrepancy might have been differing definitions of premenopausal and postmenopausal status.

In summary, although different roles for reproductive factors in the premenopausal and postmenopausal breast cancer have sometimes been demonstrated, the evidence is not strongly supportive of distinct etiologies.

TABLE 1.4: Relationship between late age at birth of first child and risk of breast cancer by menopausal status

	Risk of breast cancer	
	Premenopausal	Postmenopausal
Investigation		
Brinton et al (1979)	↑	↑
Paffenbarger et al (1980)	↑	↑
Stavraky and Emmons (1974)	..	↑
Lubin et al (1982)	..	↑
Craig et al (1974)	↑	..
Choi et al (1978)	..	..
Pike et al (1981b)	..	n.a.

Legend:

↓ = decreased risk of disease

↑ = increased risk of disease

.. = association not demonstrated

n.a. = not applicable

(ii) Dietary components, weight and Body Mass Index

Weak associations between breast cancer in both premenopausal and postmenopausal women and increased intakes of food components, especially total fat, were reported by Miller et al (1978). These authors suggested that the weaknesses may have arisen from two causes: first, the inability of their dietary methods (the use of current or short recall diet histories) to quantify accurately dietary habits of years past; and second, their use of neighbourhood "controls" - who might well have had eating habits similar to those of the cases - rather than referents from the general population.

In the largest prospective study of diet and breast cancer, Hirayama (1979) demonstrated an increased risk of breast cancer among Japanese women who ate meat daily - but the association appeared only in those 55 years of age and older.

The results of examining the effects of weight and Body Mass Index (BMI; see for example Billewicz et al, 1962; Lee et al, 1981; Lee et al, 1982) on risk of breast cancer are summarized in Table 1.5. Where an increased risk of disease associated with heavy weight or high BMI has been demonstrated, it was only in postmenopausal patients, but Burch et al (1981) failed to find such a relationship in patients aged 65 to 79 years. Further, in that part of their study concerned with premenopausal women, Paffenbarger et al (1980) reported a reverse association. These variously discrepant results may be due, in part, to the problems elderly women have in recalling weight several years previously.

Thus, the evidence for different etiological roles of dietary factors for premenopausal and postmenopausal breast cancer is not entirely convincing.

TABLE 1.5: Relationship between weight and Body Mass Index and risk of breast cancer by menopausal status

	Risk of breast cancer	
	Premenopausal	Postmenopausal
Heavy weight:		
Choi et al (1978)	..	↑
de Waard and Baanders-van Halewijn (1974)	n.a.	↑
Burch et al (1981)	n.a.	..
High body mass index:		
Paffenbarger et al (1980)	↓	↑
de Waard and Baanders-van Halewijn (1974)	n.a.	↑

Legend:

↓ = decreased risk of disease

↑ = increased risk of disease

.. = association not demonstrated

n.a. = not applicable

1.1.2 Hormonal influences on breast cancer

Despite the interest in the possibility of differing etiologies for premenopausal and postmenopausal breast cancer, the development of the assay for estrogen receptors in malignant breast tumours provided the first pathological or clinical feature that appeared to vary according to menopausal status. Breast cancers arise in tissues which are commonly affected by the action of hormones (Kirschner, 1977). There is much evidence that endogenous hormones contribute to breast cancer risk, but there is uncertainty about the exact mechanisms of their effects (Kelsey, 1979; Thomas, 1984). The influences of hormones on breast cancer are considered below only in the context of the estrogen receptors measured in the tumour. Kirschner (1977), Kelsey (1979) and Thomas (1986) discuss in detail the role of hormones in the etiology of breast cancer.

1.2 Estrogen receptors in breast cancer

The discovery of estrogen receptors, and the biochemical mechanism underlying their function in hormone-dependent tissues, were the results of experiments in which a radio-labelled estrogen was used as a marker for the receptor to which it binds. A complete discussion of the underlying biochemical mechanisms is outside the scope of this thesis, but can be found in summaries by Jensen et al (1982) and Leung (1982).

Mammalian tissues related to reproduction need the presence of sex hormones in order to grow and function properly. In experimental work involving the administration of tritium-labelled estrogen to animals, hormone-dependent tissues were found to differ importantly from tissues that were hormone-independent, in that the former attracted estradiol with high

affinity, indicating that specific estrogen binding components were present (Jensen et al, 1971). These components are subsequently referred to as estrogen receptors. [They have also been called estrophilin but for clarity this term is not used in this thesis.] Extensive in vivo and in vitro studies demonstrated the uptake of estradiol by its receptor, and led to a means of distinguishing between estrogen-responsive tissues, that contain receptor proteins, and non-responsive tissues, which it was thought did not possess these receptors. It is, however, now suggested that possibly all mammalian tissues contain small amounts of estrogen receptor, although not at detectable levels; hormone dependent tissues are distinctive in the magnitude of their receptor content (Jensen et al, 1982).

The original understanding of the mechanism of the interaction of the steroid hormone, its receptor and the target cell, was developed from research utilizing rodent and human tissues. Very simplistically, it is as follows:-

> The estrogen enters the cell (probably by passive diffusion) and binds to the unoccupied estrogen receptors.
>
> This combination of hormone and receptor results in activation to a form that binds to DNA.
>
> Interaction with the chromatin in the nucleus then stimulates RNA synthesis.
>
> This in turn ultimately results in DNA synthesis, formation of certain breast cell proteins, cell division and tissue growth (Witliff,1984; Stanford et al, 1986).

There is now debate whether the unbound estrogen receptors are indeed located in the cytoplasm or, as suggested by Schrader (1984), Welshons et al (1984), and Stanford et al (1986), mainly in the nucleus.

The first work which indicated that breast cancer tumours might possess the property of hormone interaction was that of Folca et al (1961). Ten breast cancer patients about to undergo adrenalectomy were injected with tritium-labelled estrogen. The uptake of the radio-labelled hormone by the tumour, as compared to that by the skeletal muscle, was greater in the four patients who subsequently experienced remission than in the six patients who did not respond. Skeletal muscle tissue was used as a "negative control" in that it was not expected to respond to the hormone administration. Later work by Jensen and his colleagues (1971) not only demonstrated the existence of these receptors in human breast cancer tissue, but suggested that measurement of receptors might be a valuable tool in predicting response to hormone therapy.

1.2.1 Assays of tumours for estrogen receptors

The methods adopted for the clinical measurement of estrogen receptors depend on the incubation of a cytosol preparation with radioactive estrogen and the determination of the amount of radioactive hormone bound to the receptors. This determination is by one of two procedures: multipoint titration, using dextran-coated charcoal, or sucrose gradient analysis, by ultracentrifugation [as described by Jensen et al (1982) and Witliff (1984)]. The level of receptor is usually expressed in femtomoles of radio-labelled estrogen per milligram of cytosol protein (usually abbreviated to "fmol/mg"). This quantitative value can be reported as such or can be reduced to a statement of "status" (usually ER- or ER+). The status in turn

may be determined from one cut-off point; or from two, in which case, there exists a class of "ER intermediate". In what follows, these definitions apply:-

ER level: the result of an assay for estrogen receptors, performed on the breast tumour, expressed as fmol/mg;

ER status: the classification (as ER- or ER+) of the results of an assay for estrogen receptors performed on a malignant breast tumour;

ER- tumour: a tumour for which the assay level is below the (lower) cut-off;

ER+ tumour: a tumour for which the assay level is at least as high as the (upper) cut-off.

Recently, other methods of detecting estrogen receptors have been proposed (Van et al, 1984); they include an immunocytochemical assay making use of monoclonal antibodies (King and Greene, 1984). These methods are, however, not yet in widespread use for clinical purposes; in particular, they are not employed in laboratories in Toronto.

1.2.2 Estrogen receptor status and prognosis of breast cancer

Over the past 15 years, research has indicated that the level of estrogen receptor in breast cancer is an indicator of prognosis. Criteria for patient definition, methods of endocrine therapy, and assessment of response have often differed - as have the methods of assay and the cut-off levels. Nevertheless, it has been found that women with tumours that are ER+ (or

with high ER levels) are more likely to respond to hormonal therapy and have better survival rates than patients with ER- tumours (or very low ER levels) (Allegra, Lippman, Simon, et al, 1979; Bishop et al, 1979; Cooke et al, 1979; Desombre et al, 1979; Hahnel et al, 1979; McGuire, 1979; Furmanski et al, 1980; Lippman and Allegra, 1980; Westerberg et al, 1980; Croton et al, 1981; Godolphin et al, 1981; Kinne et al, 1981; Benson et al, 1982).

The relationship between response to chemotherapy and ER status has also been examined, but with mixed results (Kiang et al, 1978; Hilf et al, 1980; Lippman and Allegra, 1980; Stanford et al, 1986).

1.2.3 Estrogen receptor status of breast cancer, and disease parameters

Associations with location, laterality, and size of tumour have been weak (Rosen et al, 1975; Allegra, Lippman, Simon, et al, 1979; Allegra, Lippman, Thompson et al, 1979; Elwood and Godolphin, 1980; Mason et al, 1982; Montgomery et al, 1985). However, Allegra, Lippman, Simon, et al (1979) did find that their ER+ patients contained a high proportion of node negative cases, or early stage of disease.

As to tumour grade, Rosen et al (1975), Maynard et al (1978), Rich et al (1978), Martin et al (1979), Elwood and Godolphin (1980), McCarty et al (1980), Fisher et al (1981), Lesser et al (1981) and Thorsen et al (1981) have confirmed that when the tumour is of low grade it tends to be ER+, when of high grade to be ER-.

With regard to histologic type, high proportions of medullary cancers have been found to be ER- (Rosen et al, 1975; Fisher et al, 1981; Lesser et al, 1981), whereas in lobular cancers high proportions were ER+. However, these results may simply reflect the fact that medullary cancers are usually

poorly differentiated (Stanford, 1986). No associations with histolgic findings were found by Hildreth et al (1983).

1.2.4 Estrogen receptor status of breast cancer and age

Early North American investigations of estrogen receptors suggested the proportions of ER- and ER+ tumours were different in premenopausal and in postmenopausal patients. In nine case series, the proportions of ER- and ER+ tumours were approximately equal among premenopausal patients, while among postmenopausal cases there were substantially fewer ER- tumours than ER+ (McGuire et al, 1975; DeSombre et al, 1978; Knight et al, 1978; McGuire, 1978a; McGuire, 1978b; Rich et al, 1978; Allegra, Lippman, Simon et al, 1979; Cooke et al, 1979; Croton et al, 1981).

There are international differences in the proportions of ER- and ER+ tumours by menopausal status. The proportions of ER- breast cancers tended to be higher in Japanese postmenopausal patients than in their counterparts in Western countries (Nomura et al, 1977; Nomura et al, 1984). In Beijing, Xu et al (1983) found a higher proportion of ER- tumours in Chinese patients than the corresponding proportions reported in the Western literature.

The ER level of the tumour has been found higher among older groups of women (McCarty et al, 1983; Thorpe et al, 1983). Within the age range containing both premenopausal and postmenopausal patients, little association has been found between menopausal status and ER status; it might thus be inferred that it is unimportant whether the menopause has been reached or not (Elwood and Godolphin, 1980; Lesser et al, 1981). Among 735 breast cancer cases presenting at a treatment institution in British Columbia, what were called "incidence rates" (but which were estimated from the numbers of cases at that one hospital and the age-specific population in

the province) of ER+ tumours were higher the older the age-group, while the corresponding rates of ER- tumours were fairly consistent at ages greater than 45 years (Elwood and Godolphin, 1980). These authors noted that their ER- and ER+ curves resembled the total breast cancer incidence curves of countries with, respectively, low and high overall risks for breast cancer.

None of these investigations included all the breast cancer patients in the population, so biases in case selection could have contributed to the apparent association between ER status and age.

1.3 Estrogen receptor status in breast cancer and age, Ontario 1981

The author assembled population-based information on all incident cases of breast cancer in Ontario, in 1981, for whom ER status was determined. This work has been summarized by McKeown-Eyssen et al (1985), but is described more fully in this section.

In Ontario, the cost of steroid receptor analysis of breast cancer tumours has been met since 1980 by the Ontario Health Insurance Plan. Surgical specimens are submitted from the hospitals to one of six assigned laboratories authorized to perform the assays. In five of these laboratories (including the two in Toronto), ER status is determined using the dextran coated charcoal method; the details of the assay techniques as provided by the two Toronto laboratories are given in Annex I. [The sixth employs the sucrose density gradient procedure, but this fact is irrelevant to the thesis research.]

The six laboratories used several different criteria for classifying a tumour as ER- or ER+; these are summarized in Table 1.6, which also

TABLE 1.6: Determination of ER status in authorized laboratories in Ontario, 1981

Laboratory code	Criterion for classifying an assay as ER-	Criterion for classifying an assay as ER+	Assay* method
H	< 3 fmol/mg	10 fmol/mg or more	DCC
K, N	< 5 fmol/mg	10 fmol/mg or more	DCC
J	< 10 fmol/mg	10 fmol/mg or more	DCC
L	< 10 fmol/mg	10 fmol/mg or more	SDG
M			
premenopausal women:	< 10 fmol/mg	10 fmol/mg or more	DCC
postmenopausal women:	< 10 fmol/mg	20 fmol/mg or more	DCC

* DCC = Dextran coated charcoal

SDG = Sucrose density gradient

indicates the mode of assay. In four laboratories, an "intermediate" (sometimes called "equivocal") classification of ER status was as follows: 3 to 9 fmol/mg in laboratory H; 5 to 9 fmol/mg in laboratories K and N; and 10 to 19 fmol/mg (postmenopausal women only) in laboratory M.

The classification of tumours into ER- and ER+ groups might therefore have been expected to differ between laboratories for three reasons: the dependence of the definition of ER+ on menopausal status in one laboratory; the varying width of the ER intermediate class; and the different assay methods. However, the directors of the laboratories have carried out interlaboratory studies which demonstrated good agreement among five laboratories in terms of the classification of ER- and ER+ (Ryan et al 1985). The sixth laboratory was not in accord but, unfortunately, has not been identified.

To receive remuneration, the laboratories submit to the Ontario Cancer Treatment and Research Foundation (OCTRF) each month a listing of every assay performed, together with some information relating to the patients. The report form (designed in 1980 by the author and Dr. Eyssen) provided the material for this investigation.

Numbers of new cases of breast cancer diagnosed in Ontario in 1981 were obtained from the Ontario Cancer Registry. There were, in total, 3908 cases of primary breast cancer in women 30 years of age or more resident in Ontario in 1981: estrogen receptor assays were requested by physicians on 3226 (or 82.5%), and laboratory reports were assembled for all these 3226 assays. Each tumour was then classified (according to the practice of the laboratory in which the assay had been performed; see Table 1.6) as ER-, ER equivocal, or ER+, and also by the age of the patient. Information on

receptor level or on age was missing for 173 women (5.4% of the tumours assayed); they were excluded, leaving 3053 tumours for study.

Table 1.7 gives details, by age, of the population of females resident in Ontario in 1981, the new cases of primary breast cancer and the incidence per hundred thousand population, together with the numbers of assays performed for which age and receptor level were available. The gradient of disease incidence with age was very steep, as expected. The final column of this table shows that the percentage of tumours assayed varied substantially with age; it was close to 85% for patients aged 30-64 years, around 75% for those 65-74, and much lower, 59%, in those over the age of 75.

Of the 3053 assay results, 219 (or 7.2%) were classified at an intermediate level of ER. The percentages of tumours classified as ER-, ER intermediate, and ER+ are given in Table 1.8. This shows the proportion of ER intermediates differing over the age-groups: it was as high as 10% at ages 45-54 years, then lower in each succeeding age group, to less than 5% in those aged 75 years or more. While it would have been possible to reclassify these intermediates as either ER- or ER+ according to some superimposed standard, this was not thought appropriate in view of the consistency among laboratories already reported (Ryan et al, 1985). This present text - and the report by McKeown-Eyssen et al (1985) - are based on the laboratories' own definitions of ER status. Further, recalculation after excluding the intermediate ER results did not change in any noticeable way the patterns reported below.

As Table 1.8 shows, among the youngest women (aged 30 - 44 years) rather over a third of the tumours were ER- and a little over a half were ER+. In each successive ten year age group, the proportion of ER- tumours

TABLE 1.7: Distribution by age of female population, breast cancer incidence and number of ER assays, Ontario, 1981

(a) Age (years)	(b) Population (thousands)	(c) No. of primary breast cancers	(d) Incidence (per 100,000)	(e) ER assays No.	%
30–44	898.8	537	59.7	466	86.8
45–54	464.9	785	168.9	672	85.6
55–64	420.8	950	225.8	803	84.5
65–74	294.6	841	285.5	646	76.8
75+	211.7	795	375.5	466	58.6
30+	2290.8	3908	170.6	3053	78.1

(a) Age at surgery for breast cancer

(b) Female population resident in Ontario; source: Statistics Canada (1982)

(c) Source: Ontario Cancer Registry

(d) Column (c) divided by column (b)

(e) Excluding a total of 173 assays where information on age or ER level was missing

TABLE 1.8: Classification of ER assays by age

Age (years)	Total*	ER assays performed: Percentages classified as: ER-	Intermediate	ER+
30-44	466	37.8	(8.8)	53.4
45-54	672	30.8	(10.0)	59.2
55-64	803	27.0	(6.2)	66.7
65-74	646	19.7	(6.2)	74.1
75+	466	15.5	(4.5)	80.0
30+	3053	26.2	(7.2)	66.7

* See footnote (e) in Table 1.7

was lower (down to 16% for women aged 75 years and over) and that of ER+ tumours was higher (80% among the oldest patients). It must be emphasized that the age distributions of cases were similar from laboratory to laboratory. Thus, the older the woman, the more likely it was her tumour would be assessed as ER+. This pattern was seen in all but one laboratory; but, there, so few assays were performed (1.3% of the total) that no reliable conclusion could be drawn. McKeown-Eyssen et al (1985) suggested that, based on the similar age distributions among laboratories, it did not appear that these patterns could have been strongly affected by interlaboratory variation in age.

Age-specific incidence rates for ER- breast cancer and for ER+ breast cancer were estimated from information on 2834 breast cancer cases (that is, 3053 less 219 intermediate), using as denominators the female population for Ontario from the 1981 Census (Statistics Canada, 1982). They are given in Table 1.9 and plotted in Figure 1.2. The rates of ER- tumours increased with successive age groups up to 60-64 years, although less steeply after age 49; from 65 years of age, the ER- incidence rate tended to decrease. The rates of ER+ tumours increased steadily over the entire age range. The ratio of the incidence rates of ER+ breast cancers to the rates for ER- cancers were:-

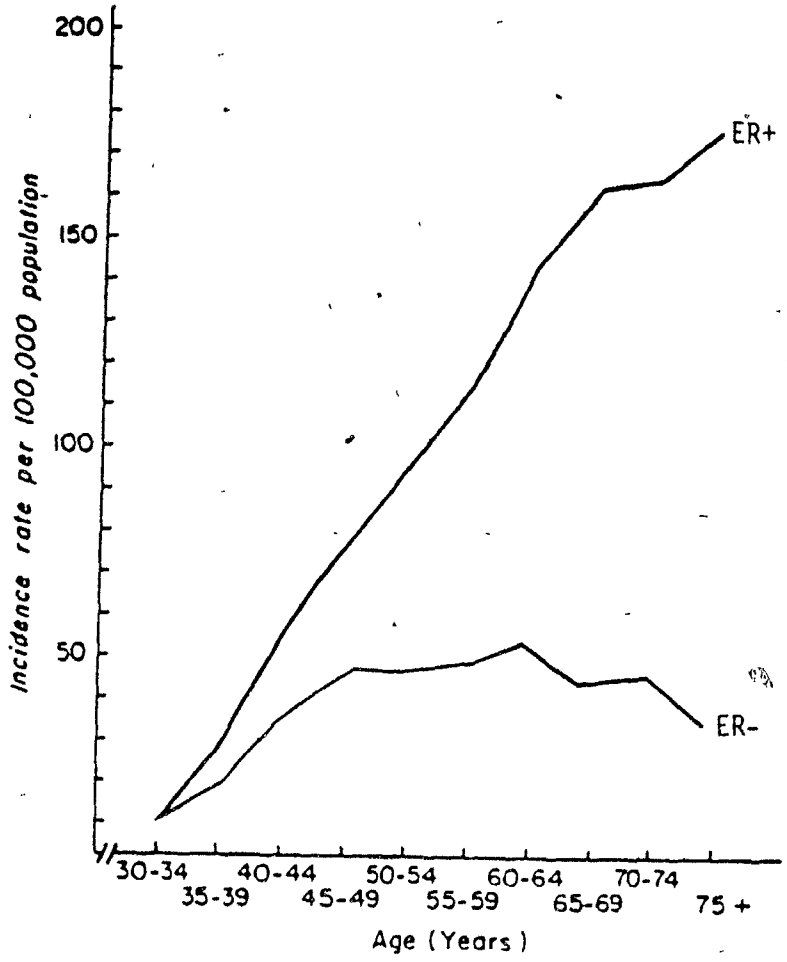
Age (years)	Ratio (ER+/ER-)
30-34	1.06
45-49	1.73
60-64	2.72
75+	5.18

TABLE 1.9: Age specific incidence rates of breast cancers assayed as ER- and ER+

Age (years)	Population * (thousands)	Incidence (per 100,000 population) of breast cancer assayed as: ER-	ER+
30-34	364.4	10.4	11.0
35-39	289.7	20.0	26.2
40-44	244.7	32.7	54.4
45-49	231.5	44.1	76.5
50-54	233.4	45.0	94.7
55-59	233.1	50.6	114.5
60-64	187.7	52.7	143.3
65-69	164.4	42.0	161.8
70-74	130.3	44.5	163.5
75+	211.7	34.0	176.2

* See footnote (b) to Table 1.7

FIGURE 1.2: Age-specific incidence rates of ER- and ER+ breast cancer in Ontario, 1981



N.B. Errors in the diagram published in McKeown-Eyssen et al (1985) have been corrected.

Explanation of the differences in the age-specific incidence curves is not straightforward. Although it is possible that the different patterns are a reflection of changes in body hormone levels with age, this is not supported by the literature on the relationship between ER levels and body hormone levels. Since the estrogen receptor assay measures unbound cytosol receptor, high endogenous hormone levels in younger women might cause the receptors to be saturated (and/or translocated to the nucleus), which would lead to lower concentrations of ER and reduced numbers of ER+ tumours. On this theory, lower endogenous hormone levels in older women might allow tumour receptors to remain unbound and would therefore be associated with increased numbers of ER+ tumours. It is true that there are occasionally reports of cases with high serum estrogen and low tumour receptor levels (Theve et al, 1978; Nagai et al, 1979), but most investigators (Fishman et al, 1977; Nomura et al, 1977; Maynard et al, 1978; Saez et al, 1978; Abul-Hajj, 1979; Edery et al, 1981; Thorsen et al, 1982; Drafta et al, 1983) have found little or no relationship between tumour receptor values and estrogen levels in serum or cytosol. Indeed, some researchers (Maynard et al, 1978; Edery et al, 1981; Drafta et al, 1983) have reported higher levels of estradiol in ER+ tumours than in ER- tumours. This is in line with the need for estrogen to stimulate the formation of its own receptor (Witliff, 1984). In addition, Sakai and Saez (1976) found that premenopausal women did not have higher amounts of bound receptors than postmenopausal women; this suggested that differences in unbound receptors detected by the assay could not be explained by the existence of "filled" sites (i.e. receptors which are already bound to hormone). That progesterone levels are higher in premenopausal than in postmenopausal women has been offered as an explanation for the differences in ER tumour status, but this has not yet been examined in detail.

Based on the evidence presented above, it was the opinion of the authors, i.e. McKeown-Eyssen, Rogers-Melamed and Clarke (1985), that the age-specific incidence curves for ER- and ER+ breast cancer seen in Ontario were not merely expressions of hormonal changes with age.

Because Figure 1.2 shows curves of true incidence of ER- and ER+ breast cancer, their resemblance to overall breast cancer incidence in, respectively, low and high risk countries, is substantially more convincing than that previously noted (section 1.2.4).

1.4 Estrogen receptor status and breast cancer risk factors

Investigations of the possible etiologic significance of estrogen receptor status have compared the characteristics often associated with breast cancer risk of patients with ER- and ER+ tumours. Most research has been concentrated on contrasts between the two disease classes ER- and ER+; Hildreth et al (1983), however, included a comparison group of women without breast cancer in order to estimate, separately, the risks of ER- and ER+ disease. Where possible, the findings reviewed here are presented in terms of the ratio of ER+ tumours to ER-, which will be referred to as the "ER+/ER- ratio".

Hildreth et al (1983) found that, for postmenopausal women, the ER+/ER- ratio was (significantly; $p < 0.05$) greater in the group of patients who were nulliparous than among those who had had a live birth. When the latter were arranged into three categories of age at which a woman delivered her first child, the ratio was higher in successive categories; similarly,

the group of patients who had breast-fed a baby had a higher ER+/ER- ratio than those who had never breast-fed a child. These authors stated that neither the number of livebirths nor the number of stillbirths was associated with ER status; the data were not presented and so cannot be considered in terms of ER+/ER- ratios. The distributions of age at menarche, age at menopause, hysterectomy and bilateral oopherectomy among the ER+ and ER- cases were reported as being similar. The ER+/ER- ratio was higher in the group of patients with a history of benign breast disease than in those without such history.

In comparison with women without breast cancer, an increased risk of ER+ breast cancer was found with nulliparity, a late age at first birth, a history of having breast-fed at least one child and a history of benign breast disease, although none of these associations quite reached statistical significance (at the conventional level, $p < 0.05$). However, the risk of ER- breast cancer was not increased by any of these factors. On the other hand, the use of estrogen replacement during menopause appeared to increase the risk of ER- breast cancer, but not of ER+ disease.

Hildreth et al (1983) suggested that their results may have been influenced by their selection of referents, who were non-cancer hospital patients, drawn from a larger case-control investigation of breast cancer - in which ER status was not considered. In that larger study, quite weak relationships were found between breast cancer and two factors, nulliparity and late age at first birth, which are generally considered to increase the risk of disease (Kelsey, 1979). The referents had been selected from a variety of services at the two hospitals where the cases were treated; the considerations which led to the referral to these institutions of the breast cancer patients and of the comparison group may not have been similar.

Three other inquiries, not restricted to postmenopausal patients, have considered associations between ER status of breast tumours and age at menarche, whether a woman ever bore a live child, the number of livebirths, the woman's age at first birth, age and type of menopause. Elwood and Godolphin (1980), Wallace et al (1980) and Lesser et al (1981) found the associations very weak with the following exceptions. Elwood and Godolphin (1980) showed the ER+/ER- ratio to have been higher in those whose age at menarche was 14 years or more than in those who started menstruating earlier (although the pattern was not monotonic); also, the ER+/ER- ratio was twice as high among women who had their first live birth at the age of at least 20 years compared to those who had given birth before age 20. As these authors stated, regrouping the data after initial examination (i.e. using the data to generate hypotheses) compromised any statistical testing, and great weight should not be given to these results.

Elwood and Godolphin (1980), Lesser et al (1981) and Hildreth et al (1983) all failed to find statistically significant associations between ER status and a reported family history of breast cancer. Ottman et al (1981) found slightly lower ER levels in familial breast cancer (defined as known breast cancer in at least one first degree relative - mother, sister or daughter) than in other breast cancer. It appears that other investigators have not separated family history by degree of relation, and this may have masked any association.

Elwood and Godolphin (1980) found little association between ER status and the use of estrogen replacement during menopause; however, Lesser et al (1981) reported the median ER level of women who had used estrogen as significantly lower than that of non-users. Because oral contraceptives

have been in widespread use by the population for less than 30 years, this factor has been examined in relation to ER status mainly in premenopausal patients. Elwood and Godolphin (1980) found no association between ER status and oral contraceptive use, but Lesser et al (1981) reported a higher ER+/ER- ratio among non-users than users. Later, Osborne et al (1983) confirmed the latter result - but only in young women with a positive family history of breast cancer.

Associations of ER status with marital status, education, religion and income have been minimal (Elwood and Godolphin, 1980; Hildreth et al, 1983).

Weight (and obesity) have been explored as risk factors for ER status of breast cancer. In a cohort investigation, DeWaard et al (1981) found that obesity [as measured by a Quetelet Index of body mass greater than 27 kg/m^2) was associated with a decreased risk of ER- breast cancer, and an increased risk of ER+ disease. Women who developed ER- breast malignancies had Quetelet indexes lower, on average, than those of healthy women. The breast cancer patients with ER+ tumours had an average BMI significantly higher than that of the referents, and hence even higher than that of the patients with ER- tumours. For breast cancer cases classified by BMI, the ER+/ER- ratio was higher the greater the BMI.

Lesser et al (1981) reported that women with ER- tumours had significantly lower mean BMI than patients with ER+ tumours. Hildreth et al (1983) reported the association between Quetelet index (or weight) and ER status as very weak; however, as mentioned earlier, this may have been due to biases in the selection of healthy women.

Some research projects have only considered weight (without taking account of height), and that may have influenced the findings. Papatestas et al.(1980) found a higher proportion of ER- tumours in breast cancer patients weighing more than 150 pounds than in patients weighing less than 150 pounds. Although Elwood and Godolphin (1980) did not report an association between ER status and weight, their data showed the ER+/ER- ratio to be lower in the lighter women and higher in the heavier. On the other hand, Mason et al (1982) found no such association.

In Japanese breast cancer patients, Kuno et al (1981) found a positive association between ER status and weight, while Nomura et al (1981) found a corresponding association with obesity, although their results were only for postmenopausal women.

It is difficult to interpret all these results; at least some of the differences between them may have arisen from varying definitions of ER status, obesity, or weight. Nevertheless, some of these investigations have suggested that diet may play a role in ER+ breast cancer. However, it can be emphasized that there has been no research on ER status in relation to diet, directly measured.

1.5 Some inferences concerning diet and ER status

It has been suggested that dietary effects on breast cancer etiology are mediated through hormonal mechanisms, although the exact relationships have not yet been established (Carroll, 1981; Hill et al, 1981; Zumoff, 1981). It has also been proposed that the effect of nutrition on carcinogenesis is one of promotion, rather than initiation (Carroll, 1981; Wynder, 1983). The

biological plausibility of the ability of diet to influence the estrogen receptor status of the tumour has been suggested by an animal experiment in which levels of estrogen receptor were higher in rats fed a high fat diet before and after challenge with a carcinogen than in those on a low fat diet similarly challenged (Ip and Ip, 1981).

The main questions to be answered by this thesis research arose from the results of the examination of population-based ER data in Ontario (section 1.3), and the findings on the relationship between ER status and risk factors for breast cancer.

1.6 Recent findings

ER+/ER- ratios have been found higher among older patients in North America in six reports (Clarke et al, 1984; Hulka et al, 1984; Montgomery et al, 1985; Rochman et al, 1985; Ballard-Barbash et al, 1986; McTiernan et al, 1986). In a seventh (Nomura et al, 1984), of Japanese women, the corresponding association was not reported.

Ballard-Barbash et al (1986) reported that a late age at first birth was positively associated with ER status. Nomura et al (1984) found the ER+/ER- ratio was high in postmenopausal nulliparous women. These workers also showed that the ER+/ER- ratio was lower (although not significantly so) among women whose first birth occurred before the age of 23 than among patients who were older when they had their first child. Two other inquiries (Hulka et al, 1984; Montgomery et al, 1985) failed to find an association between ER status and either age at first birth or parity.

Other variables studied - including age at menarche, age at menopause, family history of breast cancer, and history of benign breast disease - were only weakly, if at all, related to ER status. Hulka et al (1984) found an indication that the median ER levels were lower in users of postmenopausal estrogens, but this difference was not statistically significant. A biopsy for benign breast disease was associated with lower median ER level, while a surgical, rather than a natural, menopause tended to be associated with slightly lower ER levels (Hulka et al, 1984).

Ballard-Barbash et al (1986) did not find any evidence of a relationship of ER status with weight or obesity.

Thus, research published over the two years since this project was designed has failed to suggest any definite relationships between ER status and risk factors for breast cancer not already proposed.

It should, however, be mentioned that Micozzi et al (1986) have proposed measures of body mass other than the Quetelet index as more appropriate for use in female populations, especially in the elderly. It will be shown in section 8.3 that this suggestion was unimportant for the present research.

It must also be emphasized that methods of antibody assay of estrogen receptors have now been developed (King et al, 1985; with many articles in a supplement to Cancer Research, volume 46, no. 8, published in August 1986) - see Chapter 11.

Chapter 2

DEVELOPMENT OF RESEARCH PLANS

This chapter follows the development of the survey from the original research proposal, through necessary modifications, to the final version implemented in the field. The material is organized into six sections. The first describes the investigation which had been designed in the months before 1984.07, when a research protocol was submitted to the Ethics Committee at the University of Toronto. The simplifications of the protocol carried out between then and 1984.09 are presented in section 2.2. The third section provides details of the materials and methods, as proposed in 1984.09, extracted from the protocol of that date. Particulars of ethical approval and cooperation of surgeons are in the fourth part. Section 2.5 covers improvements to the research protocol during the early stages of data collection, and the final section outlines the protocol actually followed.

2.1 The investigation proposed, 1984.07

Review of the literature had revealed that the dietary etiology of ER- and ER+ breast cancer had not been investigated; it was therefore planned to investigate the role of diet and other factors in the occurrence of tumours of either status.

Objectives:

The general objective was to explore the role of dietary and reproductive factors in the etiology of the estrogen receptor status of breast cancer in postmenopausal women. Specific objectives were:

(1) To identify, among postmenopausal women with breast cancer, those dietary and reproductive factors which distinguish ER negative tumours from ER positive tumours.

At this stage the definitions of an ER negative and an ER positive tumour were, respectively:-

- one having an assay level less than 3 fmol/mg; and
- one having an assay level of at least 30 fmol/mg.

(2) For each factor found, in (1) above, to be associated with the ER status of the tumours, to estimate the relative risk of ER- breast cancer by comparison of patients with healthy women. Correspondingly, for ER+ breast cancer, to estimate the relative risk by comparison of patients with healthy subjects.

The variables to be investigated included:-

dietary factors: intake of total calories, dietary fat, red meat, sugar and dietary fibre;

reproductive factors: age at menarche, age and outcome of each pregnancy, parity, duration of breast-feeding, age at menopause, type of menopause;

family history of breast cancer;

use of certain hormonal medications;

socio-economic factors;

smoking history.

2.1.1 Outline of the research

The project was designed with three limbs: first, a comparison of women with ER- breast tumours against those with ER+ breast tumours; and, separately, comparisons of women with either type of tumour against healthy women who were otherwise similar.

To be eligible as a "case" a woman had to be postmenopausal and receiving surgical treatment for primary breast cancer at one of certain Toronto hospitals; also her tumour would have to have been assayed for estrogen receptors, and classified as either ER- or ER+. She had also to be an English-speaking resident of Toronto or its surrounding communities, who was at least 50 years old. Eligible cases would be included in all three limbs of the project.

For the two limbs in which cases were to be compared with referents, the latter were to be chosen from civic registries in Toronto or its surrounding communities, by age-stratified random sampling. For inclusion in the investigation, a woman would have to be postmenopausal, English-speaking and without a history of breast cancer.

All eligible patients with ER- tumours were to be selected. However, because many fewer ER- tumours than ER+ tumours were expected, not all patients with ER+ disease were to be included. The intention was to select, by means of age-stratified random sampling, the same number of ER+ patients as had ER- breast cancer. Thus the age distribution of all three groups was to be determined by that of the ER- cases.

Information on dietary and reproductive factors was to be collected on a total of 460 breast cancer patients (230 each with ER- and ER+ tumours)

and on 230 healthy women. This process was expected to take approximately three years, and a further 12-18 months were allowed for data processing and analysis, and report (in the form of a doctoral thesis).

2.2 Simplifications of protocol, 1984.07-1984.09

In order to meet the requirement (1984.07) that all field work for this thesis be completed by 1985.12, two alterations were needed to shorten the time frame over which the project was to be conducted.

First, a choice had to be made between carrying out all three limbs, as proposed but on a very small scale, or proceeding with one limb and omitting the others. The reduction of scale for the three limb project would have been so great it had to be treated as infeasible. Therefore, one limb had to be selected: it was felt by the LICR that it made most sense (as a first examination of the possible dietary etiology of estrogen receptor status) to see if differences between women with ER- and ER+ breast cancer could be detected. The study was therefore truncated to incorporate only that limb of the original plan.

Second, the sample size was reconsidered. It was still intended that the entire limb as funded by the LICR would eventually embrace 460 patients (230 with ER- tumours, and a like number with ER+); however, it was decided that this thesis would be based on material available from the first hundred or so cases of each type of breast cancer. From estimates for the year 1982 of suitable patients at the selected hospitals, it was thought that these 200 patients could be identified within one year.

Another alteration to the original design was made in the belief that it would simplify coordination of the study: the age-stratified random sampling of the ER+ cases was changed to one-to-one matching, despite the obvious disadvantage that the age match would be rather loose (within 10 years) in relation to the quite small age range of 30 years.

The questionnaire on medical, smoking and reproductive history was reviewed to ensure that only factors relevant to the research were included. In the pretesting, the time of administration had been between 25 and 35 minutes; deletion and alteration of some questions reduced this to only 10 minutes.

These four changes had, of course, to be communicated to the Ethics Committee of the University of Toronto's Office of Research Administration (ORA). The research protocol was revised by the author and resubmitted to the ORA Ethics Committee in 1984.09. Extracts from this resubmission form section 2.3.

2.3 The research protocol of 1984.09

The text of this section is extracted, without modification, from the research protocol, dated 1984.09, submitted to the ORA Ethics Committee. The Appendices to that document are reproduced, with the same labelling (A through H), in Annex II of this thesis. Portions which appear in square brackets [ ] were changed in the final version of the protocol, which evolved - through necessity - even after the field work had started.

"Design

"This study will compare women with ER+ and ER- breast cancers on dietary and reproductive factors. A total of 230 women with ER- tumours will be studied together with a sample of 230 women with ER+ tumours (see sample size calculations), [matched within 10 years of age].

"Definitions:

"(a) Cases: The cases will be incident cases of primary breast cancer (confirmed by pathology report) receiving surgical treatment for this condition at participating Toronto hospitals. Women will be included if they are 50 years of age or older (as an initial proxy measure of menopausal status), reside in Toronto or its surrounding communities, are able to speak English, have no history of a prior malignancy of the breast, and referral to the Princess Margaret Hospital is not planned within the next six months. This last criterion is to avoid overlap with another breast cancer investigation in Toronto. In addition, patients will only be included if an ER assay is performed on their tumour. This study will only include postmenopausal women because (1) it is impossible to determine accurately where a woman is in her menstrual cycle at the time of surgery, and the possibility that cyclic variation of steroid receptors related to the menstrual cycle in premenopausal women (Maynard et al, 1978; McCarty et al, 1983) could distort the results; (2) the fact that the difference in the ER incidence curves is seen mainly in older women, after the age of menopause; and (3) the small number of premenopausal patients who would be available for study in a reasonable period of time.

"(b) Menopausal status: A woman will be considered postmenopausal if (1) she has not had a menstrual period in the last year before interview or (2) she has had a bilateral oopherectomy with or without a hysterectomy.

"(c) Socio-economic status: Information will be collected on the following variables thought to reflect socio-economic status: highest level of education, total family income and recent employment history (see Appendix A).

"(d) Estrogen receptor status: For the purposes of selecting patients for study, tumours will be classified as ER+ or ER- according to whether at least 30 fm/mg protein of receptor or less than [3] fm/mg protein are identified in the tumour specimen. Women having ER levels between [3] fm/mg protein and 29 fm/mg protein (approximately [25]% of all assays) will be excluded. These classifications of positive and negative were chosen in view of (1) the high response rate to endocrine therapy exhibited by groups of patients with ER tumour levels greater than 30 fm/mg protein and (2) the lower response rate to therapy seen among women with tumours containing between 3 and 29 fm/mg protein; such tumours, although they may be classified as ER positive based on assay results, can be shown by immunocytochemistry to contain estrogen autonomous cells which are not expected to respond to treatment (E. Jensen, personal communication). Finally, it was thought that a comparison of the extremes of estrogen receptor levels should allow the most sensitive detection of an effect. Although progesterone receptor results are available, they will not be included in this research project because of measurement difficulties.

"(e) Dietary factors: Dietary factors to be studied include both direct and indirect measures of diet. Dietary factors, measured through the use of a food frequency questionnaire (Appendix A) are dietary fat, total calories, [sugar], dietary fibre and [red meat]. Indirect indicators include weight, height, and a composite measure, the Quetelet index, both at the time of diagnosis and at age 20.

"(f) Hormonal/Reproductive factors: These factors will be assessed by a questionnaire (Appendix B) and include age at menarche, age at all pregnancies together with the outcome of each pregnancy (ie., birth, miscarriage, etc), parity, duration of breast feeding for each completed pregnancy, age at and type of menopause, and family history of breast cancer.

"(g) Medical history: For each item, information will be collected on the presence or absence of the condition or treatment, together with age or date of occurrence as appropriate: breast biopsy, hysterectomy, oopherectomy (bilateral or unilateral), thyroid disease, chemotherapy, anti-estrogen therapy (Appendix B).

"(h) Type and extent of cancer: The following information on the breast cancer will be abstracted from the hospital chart and/or physician's records (Appendix C): pathological diagnosis, type of surgery, tumour laterality, location, size, stage and grade, results of nodal dissection and presence of metastases.

"(i) Medication use: Information on the type of past and current medication and the duration of its use will be collected for oral contraceptives and postmenopausal estrogens (Appendix B).

"(j) Smoking: Basic smoking information will be collected as follows: current, ex- or non-smoker, duration of smoking, amount smoked (Appendix B).

"(k) Background information: Descriptive information will be collected on ethnic origin, religion, place of birth and marital status (Appendix B).

"Sample size

"In order to determine the number of cases needed for this study, information on the distribution of dietary fat consumption (considered to be one of the important factors under study) in the Toronto population was obtained (G. Howe, personal communication). To detect a relative risk of two associated with an increase of 100g of fat consumption and setting error probabilities at $\alpha = .05$, $\beta = .10$, 230 women with ER+ and 230 with ER- tumours will be required. It is estimated that in order to assemble sufficient women, data collection will need to be carried on for about 30 months, and will involve [six] Toronto hospitals. After data have been collected for about [100] cases of each type of breast cancer, a preliminary analysis will be performed which will form the basis of a PhD thesis for Ms. Iris Rogers-Melamed. This analysis will give a first suggestion on the direction of the findings.

"Logistics

"Case identification: There will be [two] concurrent methods of case identification. [The laboratories performing the ER assay will be visited on a regular basis to identify patients whose breast tissue samples have been sent for assay.] In addition, potential cases will be

identified from operation room lists. This will be done by a member of the hospital's staff, or by a member of the Ludwig Institute for Cancer Research staff who will be responsible to a member of the hospital's medical staff, as each hospital finds most appropriate. Once a patient has been identified as potentially eligible, her surgeon will be contacted to ensure that she or he wishes the woman to be considered for the study, and to ascertain whether referral to the Princess Margaret Hospital is planned within the next six months. If the physician gives her/his consent, the eligibility criteria will be verified (age, residency, primary breast cancer) from the hospital chart before the patient is contacted. Women who meet these criteria, or if these cannot be verified from the chart, then all potential cases, will be approached by a study representative (either a member of the hospital staff or the LICR staff, as each hospital wishes). This visit will take place in the hospital a few days after surgery to explain the study briefly. A letter explaining the study and a pamphlet (Appendices D and E) will be left with the patient.

"Estrogen receptor levels will be obtained from the laboratories who perform the assay and women will be classified on the basis of these data as having an ER+ or ER- tumour. Each woman with an ER- tumour will be included in the study and [a woman with an ER+ tumour will be matched within 10 years of age with each ER- case. In this way, not all women with ER+ tumours will be required for the study.] Each pair of women to be included will be mailed a second copy of the pamphlet along with a covering letter (Appendices E and F) saying that a study representative will be calling them in the near future. Within a week of mailing these letters, the cases will be telephoned by an interviewer and an

appointment set for an interview. The interview will be scheduled as soon as possible. At the interview, a consent form (Appendix G) will be signed and information on dietary and other risk factors will be obtained. After the interview, the women will receive a letter thanking them for their participation (Appendix H)."

2.4 Ethical approval, and the cooperation of surgeons

The original protocol, of the three-limb project described in section 2.1, had been presented for ethical review to the Office of Research Administration at the University of Toronto on 1984.07.23. On 1984.09.18, some revision and clarification was requested. These changes, together with those relating to the study design (section 2.2), were submitted to the ethics committee within a week. Approval to conduct the study was given over the telephone on 1984.10.23, and the essential written permission was received on 1984.11.09.

From a review of estrogen receptor assay lists, six hospitals, which it was believed would allow the required number of patients to be collected in the time allotted, had been selected for the investigation. In July and August of 1984, an attempt was made to contact, by telephone, the chief breast surgeon at each of these institutions. The research protocol (original version) was sent to the appropriate surgeon at Hospital E on 1984.07.23 (the study had previously been presented by the author at breast rounds and the surgeons had agreed in principle to participate); the five other hospitals were contacted in writing on 1984.08.27. The letter to the senior surgeons enclosed a copy of the research protocol, and asked for their participation in the study; it also requested suggestions as to which

additional surgeons should be contacted at their institution, and advice on how to obtain the ethical approval of the hospital. The necessary approval from the separate hospitals was obtained as follows:-

Hospitals B, D and E: 1984.11

Hospital C: 1984.12

Hospital A: 1985.01.

However, the sixth (F) had still not agreed to cooperate. Despite repeated telephone calls and letters, the surgeons at this hospital never joined in, all but one having written to decline. The loss of patients from Hospital F was nearly 20% of those anticipated to be eligible.

Field work began on the same day in November in Hospitals D and E, and at various times in January at the other three (A, B and C).

It had been agreed that patients referred to the Princess Margaret Hospital for radiotherapy within six months of surgery would not be included. This was so that we would not interview patients who would later be asked to participate in another study of breast cancer (because its Principal Investigator believed that our interviews of her subjects would seriously inflate her refusal rate). At the time of this agreement, the coordinator of the other study had found no more than three or four eligible women per month in all six hospitals which, at that time, were to be included in the present investigation. However, it became apparent very soon after field work had started that, at Hospital B, almost every surgery for breast cancer was a lumpectomy (less radical than mastectomy) and involved early referral to the Princess Margaret Hospital for radiation therapy. In fact, for this reason, almost 20% more of the originally eligible cases had to be excluded.

The intention had been that patients would be identified at six hospitals during the 12 months 1984.10.01 to 1985.09.30. The loss of two-fifths of the planned intake of subjects would have meant extension of this period by approximately eight months if the proposed numbers of cases were to be recruited. The late starts in the hospitals where cooperation was obtained, coupled with hospital-related and seasonal postponements, meant that the period for subject identification did not commence until nearly four months behind schedule. Thus, it would have taken until at least 1986.09 to recruit the planned 200 patients.

This additional year was unacceptable, and the difficult decision had to be made to stop subject identification early. There was no alternative if the thesis research was to be completed in admissible time.

2.5 Enhancements of protocol (1984.11-1985.02)

Even before field work could begin, the advantages of including all eligible women whose tumours had been assayed, regardless of assay result, were apparent and the possibilities were explored.

Initially, the LICR, as granting agency, required that no change in principle be instituted; in particular, it wished equal numbers of subjects with ER- and ER+ tumours to be maintained. As substantially more ER+ cancers were expected than ER-, only a proportion of the former could be included. The intention (see section 2.3) was to find, for each subject with an ER- tumour, another with an ER+ tumour matched with the former on age (at least within 10 years). However, for matching to have been

effective, it would have had to be closer on age, and additional factors (e.g. month of surgery and hospital) would have had to be taken into account. Such fine matching could not be achieved unless the numbers of ER+ subjects available within a defined period of time were very large, and this could not be so. Indeed, even the rather coarse proposed scheme of matching would inevitably have led to logisitic difficulties; in particular, if it had been followed strictly, some ER- patients would have been lost. Such loss was to be guarded against at all costs. Further, the planned matching could offer no advantages for the analysis over "balancing" by age: this, then, was the method finally adopted for selecting ER+ subjects [but see below for later agreement that sampling of ER+ subjects could be abandoned].

The age distributions of women with ER- and ER+ breast cancers, from five of the six hospitals to be included in the survey, had been obtained early on from lists of ER assays in previous years. The patients were classified by their assay results - according to the definitions of this project - as ER- or ER+ and then divided into age-groups (or strata). The sampling fraction for the ER+ patients in each stratum was calculated so that the number of ER+ subjects selected could be expected to be close to the number of ER- patients included. This age-stratified random sampling scheme was fully devised before case identification began.

Very early, it became apparent that case recruitment was much slower than expected, even bearing in mind that patient identification had not yet started in all institutions. In two periods - November and December 1984, and January 1985 - 46 and 38 women were identified as having had breast surgery, but only 11 and 16 met the criteria of eligibility for the study. Two assays were not carried out, but the findings on the other 25 tumours are presented in Table 2.1. The case enrollment plan outlined above would

TABLE 2.1: ER status of tumours of eligible women, 1984.11 - 1985.01

Number of patients eligible for study:		27
Number of eligible cases assayed:		25
By ER level (fmol/mg):		
ER < 3	(ER-)	2
3 ≤ ER < 10		2
10 ≤ ER < 30		4
30 ≤ ER	(ER+)	17

have yielded only the 2 patients with ER assays less than 3 fmol/mg and about half, say 9, of those with assay results 30 fmol/mg or more, i.e. a total of 11, less than 50% of the eligibles. On this basis either the patients recruited in a year would be less than half of those planned or the research would have to be continued for an extra year. Thus action had to be taken immediately to try and improve the situation.

The first step was to redefine ER- cases more liberally. On 1985.01.14, the author met with Dr. E. Jensen, medical director of the Ludwig Institute for Cancer Research (Zurich, Head Office), and an expert in the field of estrogen receptor assay. He had originally supported the ER- definition as less than 3 fmol/mg, but agreed that raising it to less than 10 fmol/mg was advisable for this research, in the light of the recent local evidence. Thus, the number of subjects whose tumours could be considered ER-, and so could be included in the study, would be roughly doubled (see Table 2.1, and also Table 4.2).

The second step was to include all (rather than a sample of) ER+ subjects; considerations of statistical efficiency are dealt with in section 7.6. Of immediate importance was that, on the basis of Table 2.1, the patients who could be included were increased to over 80% of the eligibles (i.e. the 4 ER-, on the new definition, together with all 17 ER+, or 21 out of the total of 25). A request to the LICR to include also the "ER intermediates", and include every eligible, was not granted.

The final attempt to improve recruitment was to expand the catchment area for the cases' residence. Initially, one qualification required of a subject was residence in Metropolitan Toronto, that is, the City of Toronto and the surrounding cities (and the one borough). However, because the

hospitals in the survey (all downtown teaching institutions) attract patients from all over Ontario, a substantial proportion of otherwise eligible patients were being excluded. It was not feasible to arrange interviews throughout the province, but the catchment area was extended to embrace all residences within a 30 mile radius of the city centre.

Early in 1985 it was agreed that, given the poor accrual rate, enrollment of subjects could continue until 1985.12. Even so, it became clear that numbers would remain seriously diminished, to, at most, 100 patients in all; statistical power would be radically reduced.

2.5.1 Work plans - approaches in the hospitals

The logistics of patient identification were discussed with the surgeons in all five participating hospitals; some preferred approaches other than that planned. In Hospitals B, C and E, the surgeons wished their patients to be identified through the breast trial coordinator at their respective institution. The author telephoned these persons on a regular basis to obtain information on eligible women. At Hospital D, the author went twice weekly to the operating room to review lists and then checked the eligibility criteria at the surgeons' offices. Surgeons at Hospital A preferred to have their patients identified directly through their office secretaries; arrangements were made to telephone the offices at least once a week. The letters sent to these patients are illustrated in Annex III.

These channels provided the information rapidly, and no advantage could be gained by searching the estrogen receptor lists for patients that might have been overlooked. Indeed, it was felt that the coordinators and

secretaries would probably have felt their competence was being doubted if, for example, information was requested on a patient whom they had not enrolled.

Early in 1985, a modification became necessary at Hospital C where the breast trial coordinator found that identifying the patients consumed too much of her time. She suggested that the surgeons' offices be contacted directly by the author, and, as this system was working satisfactorily at Hospital A, her proposal was adopted. The work plans are summarized in Table 2.2.

The patients were to have been visited while still in the hospital by the author (or a study representative) a few days after surgery, and every effort was made by the author to visit subjects in Hospitals A and C. At two other hospitals, D and E, this was seldom possible, because the delay in obtaining signed individual consent from the surgeons usually meant the patient was discharged before she could be seen. At Hospital B, it was known from the outset that the delay in obtaining information on pathology would eliminate the possibility of hospital visits.

The majority of patients who were seen in hospital were eager to be involved in the study. Some of these women could not be included because their tumour receptor levels were not in the appropriate ranges, but the author felt that these patients should nevertheless receive some thanks for their interest. From February 1985, any woman who had been visited but who was not eventually included in the study was sent a letter signed by the author (Annex III).

TABLE 2.2: Work plans in hospitals

	Hospital				
	D	E	B	C	A
Hospital's coordinator of breast trials		1984.11 - end	1985.01 - end	1985.01 -1985.02	
Author's review of surgery lists	1984.11 - end				
Surgeons' office secretary				1985.02 - end	1985.01 - end

2.6 The final research protocol

The modified protocol described in sections 2.4-2.5 was never written out in full, but the following paragraphs summarize the changes from the protocol presented in section 2.3.

Definitions of cases: The criterion for ER- was changed from less than 3 fmol/mg to less than 10 fmol/mg [section 2.5 - paragraph 5]. This meant that the proportion of ER intermediates was decreased from roughly 25% to about 15% [Table 2.1].

Initially not all ER+ cases were to be included. The protocol of 2.3 proposed sampling by one-to-one matching, but this was replaced for the first months of the survey by age-balancing [section 2.5 - paragraph 3]. However, it was soon decided to include all ER+ cases [section 2.5 - paragraph 6].

The catchment area of subjects' residence was expanded [section 2.5 - paragraph 7].

Definitions of dietary variables: The intake of sugar and red meat could not be measured for this survey [section 8.2.2].

Numbers of cases: The protocol of section 2.3 mentioned 230 ER- subjects and a like number of ER+ subjects, but stated that this thesis would be based on the first 100 or so cases of each type of tumour. Although six hospitals were specified for inclusion, the surgeons at one did not wish to participate [section 2.4 - paragraph 3]. Almost all cases at a second hospital had to be excluded because they were to be recruited for a competing investigation [section 2.4 - paragraph 5].

Time constraints led to an early decision to stop recruiting subjects in 1985.10 [see Preface], by which time it was expected that 100 ER- and 100 ER+ cases would have been included. Rates of accrual were so low that it was agreed that recruitment could be continued until 1985.12 [section 2.5 - paragraph 8].

Work plans: Laboratories were to be visited to obtain subjects' names, but instead work plans were set to suit the surgeons, hospital by hospital [section 2.5.1].

[It should be noted that for many reasons, known and unknown - see Chapter 6 - the subjects eventually available for study totalled only 78.]

Chapter 3

FIELD MEASUREMENTS

This chapter describes the systems and instruments used to collect information on (1) the subjects' diets, (2) reproductive, general medical and smoking histories, and (3) medical facts relating to the breast cancer. The primary objective required (1); both (2) and (3) were needed to characterize the patients in terms of the breast cancer itself, but more importantly so that the roles of germane factors might be examined in relation to ER status.

Section 3.1 presents details of the Canadian Nutrient Data Analysis - Toronto system (acronym: CANDAT), which was used to calculate the patients' intakes of the food components of interest. The expansion of one of the basic elements of CANDAT, the food frequency questionnaire, is also described, as is the discovery of errors in another, and their correction.

The development and pretesting of the questionnaire on reproductive, general medical and smoking history, followed by its shortening and retesting, are presented in part 3.2. The feasibility of obtaining details from the patients' medical charts, and the collection of this material, is dealt with in section 3.3.

3.1 The CANDAT system

CANDAT is a food and nutrient calculation system (with a computerized component) which is used for evaluating a subject's dietary intake of selected nutrients (or food components) (Bright-See et al, 1986). The principles are described in section 3.1.1. Thereafter the essential elements of the system are presented as follows:-

element (a): a means of obtaining information on a subject's food intake [section 3.1.3];

element (b): entering that information into the computer system [section 3.1.4];

element (c): three "food component files", which contain the values of around 20 "nutrients" in 100 g of each of many individual foods [section 3.1.2]; and

element (d): a program to calculate the intakes, subject by subject, of each of the various "nutrients" [section 3.1.5].

The rest of section 3.1 deals with the expansion of the food frequency questionnaire to include the measurement of total energy [section 3.1.6], corrections to the stored information, found from preliminary enquiry to be necessary [section 3.1.7], and coding and further expansion of the food frequency questionnaires [section 3.1.8]. An example to illustrate how the CANDAT system works is introduced in section 3.1.9, but given in detail in Annex IV. Finally, the training of the interviewer is dealt with in section 3.1.10.

3.1.1 Calculation of nutrient intakes

It is first necessary to consider the principles of calculating each subject's dietary intakes. For this, the "food component files" [element (c)] have to be described. Thereafter, elements (a), (b) and (d) fall more easily into place.

Three definitions are required at the outset:

Nutrient: is the term used throughout the remainder of this thesis in a generic sense to represent any food component. [While this is not a standard practice, it is used in the thesis for purposes of simplification.]

Food component files: are composed of (machine-readable) rectangular arrays (matrices), in which the columns are allocated to the nutrients (n) and the rows to the foods (f), and the entries are quantities of nutrient per hectogram of food.

Standard portions: are intended to allow the actual serving size of each particular food to be judged. A standard portion can take one of three forms, either: a natural unit (such as an egg or an apple); a kitchen measure (e.g. a measuring utensil - spoon, cup, bowl - of specified capacity); or a plastic model of a specific food, in a pre-determined amount considered to represent a quantity commonly served.

For each subject (i), the intake of each specified nutrient (n), here called $I(n)_i$, is obtained. The requirement is to sum, over every food (f) consumed, the products

$$(t_{if}) \times (s_{if}) \times (a_{fn}),$$

where t_{if} is the usual number of times per day subject (i) consumes food (f);

s_{if} is the serving size of food (f) normally eaten by subject i [note that s_{if} is expressed as a multiple (or fraction) of the standard portion of that food];

a_{fn} is the amount of nutrient (n) in a standard portion of food (f).

In other words,

$$I(n)_i = \sum_f \{([t_{if} \times s_{if}] \times a_{fn})\}.$$

Each of the products to be summed consists of two parts. The first, in square brackets, requires the two values t_{if} and s_{if} to be obtained from element (a) (see 3.1.3 below). The amounts (a_{fn}) are compounded of the weight, in hectograms (hg), of a standard portion, and the quantity (q_{fn}) of nutrient per hg; both have to be stored permanently in machine-readable form, the former in the Model Table (see definition in 3.1.5 below), and the latter in the food component files (3.1.2).

3.1.2 The food component files [element (c)]

The second part of each product within curly brackets { } to be summed for $I(n)_i$ requires the quantity q_{fn} which is stored in the food component files, of which there are three, referred to as the Master File, the Institute File, and the User File. In each file, there is a matrix of values q_{fn}, the quantity of nutrient (n) per hg of food (f); many of the q_{fn} are zero, and are stored as such.

The Master File is the Canadian Nutrient File (Verdier and Beare-Rogers, 1984), and contains nutrient data on over three thousand foods. The Institute File includes nutrients (e.g. values of dietary fibre) not in the Master File. The User File incorporates data on nutrients for foods which

are in neither of the other two files, but which may be necessary for a specific investigation (Bright-See et al, 1986).

3.1.3 The food frequency questionnaire [element (a)]

Information on diet for this survey was collected with the use of a food frequency questionnaire; this is given in full as Annex II, Appendix A. The principle behind the interviewing is straightforward. The respondent is asked to recall her intake, over a specified period, of certain foods. These can be identified in one of three ways. First, there are show cards (generally one for each sheet of the questionnaire), listing between them all foods deemed necessary (Bright-See et al, 1986) for the estimation of the nutrients of interest. Second, there are "add-ons" (i.e. foods which are often eaten in conjunction with other foods, such as margarine on bread), asked about as appropriate. Third, certain categories of foods, including cereals and cookies, are not listed on the show-cards, but answers to open-ended questions about them are recorded.

Each food on the questionnaire is identified by an "item code", consisting mainly of page and line number. Also printed on the document is a code identifying the physical model used to define the standard portion of each food.

The respondent is asked to report, with respect to each of the foods, whether she consumed it during the relevant period (in the present project the four months before her surgery). If she states that she did, the item is checked (✓), and she is asked whether on a Daily, Weekly or Monthly basis, and the frequency with which it was consumed; she is also asked to provide an estimate of the size of serving usually eaten on each occasion, guaged as a multiple or fraction of a standard portion. The use of each

recognized "add-on", i.e. those printed on the questionnaire, is also enquired about.

3.1.4 Data entry [element (b)]

The entry of the information recorded on the food frequency questionnaire is through an inter-active computer system; for each checked item, the item code is entered, together with the number of servings (i.e. D, W or M, followed by the number of times consumed), and the serving size. Where appropriate, information is entered about the add-on(s).

3.1.5 The program [element (d)]

Two further definitions are now required.

The Questionnaire Table is a list (stored in the CANDAT system) giving (for each food item, identified by the item code): the "food code" (as stored in the food component files); the "model code" (for use in the Model Table, see below); and information [including a food code and a model code (in the same senses as before) for each relevant category] about up to three "add-ons" to the specific food.

The Model Table is a listing (also stored in CANDAT) of each model code, and the weight [in grams (g)] of a standard portion of such food.

Brief extracts from the Questionnaire Table and Model Table which provide information in relation to the illustrative example in Annex IV are given in that Annex.

Each item code is translated (by the program, using the Questionnaire Tables) into the relevant food code, and the specific model code is obtained. The latter is then related to the Model Table which supplies the appropriate weight for a standard portion of each food. Information on the

nutrients for each food is obtained by a search for food codes through the User File, then the Institute File, and finally the Master File (Bright-See et al, 1986).

The program can now calculate $I(n)_1$, in other words the intake of each nutrient, given the t_{1f} and s_{1f}, and bearing in mind that the a_{fn} are available by cross-multiplication of entries in the Model Table (converted to hg) and the appropriate food component file (i.e. the q_{fn}).

3.1.6 Extension of the questionnaire

The food frequency questionnaire had been designed (by Dr. E. Bright-See and Mrs. V. Jazmaji, at the LICR) for use in studies of colon polyps, where the aim was to assess normal consumption of certain nutrients, including fat and dietary fibre, but not total energy. Review of the literature on the relationship between diet and breast cancer suggested the importance for the present research project of including total energy intake in the measurements. This meant that the food frequency questionnaire had to be expanded accordingly.

The author spent more than two months reviewing the food items in the Canadian Nutrient File (Verdier and Beare-Rogers, 1984) to decide which foods had to be added to the questionnaire to permit the estimation of total energy intake. Additionally, several food companies and trade associations were asked to provide information on the nutrients in their products. In order to measure total energy, it was necessary to include sugars, which in turn meant that certain foods (for instance, desserts, jam, sugar in coffee) had to be added to the questionnaire. Further, some of the existing food

categories had to be reorganized. For instance, each type of fruit had to be treated separately according to whether it was consumed fresh or canned, with or without syrup.

3.1.7 Correction of information stored in CANDAT

The author generated listings of three nutrients - fat, dietary fibre, and energy - in a standard portion of every food included on the original questionnaire, and it was obvious to her that errors existed in some of the values (a_{fn}) obtained making use of each of the three food component files. An essential step became to identify these errors; most were traced to incorrect entries of the "food codes" in the Questionnaire Table (see 3.1.5). All identified errors were notified to the nutritionists at the LICR, and the relevant computer-stored tables were corrected.

3.1.8 Management of completed questionnaires

As the food frequency questionnaires were received, they were reviewed with the interviewer to ensure all entries were clear to the author. In some instances, this necessitated a further contact between the interviewer and the respondent.

The second step was to ensure that all entries on the completed questionnaires were coded in the form which would allow entry through the standard interactive process; most entries were already in that form, but a substantial proportion had to be translated into it by the author.

During this procedure, it was not unusual to find foods for which no "item code" was immediately available. The majority of these foods were those which had been added to the questionnaire in the extension described in section 3.1.6, but another substantial proportion came from items which had been "written in".

Some of these foods could be identified in the questionnaire manual being prepared during the course of this research (but only fully available in 1986.07). For certain other foods, consultation with the nutritionists at the LICR provided a means of bringing the food within the CANDAT system. However, in many instances, recipes or actual products had to be acquired in order to determine the ingredients and nutrients. The respondents' personal recipes were in every instance broken down into their ingredients and each item assigned an individual item code and serving size.

3.1.9. An example of the CANDAT process

In order to illustrate the workings of CANDAT, an example (grossly over-simplified) is provided in Annex IV, and the calculations of two nutrients for a commonly eaten food are worked through in detail, thus emphasizing the complexity of the system and the need for computerization. Short extracts from the Questionnaire and Model Tables (see section 3.1.5 above) are included in this Annex.

3.1.10 Interviewer training

In the fall of 1984 an interviewer, Mrs. V. Hunter, was hired to collect the information for this study. Mrs. Jazmaji, who had acquired great expertise in working with the food frequency questionnaire and interviewers, had the responsibility for training Mrs. Hunter, but the author herself assisted throughout the training process. The questionnaires were reviewed item by item with Mrs. Hunter, and she seemed to grasp easily the concepts of its administration. Many practice sessions were carried out: first the interviewer herself was interviewed; then she carried out interviews of

volunteers from the LICR. By the time the first case was identified for the study (1984.11), all involved felt confident that Mrs. Hunter was well prepared to carry out the interviews.

3.2 Questionnaire on reproductive, medical and smoking histories

A questionnaire was needed to elicit information, in the same form for each subject, on factors relevant to the research objectives which could not be included in the food frequency questionnaire and would not be recorded in the patients' medical charts.

3.2.1 Content

The items the author considered of interest were:

(1) menstrual history;

(2) pregnancy and birth history;

(3) medical history;

(4) family history of breast cancer;

(5) use of certain medications;

(6) smoking history;

(7) demographic details; and

(8) socio-economic information.

All have been inquired about in many studies of breast cancer, and several investigators were kind enough to let the author review their questionnaires. Most of these had individual items which were appropriate, but no single questionnaire was suitable. Further, these questionnnaires enquired about several factors using various forms of questions which could have led to different "measurements". For example, a mother's age at the

birth of her first child can be "measured" by means of a single direct question or from the difference between the dates of the mother's birth and of the birth of her first baby, and again the dates could be asked for simply as years or to the actual day.

Thus, a questionnaire had to be developed afresh. The author designed the new instrument in sections corresponding to factors (1) through (8) above, with the questions on socio-economic factors placed at the end of the complete interview, as they were thought to be particularly sensitive. The demographic factors, (7) above, included reports on current height, weight six months before surgery, and weight at age 20 years; height and weight were converted to SI units as necessary.

A good instrument of this nature requires not only precise and unequivocal questions, but also "probes". A further requirement is a clear set of rules for the administration of the questionnaire, and an interviewer manual was prepared (Annex V).

3.2.2 Development and testing

The author prepared a first edition of the new questionnaire, and tested it by administering it to volunteer female staff members of the LICR. As these women were all younger than the subjects for which the instrument was intended, further testing was carried out, this time on postmenopausal members of the author's family. As a result, some questions were refined and several probing queries added.

From this preliminary use of the questionnaire the time for its administration was estimated as between 25 and 35 minutes. Following the well-known principle formulated by Sir Austin Bradford Hill (see also Social and Community Planning Research, 1972) that questionnaires should be as

short as possible, this one was reviewed to ensure that factors not strictly relevant to the research objective were not enquired about. Deletions and alterations resulted in an interview requiring only 10 minutes.

In order to test this edition of the questionnaire as objectively as possible, it was necessary to find subjects without personal or professional interest in the research. An appropriate source was considered to be the ladies who provide volunteer services at a cancer treatment centre near the LICR. Eight women, ranging in age from 50 to 78 years, responded to an approved notice inviting participation. Five were interviewed by the author; the other three by the interviewer who had now been hired for the main survey. Two of the women had previously suffered from breast cancer, and one from uterine cancer.

The questionnaire seemed to be well received by the volunteers, and was easy to administer, except that one question (relating to ethnic status) was found unclear. This question had been taken, with only minor adaptation, from the 1981 Census of Canada, and was retained in the hope that ethical problems (which might otherwise have arisen when asking about religion or ethnicity) would be avoided. As clarification, an example was added of the type of answer sought, even although the question was already rather long.

The final version of the questionnaire (Annex II, Appendix B) was ready well before the scheduling of the first interview of a subject within the project. [The earlier, longer, version is not reproduced in this thesis.]

3.2.3 Practice

In the interviews this questionnaire was administered before the food frequency questionnaire. No problems were encountered except for a few questions left unanswered by one subject because of her poor memory. Some of the information which was collected was, in the event, not included in the final analysis; these issues are discussed later (section 8.4).

3.3 Information from patients' medical charts

3.3.1 Feasibility

In 1981, the author had conducted a preliminary investigation (in collaboration with the OCTRF) to discover what information on breast cancer patients could be gleaned from the medical records of Ontario hospitals.

The lists, from all six Ontario laboratories, of ER assays carried out during the month 1980.12 formed the sampling frame, which was found to contain over 250 names. A systematic sample (Cochran, 1963; Yates, 1981) was selected as every second woman listed after a starting point chosen at random; the sample consisted of 129 patients. Letters were sent to 69 hospitals requesting, for each patient in the sample, a copy of:

- the admitting notes;
- the operating room report;
- the pathology report; and
- the discharge summary.

Some response relating to 120 women (93%) was received from 66 hospitals (96%), but for only 40 patients (31%) were all four documents received.

All the documentary material was reviewed by a pathologist, who

extracted information on the breast cancer. The two factors most often recorded were the diagnosis and whether or not there had been nodal involvement. However, the material which had been supplied by the hospitals often did not cover the following: the grade of tumour; the stage of the disease; whether or not metastases had been present; details of medication use. Likewise, reproductive histories were seldom provided.

Thus, it was anticipated that the information desired for this research might not have been recorded in full detail in some instances, and would certainly not be available "on request".

3.3.2 Practice

An attempt had nevertheless to be made to record all those items that could not be obtained by interview. The form first designed for this purpose is in Annex II, Appendix C, but the version actually used was a revision, which forms Annex VI.

At the end of the survey, after all subjects had been interviewed, the author visited the cooperating hospitals and transcribed on to this form, from the patients' medical charts (including the four documents listed in 3.3.1 above), whatever relevant information they contained.

Chapter 4

FINDINGS

This chapter presents the findings from the survey, starting (section 4.1) with the enrollment of subjects. Section 4.2 provides the distributions of the interviewed patients according to each of the factors measured (treated singly), and by ER status: wherever appropriate, the ratio of the numbers of ER+ subjects to those of ER- patients [or the "ER+/ER- ratio"] is shown. In the various tables, the number of interviewed patients occasionally differs from 78; the reason is given in the relevant footnote. There are five sub-sections dealing with: socio-demographic factors (4.2.1); medical and reproductive histories (4.2.2); smoking habits (4.2.3); adiposity (4.2.4); and dietary intakes (4.2.5).

4.1 Enrollment of subjects

The following definitions are required:

Potential case: a woman who had a surgical operation for the treatment of primary breast cancer, at one of the five participating hospitals;

Eligible case: a potential case who met the additional eligibility criteria of: menopausal status and age at surgery; the fact that the cancer was primary; and residence;

Provisionally included case: an eligible case whose tumour had been assayed and classed as either ER- or ER+, thus justifying invitation to be included in the survey;

Accepted case: a provisionally included case who was interviewed, and not subsequently rendered ineligible (as a result of information obtained during interview or from the medical chart).

The identification of cases for this study began in two hospitals in 1984.11, but patients were not being recruited from all institutions until 1985.01.15. Table 4.1 gives the numbers of potential, eligible, provisionally included and accepted cases, over the 14 months of subject identification. The monthly counts of potential cases at the five participating hospitals fluctuated considerably. Over the eleven-month period, 1985.02 - 1985.12, throughout which all hospitals were participating fully, a total of 440 surgeries were carried out; instead of there having been approximately 40 operations each month, the numbers ranged from 22 (in 1985.12) to 75 (in 1985.06).

Only 160 patients were found to be eligible for the project. This represents 30% of the total of 524 potential cases, but the proportion varied month by month - from 20% (in 1985.03) to 50% (in 1985.09).

Results of estrogen receptor assay were sought for all the eligible patients, but 52 could not be included even provisionally. One reason (for 30) was that the tumour had not been assayed or the assay results were considered inaccurate [for example, because the tissue sample had been contaminated in handling]. A further 22 were disqualified because the ER asssay results were "intermediate" (10 to 29 fmol/mg).

TABLE 4.1: Distribution of breast cancer surgeries by month of operation, eligibility and inclusion in the study

Date of surgery:	1984 Nov *	1984 Dec *	1985 Jan *	1985 Feb	1985 Mar	1985 Apr	1985 May	1985 June	1985 July	1985 Aug	1985 Sept	1985 Oct	1985 Nov	1985 Dec	Total
Potential cases:	23	23	38	43	29	52	50	75	28	30	32	37	42	22	524
Eligible cases:	5	6	16	10	6	11	14	22	13	9	16	11	11	10	160
Number of eligible cases according to ER assay:															
assay invalid or not done:	1	3	1	2	0	2	3	6	3	1	4	1	1	2	30
intermediate (10-29 fmol/mg):	1	1	2	1	2	2	1	3	2	2	1	1	1	2	22
Provisionally included in the study:	3	2	13	7	4	7	10	13	8	6	11	9	9	6	108
Interviewed and accepted:	2	2	10	6	4	6	9	9	6	4	8	5	6	1	78

* Months during which not all hospitals were fully enrolled.

A total of 108 women (160 eligibles less the 52 just mentioned) were provisionally included in the project. However, two were known to the interviewer, so that ethical issues precluded her approaching them; six others could not be interviewed because of the interviewer's resignation from the LICR. All eight of these losses were from the last three months.

Letters were sent to the remaining 100 provisionally included cases asking for their participation in the investigation. For a variety of reasons, 18 women refused to participate; 12 refusals occurred in the second half of the period in which potential cases were identified.

These 26 losses of provisionally included cases were 6/46 (13%) and 20/62 (32%) in the two halves of the case identification period.

Despite the efforts made to contact patients while still in hospital after surgery, only 47 of the 100 provisionally included cases were so visited. Of these 38 (or 81%) agreed to participate in the project; of the 53 not visited, 44 (or 83%) participated.

Mrs. Hunter interviewed 82 patients in their homes but, subsequently, four had to be excluded from the analyses: information obtained at the interview, or from the chart review which followed, showed that two were not postmenopausal and two had had previous breast cancer surgery. So only 78 patients were "accepted" and so could be included in the analyses; just one-third of these cases had ER- tumours and two-thirds had ER+ disease.

The 30 losses just described were 21% of the ER- subjects provisionally included (7/33) and 31% of the ER+ (23/75).

Table 4.2 shows the distribution of the 78 accepted cases according to the laboratory performing the assay and the hospital at which the surgery took place, as well as by their ER assay result, further sub-classified as:

"very low" (no more than 2 fmol/mg);

"low" (greater than 2 fmol/mg, but less than 10 fmol/mg);

"high" (greater than 29 fmol/mg, but less than 141 fmol/mg); and

"very high" (greater than 140 fmol/mg).

As can be seen, the ER+/ER- ratio was roughly 2:1 for each hospital. However, for Hospital E (served by Laboratory M), there were no "very low" ER assays; for the other hospitals (which used Laboratory N), 11 of 16 tumours fell in this class. Correspondingly, the proportion of ER+ tumours subclassified as "very high" was 15/23 for Hospital E, but only 11/29 in the other hospitals.

Despite the attempt to interview each subject as soon as possible after she had been identified as eligible for the investigation, an average of 9.5 weeks elapsed from the date of surgery to the date of interview. This interval varied greatly: its standard deviation was 4.3 weeks; the shortest delay was 2.6 weeks, the longest over 28 weeks.

4.2 Descriptive findings

4.2.1 Socio-demographic factors

From Table 4.3, it can be seen that patients with ER- tumours were slightly younger than those whose tumours were ER+; mean ages were 61.3 years

TABLE 4.2: Distribution of subjects according to ER assay results; laboratory and hospital

		ER negative			ER positive			
Laboratory	Hospital	very low	low	Total	high	very high	Total	Total subjects
M	E	0	10	10	8	15	23	33
N	D	4	3	7	7	5	12	19
	A	4	2	6	7	2	9	15
	C	2	0	2	3	2	5	7
	B	1	0	1	1	2	3	4
	Sub-total	11	5	16	18	11	29	45
	Total	11	15	26	26	26	52	78

* Throughout this and succeeding chapters, the following definitions apply to ER assay results:
very low: less than 3 fmol/mg
low: at least 3 fmol/ g, but less than 10 fmol/mg
high: at least 30 fmol/mg, but less than 141 fmol/mg
very high: at least 141 fmol/mg

TABLE 4.3: ER status and age

Age at surgery: (years)	ER status: Negative	ER status: Positive	ER+/ER- ratio	Total subjects
50-54	5	5	1.00	10
55-59	6	14	2.33	20
60-64	9	13	1.44	22
65-69	4	8	2.00	12
70-74	1	8	8.00	9
75-79	1	4	4.00	5
Total	26	52	2.00	78
Mean age (yr):	61.3	63.7		
s.d.	6.4	7.2		

(standard deviation 6.4 years) and 63.7 years (s.d. 7.2 years), respectively.

Forty-eight of the 78 subjects in the study (61.5%) lived in the City of Toronto, 17 (21.7%) in the City of North York (which is part of Metropolitan Toronto), and the remaining 13 in several other cities, etc. The 78 women interviewed reported a total of 18 different countries of birth: 47 (or 60%) were born in North America (all but one in Canada); 13 of the women had been born in Western Europe, 11 in Eastern Europe, and 7 in other places (India, Syria, Guyana, Jamaica). The ER+/ER- ratio varied only slightly according to country of birth, and without discernible pattern.

Altogether, 14 different religions were reported; 11 of the subjects (14%) were Jewish. The ER+/ER- ratios for Jewish and Gentile women were similar.

Table 4.4 shows that the ER+/ER- ratio differed somewhat according to the highest certificate of education obtained, but there was no semblance of trend.

At least partly because the question about occupation did not specify clearly whether present or former occupation should be reported, 28 subjects called themselves retired or unemployed or homemakers. Present or former employment outside the home was reported by the other 50 women. The ER+/ER- ratio of the 28 and the 50 were both close to 2:1.

Information on marital status was available for 77 subjects. As can be seen from Table 4.5, for the separated and divorced women the ER+/ER- ratio was 3:8 (= 0.38); among all the other patients the ratio was 48:18 (or 8:3 = 2.76), highly consistent for the single, married and widowed.

TABLE.4.4: ER status and education

	ER status		ER+/ER- ratio	Total subjects
	Negative	Positive		
Highest certificate of education obtained:				
None	8	9	1.12	17
High School	10	22	2.20	32
Trade or non-university	2	12	6.00	14
University	5	8	1.60	13
Total	25	51	2.04	76 *

* Level of education was not determined for two subjects (one each ER- and ER+)

TABLE 4.5: ER status and marital status

	ER status			
	Negative	Positive	ER+/ER- ratio	Total subjects
Marital status:				
Single	4	11	2.75	15
Married	11	29	2.64	40
Widowed	3	8	2.67	11
Separated or Divorced	8	3	0.38	11
Total	26	51	1.96	77 *

* Marital status was not determined for one subject (ER+)

It was not surprising that nine women refused to answer the question on family income; it was probably polite refusal that caused a further 19 to say they "did not know". The ER+/ER- ratio of these 28 "refusals" was 19:9, or as close to 2:1 as possible (Table 4.6). Among the 50 subjects who did report their income, the ER+/ER- ratio varied considerably, but no pattern was evident.

4.2.2 Medical and reproductive histories

Most of the tumours had been unilateral (38 in the left breast, 35 in the right), but four patients had bilateral surgery: and laterality had not been entered on the chart of one patient (whose tumour was ER+). All eight of the bilateral tumours were ER+, so in terms of tumours (as distinct from patients), the ER+/ER- ratio was 56:26 (= 2.15). Location of the tumour in the breast could not be classified for 18 subjects (23%). The specified locations were: the upper-outer quadrant for 28 patients; near the nipple for 13; in the upper-inner quadrant for 9; while the remaining 10 were distributed across four other locations. The ER+/ER- ratio was 43:25 (not far from 2:1) except for tumours from the upper-inner area, for which the ratio was 8:1.

My enquiry in 1981 had indicated that information from various sources comprising any one patient's chart could be discordant as to tumour size; in the present enquiry, major disagreements on size of tumour were recorded for 19 out of the 78 subjects (24%).

The most common single pathological diagnosis of the breast cancer was ductal carcinoma (54 tumours; see Table 4.7), which was also recorded, with another diagnosis - never adenocarcinoma - for a further six tumours. Adenocarcinoma (alone) was the diagnosis for 8 tumours. The ER+/ER- ratios

TABLE 4.6: ER status and family income

Annual family income (thousand $)	ER status: Negative	ER status: Positive	ER+/ER- ratio	Total subjects
< 15	3	10	3.33	13
15, < 35	9	3	0.33	12
35, < 55	4	17	4.25	21
55 or more	1	3	3.00	4
Subtotal	17	33	1.94	50
Not determined	9	19	2.11	28
Total	26	52	2.00	78

TABLE 4.7: ER status and pathological diagnosis

	ER status		ER+/ER- ratio	Total subjects
	Negative	Positive		
Pathological diagnosis of breast tumour:				
Ductal carcinoma (alone)	18	36	2.00	54
Ductal carcinoma, with at least one other diagnosis (not adenocarcinoma)	3	3	1.00	6
Adenocarcinoma	4	4	1.00	8
Other known single diagnosis	1	8	8.00	9
Total	26	51	1.96	77 *

* A pathology report was not available for one subject (ER+)

were 2:1 (as for all patients) among the ductal carcinomas, and 1:1 among both multiple diagnoses (including ductal carcinoma) and adenocarcinoma. The nine other known single diagnoses were: ductal in situ (2); lobular cancer (2); tubular cancer; mucinous cancer; and three that did not fall into the LICR classification. All were ER+, except for one case of lobular cancer.

Although in 30 of the 78 patients (38%) the disease had spread to the nodes by the time of surgery, the ER+/ER- ratio remained close to 2:1. As had been expected from the 1981 survey, grade of tumour and stage of disease had been entered on few charts (20% and 55%, respectively).

Reported ages at menarche ranged between 8 and 19 years, but for over three-quarters it was 12, 13 or 14; see Table 4.8(a). Only in the women reporting menarche at age 15 or over was the ER+/ER- ratio substantially less than 2:1. There was little consistent association between ER status and reported age at menopause; see Table 4.8(b). The menopause was reported as natural by 78% and surgical by 22% of the patients, and the corresponding ER+/ER- ratios were both close to 2:1. Associations were weak between ER status and both years of menstruation and years since menopause.

In Table 4.9, the 78 subjects are classified by their gravidity (i.e. the number of times they had been pregnant) and the ER status of their tumours; for ease of interpretation the number of pregnancies are listed in descending order. Among the 61 women who had been pregnant, there was a clear (effectively linear) relationship between ER status and the number of pregnancies: the ER+/ER- ratio was higher the fewer the pregnancies.

TABLE 4.8: ER status and (a) age at menarche and (b) age at menopause

	ER status		ER+/ER- ratio	Total subjects
	Negative	Positive		
(a) Age at menarche (years):				
11 or younger	4	7	1.75	11
12	5	15	3.00	20
13	6	15	2.50	21
14	7	11	1.57	18
15 or older	4	3	0.75	7
Total	26	51	1.96	77 *
(b) Age at menopause (years):				
39 or younger	1	3	3.00	4
40-44	5	5	1.00	10
45-49	7	17	2.43	24
50-54	11	21	1.91	32
55 or older	2	5	2.50	7
Total	26	51	1.96	77 *

* One subject (ER+) could recall neither the age at which she started menstruating nor the age at which menstruation ceased

TABLE 4.9: ER status and gravidity

Number of pregnancies:	ER status: Negative	ER status: Positive	ER+/ER- ratio	Total subjects
7	3	0	0.00	3
6	2	1	0.50	3
5	2	2	1.00	4
4	5	5	1.00	10
3	6	7	1.17	13
2	2	19	9.50	21
1	1	6	6.00	7
1-7	21	40	1.90	61
0	5	12	2.40	17
Total	26	52	2.00	78

However, when all 61 subjects were considered together the ER+/ER- ratio was 1.90. This did not differ greatly from the ratio of 2.40 for those 17 women who were never pregnant.

The tumours of these 17 subjects were further sub-classified as in Table 4.2, and it was found that none of the 5 ER- tumours had "very low" assays. On the other hand, of the 21 women who had been pregnant and had ER- tumours, 11 assays were "very low".

At least one incomplete pregnancy was reported by 28 of the 61 women who had been pregnant; the ER+/ER- ratio of these 28 was 1.33, compared with 2.67 for the other 33 women.

Of the 61 women who had been pregnant, all but five had given birth to live children. The association between ER status and parity (number of livebirths) was so similar to that shown in Table 4.9 it is not illustrated here.

In the upper portion of Table 4.10(a), which concerns the 56 parous women, it is clear that the younger the woman at the time of her first baby's birth the lower the ER+/ER- ratio. The lower portion shows that the 22 nulliparous women had an ER+/ER- ratio corresponding to a first birth at "mother's age" somewhat over 30 years. Table 4.10(b), dealing only with parous subjects, shows that the ER+/ER- ratio was rather higher in the women who had never breast-fed than in those who had.

Of the 22 women who reported that a female relative had suffered from breast cancer, 10 were ER-, yielding an ER+/ER- ratio of 12:10 (= 1.20); for the 52 subjects whose families were apparently without a history of the disease the ratio was 38:14 (= 2.71) (Table 4.11). The ER+/ER- ratios for

TABLE 4.10: ER status and (a) age at first birth and (b) breast-feeding a baby

	ER status		ER+/ER- ratio	Total subjects
	Negative	Positive		
(a) Age at first birth:				
less than 20	2	0	0.00	2
20-24	9	12	1.33	21
25-29	9	17	1.89	26
30 or older	1	6	6.00	7
All parous	21	35	1.67	56 *
Nulliparous	5	17	3.40	22
Total	26	52	2.00	78
(b) Breast-feeding:				
No	6	15	2.50	21
Yes	15	20	1.33	35
Total	21	35	1.67	56 *

* The 56 parous subjects

TABLE 4.11: ER status and family history of breast cancer

	ER status			
	Negative	Positive	ER+/ER- ratio	Total subjects
Reported familial history of breast cancer:				
Breast cancer in a first degree relative	6	10	1.67	16
Breast cancer in a second degree relative	4	2	0.50	6
Family history of breast cancer reported	10	12	1.20	22
No breast cancer reported	14	38	2.71	52
Total	24	50	2.08	74 *

* Family history of breast cancer was not determined for four subjects (two each ER- and ER+)

patients reporting breast cancer in first-degree (i.e. mother, sister or daughter) and second-degree relatives (i.e. grandmother or aunt) were 1.67 and 0.50, respectively; no explanation has been found for this pattern.

Eighteen women (23%) reported having had a hysterectomy; their ER+/ER- ratio was the usual 2:1. When asked about bilateral oopherectomy, two of the women (whose tumours were assayed as ER+) did not know whether or not their ovaries had been removed. Among the other 76, ten reported having had this operation; the ratio of ER+/ER- was again close to 2:1. Seventeen subjects (22%) gave a report of thyroid disease; their ER+/ER- ratio was 1.83. Any surgical biopsy for lumps in the breast before the cancer surgery [a crude surrogate for history of benign breast disease] was reported by only seven subjects: 4 were ER-, 3 ER+; for the other 71 patients the ratio was close to 2:1.

As Table 4.12 shows, the use of oral contraceptives was reported by 20 patients, and the postmenopausal use of hormone replacement by 26. The ER+/ER- ratios in users and non-users of the two medications were 2.22 and 1.50 (oral contraceptives) and 2.25 and 1.60 (postmenopausal hormones).

4.2.3 Smoking habits

Almost half of the women interviewed (37/78) reported that they had never smoked, and only 9 women (12%) stated they were current smokers (Table 4.13). The ER+/ER- ratio was not far from 2:1 for the non-smokers and ex-smokers, but was 3.50 for the current smokers.

4.2.4 Adiposity

Table 4.14 gives means (with standard deviations) of: (a) height, as reported at interview; (b) weight (with corresponding Body Mass Index, BMI), six months before surgery; (c) weight and BMI at age 20 years; and

TABLE 4.12: ER status and use of (a) oral contraceptives and (b) postmenopausal hormones

	ER status		ER+/ER- ratio	Total subjects
	Negative	Positive		
(a) Use of oral contraceptives:				
No	18	40	2.22	58
Yes	8	12	1.50	20
Total	26	52	2.00	78
(b) Use of postmenopausal hormones:				
No	16	36	2.25	52
Yes	10	16	1.60	26
Total	26	52	2.00	78

TABLE 4.13: ER status and smoking history

	ER status		ER+/ER- ratio	Total subjects
	Negative	Positive		
Reported smoking history:				
Never smoked	13	24	1.85	37
Ex-smoker	11	21	1.91	32
Current smoker	2	7	3.50	9
Total	26	52	2.00	78

TABLE 4.14: ER status and height, weight and Body Mass Index
(Figures quoted are means, with standard deviations in brackets)

[Numbers of subjects for parts (a) and (b) were 26 ER- and 52 ER+; for parts (c) and (d) they were 25 ER- and 49 ER+, because four subjects could not recall their weights at age 20.]

	ER status	
	Negative	Positive
(a) Height (cm.)	160.0 (6.8)	161.8 (6.8)
(b) Six months before surgery:		
Weight (kg)	63.9 (14.6)	64.6 (12.8)
Body Mass Index (kg/m²)	25.0 (5.8)	24.7 (5.0)
(c) At age 20 years:		
Weight (kg)	52.7 (7.6)	52.4 (7.6)
Body Mass Index (kg/m²)	20.5 (2.9)	20.1 (2.6)
(d) Change from 20 years to six months before surgery:		
Body Mass Index	4.4 (5.2)	4.8 (4.8)

(d) change in BMI from age 20 to six months before surgery. The mean heights of the two groups were closely similar, and the differences between eventual ER- patients and ER+ cases were minuscule, in weight and in BMI, both at age 20 and six months before surgery.

Body mass indices, which have been in use at least since the days of Roger Quetelet (1796-1874), are predicated (inter alia) on the positive association of a person's weight with her height; in other words it is normally assumed that "height is an important determinant of weight" (Rosenbaum et al, 1985). However, this association is not as great as might be expected from this last quotation: indeed, the coefficient of correlation between weight and height for a representative sample of British females aged 50-64 years in 1980 can be estimated from Table 16 in Rosenbaum et al (1985) as +0.31. In the present material, the correlation between reported weight six months before surgery and reported height, was rather lower at +0.19.

4.2.5 Dietary intakes

The intakes of total fat, dietary fibre, protein and total energy, estimated from the food frequency questionnaire as daily averages, are shown in Table 4.15. The means of the daily average intakes of each nutrient were marginally lower for the ER- subjects than for the ER+ patients.

Table 4.16 presents the frequency distribution of the 26 ER- and 52 ER+ subjects according to their reported daily caloric intake. The

TABLE 4.15: ER status and nutrient intakes (Figures quoted are means with standard deviations in brackets)

Nutrient intake	ER status: Negative	ER status: Positive
Energy (kcal/day):	1946 (705)	2122 (805)
Fat (g/day):	75 (24)	82 (37)
Protein (g/day):	80 (27)	82 (35)
Fibre (g/day):	17 (8)	19 (10)
Number of subjects:	26	52

TABLE 4.16: ER status and caloric intake

Caloric intake (kcal/day):	ER status: Negative	ER status: Positive	Total Subjects
< 1000	0	3	3 (3.8%)
1000, < 1500	8	8	16 (20.5%)
1500, < 2000	6	14	20 (25.6%)
2000, < 2500	8	15	23 (29.5%)
2500, < 3000	3	3	6 (7.7%)
3000 or more	1	9	10 (12.8%)
Total	26	52	78 (100%)

coefficients of correlation between the total caloric intake and Quetelet index were as follows:

ER- cases (26): -0.273

ER+ cases (52): +0.075

All subjects (78): -0.042

Chapter 5

ANALYSIS

This chapter describes the analyses carried out to achieve the stated objective [section 2.1], viz:-

> To identify, among postmenopausal women with breast cancer, those dietary and reproductive factors which distinguish ER negative tumours from ER positive tumours.

This aim called for one or more of the group of statistical techniques known generically as "discriminatory analysis". The first such technique was introduced in 1936 by R. A. Fisher; much more recently (in 1962), J. Cornfield described what he called a discriminant function analysis (of the risk of coronary heart disease in relation to serum cholesterol and blood pressure), which has since been termed logistic regression analysis. Both forms of discrimination were used here, as described in section 5.2.

However, as a preliminary, it was necessary to decide upon an approach to the aims of the research, and this is outlined in section 5.1. The approach adopted had three stages, the findings from which are presented in sections 5.3, 5.4 and 5.5. The final section (5.6) indicates the importance of the degree of discrimination between ER- and ER+ tumours achieved by the various factors.

5.1 The approach adopted

As the original component of this research was to determine whether dietary intakes were associated with ER status, Stage 1 was to introduce into the discriminatory analyses only the four nutrients (food components), regardless of other factors. The food intakes were incorporated both separately and all together. A device for "adjusting" specific nutrients in relation to total energy was also investigated. This stage was performed including all 78 subjects, and the findings in section 5.3 are on this basis. (For the later stages, it was necessary to exclude one subject, so Stage 1 was repeated on the remaining 77 subjects.)

Stage 2 was concerned with variables other than dietary. These variables were introduced "stepwise", as will be described in section 5.4. One subject could recall neither when she started nor when she finished menstruating. To maintain consistency as to the number of subjects included in this stage, the analyses were carried out only on the other 77 women. (As will be explained in section 5.4, certain variables were excluded from discriminatory analyses because there would otherwise have been substantial variation, from factor to factor, in the numbers of subjects; this would have vitiated the step-wise process.)

Thereafter, in Stage 3, both sets of variables were entered into the discrimination process. Again, a stepwise procedure was adopted, and, as for Stage 2, only 77 subjects were incorporated.

5.2 Forms of discriminatory analysis

As explained by Armitage (1971), the most widely used multivariate method was that of the "linear discriminant function". For the variables x_1, x_2,... x_k, such a function is of the form:-

$$z = b_1x_1 + b_2x_2 + \ldots + b_kx_k$$

where the b_1, b_2,..., b_k are coefficients chosen so that the mean values of z in the two groups (here ER- and ER+) are as far apart as possible in comparison with the variation of z within groups. This discriminant function is likely to be a good indication of the relative likelihoods of the two groups (Armitage, 1971). It has been shown (Liddell, 1988) that the method is usually robust to departures from the postulated multivariate normality of the distributions of the variables.

Schlesselman (1982) pointed out that logistic regression analysis can be used for case-referent studies - and so also in the present circumstances - citing four references from the 1970's. The principles are broadly similar to those just described, but the criterion for the choice of the coefficients is to provide the best indication of the relative likelihoods of the two groups (Schlesselman, 1982). It will be clear, if only from analogy, that logistic regression will also separate the groups well. In fact, the form of the function appears broadly similar, i.e.

$$\log[p/(1-p)] = \underline{b}_1x_1 + \underline{b}_2x_2 + \ldots + \underline{b}_kx_k$$

where p is the empiric probability of belonging to one of two groups. Logistic regression has been widely adopted in epidemiologic research, but with "no overriding biological rationale" (Schlesselman, 1982), and despite the fact that the methods of calculation can only be iterative.

It is important to appreciate that the coefficients $b_1, b_2, \ldots, b_k$ and $\underline{b}_1, \underline{b}_2, \ldots, \underline{b}_k$ in the two types of function are not strictly equivalent. However, the two modes of analysis will usually yield similar results, with parallel means of evaluating the degree of discrimination. The first, which (in this thesis, as usually elsewhere) is called simply "discriminant analysis", yields an F-statistic, with p and (N - p - 1) degrees of freedom (df) [where p is the number of variables in the discriminant function, and N is the number of subjects in the two groups combined], and the second ("logistic regression analysis") a χ^2-statistic, with p df. For large N, the distribution of F [with p df for the numerator] is close to that of $(\chi^2)/p$ [with p df for the χ^2] (Liddell, 1983).

Both forms of discrimination were adopted in each stage; in view of the immediately preceding paragraph, the findings are presented in parallel. The actual analyses were carried out as follows:-

Discriminant analysis - using the regression analogue, with ER status represented by a dichotomous variable (Armitage, 1971); the program was taken from SAS package (SAS Institute Inc., 1985).

Logistic regression analysis - using the program in the SAS package (SAS Institute Inc., 1985).

Finally, advantage was taken of an extension of the regression analogue to discriminant analysis to explore the relationships between ER levels (in fmol/mg) and the "explanatory" variables. However, this produced so little additional information that it has not been considered worth reporting.

5.3 Stage 1: dietary intakes

The variables entered into the two forms of discriminatory analysis (together with the differences in mean intakes [Table 4.15] ranked according to the values of the [univariate] t-statistics) were:-

Nutrient	Overall mean intake (78 subjects)	Differences in mean intake (ER+ less ER-)	t-statistic (76 df)
Fibre	18.3 g/day	2.2 g/day	1.04
Fat	79.9 g/day	7.6 g/day	0.95
Energy	2063 kcal/day	175 kcal/day	0.94
Protein	81.2 g/day	2.4 g/day	0.31

These four variables were entered separately, one by one, and the results of both analyses are in Table 5.1. Each F-statistic is the square of the t-statistic quoted above (any discrepancies in the figures being due to rounding). See Chapter 9 for comment on the agreement between the differences in mean intakes found indirectly, by manipulation (McKeown-Eyssen and Thomas, 1985) of the findings from logistic regression, and those found directly; also on the accord of the χ^2 and F statistics.

As planned, the analyses were repeated with all four variables incorporated into the discrimination. The test statistics were: F = 0.56 (df: 4 and 73), and χ^2 = 2.52 (df:4); this last statistic, divided by the number of variables, yields (χ^2)/4 = 0.63.

All these analyses were repeated on the 77 subjects used in Stage 2, but - as anticipated - results were virtually identical, and therefore are not reported.

TABLE 5.1: Discrimination of ER status by dietary intakes

Nutrient	Differences in mean intakes (ER+ less ER-)		Test - statistic *	
	Discriminant Analysis	Logistic Regression	F	χ^2
Fibre (g/day)	2.2	2.3	1.07	1.07
Fat (g/day)	7.6	8.2	0.91	0.91
Energy (kcal/day)	175	186	0.89	0.89
Protein (g/day)	2.4	2.5	0.10	0.10

* df for F-statistic (discriminant analysis): 1 and 76;

df for χ^2-statistic (logistic regression): 1

One accepted analytical practice (Willett et al, 1985) has been to "adjust" dietary intakes for the intake of total energy. This practice was adopted here, but is not reported because it provided no further insight. Recently, Willett and Stampfer (1986) have proposed a variation of considerable complexity. In view of the extremely weak discrimination achieved by total energy intake or by the other nutrients, this more complex approach was not considered necessary.

5.4 Stage 2: variables other than dietary intakes

As already explained, one subject had to be excluded; her tumour was ER+, so that throughout this stage the discrimination is between 26 ER- patients and 51 ER+. The variables included in Stage 2 are listed in Table 5.2, which also indicates the units in which they were measured.

Two variables, marital status and level of education, were excluded because of the obscurity of their relationship with ER status. Four others - parity itself, incomplete pregnancies, age at birth of first child, and breastfeeding - were, of course, available only for the 56 parous women; these factors could not be incorporated into the discriminatory analyses. To eliminate nulliparous women from these analyses would have reduced the sample size drastically (i.e. to 56); much worse, it would have masked the complex inter-relationship (see Table 4.9) of ER status and number of pregnancies.

To maintain the necessary comparability of the ability to discriminate, the same number of subjects had to be anlysed throughout. However, certain variables were incompletely recorded, viz:- family history

TABLE 5.2: Variables for Stage 2

Age at surgery	years
Religion	0=non-Jewish; 1=Jewish
Age at menarche	years
Age at last menstrual period	years
Type of menopause	1=natural; 2=surgical
Years of menstruation	years
Number of pregnancies	0 - 7
Thyroid disease	0=no; 1=yes
Benign breast disease	0=no; 1=yes
Oral contraceptive use	0=no; 1=yes
Postmenopausal hormone use	0=no; 1=yes
Smoking habits	1=current; 2=ex; 3=never
Weight (6 months before surgery)	kilograms (kg)
Body mass index (6 months before surgery)	kg/m^2

of breast cancer (for 4 patients), weight at age 20 years - and hence BMI at age 20 and change in BMI from age 20 - (4 different women), and family income (28 subjects). These five variables were, therefore, also excluded from the main discriminatory analyses. Thus, the number of subjects included throughout Stages 2 and 3 was maintained at 77. [Earlier analyses had included several of these variables on subsets of the subjects, without indication of their discriminatory ability.]

The first steps of both analyses identified number of pregnancies as an important discriminatory variable (see Table 4.9). Whereas those women who had been pregnant had ER+/ER- ratios varying from 0, for gravidity 7, to over 8 for gravidity 2 and 1, those who had never been pregnant had ER+/ER- ratio of 2.40. Therefore, gravidity could not be introduced in the usual (linear) sense.

Inspection of Tables 4.9 and 4.10 indicated that neither women of gravidity 0, nor the nulliparous, fitted the corresponding patterns for the rest of the patients. [A parallel is found in relation to risk of breast cancer (without regard to ER status) by age at first birth (Kelsey, 1979).] It is impossible to use the usual linear form in any of these situations, despite the fact that (for instance), the relationship of gravidity with ER status was close to linear for those who had been pregnant. There are no biological reasons for fitting a functional transform - even if there were a transform that fitted the data adequately.

The phenomenom was studied in terms of the number of pregnancies. This number, when allowed to enter both as no/yes and numerically, dominated the discrimination. Three methods of overcoming the discontinuity of the relationship were tried. Despite obvious drawbacks a functional transform

(namely, the square of the number of pregnancies) was used: the degree of discrimination is indicated by an F-statistic of 13.1 (with 1 and 75 df). The second method was to use as surrogate for the number of pregnancies the observed value of the proportion of subjects who were ER-. By definition, this would give the best discrimination - in fact F = 20.6 (also with 1 and 75 df). However, this degree of discrimination might well be artifactually high, removing more of the variability than could be justified. This could inhibit the demonstration of the discriminatory abilities of other variables.

The chosen method was to use the actual number of pregnancies (1 through 7), replacing 0 by a "notional number of pregnancies" in conformity with the observed ER+/ER- ratio (2.40) of these subjects. A regression analysis on the 61 subjects who had been pregnant provided the following linear equation:

$$\text{(Proportion of subjects whose tumours were ER-)} = -0.0984 + 0.1444\ \text{(number of pregnancies)}$$

Substituting the proportion corresponding to an ER+/ER- ratio of 2.40 (i.e. 0.2941) in this equation yielded the notional number of pregnancies 2.72, found from (0.2941 + 0.0984)/(0.1444). Although this is only an estimate, it might be considered too precise, and so several other values (lying between 2 and 3; see Table 4.9) were also substituted independently.

The discriminatory power of this device is shown by the following values of the F-statistic (all with 1 and 75 df):

Value substituted for zero gravidity	F-statistic
2.25	16.1
2.50	16.5
2.72	16.7
2.90	16.7

All these F-statistics were in close conformity, lying between the values of 13.1 (for the - inadequate - functional transform) and 20.6 (for the device yielding maximal discrimination). Further, the rest of each analysis was virtually unchanged. It therefore seemed reasonable to adopt as "definitive" the analysis using the regression-determined substitute of 2.72. Although all this exploration could have been confirmed using logistic regression analysis, in view of very close parallels seen throughout this chapter (and discussed in Chapter 9), only the "definitive" analysis was repeated by logistic regression.

Table 5.3 presents the parallel findings of the two discriminatory analyses of Stage 2. Even with the p-value for the extra discrimination of a new variable set as high as 0.30, only two were included, viz:- age at menarche and a history of benign breast disease. Table 5.3 gives the "stepwise" test statistics (with the corresponding degrees of freedom, df) for the three variables which contributed to the discrimination between ER- and ER+ cases.

TABLE 5.3: Discrimination of ER status by variables other than dietary

Variable	Stepwise test statitics, with df			
	Discriminant Analysis		Logistic Regression	
	F	df	χ^2	df
Number of pregnancies *	16.67	1,75	14.00	1
Age at menarche	1.91	1,74	1.66	1
History of benign breast disease	2.34	1,73	2.94	1

* For subjects who had never been pregnant, the number of pregnancies was replaced with the value 2.72 (see text).

5.5 Stage 3: dietary intakes and other variables

In this stage the four dietary intakes used in Stage 1, and the variables listed in Table 5.2, were incorporated. The numbers of subjects were 26 ER- and 51 ER+ (as they had been for Stage 2).

The first three steps of both analyses were identical to those of Stage 2. In the fourth step, the intake of dietary fibre was deemed, by the same criterion as before, to contribute to the discrimination. No additional variable met this criterion, so there was no fifth step.

Thus, a table giving the stepwise test statistics with degrees of freedom for all four steps of Stage 3 would have as the first three entries the material in Table 5.3. The only new information is that relating to the fourth step, and is given in Table 5.4.

5.6 Factors discriminating ER status

It will be recalled that the coefficients (b_1, b_2,... b_k) in the discriminant function $z = \Sigma\, b_i x_i$ had been chosen so that the mean values of z in the ER- and ER+ groups were as far apart as possible in comparison with the variation of z within the groups (section 5.2). After Stage 3 had been completed, the values of the discriminant function for all 77 subjects were obtained. The means of z for the 26 and 51 patients differed by 0.2418 in the arbitrary units provided by the specific program. The within group variance was calculated, and from it the standard error (se) of the difference in the means; the value of se was 0.0494, so that the separation of the means was by a factor of 0.2418/0.0494 se = 4.89 se. [It must be emphasized that this ratio does not form a valid t-test; nor do any of the

TABLE 5.4: Fourth step of discrimination of ER status by all variables

	Stepwise test statistics, with df			
	Discriminant Analysis		Logistic Regression	
Variable	F	df	χ^2	df
Fibre	1.39	1,72	1.28	1

other ratios that will be presented below. Nevertheless, some rough indication of statistical significance may be obtained by referring these ratios to the t-distribution, with df around 70.]

Table 5.5 shows, for the four factors which were selected in Stage 3 as contributing to the discrimination, their mean values for the 26 ER- patients and for the 51 ER+ subjects, the difference in their means, the value of the coefficient, b, adjusted to allow comparison with the variation of z within groups (i.e. amending from arbitrary units to units of Se), and the contribution (viz: the product of the adjusted coefficient and the difference between means) that factor made to the discrimination (or in other words, to the separation of the mean values of the discriminant function z for the two groups of subjects).

It can be seen that the contributions in the final column of Table 5.5 are dominated by that from the number of pregnancies (bearing in mind the device of substituting the value 2.72 - instead of 0 - for women who had never been pregnant). -The ER+ group had, on average, fewer pregnancies, a marginally lower age at menarche, less benign breast disease, but consumed slightly more dietary fibre.

Because the coefficients in the two forms of discriminatory analysis are not equivalent, the findings from the logistic regression analysis are presented rather differently; see Table 5.6. Here, for the same four factors, the differences in group means are again presented, along with each corresponding coefficient b in the discriminant function (from logistic

TABLE 5.5: The discrimination of ER status: discriminant analysis

[Each figure in this table has been correctly rounded before entry.]

	Means		Difference in means	Adjusted † coefficient	Contribution † to the discrimination
	ER-	ER+	ER+ less ER-		
Number of pregnancies *	3.83	2.57	-1.26	-2.85	3.60
Age at menarche	13.15	12.73	-0.43	-1.09	0.47
Benign breast disease	0.15	0.06	-0.10	-5.25	0.50
Fibre (g/day)	16.79	19.23	+2.44	+0.13	0.32
					4.89

* See footnote to Table 5.3.

† See text for definitions.

TABLE 5.6: The discrimination of ER status: logistic regression

[Each figure in this table has been correctly rounded before entry.]

	Difference † in means (ER+ less ER-)	Coefficient §	Odds Ratios ER-	ER+
Number of pregnancies *	-1.26	-0.77	2.64	0.38
Age at menarche	-0.43	-0.32	1.15	0.87
Benign breast disease	-0.10	-1.47	1.15	0.87
Fibre (g/day)	+2.44	+0.01	1.09	0.91
			3.81	0.26

* See footnote to Table 5.3.

† Repeated from Table 5.5.

§ Coefficient, $\underline{b}_1$, in the linear discriminant function from logistic regression analysis.

regression). The final two columns give the Odds Ratio (OR), calculated from this material, for ER- tumours and for ER+ tumours, corresponding to the observed difference in the means for each variable. [The Odds Ratios for all four factors combined are the products of those for the factors separately.]

Again it can be seen that the discrimination between ER- and ER+ patients is dominated by the number of pregnancies.

Chapter 6

DISCUSSION I: FIELDWORK

The first part of this chapter deals with the vital issue of the scarcity of eligible patients. Thereafter, there are nine sections dealing with delays in getting field work under way (6.2); inadequacies of planning information (6.3); unwillingness of the surgeons at one hospital to cooperate (6.4); a "competing" investigation (6.5); patients' refusals to participate (6.6); other reasons for loss of patients (6.7); benefits from redefinition of ER- and ER+ patients to be included (6.8), and from widening of the catchment area (6.9); and, finally, the work plans (6.10).

6.1 Scarcity of eligible subjects

Poor case accrual is a problem in many investigations. A close eye was kept on the numbers of patients available for study, and shortfalls were identified early on. The processes of estimating numbers are outlined in the following paragraphs.

The preliminary estimates of patient accrual were based on various sources, as follows:-

(1) The surgical procedures for treatment of breast cancer for women aged 50-79 years in the six Toronto hospitals performing the most breast surgery

(Table 1.2). This table showed a steady increase from 1981-82 to 1984-85, and the estimate for the year 1985 was nearly 500.

(2) Although ER assay lists for women of appropriate ages for 1981 (Table 1.7) indicated that by no means all these tumours would be subject to assay, no allowance was made for any shortfall. This was primarily because the material was rather dated, and it was thought reference rates would probably have become substantially higher in 1985.

(3) The corresponding ER assays for 1982 (collected by the author from the laboratory) gave the distribution of tumours at four of the selected hospitals as:

fmol/mg	< 3	3 - 9	10 - 29	30+	Total
tumours	69	40	47	133	289

Extrapolating from these figures to the 500 cases in (1) above, provided estimates of 500(69/289) = 119 ER- cases [on the basis of ER- status given in the protocol of section 2.3] and 500(133/289) = 230 ER+ cases [from which it was intended to make a selection].

(4) Refusals of cooperation, and failures to meet the criteria for inclusion, would inevitably reduce these figures somewhat, but it did not appear unreasonable that 100 ER- cases could be recruited for interview within a year or a little longer. Clearly, there would be - on this basis - more than sufficient ER+ cases to permit a one-for-one selection.

These estimates had to be adjusted downwards for several reasons.

(5) The facts that patients from Hospitals F and B could not be recruited (because of lack of cooperation at Hospital F and early referral for

radiotherapy at Hospital B) meant that, on the basis of 1982 assay lists, there wold be a shortfall amounting to 37%. The estimates in (3) thus became 75 ER- and 145 ER+ patients.

(6) During 1985, it was revealed that the number of ER assays carried out the previous year (241) was only 83% of those (289) performed in 1982. At this stage, it appeared necesary to make some allowance for shortfall of assayed cases, although this had not been done at (2) above. No reliable allowance could be determined, but the numbers at (5) above would have to be reduced, say to 60 ER- and 120 ER+ cases.

(7) At least initially, few patients refused to cooperate, but - even from the outset - substantial proportions of subjects were lost for other reasons; these included recurrent disease, residence outside the defined catchment area, inability to communicate in English, and not having reached the menopause (although over 50 years of age). The shortfall was very high, around two-thirds. Thus the estimates at (6) had to be reduced to around 20 ER- and 40 ER+ (of which only 20 were to be included).

It was clear that immediate action had to be taken to improve the situation, if anything like 100 patients, in all, were to be recruited within one year (see section 2.5). The major changes were as follows:-

(8) The redefinition of ER- status as less than 10 fmol/mg would, according to the information at (3) above, increase the ER- cases in roughly the proportion (40 + 69)/69, i.e. by 58%, or to approximately 32 ER- cases in all.

(9) The decision to include all ER+ subjects increased the expected number available to the 40 mentioned in (7) above - instead of only 20.

A final attempt to improve recruitment of cases was:

(10) An extension of the catchment area, although this was not expected to have a major influence on the numbers of subjects.

It must, of course, be clear that all the estimates in (1) through (9) above could provide orders of magnitude only. In this light, the numbers of interviewed cases (26 and 52) were in fair agreement with the figures in (8) and (9), i.e. 32 and 40.

6.2 Delayed start

Before field work could start, much longer delays than anticipated were encountered in obtaining not only ethical approval from the University of Toronto and the hospitals, but also the surgeons' cooperation. Indeed, this lead-time in the present research was effectively six months.

6.3 Inadequacy of planning information

The only information on ER status of elderly breast cancer patients in Toronto was contained in lists which related to only four of the participating hospitals; at the planning stage, the latest available lists were for 1982. Therefore, estimates could only be crude. Further, as these lists did not provide detail concerning menopausal status, whether the disease was primary or recurrent, residence or language, it would not have been possible to foresee any of the shortfalls related to these factors.

6.4 Surgeons' refusal to cooperate

At one hospital, F, which it had been estimated would have provided about 20% of the patients, participation was not forthcoming. It might appear that an obvious solution would have been to replace this institution; however, this would have entailed enrolling at least two additional hospitals. The six hospitals originally selected (A through F) treated, between them, fifty percent of all eligible Toronto patients (Table 1.2), and no other single institution treated enough patients to be adequate as a replacement for Hospital F. The effort needed to enroll two additional hospitals - obtaining ethical approval and support from the surgeons (and secretaries) - and the associated delays before patients could be identified would have meant that information obtained in time for inclusion in the thesis would have been on so few patients as to render the exercise futile.

6.5 The "competing" investigation

The conflict, mentioned in section 2.4, with another breast cancer study led to the loss of virtually all cases from Hospital B (nearly 20% of the original estimate of eligibles) and also of a further not insubstantial number from the other four participating hospitals.

The problem of research projects vying for the same patients is indeed important and by no means uncommon. Usually, either one Principal Investigator is given priority (to the serious detriment of others), or the cases of interest are "shared" in a way which tends to leave each investigator unhappy. Mechanisms of collaboration should be considered in all such conflicts of research interest. A proposal was made, in the Spring

of 1985, to introduce to patients, at one time, both the present research and the "competing" investigation. However, by the time it would have been possible to proceed on these lines, additional recruitment for the thesis research could only have been very slight. In any case, it was thought that ensuing complications would have caused considerable difficulties to both studies. Thus, the proposal was abandoned.

Another reason for not proceeding with the collaborative proposal was the impact on the patient of being involved in more than one study of the same condition. During a most difficult and stressful time, she can be besieged by investigators. Although many women in the present research appeared to appreciate the attention paid to them, and stated they felt they were making a significant contribution to science, it is not unlikely that too many approaches could be counter-productive, making the patients "feel like guinea pigs".

6.6 Patients' refusals to participate

Overall, only 18% of patients who were approached refused to cooperate. This rate was a little over half that allowed for in the original study design, and could not be taken as a major factor responsible for the low enrollment of subjects.

It is, however, of interest that two-thirds of the refusals of women to participate occurred during the latter half of the field work, which related to 54% of the subjects included. This is difficult to explain, because there was no evidence of a lessening of enthusiasm on the part of study personnel.

6.7 Other reasons for loss of patients

The author had hoped to explore fully the low number of enrolled patients by hospital, by means of a review, during the fieldwork period, of ER assay lists, which included the surgeon's name and ER level, and would have allowed comparison with provincial surgery counts. However, the ER information could not be made available.

During a period when the overall number of operations was not decreasing, the number of eligible patients was low. This could have been a reflection of the proportions of eligible patients according to: primary or recurrent disease; menopausal status; residence; and language.

A review of the numbers enrolled from each hospital showed interesting results, particularly at two institutions (A and C). The numbers of surgeries at these hospitals had been steadily increasing but the enrollment rates were surprisingly low, even when compared with estimates of total number of surgeries from the previous year. One reason for the low rates was that some surgeons at these hospitals did not participate. For example, at Hospital A there were four surgeons who treated breast cancer patients; only three of them participated in the study, and one of these would enroll a woman in our study only if she was not to participate in one of the many drug trials in which he was involved. Other possible reasons for low enrollment rates included high proportions of patients who could not speak English well enough to be interviewed, and of patients travelling from outside the project catchment area for treatment.

There was considerable fluctuation in surgical activity during the fourteen months of subject identification; low numbers may have arisen in

the months during which surgeons took their vacations. There were also wide fluctuations by month in the proportion eligible, but no explanation has been found.

6.8 Benefits from redefinitions of ER- and ER+ patients to be included

In (8) and (9) of section 6.1, it was explained how the definition of ER- status was relaxed, and how all ER+ cases - rather than only a sample of them - were to be included. In retrospect (through Table 4.2), it is possible to see the effects of these changes on the numbers of subjects who would have been included in the discriminatory analyses of Chapter 5.

Taking n_1 and n_2 as the numbers of ER- patients and ER+ subjects, the original plan would have yielded only $n_1 = 11$, together with $n_2 = 11$ also (sampled from the 52 patients who were ER+). The redefinition of ER- status would have increased the values of n_1 and n_2 to 26 each. The final inclusion of all ER+ cases retained n_1 as 26, but increased n_2 to 52.

Statistical "efficiency" is inversely proportional to $(1/n_1 + 1/n_2)$ (Ury, 1975; Yates, 1981). Taking as 100 the efficiency based on the numbers in the original plan, the efficiencies of the other plans were 236 and 315, respectively. These are equivalent to having 2.4 times, and 3.2 times, as many cases available as in the first plan.

6.9 Catchment area

Although the catchment area for the project was enlarged, only four more patients were included. That the improvement was so small is not surprising

in view of the arguments in section 2.5. Had it been possible to include all Ontarian patients at the participating hospitals there could have been a further improvement in recruitment of approximately 30 subjects, which might have led to efficiency (in the sense of section 6.8) of well over 400.

6.10 Work plans

The original plan had been to identify eligible patients from assay and surgery lists. In practice, the ER assay lists could not be obtained as a source of subjects. Fortunately, this did not matter; it was discovered that the information these documents contained could be up to at least six weeks old, so that the delay from surgery to interview would, in many cases, have been excessive.

Table 2.2 shows how various work plans had to be introduced, and in some hospitals, modified. Without keeping a constant check on the surgery lists (which was impossible as permission was not obtained to see these documents in all institutions) it is difficult to ascertain whether these different methods of case identification led to subject losses.

Although plans can be laid for methods of subject recruitment, one must be flexible in altering these approaches to meet the wishes of the cooperating institutions.

Chapter 7

DISCUSSION II: STATISTICAL POWER AND RELATED CONSIDERATIONS

Much of this chapter is based on a monograph: "Practical Considerations of Statistical Power", being prepared by F. D. K. Liddell and I. Rogers-Melamed; it will be referred to here as "LRM". Considerable reliance is also placed on a recent paper by McKeown-Eyssen and Thomas (1985), cited in this chapter as "MET".

The general principles of sample size calculations are discussed in section 7.1, which compares the traditional approach to that recently introduced by MET, drawing the parallels and setting the stage for what follows. The nomenclature used in this chapter (and by LRM) differs in some important particulars from that of MET, and Table 7.1 provides an appropriate guide.

An essential preliminary in any specific problem is the selection of the hypothesized effect; see section 7.2. How sample sizes for the submitted protocol were determined is explained in section 7.3; the following two sections discuss power in two circumstances, in each of which the sizes of the groups compared are taken as equal.

The important issue of unequal sample sizes forms the subject of section 7.6. Section 7.7 considers issues of statistical power for the anticipated sample sizes, allowing for the imbalance in their numbers. There

TABLE 7.1: Nomenclature

This chapter (and LRM)	Meaning	MET
α	probability of Type I error (2-sided)	α
β	probability of Type II error (1-sided)	β
t_α, t_β	t-statistics corresponding to α and β (large positive t_β indicates low Type II error; this is the older convention not followed by MET)	t_α, $-t_\beta$
z_α, z_β	normal deviates corresponding to α and β (z_β taken with same sign as t_β)	--
x μ, σ	any value of exposure variable (such as dietary fat), together with population mean and variance	x μ_o, σ_o
δ	difference in mean exposures of the compared populations	δ
A, G	parameters of exponential model of risk $\ell n(\text{Odds}) = A + G(x/\sigma) = a + bx$	a, $b = G/\sigma$
Gradient	"exponential risk gradient" $G = b\sigma$	--
Δ	predetermined range of exposure	Δ
θ	$\theta = \Delta/2\sigma$	--

TABLE 7.1: Nomenclature (continued)

This chapter (and LRM)	Meaning	MET
*	indicator of hypothesized true effect	--
OR^*	hypothesized Odds Ratio or Relative Risk, at Δ	RR
Effect	$G^* = \delta^*/\sigma = \ell n(OR^*)/2\theta$	--
$[OR \mid \theta]$	Odds Ratio for a specified θ	--
n_1, n_2	the sizes of the two samples	--
$n_1 = n_2$	equal sample sizes	n
$n_1 = n$ $n_2 = rn$	unequal sizes of samples n and rn, $(r > 1)$	--

are two sub-sections, 7.7.1 reverting to small sample theory and 7.7.2 explaining how power can be increased at the expense of Type I error. Then, in section 7.8 there is a note on the situation arising with the sample size actually attained. Finally, there is a short summary.

All the calculations were carried out to high precision, but numerical values in text and tables are presented with only a reasonable number of significant figures. Any discrepancies can be taken as due to rounding.

7.1 General principles

All discussions of sample size and statistical power require definition or decision on four quantities: the variability (σ) of exposure (x) among the population sampled, the "effect" (often termed δ), and the probabilities (α and β) of Type I and Type II errors. Only the first of these quantities is not determined arbitrarily.

Most authorities define the effect in terms of the magnitude of a true difference (δ, say) in the mean exposures of the compared populations, here all ER- and ER+ cases. Armitage (1971) proposed the possibility of specifying a value of δ, say δ^*, which one did not wish to overlook [in a certain sense]. Throughout this chapter " * " is used to indicate what is called the "hypothesized true effect".

MET's proposal was a specified hypothetical odds ratio (OR^*) associated with an arbitrarily predetermined range (Δ) of exposure. Relationships between the two specifications of effect are simple, provided:

(i) the distribution of exposure (x) in the population to be sampled is normally distributed about the mean (μ); and [7.1]

(ii) an exponential model of risk can be assumed, i.e.

$$\ell n(\text{Odds}) = A + G(x/\sigma). \qquad [7.2]$$

Any "working" range of exposure can be defined (predetermined): it will usually be found convenient to write $\Delta = 2(\theta)\sigma$, where θ is simply $\Delta/2\sigma$.

Write: $O^*(L)$ for Odds on "ER+" at $(\mu - \theta\sigma)$, and

$O^*(U)$ for Odds on "ER+" at $(\mu + \theta\sigma)$.

Then OR^* is the Odds Ratio, $O^*(U)/O^*(L)$, which indicates the hypothetical relative risk at the extremes of the selected working range of exposure, Δ.

Now, on the model of [7.2],

$$\ell n[O^*(U)] = A + G^*(\mu + \theta\sigma)/\sigma, \text{ and}$$

$$\ell n[O^*(L)] = A + G^*(\mu - \theta\sigma)/\sigma,$$

so that $\ell n(OR^*) = \ell n[O^*(U)] - \ell n[O^*(L)] = 2G^*\theta$. [7.3]

In their appendix, MRFIT stated (in the terminology of LRM) that $\delta^* = G^*\sigma$ and $G^*/\sigma = \ell n(OR^*)/(2\theta\sigma)$. Thus, $G^* = \delta^*/\sigma$; but also, from expression [7.3], $G^* = [\ell n(OR^*)]/2\theta$. In other words, G^* is just one hypothesized effect expressed in different terms. [It is important to note that the apparent double choice of OR^* and Δ is in fact only a single selection of "(exponential risk) gradient" G^*.]

In the present situation assumptions [7.1] and [7.2] appeared reasonable. This is fortunate because the effect can probably be understood

more easily in terms of Odds, although the formulae for calculating sample sizes and power have traditionally been in terms of δ^*, or preferably δ^*/σ.

7.2 The selection of the hypothesized effect

The predetermined Δ fixes the value of θ. In fact, there seem good reasons for taking $\theta = 2$ (together with a corresponding OR*), and for judging the OR at the extremes of what can be thought of as the real range of exposure (in a comparatively small population). However, there is also much to be said for a recommendation of MET, viz: "comparing the risks at the average exposures μ_+ and μ_- of the subpopulations above and below [the mean exposure in the community, i.e.] μ." MET show that, on assumption [7.1], μ_+ and μ_- are $\mu \pm 2\sigma/\sqrt{(2\pi)}$; their recommendation is of course equivalent to taking $\theta = \sqrt{(2/\pi)}$, or effectively 0.80.

7.3 Sample sizes for the submitted protocol

The sample size calculations at the outset of this research were based on the evaluation of the Odds of a tumour being ER+ in relation to daily fat intake. [Although the objectives were obviously of multivariate nature, as is usually the case, the problem was treated as though it were univariate.] For the protocol submitted to the Office of Research Administration at the University of Toronto (section 2.3), the recommendation of MET was followed in predetermining Δ = 100 grams of fat (50 g below and above the mean), together with the hypothesized (hoped for) OR* of 2.00 at the extremes of this range. An estimate of the population standard deviation of daily fat

consumption (i.e. σ) was provided as 43.64 g/day (G. Howe, personal communication), so that $\theta = 50/43.64 = 1.15$. It is at once obvious that $G^* = (\ln 2)/(2 \times 1.15) = 0.30$; as $G^* = \delta^*/\sigma$, the value of δ^* is found as 13.2 g/day.

The probability of rejecting the null hypothesis when true [or what is called the "size of the test"] was set at $\alpha = 5\%$ (two-sided), for which t_α was approximated by the normal deviate $z_\alpha = 1.96$. The probability of failing to reject - by a test of size α - the null hypothesis if the hypothesized effect exists was set at $\beta = 10\%$ (one-sided), and t_β was taken as $z_\beta = 1.28$. [It is common practice in the early stages of an exposition of this nature, to use normal deviates z_α and z_β as surrogates for t_α and t_β.]

The submitted protocol required the two classes of patients to be equal in number, say n. The MET equation for calculation of the sample size (restated in the terminology of LRM) is:

$$n = 8(t_\alpha + t_\beta)^2(\theta)^2/(\ln OR^*)^2 = 2(t_\alpha + t_\beta)^2/(G^*)^2 \qquad [7.4]$$

which yields n a little more than 229. The number of subjects required were, thus, 230 ER- patients and 230 ER+, or 460 in all. [The identical sample sizes could have been inferred just as well from the traditional equations (Armitage, 1971), using the same α, β, and σ, but with $\delta^* = 13.2$ g/day.]

Clearly from expression [7.4], the more definite the effect (i.e. the larger the value of G^*, either as δ^*/σ, or as gradient), the easier it is to detect, and the smaller the sample required for specified α and β.

7.4 Power in relation to samples of 230 subjects

The "power" of the test is the probability of rejecting the null hypothesis (by a test of size α) when a specific effect exists; this specification will be expressed as the Odds Ratio for a selected θ, or in algebraic terms $[OR|\theta]$. Substituting this expression for OR* in expression [7.4] yields:-

$$8(t_\alpha + t_\beta)^2 = n(\ell n[OR|\theta])^2 / \theta^2$$

$$\text{or} \quad t_\beta = (\sqrt{n})(\ell n[OR|\theta])/(2\theta\sqrt{2}) - t_\alpha , \qquad [7.5]$$

from which power can be ascertained readily. Table 7.2 gives $[OR|\theta]$ for selected values of power (determining z_β) in the following circumstances: $n_1 = n_2 = 230$; $\alpha = 5\%$ (with z_α taken as 1.96); and with two values of θ, namely 2 and $\sqrt{(2/\pi)}$. The appropriate values of (the dimensionless) δ/σ are also quoted, as are those of δ, taking $\sigma = 43.64$ g/day. It can immediately be confirmed that these sample sizes have 90% power of detecting $\delta = 13.2$ g/day, or alternatively $[OR|2] = 3.35$, or equivalently, in relation to the original specification, $[OR|1.15] = 2$.

7.5 Power in relation to samples of 100 subjects

The decision (1984.09) to include only 100 each of ER- and ER+ cases was based solely on the number of patients that it was expected could be accrued in the time allotted. Table 7.3 is in the same form as the preceding table, but for $n_1 = n_2 = 100$. The hoped for $[OR|1.15] = 2$, or equivalently $[OR|2] = 3.35$, could have been detected only with power between 50% and 60% (according to the table), or 57.1% (calculated from expression [7.5]). The $[OR|2]$ would have had to have been much larger for what is usually

TABLE 7.2: Values of Odds Ratio for selected values of power:

$n_1 = n_2 = 230, \quad \alpha = 5\%$

Power (%)	Odds Ratio for specified θ [OR | θ]		Difference between groups in mean exposure	
	$\theta = 2$	$\theta = \sqrt{(2/\pi)}$	Dimensionless δ/σ	Intake of fat (g/day) δ
99.5	5.43	1.96	0.423	18.5
99	4.95	1.89	0.400	17.4
95	3.84	1.71	0.336	14.7
90	3.35	1.62	0.302	13.2
85	3.06	1.56	0.279	12.2
80	2.84	1.52	0.261	11.4
70	2.53	1.45	0.232	10.1
60	2.28	1.39	0.206	9.0
50	2.08	1.34	0.183	8.0
25	1.62	1.21	0.120	5.2

TABLE 7.3: Values of Odds Ratio for selected values of power:

$n_1 = n_2 = 100$, $\alpha = 5\%$

Power (%)	Odds Ratio for specified θ [OR | θ]		Difference between groups in mean exposure	
			Dimensionless	Intake of fat (g/day)
	$\theta = 2$	$\theta = \sqrt{(2/\pi)}$	δ/σ	δ
99.5	13.01	2.78	0.641	28.0
99	11.30	2.63	0.606	26.4
95	7.68	2.26	0.510	22.2
90	6.26	2.08	0.458	20.0
85	5.45	1.97	0.424	18.5
80	4.88	1.88	0.396	17.3
70	4.08	1.75	0.351	15.3
60	3.50	1.65	0.313	13.7
50	3.03	1.56	0.277	12.1
25	2.07	1.34	0.182	7.9

considered adequate power, e.g. for 80% power [OR|2] would have had to be nearly 5. However, with $\theta = \sqrt{(2/\pi)}$, $[OR|\theta] = 2$ could have been detected with power between 85% and 90%.

7.6 Power in relation to samples of unequal size

Sample size and power calculations are all based on the fact that the standard error of the difference between two means, in samples of sizes n_1 and n_2, is $se(\delta) = \sigma\sqrt{(1/n_1 + 1/n_2)}$. This can be restated as: $var(\delta/\sigma) = (n_1 + n_2)/n_1 n_2$. When $n_2 = rn_1$ (taking r greater than 1 and writing n for n_1), $var(\delta/\sigma) = (r + 1)/rn$. This means that expression [7.4] can be replaced by

$$n = [8(t_\alpha + t_\beta)^2(\theta)^2/(\ell n\ OR^*)^2][(r + 1)/2r] \qquad [7.6]$$

bearing in mind that, in expression [7.6], the sample sizes are n and rn. It is easy to see that, as r increases from its minimum value of unity, the required value of n [for fixed α, β, θ, and OR^*] decreases; this means that, in broad terms, the larger the value of r the better - but not simply pro rata, as is well known from corresponding situations (Ury, 1975; Yates, 1981). In the present research, the r could not have been greater than about 2 or 3. However, to sample from the ER+ cases to reduce r to effectively 1 would have meant an unwarranted increase in the number of ER- cases for any specified conditions.

The effects on power of increasing r from 1, by stages, to 3, with n = 100, and hence rn = 100, 125, 150, 175, 200, 250, 300 are shown in Table 7.4. However, to see how sample size is affected by varying r it is necessary to consult expression [7.6]. For hypothesized OR^* and specified θ (and given α and β), the first term in square brackets is fixed; thus, n

TABLE 7.4: Power of detecting $[OR \mid \sqrt{(2/\pi)}] = 2$:

$n_1 = n = 100$; $n_2 = rn = 100(25)200(50)300$

r	rn	power (%)
1	100	86.7
1.25	125	89.9
1.5	150	92.0
1.75	175	93.3
2	200	94.4
2.5	250	95.6
3	300	96.4

is proportional to $(r + 1)/2r$. For $r = 2$, n is only three-quarters of the value required for $r = 1$. For example, taking $\alpha = .05$, $\beta = .10$, $\theta = \sqrt{(2/\pi)}$ and $[OR^{*} | \theta] = 2$, two equal samples of size 112 would have been required, whereas, with $r = 2$, the sample sizes would have become 84 and 168. The total number of subjects required would thus have been 252 compared with 224 (i.e. an increase of one-eighth) - but the time taken to recruit the ER- cases would have been reduced by a quarter.

7.7 Power considerations for samples of 34 and 66 subjects

When it became apparent that in the time allotted for case identification, the total number of patients who could be enrolled would be around 100, power calculations were repeated, and are presented in Table 7.5. The values of n_1 and n_2 were taken as 34 and 66 respectively, and power is given for selected values of $[OR \mid \sqrt{(2/\pi)}]$. Column (2) gives values of power, for $\alpha = 5\%$ calculated in the conventional way, i.e. using the normal deviate surrogates, z_α and z_β.

7.7.1 Reverting to small sample theory

The opportunity was taken to refine the calculations by replacing the normal deviates with the appropriate t_α and t_β, each with $(n_1 + n_2 - 2) = 98$ df. Column (3) of Table 7.5 shows how power on this more correct basis is slightly lower than when the asymptotic surrogates are used. However, as df were as high as 98, the effects were small. (This is, of course, the justification for using the normal deviates - valid only provided df are adequately large.)

TABLE 7.5: Power of detecting selected values of $[OR \mid \sqrt{(2/\pi)}]$: $n_1 = 34$; $n_2 = 66$.

OR	α = 5%		α = 10%	α = 20%
	From z_β	From t_β	From t_β	From t_β
(1)	(2)	(3)	(4)	(5)
3.0	90.3	89.8	94.4	97.4
2.8	86.4	85.7	91.7	96.0
2.6	81.0	80.2	87.9	93.7
2.4	73.9	73.0	82.5	90.3
2.2	64.8	63.9	75.1	85.2
2.0	53.9	52.9	65.4	77.8
1.8	41.5	40.6	53.3	67.5
1.6	28.6	27.9	39.6	54.2
1.5	22.5	21.8	32.4	46.6

(1) Postulated values of $[OR \mid \sqrt{(2/\pi)}]$

(2) Power calculated from $z_\alpha = 1.96$ and z_β

(3) Power calculated from $t_\alpha = 1.98$ and t_β, both with 98 df

(4) Power calculated from $t_\alpha = 1.66$ and t_β, both with 98 df

(5) Power calculated from $t_\alpha = 1.29$ and t_β, both with 98 df

7.7.2 Variation in Type I error

Another important consideration in all power calculations, as mentioned in section 7.1, is the arbitrary nature of the selection of α. Columns (4) and (5) of Table 7.5 show how the power to detect specified ORs can be increased as the value of α itself was increased, here to 10% and 20%. It must be emphasized that the power is that of detecting the effect by a test of the null hypothesis of the size indicated by the stated α. It can be seen that, where power was already high for α = 5%, it could be increased only slightly for greater α. However, where power associated with the conventional α of 5% was low, important gains in power could be achieved by increasing the rate of "false positives", or Type I (or α) error.

7.8 Attained sample sizes

With the even smaller achieved sample sizes, power was inevitably even less. Although it is not usual to calculate power post hoc, it seems appropriate in a chapter of this nature to provide some information in relation to $n_1 = 26$ and $n_2 = 52$. Thus, for $[OR \mid \sqrt{(2/\pi)}] = 2$, the power (based correctly on t-statistics with 76 df) can be stated as follows:-

α (two-sided)	$1 - \beta$ (power)
5%	42.8%
10%	55.7%
20%	69.6%

The nutrient intake with highest discriminatory ability (fibre) was associated with $[OR \mid \sqrt{(2/\pi)}]$ approximately 1.5, the corresponding p-value being around 0.26.

7.9 Summary

This chapter is long because none of the issues discussed could be ignored, particularly in the light of the methodological emphasis of the thesis.

The effect originally selected ($OR^* = 2$, when $\Delta = 100$ g/day) has been shown to be rather inappropriate; a dimensionless specification would be more suitable in most cases. Of course, the statistical power was much worse for two samples of 100 subjects than for 2 x 230 subjects. However, as may not always be appreciated, unequal samples, of 100 and 200 subjects, would have been considerably superior to samples of 100 each. The much smaller plan of having 34 and 66 women had, inevitably, less power; but it has been shown that power can be gained at the expense of α, a useful device when, as here, power $(1 - \beta)$ was small for α of conventional size.

The common belief that unequal sample sizes are inefficient is valid only in experimental situations. However, where the number of cases of one sort is limited, as in many situations, particularly a "case-referent-within-a-cohort" analysis, efficiency is improved the greater the included proportion of subjects of the more common type (Ury, 1975).

Chapter 8

DISCUSSION III: MEASUREMENT

This chapter reviews issues related to measurement, dealing in the first three sections with Estrogen Receptors, dietary factors, and Body Mass Indices. The reproductive, medical and smoking histories and the patients' medical charts are discussed in sections 8.4 and 8.5, respectively.

8.1 Estrogen Receptors

The first part of this section is concerned with the definitions of ER status used in this survey, while their comparability with previous research is dealt with in 8.1.2. Inter-laboratory variation is the subject of 8.1.3, and the section finishes with discussions of seasonal variation in ER+/ER- ratios, and differential loss of cases who had been included provisionally.

8.1.1 Definitions of ER status

In the initial plans, tumours could not be classed as ER- unless the assay gave a value less than 3 fmol/mg protein. This cut-off had been selected because according to the experts consulted, particularly Dr. Jensen, it was felt to delineate "true negatives". However, only 11 tumours fell into this class, and (as already explained; section 6.8) the change to a definition of less than 10 fmol/mg increased the number of subjects to 26.

The corresponding definition of ER+ (i.e. at least 30 fmol/mg protein) was to ensure that all tumours in this class were "true positives". This definition was retained throughout the survey. Because of the unusually high cutoff (compared with the more common 20 fmol/mg or 10 fmol/mg or even lower), the differentiation between negative and positive was thought, by all concerned, to remain uncompromised.

8.1.2 Comparability with previous research

The levels of estrogen receptor adopted in earlier research have included the following:-

ER- < 3 fmol/mg; < 5 fmol/mg; < 10 fmol/mg;

ER+ ≥ 3 fmol/mg; ≥ 5 fmol/mg; ≥ 10 fmol/mg; ≥ 20 fmol/mg; ≥ 30 fmol/mg.

The percentage distributions of 289 assays at one laboratory in 1982 [see (3) in section 6.1] and in the present survey [extended from Tables 4.1 and 4.2] were as follows:-

fmol/mg	< 3	3 - 9	10 - 29	30+	Total
Laboratory N, 1982	23.9	13.8	16.3	46.0	100
Present survey	12.3	13.1	16.9	57.7	100

There are major differences between these distributions, perhaps arising mainly from the void class of <3 fmol/mg at Laboratory M in the present survey. In the investigation reported in section 1.3 (see also McKeown-Eyssen et al, 1985), the proportions of patients by ER status (aged 50 years and older) were 23% ER- and 71% ER+, using the definitions applied by the laboratories themselves. There was so much variation in the definitions of ER- that is is difficult to draw any conclusion, although 23%

is perhaps lower than could reasonably be expected had the findings from the present survey been on similar subjects. However, all laboratories used 10 fmol/mg as the cut-off for ER+ subjects (except for postmenopausal patients at Laboratory M, where it was 20 fmol/mg). Therefore, the status of the ER+ subjects of the population survey and that of the subjects whose tumours were either ER+ or ER intermediate in the present research were reasonably comparable: proportions of 71% and 75% are in accord.

8.1.3 Inter-laboratory variation

Table 4.2 shows that, for whatever reason, the distribution of patients by ER assay level were rather different in Laboratories M and N. Fortunately, when the subclassifications of ER- (into very low and low) and of ER+ (into high and very high) are ignored, the differences were much less marked. It is possible to speculate that Laboratory M was the one out of line with the other five Ontarian laboratories in the investigation by Ryan et al (1985).

8.1.4 Seasonal variation

There was some seasonal variation in the proportion of ER+ tumours amongst all those assayed: 56% (Jan.-Mar.), 43% (Apr.-June), 42% (July-Sept.) and 49% (Oct-Nov). No explanation has been found, nor reason for assuming the introduction of bias.

8.1.5 Losses of provisionally included cases

Of the 108 patients who had met sufficient of the inclusion criteria to justify an approach to participate, 30 were lost either because of refusal (18), subsequent discovery of ineligibilty (4), or interviewer-related difficulties (8). The losses were 7 out of 33 ER- patients and 23 out of 75 ER+ subjects. This differential loss remains a possible source of bias. However, it cannot be attributed to differences in time to interview or to

differences in stage of disease. Further, the interviewer was unaware of the hypothesis of the research and of the assay results, and it is unreasonable that the manner in which the subjects were approached affected the loss rates.

8.2 Dietary factors

This section starts with a discussion of some principles of measuring dietary intakes. The CANDAT system is the subject of 8.2.2, with some comments on flexibility and on interviewing. The final section deals with caloric intake, particularly in relation to previously published survey results.

8.2.1 Assessing past eating habits

A major concern of this investigation - and of many others - is whether measures of recent diet are an accurate reflection of past eating habits. A study of the reliability of dietary history as recollected from the distant past (Byers et al, 1983) found that recalled diets were better estimates of originally recorded information than were current diets; however, the investigators also found that the recalled diet was influenced by current diet.

For the present survey, women were asked to recall, several weeks after surgery for breast cancer, their food consumption in the four months before the surgery took place. It is recognized that, even if accurately recalled, such consumption may not be a true reflection of relevant former dietary habits, but there is uncertainty regarding which diet is relevant

(e.g. that of 5, 10, 20 or more years ago). Recent intake was adopted as the most appropriate solution.

While other research has often employed food records, and biochemical measurements on blood, urine and feces as markers of intakes of certain food components, there was no point in incorporating such measurements into the present investigation because they would only reflect diet weeks after (and so possibly affected by) breast cancer surgery.

8.2.2 The CANDAT system

The CANDAT system was improved by the author in that she incorporated certain foods to allow estimation of total energy intake. Although this caused substantial delay during the present research, recurrence is unlikely.

On the other hand, the system allows for foods which the subjects "write in". With the expenditure of great effort, the appropriate computer-readable files were correspondingly extended. However, consideration should be given as to the utility of including these extra items. For example, in the original design of the instrument cheeses were already categorized into low, medium and high fat, and perhaps the amount of detail incorporated for the present research was too refined for the rest of the instrument. An examination of the calculated intakes including and excluding the newly added food items could shed light on this issue.

The CANDAT system allows for the calculation of many more nutrients (food components), such as vitamins, than were asked for in this work. It also permits the reporting of the nutrients by selected food groups [e.g.

red meat, dairy products, fruit, vegetables (by type), alcohol] (Bright-See et al, 1986). The delays to the system's development meant that neither of these facilities could be utilized for this thesis research.

In the planning of this project, the LICR decided that one interviewer should be hired, on a fee-for-interview basis. Having a single interviewer eliminates the need to consider interviewer differences, but can lead to its own difficulties. First, there is the possibly unsurmountable problem of intra-interviewer variation over time. Further, when the interviewer is acquainted with a patient she cannot ethically approach her; two such subjects had to be excluded. Again, if the interviewer chooses to resign, problems are inevitable; in the present case, six of the subjects who had been identified during the final two months of case identification could not be interviewed.

8.2.3 Caloric intake

In Canada, the Bureau of Nutritional Sciences (1983) quoted recommended caloric intakes for females as 1800 kcal/day for those age 50-74 years and 1500 kcal/day for those 75 and over. The patients in this survey reported that, on average, their energy intakes were just over 2000 kcal/day (Table 4.15); however, 19 of the 78 subjects (24%) reported caloric intakes less than 1500 kcal/day (Table 4.16). Meanwhile, Bowman and Rosenberg (1982) have stated "...most elderly persons have energy intakes below the recommended".

It is therefore instructive to compare the distribution of Table 4.15 with that taken from a nutrition survey of the elderly in Great Britain (Panel, 1972); see Table 8.1. There are many reasons why those distributions should not be compared too closely; however, it would appear

TABLE 8.1: Percentage distributions of caloric intake

Caloric intake (kcal/day):	78 breast cancer patients, 50-79 Ontario, 1985	383 women, 65 and over Great Britain, 1972
< 1000	3.8	4.5
1000, < 1500	20.5	26.2
1500, < 2000	25.6	47.5
2000, < 2500	29.5	17.8
2500, < 3000	7.7	4.0
3000 or more	12.8	0.0
Total	100	100

that the breast cancer patients in Toronto had, on average, higher caloric intake than female members of the general British population in the 1970s.

8.3 Body Mass Indices

In this investigation, the estimation of the body mass index may also have been unreliable: it was constructed using heights and weights reported by the subjects. Possible under- or over-reporting of weight is recognized as a source of error. Further, it has been indicated that height can decrease more than trivially with age (Bowman and Rosenberg, 1982). Thus, subjects who had not recently been measured may have been reporting their height at a younger age; even greater problems may have arisen over reports of weight (a) up to eight months before interview and, especially, (b) at age 20.

It has already been shown, at the end of Chapter 4, that the correlations between caloric intake and Quetelet's BMI (kg/m^2) were very low, the coefficient for all 78 subjects being -0.042.

Recent work (Micozzi et al, 1986) has suggested that Quetelet's index may not be the best BMI for elderly women. Stage 3 of the discriminant analysis was carried out incorporating, separately, four versions of BMI, namely: kg/m^p where p took the values 0, 1, 1.5 and 2. The first "BMI" is weight without any correction for height, the middle two are the indices recommended by Micozzi et al (1986), and the fourth ($p = 2$) the usual Quetelet index. The values of "F-to-enter" the discrimination are given below (with associated probabilities) (a) before any variables had been

entered into the discrimination and (b) after the analysis had been completed [because all variables with the recognized criterion had already been entered].

	p = 0	p = 1	p = 1.5	p = 2
(a) Before the first step of analysis:				
F-to-enter:	0.0108	0.0122	0.0465	0.0998
(associated p-value)	(0.92)	(0.91)	(0.83)	(0.75)
(b) After last step of analysis				
F-to-enter	0.6379	0.3686	0.2558	0.1640
(associated p-value)	(0.43)	(0.55)	(0.61)	(0.69)

The higher the F-to-enter at (a) the lower at (b); and in every case the value at (b) is greater than that at (a). However, BMI (in any variant) was clearly not a variable of importance in discriminating the ER- and ER+ subjects.

It remains clear that body mass and caloric intake were poorly correlated. This is not a surprising result: it has been reported earlier; so has a very weak association between food intake and "obesity" (Rolland-Cachera and Bellisle, 1986; Willett and Stampfer, 1986). Also, in the nutrition survey of the elderly in Great Britain, it was reported that "in general fat people have a lower energy intake than thin people" (Panel, 1972). Possible reasons quoted by the Panel included differences in: insulation; dietary regimens; activity; efficiency of mastication.

8.4 Reproductive, medical and smoking histories

Although this questionnaire was reduced in length in an attempt to include only those items of importance to the research objectives, some information was recorded that could not be used in the analyses. As instance, biopsy for benign breast disease was so rare that details had to be excluded. Other questions, which were intended only as lead-ins to sensitive issues (e.g. ethnicity leading to religion), had been included merely to verify related items.

Some of the information collected on this questionnaire was affected by the mode of measurement. It was hoped to obtain the exact age of menarche. During the pre-testing of the questionnaire, more than half the subjects could remember at least the year and the season; however, most of the survey subjects could remember only the year they started menstruating. Thus, age at menarche had to be calculated as the difference between the year of menarche and year of birth.

8.5 Information from the patients' medical charts

A thorough examination of the patients' medical records failed to disclose some of the information needed. Even in such information as was available, there were often contradictions, particularly with regard to size of tumour and stage of disease. In order to obtain more accurate information for these factors, it might be appropriate to enlist the active cooperation of pathologists and oncologists.

Chapter 9

DISCUSSION IV: ANALYTICAL PROCEDURES

A fundamental question at the outset was which form of discriminatory analysis to use: a discussion of the basis for the selection between discriminant analysis and logistic regression analysis is in section 9.1. Detailed comparison of the sets of findings from both forms of analysis (as reported in Chapter 5) is in section 9.2. The final section deals with some of the problems of proceeding in "stepwise" fashion.

9.1 Selection between forms of discriminatory analysis

The selection of logistic regression analysis over discriminant analysis has often been based on the assumption that the former is robust, i.e. it does not depend on the distributions of the predictor variables. This implies the further assumption that discriminant analysis is not robust; however, there is much evidence to the contrary.

Although both techniques obtain what are called linear discriminant functions, the criteria for the choice of coefficients are not identical. Logistic regression analysis is best for indicating the relative likelihoods of the two groups; discriminant analysis for separating the groups in terms of the mean values of the discriminant function. The former can also be

expected to separate the groups (although not optimally), and the latter to indicate the odds of correct classification (again not optimally). The criteria are not interchangable, and there are situations where one analytical method is clearly preferable in that its objectives meet more closely those of the study design. In the present situation, such preference was not obvious.

It was therefore decided to use both methods, in as closely parallel a fashion as possible. Any major differences in findings might well be instructive. On the other hand, similarities - which were to be expected - would perhaps help to resolve some of the controversies over the selection between methods.

9.2 Comparison of findings from the two forms of analysis

In all three stages described in Chapter 5, both discriminant analysis and logistic regression analysis produced closely equivalent findings: they nominated in precisely the same orders the variables according to their power to discriminate; they indicated similar differences between the ER- patients and the ER+ group in mean values of the variables included; and they yielded test statistics leading to similar levels of probability.

In Table 5.1, when the four nutrients were treated separately, the differences between the groups in mean daily intakes were only slightly dissimilar. To two decimal places, the F- and -statistic were the same, for each nutrient; this means that the former were associated with slightly higher p-values than the latter (Liddell, 1983). Thus, there might appear slight contra-indication for discriminant analysis.

When the four nutrients were entered together (in a single "step") the test statistics (see section 5.3) had associated p-values of 0.69 (for F) and 0.64 (for χ^2).

The findings from the Stage 2 and 3 analyses (Tables 5.3 and 5.4) were in accord, in terms of: (a) the order of entry of the four factors; (b) the fact that no fifth factor was included; (c) the test statistics; and (d) the associated probabilities. With both analytical methods, although age at menarche was entered second, the test statistic associated with benign breast disease became larger at the third step than that of the previous factor. The p-values for the F-statistics relating to number of pregnancies, age at menarche and fibre intake were slightly lower than those for the corresponding χ^2-statistics. It must be emphasized that the intake of dietary fibre contributed very little to the degree of discrimination ($p = 0.24$, for F with 1 and 72 df; $p = 0.26$ for χ^2 with 1 df).

Both analytical techniques showed (Tables 5.5 and 5.6) that the number of pregnancies dominated the discrimination; the contribution of this factor can be estimated (very crudely) as over two-thirds (i.e. 3.60/4.89 = 0.74 in discriminant analysis or 2.64/3.81 = 0.69 in logistic regression analysis). The other "third" of the contribution to each discrimination was shared between history of benign breast disease, age at menarche and, to only slightly less degree, intake of dietary fibre.

9.3 Stepwise procedures

While both methods were conducted stepwise, the shortcomings of this approach were fully recognized and every attempt was made to minimize them.

Stage 1 of the discriminatory analyses was performed in order to consider the dietary intakes (separately and together) without allowing any other factors to interfere. In fact, no nutrient by itself or in combination had adequate discriminatory ability, and the problems of stepwise procedures were irreleyant.

Stage 2 was carried out on the other variables, without permitting the interference of dietary factors. However, here stepwise procedures were necessary, unless what is called "best subsets analyses" had been undertaken. However, the predominance of the number of pregnancies, and the weakness (in discriminatory ability) of all other variables meant that most of the common shortcomings of the stepwise approach were negligible. This is not to say that stopping rules could be applied blindly, but there is no indication that seriously different findings could have emerged.

Stage 3 was, in some senses remarkable, in that it reproduced Stage 2 precisely, simply adding a fourth step, that of incorporating fibre intake into the discriminant function(s). It is of interest that not only did fibre have the highest test statistic (in either form of analysis) in Stage 1, but the probability associated with fibre intake in Stage 3 was (in both analyses) marginally lower, indicating that its discriminatory power had been improved by the inclusion of the other factors.

When all variables were considered, because the stepwise process emphasizes tests of statistical significance (which are dependent on the size of the study sample), the "p-to-enter" was set high ($p = .30$) in order to allow identification of variables which might otherwise have been overlooked, but not so high that entirely trivial differences would appear.

In addition, care was taken that the order of factors was not determined by very small differences in the statistics-to-enter. However, because this was a "searching" rather than a "testing" approach, any p-values quoted here are only for purpose of comparing the analytical techniques; they are not meant as statements of probability, for which they are not appropriate. Correspondingly, confidence limits were not calculated.

Chapter 10

DISCUSSION V: FINDINGS

The principal aim of a discussion of the findings from thesis research is, usually, to assess whether the study objectives have been achieved. To do so, one must consider whether the subjects investigated were representative of the "target population", and if not why not. In the present circumstances, the target population was not all cases of primary breast cancer in postmenopuasal women, and so it is also necessary to consider the extent to which the study patients were representative of postmenopausal breast cancer patients (regardless of their estrogen receptor status).

The next stage would be to evaluate what can be learnt from the present research about the etiology of breast cancer, and whether such findings are in conformity with current epidemiologic theory.

Only then is it appropriate to examine findings concerning ER status and its relationships with the factors of prime interest, and where appropriate with those of secondary interest also. It is necessary to weigh whether such relationships are in accord with earlier work, and to what extent any findings can be considered novel. The summary becomes a statement as to whether the thesis objectives have been met.

In the present thesis, the stated objectives were concerned with the discrimination of ER- tumours from ER+ tumours by means of dietary,

reproductive and other factors; it is these aims which are considered in this chapter. However, it has to be borne in mind that the previous four chapters have been examining objectives, which remained implicit, related to the conduct of epidemiologic surveys of this nature.

The chapter is arranged in a number of sections. The first deals with the problems mentioned in the first paragraph, i.e. how representative the subjects were of the target population, on the one hand, and of all primary breast cancer patients, on the other.

The next four sections (10.2 through 10.5) deal with variables for which there is information with respect to the general population and/or breast cancer risk. In each of these sections questions about etiology are examined first, and a discussion of relationships with ER status follows.

Sections 10.6 and 10.7 discuss how certain factors were related to ER status; the grouping is principally that section 10.6 presents findings in general accord with earlier reports, while the later section deals with variables where there has been considerable inconsistency.

Section 10.8 returns to the factors of principal interest, i.e. dietary intakes, preceded by a commentary on Body Mass Indices; and the final section (10.9) is a brief summary statement.

For brevity in the presentation, the phrases "ER- group" and "ER+ group" are used to indicate, respectively, the 26 breast cancer patients whose tumour levels of ER receptors were less than 10 fm/mg and the 52 patients with ER levels at least 30 fm/mg. Much use will also be made of the ER+/ER- ratio, i.e. the ratio of the number of ER+ subjects to ER- patients.

It must be emphasized at the outset that the ER+/ER- ratios in this survey were lower than reported elsewhere simply because of the definitions of the ER classes.

10.1 "Representativeness" of study subjects

A target population "... is that population about which an investigator wishes to draw a conclusion" (Colton, 1974). In this research, it could be defined as: all postmenopausal women in Canada with primary breast cancer whose tumours were Estrogen Receptor negative or Estrogen Receptor positive - whether the tumour was assayed or not. Clearly, this target could not be reached. The following restrictions had to be imposed:-

1. The women had to be surgical patients at one of several teaching hospitals in Toronto.

2. The women had to be between 50 and 79 years of age.

3. The ER assay had to be performed, satisfactorily.

Further restrictions arose, for several reasons, including the following. First, certain surgeons were unwilling to participate, eliminating one of six selected hospitals, and reducing the numbers of patients available for selection at other hospitals. Second, a competing research eliminated a further large proportion of patients. Third, otherwise eligible patients who could not speak English or who lived outside a defined catchment area had to be excluded. Fourth, almost one-fifth of the patients invited to participate refused to do so. Fifth, the interviewer was unable to carry out some of the interviews to which she had been allocated.

Clearly, then, the subjects investigated were by no means a random sample of the target population. The most important sources of potential bias were the concentration on teaching hospitals in Toronto, the fact that ER assays are not requested evenly over the age range, the loss of patients who did not speak English (which may have introduced bias in terms of ethnicity, etc.), and of those outside the catchment area (who may have lived largely in rural rather than urban areas), and, as so often, the reliance on "volunteers".

It must be even more obvious that the 78 subjects investigated were not truly representative of all postmenopausal breast cancer sufferers, particularly as those investigated had, by definition, undergone breast surgery. Also, the target population excluded all women with "ER intermediate" tumours.

10.2 Age at surgery

The one variable on which most reliance can be placed is age at surgery. The material reported in section 1.3 of this thesis (see also McKeown-Eyssen et al, 1985) was obtained from similar sources, although covering different geographical areas and years, and the distributions by age (50 - 74 years) of all subjects classed ER- or ER+ in the 1981 population and from this survey are presented in Table 10.1. (Those over 75 were excluded because of different definitions.) The inclusion criteria for the two surveys were by no means identical, and the definitions of ER intermediate (who are excluded from both series) were different. In all these circumstances, the percentage age distributions were not grossly dissimilar.

TABLE 10.1: Distribution by age of patients whose tumours were assayed as ER- or ER+ for the 1981 population and the present survey

	1981 population		1985 survey	
	number	%	number	%
Age at surgery:				
50 - 54	326	19.3	10	13.7
55 - 59	385	22.8	20	27.4
60 - 64	368	21.8	22	30.1
65 - 69	335	19.9	12	16.4
70 - 74	271	16.1	9	12.3
Total	1685	100	73	100

The ER+/ER- ratio has been found to be higher the older the subjects (Elwood and Godolphin, 1980; Lesser et al, 1981). The most convincing evidence has been reported in detail in Chapter 1 (and summarized by McKeown-Eyssen et al, 1985). In the present research the tendency was also for the ER+/ER- ratio to be higher the greater the age at surgery - but not consistently so. Table 10.2 compares the ratios from these last two studies. In view of all the differences between the two projects, the patterns of the ratios were no more dissimilar than might have been anticipated.

10.3 Marital status

According to Statistics Canada (1986), 5% of the Canadian female population aged 50 - 79 years had never been married, whereas 15 of the 78 subjects in the present research reported they were single. Bayes' theorem (Colton, 1974) allows the estimation of the relative risk (R) of breast cancer in single women compared with the risk in other women; the calculated value of R was 4.75.

Kelsey (1979) gives a relative risk of 1.5 for "... women throughout the world... who have never been married". The three-fold inflation of R over Kelsey's estimate suggests strongly that there were major inconsistencies in the classification of marital status in the Canadian population and in the present study. These may well be sufficient to account for the extraordinary pattern of ER status with marital status (Table 4.5).

TABLE 10.2: ER+/ER- ratios by age, for the 1981 population and the present survey

Age at surgery:	ER+/ER- ratio	
	1981 population	Present survey
50 - 54	2.10	1.00
55 - 59	2.26	2.33
60 - 64	2.72	1.44
65 - 69	3.86	2.00
70 - 74	3.67	8.00

As, in any case, it had been intended to view marital status mainly as a surrogate for parity, and as information on parity was available, the reports of marital status (as such) should be given no weight.

10.4 Pregnancy and parity

The percentage of nulliparous women in this survey (28%) was at the upper end of the range generally reported in the literature (Elwood and Godolphin, 1980; Hildreth et al, 1983; Ballard-Barbash et al, 1986; McTiernan et al, 1986).

In this investigation the most striking association found was that related to the number of pregnancies reported by the subjects, in which although (among the subjects who had been pregnant) the ER+/ER- ratio was lower the greater the number of pregnancies, the ratio for those who had reported never having been pregnant was similar to those with 2 or 3 pregnancies. Previous findings on the relationship between pregnancy and ER status have been mixed; however, any associations found among those who had been pregnant were not discordant with that reported here.

10.5 Smoking

Table 10.3 is the best comparison that can be made between the reported smoking habits of Canadian women and the study subjects. However, the ratios of "never smoked" to "ex-smokers" are at such variance, at both ages, that little confidence can be placed in the comparability of the classifications of these categories in the two surveys.

Table 10.3: Percentage distributions of Canadian women* and study subjects by smoking habits, by age

	Canada	Study	Canada	Study
age (years):	55-64	50-64	65+	65-79
Never smoked	53	46	69	50
Ex-smoker	18	38	16	46
Current smoker	29	16	15	4
	100	100	100	100

*
Source: Millar (1985).

On the assumption that current smoking has been reported with similar reliability, and noting from Table 4.13 that 9 of the 78 subjects in the present research were current smokers, it is again possible to use Bayes' theorem to estimate the relative risk (R') of breast cancer in current smokers compared with the risk in other women; the calculation gave $R' = 0.52$. This is not grossly out of line with current thinking (Baron, 1984; Michnovicz et al, 1986).

Whatever support this may give to the similarity of the classification of current smokers in the two surveys, it does not provide any confidence in the other classifications. Further, the close similarity of the ER+/ER- ratios for "never smoked" and "ex-smokers" (Table 4.13) is in line with the belief that these reports are undifferentiable.

In the present survey, in which only 9 women reported smoking currently, the ER+/ER- ratio was almost twice as high in these women than in the others. This was surprising in view of the current belief (Baron, 1984) that smoking has an anti-estrogenic effect. An earlier investigation reported an inverse association between cigarette smoking and ER+ breast cancer, but there was some suggestion that these results may have been confounded by other factors such as age, menopausal status or weight (Stanford et al, 1986).

10.6 ER findings in accord with earlier results

All of the findings mentioned in this section were in accord with previous reports.

The ER+/ER- ratio did not vary in any systematic way with any of the following demographic variables: country of birth, religion (Jewish or not), and certificate of education obtained. Nor did it vary consistently with laterality of tumour and nodal involvement. Again, there was no indication of an association with a history of hysterectomy or bilateral oopherectomy. Finally, ER status was related minimally to each of the following reproductive factors: age at last menstrual period, natural or surgical menopause, years since menopause and years of menstruation.

10.7 ER findings where there has been inconsistency

Only seven patients reported having had benign breast disease (in terms of a surgical biopsy for a lump in their breast before the time of their breast surgery); their ER+/ER- ratio was less than half that in other subjects. A similar association was found by Hulka et al (1984), but Hildreth et al (1983) reported an inverse relationship. This discrepancy may be due largely to different definitions of non-malignant breast disease.

Among the parous group of cases in the present research, the ER+/ER- ratio tended to be higher the greater the age at first birth. This was in accord with an association demonstrated by Hildreth et al (1983), but the findings of other investigators have been inconsistent. Among the same cases, patients who had never breast fed an infant had an ER+/ER- ratio nearly twice that of those who had nursed a baby. A similar association has been reported previously, but not by most investigators. For subjects who had experienced an incomplete pregnancy, the ER+/ER- ratio was low; no corresponding information could be found in the literature.

An association between ER status and a family history of breast cancer was found in the present study and in one other (Ottman et al, 1981), but not in the other three earlier studies that have looked for this association. As family history of breast cancer is a definite risk factor for breast cancer in general, its role in ER status requires elucidation.

Single pathological diagnoses other than ductal or adenocarcinoma tended to be ER+, but this finding was the result of "data dredging" (Armitage, 1971) and cannot be given much weight. However, some, but by no means all, previous studies have reported similarly.

The association between ER status and use of exogenous hormones was weak; findings from earlier studies have been equivocal. A history of thyroid disease has not previously been looked for in relation to ER status; here, the association was minimal.

For the few patients who reported their menarche at ages greater than 14 years the ER+/ER- ratio was only about one-third of average; previous findings have been in some disagreement.

10.8 ER findings, diet and Body Mass Index

Weight and Body Mass Index at age 20 were very slightly greater in the ER- group, but these women were slightly lighter at the time of surgery. Both groups had gained weight on average, since age 20, the gain being greater in the ER+ group. Of the 11 other investigations which examined the association between ER status and weight, five reported weak relationships, although findings from the other six were more definite.

The mean intakes of total energy, fat, protein and dietary fibre were all very slightly lower in the ER- group compared to the ER+. Of these nutrients, dietary fibre led to the highest test statistic, but this was extremely small, even after incorporation of other factors which improved its ability to discriminate. The relationship between diet and ER status had not been examined previously.

10.9 Summary

The most important, and clearly significant, factor discriminating the ER- patients from the ER+ was the number of pregnancies. Age at menarche, history of benign breast disease and intake of dietary fibre gave slight indication of discriminatory ability, but it must be stressed that the number of pregnancies played the dominating role.

As to whether the research objectives have been met, it is clear, from the early sections of this chapter, that the findings cannot be generalized to the target population. However, for this select group of subjects, factors were identified which distinguished the ER- patients from the ER+.

The dominance among these factors of the number of pregnancies, and the poor discriminatory ability of fibre intake - let alone other dietary variables - suggests that although a much larger study might lead to statistically significant discrimination in terms of dietary intakes, this is likely to be "negligible" in the sense which Sir Austin Bradford Hill has stressed for over 25 years (see also, Hill, 1984).

Chapter 11

CONCLUSIONS AND RECOMMENDATIONS

This chapter presents the main conclusions I drew from the research and makes some recommendations for future work. There are three sections, dealing with: measurement (11.1); principles of design (11.2); and then the recommendations (11.3).

11.1 Measurement

A basic lesson drawn from this research was that each of the measurement systems was less than perfect. Before the initiation of any further research on the etiology of Estrogen Receptors in breast cancer, it would seem vital to investigate and improve as far as possible every means of measuring the relevant variables. The remainder of this section deals, separately, with ER assay (11.1.1), dietary intakes (11.1.2), Body Mass Indices (11.1.3), histories of reproduction and associated factors (11.1.4), medical histories (11.1.5), information available only in the patients' medical charts (11.1.6), and socio-demographic variables, including marital status and smoking (11.1.7).

11.1.1 ER assay

It is a fundamental of good research that the best possible means of determining the essential variables be employed, and here the most important variable was the level of estrogen receptor. At present, in Ontario, only the two laboratory methods mentioned in section 1.3 are in use for routine purposes, but what are considered much more precise assays are now available (King et al, 1985; see also supplement to Cancer Research, volume 46, no.8). Every effort should be made to employ the most reliable possible assays in future research.

It would also be highly desirable to have assays carried out for all breast cancer patients, not just those ordered (on clinical grounds) by the surgeons.

Further, as part of the general epidemiologic principle that no case should be discarded without exceptional reason, all assayed tumours should be incorporated into the research design. The use of the actual ER level, as distinct from a grouping by status at arbitrarily-set cut-off points, is to be recommended. If, even so, there should be reasons - perhaps of a "political" nature - for insisting on grouping, it remains essential (a) that ER intermediates are not discarded and (b) that actual ER levels be recorded.

Table 4.2 demonstrated what may well have been serious inter-laboratory variation in the ER assay findings. It would be vital to investigate such variation in advance, and, if it cannot be eradicated, to make allowance for it. Neither can intra-laboratory variation be disregarded.

11.1.2 Dietary intakes

Perhaps the most important unresolved issue is the period of each patient's life for which her dietary habits should be determined. It may be impossible to find a definitive answer to this question, but care must be taken to avoid potential biases of great importance. Recent work by Byers et al (1987) and no doubt others could be of great relevance.

Current interest in the media regarding diet and cancer - with suggestions for dietary changes that may have affected the habits of the general population - makes it clear that present intakes may be different from those, say, five years ago, even in the healthy.

Meanwhile, there is still debate on the best methods of "measuring" past dietary intakes. Is the approach through a food frequency questionnaire the most appropriate? If it is, does it need the refinements that were introduced by the author, especially in relation to foods that were "written in"?

Whatever method of measurement is eventually decided upon, it has to be borne in mind that it will be subject to the equivalent of instrument-variation. For instance, the food frequency questionnaire requires at least one interviewer, and both intra- and inter-interviewer variation would have to be measured and allowed for.

An additional open question is which nutrients (or food components) to measure, and so to include in the appropriate statistical analyses.

11.1.3 Body Mass Indices

In the current research, the subjects' heights and weights were self-reported, with inevitable inaccuracy. This suggests the need for more

objective measures. Perhaps the interviewer could be provided with portable instruments to carry out at least some measurements at the time of interview. There are some precedents, such as those reported by Barnes (1987) in relation to surveys carried out, in the United Kingdom, by the Office of Population Censuses and Surveys. It would also be important to inquire about change of weight in the time period prior to surgery, to avoid recording the weight which may have been altered by a concomitant of the cancer surgery.

Quetelet's index of body mass (kg/m^2) has been of inestimable value for well over a century, but there has now been a suggestion (Micozzi et al, 1986) that it may not be the most suitable for elderly women. Although these latest suggestions did not affect any current findings, the issue requires resolution.

11.1.4 Reproductive history

It is not unlikely that ages at menarche and at last menstrual period could be of importance. The evaluation in the present survey of both these ages could have been improved, although probably nothing can be done when a patient's memory fails in these regards. Careful attention should be paid to getting the information in as precise a form as possible.

On the other hand, there is reason to believe that information on pregnancies and parity was reported with satisfactory reliability. Nevertheless, one must not be over-confident, believing that questions on these matters (Annex II, Appendix B) cannot be improved.

11.1.5 Medical history

It is impossible to tell whether the family histories of breast cancer were accurately reported. However, it is important that the degree of

relationship between the case and any affected family member be recorded.

In this regard, the definition of "first degree relatives" requires review. In the consideration of breast cancer risk, daughters have traditionally been included as first degree relatives. However, the genetic pathways from mother-to-daughter, or between sisters, are different from those from daughter-to-mother. The genetic interpretation of this issue is beyond the scope of this thesis, but merits further consideration.

The importance of benign breast disease in the etiology of breast cancer suggests that it should be assessed as accurately as possible. In the present research, this factor could only be estimated crudely by a reported surgical biopsy for a lump in the breast before the time of cancer surgery. This is an appropriate surrogate (N. Boyd, personal communication) when more precise information cannot be obtained, but effort should be expended on acquiring a more direct assessment.

Information on the use of exogenous hormones could not be documented satisfactorily. The role of these medications is potentially important for the ER status of breast cancer, and much more attention should be paid to them.

11.1.6 Patients' medical charts

Much detail concerning the breast cancer itself can only be obtained from the patients' so-called medical charts. These are maintained solely for clinical purposes, and are of highly variable quality; it is unlikely in the extreme that the quality could be improved for research purposes. A device such as that adopted here (as explained in section 3.3 and Annex VI) would still be necessary.

11.1.7 Socio-demographic factors

There was strong evidence that, in the present research, variables such as marital status and smoking habit were reported rather differently than in surveys carried out by government agencies. Careful attention to this problem is clearly essential.

11.2 Design principles

Before making any recommendations as to future research, it is essential to consider certain principles of design. A fundamental of all epidemiologic survey is that no potential subject should be discarded without overwhelming justification. Section 11.2.1 elaborates on this principle. The ability to generalize from research findings is one of the most desirable considerations; some reflections on this issue are in section 11.2.2. The final section (11.2.3) deals with some miscellaneous matters.

11.2.1 The inclusion of all possible subjects

The original design of the study (section 2.1) was to include all patients with ER- breast cancers, a sample of equal size from among the ER+ patients, and a further equal number of healthy women, both samples to be age-stratified. This would have meant that the age distributions of the two samples (i.e. of ER+ breast cancer patients and of healthy women) would have been determined by that of the ER- patients. Therefore, not only would roughly half the ER+ patients have been discarded, but those selected would have been far from representative, at least in terms of age, of ER+ breast cancer. It is not unlikely that any findings with respect to the risk of ER+ disease would have been affected by this. (It has already been shown -

see section 7.6 - that the discard of ER+ cases would have been statistically inefficient.)

Further, the use of a single group of healthy women (with the same age distribution as that of the ER- subjects), and the planned comparison of ER- against healthy, and of ER+ against healthy, would have broken the fundamental statistical principle of contrasts being kept "orthogonal", i.e. independent. (For instance, if the sample of healthy women turned out to be unrepresentative in some important variable, the differences of the mean of that variable from the means in both the ER- and ER+ groups might both be assessed as, say, significant, although this double effect would be an artifact of the lack of independence.) See also Li (1964) and Campbell (1974).

Thus, it would be necessary to include one group of healthy referents for each class of patient. An attempt at this design was made by Stanford et al (1987), although they had some difficulties in finding the best possible selection of referents for the ER- and ER+ patients. However, they did proceed correctly by "stratifying" their study into two independent comparisons, of ER- against specific referents, and of ER+ against a separate selection of referents.

The tenet of retaining all subjects implies no selection from among the larger groups. This, of course, means that no matching of patients would be required. It would still probably be necessary to match referents to patients in the various classes. This is possible only on two assumptions: first, the "pool" from which referents can be drawn must be very large compared with the number of cases; second, only a very few matching factors can be considered.

Finally, the same tenet requires the inclusion of the nulliparous in any form of analysis.

11.2.2 Generalizability

As explained in section 10.1, the subjects in the current study were representative neither of the target population nor of the population of all postmenopausal breast cancer in Ontario. Some suggestions have been made above about the inclusion of patients who are ER intermediate, and the desirability of having an ER assay result for all breast cancers.

However, serious problems still remained in the concentration on Toronto teaching hospitals, and through the failure to cooperate of certain selected hospitals and of some surgeons even within the hospitals where cooperation was forthcoming. In particular, the exclusion of the high proportion of women who had had lumpectomies may have introduced important biases. Future research would have to pay especial regard to these problems.

It is inevitable that some proportion of subjects will refuse to cooperate. No emphasis is required on the need to keep this proportion to the absolute minimum.

11.2.3 Other issues

Another important lesson I learnt was the need to keep detailed records of every deviation from the research protocol, and of reasons for ineligibility, etc. Only because detailed documentation had been maintained at all stages of fieldwork in the present enquiry was it possible to explain such matters as the reasons for the shortfalls in numbers.

One other device to be recommended is the specification (for calculations of sample sizes, power and related issues) of hypothesized effect in dimensionless terms, e.g. with a hypothesized difference in the means of exposure expressed as a multiple (or fraction) of the standard deviation of the population distribution of exposure. This would mean that the calculations would remain valid whatever variable was being considered. However, it must be emphasized that - so far - each variable has to be considered separately in such calculations; there is need for a theory for the common multivariate problem.

11.3 Recommendations

The following recommendations are based on the premise that amelioration of all problems of measurement can be achieved before any plan is put forward. There are two sub-sections, dealing with: (11.3.1) the need to confirm and clarify existing findings; and (11.3.2) additional research.

11.3.1 Confirmation and clarification of existing findings

The p-value associated with the ability of the number of pregnancies to discriminate between ER- patients and ER+ subjects cannot be taken directly from the output of either program of discriminatory analysis, because of the "stepwise" selection of this variable into the leading position (a form of "data-dredging"; Armitage, 1971). However, even the use of a conservative form of the Bonferroni inequality yielded a p-value very much less than 0.01. It would therefore seem likely that this finding would be repeated - unless it was due to unsuspected biases. Therefore, even a project involving a very large number of subjects, which might increase the statistical significance of the discriminatory ability of other factors,

including dietary fibre (or other intakes), could not be expected to affect greatly the relationship between ER status and the number of pregnancies. The "significance" associated with any other factor could then be due essentially to the large numbers but the difference remain "utterly negligible" (Hill, 1984).

Nevertheless, given the importance of ER status with respect to prognosis in breast cancer, its etiology should be more fully explored. In particular, the findings of this survey, both suggestive and negative, require confirmation or clarification. Further, inconsistent findings require elucidation. A factor of potential importance is smoking.

11.3.2 Additional epidemiologic research

One unresolved issue is the consistency of ER status in bilateral tumours; these can have different levels, and might well therefore not be classed as in the same ER status.

All the factors studied could also be examined in younger women with breast cancer, to ascertain whether they played similar roles in premenopausal disease.

Another possibility would be an attempt to understand whether ER status is a characteristic of the breast tissue or of the tumour. Further information might be sought on the ER status of healthy breast tissue (perhaps from breast reduction surgery).

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Annex I

ASSAY PROCEDURES

The author requested descriptions of the ER assay procedures followed by the two participating laboratories.

The first page of this Annex is the complete statement from Laboratory M.

Laboratory N provided the detail on the remaining three pages.

Laboratory M

PREPARATION OF CYTOSOL

Tumour specimens (usually 0.1 to 0.5g) were trimmed of fat and extraneous tissue and a respresentative slice was fixed for histological examination. The remainder was snap frozen in foil containers and stored in liquid nitrogen for not longer than two weeks. All tissue handling and assay procedures were carried out at 0-4° with pre-cooled equipment, glassware and buffer solutions unless otherwise specified. Frozen tissue was pulverized in an Auto pulverizer (Redi Industries Corp., Hempstead, N.Y.) cooled with liquid nitrogen, and then homogenized in buffer (10mMTris, 1.5 mM EDTA, 1 mM dithiothreitol, pH 7.4) using a Polytron P-10 homogenizer (Brinkman Instruments Inc.) for 2 bursts of 20 sec. (setting 3-5) with a 60 sec. cooling interval. The homogenate was centrifuged at 3000g for 10 min. to yield a crude supernatant (SN) which was centrifuged at 100,000g_{av} for 1 hour to separate the cytosol. An aliquot of cytosol (25μl) was taken for cytosol protein assay.

ER ASSAY

Replicate 200μl aliquots of cytosol (1-3 mg protein/ml) were added to 200μl of buffer A, containing [^{3}H] estradiol + DES to give a final concentration of 1 nM [^{3}H] estradiol + 100 nM DES. Incubation was at 0-4°C for 18 hours. Unbound steroid was removed by the addition of 400μl of DCC to each tube, in ice, for 30 min. with intermittent vortexing, followed by 2 x 10 min. spins at 1,500g_{av} (Beckman Model TJ-6). An aliquot (500μl) of SN was counted (10 min. or to 2% error) in 10 ml of PCS: toluene.

Laboratory N

STERIOD-BINDING ASSAY ---- JUN 85

Tissue Homogenization

-Store tissue at -70oC before assay
-Tumor tissue of approx. 0.5gm. is weighed out and pulverized with liquid N2
-15ml. buffer A is added per gram of tissue powder
-Homogenize on ice with two 5 sec. bursts on polytron, at setting 5
-Homogenate is spun for one hour at 105,000xg. in an ultracentrifuge at 2oC
-The top lipid layer is removed by suction
-Lowry protein assay is used to measure protein content of cytosols and adjusted to a concentration of 2mg./ml. using buffer A

Reagents

Stock Buffer Solution: 0.10M Tris/HCL
0.015M EDTA
-6.059gm. Tris (m.w.=121)
-2.79gm. diNa+ EDTA (m.w.=372)
-Dissolve in dis. water
-Make up to 500.0ml. with dis. water
-Store at 4oC

Buffer A. 0.01M Tris/HCL
0.0015M EDTA
0.87mM Monothioglycerol
-Buffer prepared fresh daily
-Add 25ml. stock buffer and make up to 250ml. with dis. water
-Add 25ul. of monothioglycerol
-Cool to 4C and ph to 7.4
-Store at 4C

Buffer B: 0.01M Tris/HCL
0.0015M EDTA
26.7% w/w Glycerol
0.87mM Monothioglycerol
-Add 66.75ml. of glycerol and make up to 250ml. with buffer A
-Cool to 4C and readjust to ph 7.4
-Store at 4C

Buffer C: 0.01M Tris/HCL
0.0015M EDTA
-25ml. stock buffer and make up to 250ml. with dis. water
-Cool to 4C and ph to 7.4

Stock DES- 531uM
-Sigma DES (m.w.=268)
-Dissolve 1.43mg. in 10ml. of 100% ethanol
-Store in 4C

Stock R5020: 531uM
-NEN R5020

-Dissolve 1.68mg. in 10ml. of 100% ethanol
-Store in 4C

Stock cortisol: 1327.5uM
-Sigma hydrocortisone
-Dissolve 4.81mg in 10ml. of 100% ethanol
-store in 4C

Stock DHT: 132.8uM
-Sigma DHT
-DIssolve 0.39mg. in 10ml. of 100% ethanol
-store in 4C

Working DES buffer 531nM
-Add 10ul. of stock DES and 20ul. of 100% ethanol to 10ml. buffer A
-Store in 4C

Working R5029 buffer: 531nM
-Add 10ul. of stock R5020, 10ul. of stock DHT and 10ul. of stock cortisol
-To 10ml. of buffer B
-Store in 4C

Working Estradiol Tracer· [2,4,6,7-3H(N)] 8.0nM
-Stock tracer NEN 2,4,6,7-3HE2 (0.25mci/0.25ml)
-Add 12.5ul. stock tracer and 87.5ul. of 100% ethanol to 10ml buffer A
-Count an aliquot and adjust to a final concentration of 8.0nM
-Store at 4C

Working R5020 tracer [17-methyl-3H] 16nM
-Stock tracer NEN 17-methyl-3HR5020
-Add 25.0ml. and 75ml. of 100% ethanol to 10ml. buffer A
-Count an aliquot and adjust to a final concentration of 16nM
-Store at 4C

Working Estrogen Buffer
-Add 30ul. of 100% ethanol to 10ml. of buffer A
-Store at 4C

Working Progestrone Buffer
-Add 10ul stock DHT, 10ul stock Cortisol and 10ul. 100% ethanol to 10ml. of buffer B
-Store at 4C

Dextran Coated Charcoal· (DNC)
-4.0gm of norit A charcoal in 40ml of buffer C
-Shake and centrifuge for 15 min at 2500 rpm
-Remove fines
-Resuspend pellet in 40ml of buffer C
-Shake and centrifuge for 15min at 2500 rpm
-Remove fines
-Dissolved 400mg of dextran T70 in 10ml of buffer C and add to pellet
-Resuspend pellet and make up to 40ml with buffer C
-Keep overnight at 4C before use
-Dilution of 1·20 with buffer C before use

Receptor Assay

-Set up total binding (1a and 2a) and non-specific binding (1b and 2b) tubes in triplicate in 10 X 75 m.m. polystyrene tubes for each cytosol

	ESTROGEN RECEPTOR		PROGESTRONE RECEPTOR	
	1a	1b	2a	2b
estrogen working buffer	150ul.			
progestrone working buffer			150ul.	
working DES		150ul.		
working R5020				150ul.
working 3HE2	50ul.	50ul.		
working 3HR5020			50ul.	50ul.
cytosol	200ul.	200ul.	200ul.	200ul.

-Mix well and incubate at 4C for 16-18 hours
-Add 400ml. D.C.C. to all tubes and incubate at 4C for 30min.
-Centrifuge at 3000 xG for 15 min. at 4C
-Remove 500ul. aliquot from each tube and add 5ml. of scintillation fluid and count for 10 min. or 2% sigma

Annex II

APPENDICES TO THE RESEARCH PROTOCOL OF 1984.09

This Annex contains the Appendices, with the same labelling (A through H), to the research protocol dated 1984.09; see section 2.3. There is an index below, and a separate title page for each Appendix.

Appendix A: Food frequency questionnaire

B: Medical, smoking and reproductive history questionnaire

C: Chart information

D: Letter of invitation given to patient when visited in hospital

E: Breast diseases pamphlet

F: Mailed letter of invitation

G: Consent form.

H: Letter of thanks for participation

Appendix A: Food frequency questionnaire

APPENDIX A

Subject ID

LUDWIG RESEARCH INSTITUTE

TORONTO BRANCH

BREAST DISEASES STUDY

Participant _____

Study Representative _____

Date _____

Appendix A

BREAST DISEASES STUDY

FOOD FREQUENCY QUESTIONNAIRE

Neighbourhood Study Rep

Time Began _____ a.m. p.m.

Subject ID

Year Month Day

INTRODUCTION

We are studying the types of food people eat and how this may influence health. I would therefore like to ask you some questions about your usual diet. Please keep in mind that the information you give will be kept strictly confidential.

We would like to know what you have been eating and drinking over the 4 months prior to your recent breast surgery. We will be talking about many different kinds of food such as: dairy products, meat, fish, poultry, breads, cereals, vegetables and fruit.

Your surgery took place in _____ 4 months before would have been _____

Surgery	Jan	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept	Oct.	Nov,	Dec.
4 Months Ago	Sept.	Oct.	Nov.	Dec	Jan.	Feb	Mar.	Apr.	May	June	July	Aug.

I would like you to think back over this 4 month period.

I will ask you to look at some cards and tell me which foods you ate during these last four months.

Then I will ask you whether you ate those foods

- Daily, that is, every day
- Weekly, at least once a week, but not every day
- Monthly, at least once a month, but not every week

These divisions are shown at the top of the cards (hand SHOWCARDS to respondent).

Let's begin by talking about your use of dairy products.

QUESTION	CODE	ITEM	✓	MODEL	NO. OF SERVINGS DWM	SERVING SIZE	COMMENTS
*SHOWCARD 1		DAIRY PRODUCTS					
Please look at the list of types of milk on the first CARD During the last 4 months what types of MILK did you drink, not including milk used in coffee, tea or on cereal?	01.01	White milk - Whole		11			
	01 02	- 2%		11			
	01.03	- Skim		11			
	01.04	- Buttermilk		11			
A Did you drink .. milk daily, weekly or monthly? (D/W/M)	01.02	- Unspecified		11			
B How many glasses of . . did you drink per .?		Chocolate Milk					
		(commercial or home made with mixes and milk)					
C Compared to this model how large was your usual serving?	01.11	- Whole		11			
	01.12	- 2%		11			
	01.13	- Skim		11			
	01 12	- Unspecified		11			
							SUGGESTED
Hot chocolate was your hot chocolate mix sugar-free? Y N	01 15	Hot chocolate		30			
		(made from mix and hot water)					
		Other?		11			

QUESTION	CODE	ITEM	✓	MODEL	NO. OF SERVINGS DWM	SERVING SIZE	FRUIT	COMMENTS
*SHOWCARD J		YOGURT						
From this list of YOGURTS which types did you eat?	01.33	- Whole Milk		12				
	01 34	- 2%		12				
A (D/W/M)	01 35	- Skim Milk		12				
B Servings	01 34	- Unspecified		12				
C								
Was the yogurt you had usually fruit flavoured? (Y/N)								
I would like to ask about your use of EGGS. In answering the question please include eggs eaten alone, in omelettes or in sandwiches, but not in baking or other cooking.	01.66	EGGS - Cooked in Fat		Unit				
	01 67	- Other?		Unit				
A. (D/W/M)								
B Eggs								

QUESTION	CODE	ITEM	✓	MODEL	NO. OF SERVINGS DWM	SERVING SIZE	COMMENTS
*SHOWCARD 3		CHEESE					
From this list of CHEESES which types did you eat?	02 01	Cottage Cheese-Creamed		13			
A (D/W/M)	02.02	- 2%		13			
B Servings - Cottate	02.01	- Unspecified		13			
- Hard		- Other ? e.g. Skim Milk		13			
- Soft	02.05	Hard Cheese e.g Cheddar		14			
Tbsp - Cream Cheese	02 06	Soft Cheese e.g Brie, Camembert		14			
- Cheese Spread	02.07	Cream Cheese e.g. Philadelphia		Tbsp			
Slices - Processed Cheese	02 08	Cheese Spread e.g. Cheeze Wiz		Tbsp			
	02.09	Processed Cheese Slices		Unit			
		Other? e.g. Skim Milk		•			
PEANUT BUTTER (a) You eat peanut butter?							
A (D/W/M)	02 33	PEANUT BUTTER		Tbsp			
B Servings - tbsp							

QUESTION	CODE	ITEM	✓	MODEL	NO. OF SERVINGS DWM	SERVING SIZE	CHEESE	G/S/M	COMMENTS
Now I would like to ask you about MEAT. At this point, I only want to discuss individual portions of meat We will be discussing mixed dishes such as casseroles later.		MEAT							
		BEEF							
		Ground Beef							
		Hamburgers							
*SHOWCARD A	03.01	- Commercial - Regular (McDonald)		Unit					
Here is a list of types of BEEF. During the last four months which did you eat?	03.02	- (Big Mac, Whopper) Large		Unit					
A (D/W/M)									
B . . Hamburgers									
C. Did you usually have these with CHEESE? (Y/N) Did you usually add MAYONNAISE? (Y/N)									
Were the HOMEMADE HAMBURGERS usually prepared with Regular or Lean Beef?	03.33	- Homemade - Regular Beef		15					
	03.34	- Lean Beef		15					
A (D/W/M)	03.33	- Unspecified		15					
B Homemade Hamburgers									
C. Did you usually have these with CHEESE? (Y/N) Did you usually add MAYONNAISE? (Y/N)									

QUESTION	CODE	ITEM	✓	MODEL	NO. OF SERVINGS DWM		SERVING SIZE	CHEESE	G/S/M	COMMENTS
Was the MEATLOAF you had usually made with Regular or Lean Beef?	03.66	Meatloaf - Regular Beef		16						
A (D/W/M)	03 67	- Lean Beef		16						
B Servings	03 66	- Unspecified		16						
C Did you usually add CHEESE? (Y/N)										
Did you usually add GRAVY or SAUCE? (Y/N)										

QUESTION	CODE	ITEM	✓	MODEL	NO. OF SERVINGS DWM	SERVING SIZE	FAT G/S	COMMENTS
A. (D/W/M) B. Serving - Roast, Steaks C. Did you usually eat the FAT? (Y/N) Did you usually add GRAVY or SAUCE? (Y/N)	04.01	Roast		17				
	04 02	Steak		18				
		Other?		*				
SHOWCARD 5 From this list of PORK which types did you eat over the last 4 months? A. (D/W/M) B. Servings - Roast Chops Spareribs Ham Slices - Bacon Cold Cut C (Spareribs) Using this ruler, please show me the length and breadth of your usual serving of spareribs Did you usually eat the FAT? (Y/N) Did you usually add GRAVY or SAUCE? (Y/N)		PORK						
	04.33	Chops		19				
	04.34	Roast		17				
	04.35	Spareribs		Rule				
	04.36	Bacon - Side (regular)		Unit				
	04.37	- Back, Peameal		Unit				
	04 38	Ham - Cured		33				
	04.39	- Cold Cut Slices		Unit				
	04.40	- Picnic, Cottage Roll		33				
		Other?		*				

QUESTION	CODE	ITEM	✓	MODEL	NO. OF SERVINGS DWM	SERVING SIZE	FAT	G/S	COMMENTS
* SHOWCARD 6 During the last 4 months which items on this list did you eat? A (D/W/M) B Chops Cutlets Other? - roast Did you usually eat the FAT? (Y/N) Did you usually add GRAVY or JUICE? (Y/N)		VEAL							
	05 01	Chops		19					
	05 02	(Schnitzel Cutlets Scallopini)		19					
	05 03	Roast		17					
		Other?							
		LAMB							
	05 33	Chops		19					
	05 34	Roast		17					
		Other?							
* SHOWCARD From this list of ORGAN and GLANDULAR MEATS which did you eat? A W M Livers Did you usually add GRAVY or JUICE? Y N		ORGAN AND GLANDULAR MEATS							
	05 66	Heart		17					
	05 67	Liver - Beef, Pork		17					
	05 68	- Calf, Lamb		17					
	05 69	- Chicken, Turkey		40					
	05 70	Tongue		?					
		Other Organ Meats?		*					
				L				G	

QUESTION	CODE	ITEM	✓	MODEL	NO. OF SERVINGS D	W	M		SERVING SIZE		Skin	G/S	COMMENTS
* SHOWCARD 8 From this list of POULTRY which did you eat over the last 4 months? A. (D/W/M) B. Which pieces did you usually eat and how many did you have per (D/W/M)? Did you usually eat the SKIN? (Y/N) Did you usually add GRAVY or SAUCE? (Y/N)		POULTRY											
		Chicken-Commercial Fried		*									
		- Home Fried-Breaded		*									
		- Home Fried-Unbreaded		*									
		- Barbecued		*									
		- Baked, Roasted		*									
		- Chicken salad		½C									
		- Other?											
* SHOWCARD 9 From this list of FISH which did you eat over the last 4 months? A. (D/W/M) B. Portions-Breaded Sticks -Breaded Servings-Salmon -Other Fish -Canned -Shellfish Did you usually add a sauce such as TARTAR SAUCE, MAYONNAISE or any other FAT-BASE SAUCE? (Y/N)		FISH											
	06.66	Breaded/-Battered Portions		Unit									
	06.67	Breaded/Battered Sticks		Unit									
	06.75	Salmon-fresh, frozen		20									
		Other fish- fresh,frozen											
	06.68	- fried		20									
	06.69	- baked,broiled		20									
	06.70	Canned-Oil Pack		½C									
	06.71	-Water Pack		½C									
	06.73	Shellfish-Fried		½C									
	06.72	Shellfish-Other		½C									
		Fish salad (tuna, salmon)		½C									

QUESTION	CODE	ITEM	✓	MODEL	NO. OF SERVINGS DWM	SERVING SIZE	G/S/M	COMMENTS
• SHOWCARD 10		PREPARED MEATS						
From this list of PREPARED MEAT which did you eat over the last 1 month?	07 01	Bologna & Other Cold Cuts		Unit				
	07 02	Salami		Unit				
	07 03	Luncheon Meat, Canned		Unit				
	07 04	Liverwurst, Pate		Tbsp				
	07.05	Sausages - Regular		Unit				
	07	- Large		Unit				
	07	Wieners - Regular		Unit				
	07	- Large		Unit				
	07	Meat Salad		1C				
		Other?		•				
		Other Meat?		•				

RARE | MEDIUM RARE | MEDIUM | WELL DONE | D K

Would you please estimate what percentage of all the meat, fish and poultry you ate was usually FRIED? [ ] %

QUESTION	CODE	ITEM	✓	MODEL	NO. OF SERVINGS DWM	SERVING SIZE	SUGAR	HONEY	COMMENTS
I would now like to discuss the CEREALS and BREADS you have eaten over the last 4 months. SHOWCARD 11 From this list of CEREALS which kinds did you eat? Please include hot cereals. A. (D/W/M) B. Bowls		CEREALS (Please list)							
				Bowl					
Did you usually add SUGAR or HONEY to your cereal? Which cereals did you add SUGAR to?		Other?		Bowl					
Which cereals did you add HONEY to?									
WHEAT BRAN A (D/W/M) B. Tbsp.		WHEAT BRAN		Tbsp					

What type of MILK or CREAM did you usually use on your cereal? (Please Check)

Whole Milk	(1)	Table Cream	(5)
2% Milk	(2)	Half & Half Cream	(6)
Skim Milk	(3)	Unspecified Cream	(5)
Unspecified Milk	(2)	D. K	(2)

QUESTION	CODE	ITEM	✓	MODEL	NO. OF SERVINGS DWM	SERVING SIZE	FAT	SWEET SPREAD	COMMENTS
* SHOWCARD 12		BREADS, BUNS, ROLLS							
From this list of BREADS, BUNS and ROLLS which did you eat over the last 1 month/s?	09 01	White		Unit					
	09 02	Whole Wheat (40-60%)		Unit					
A (D/W/M)	09.03	Whole Wheat, (100%)		Unit					
B Slices - Bread Rolls Buns Bagels Croissants	09 02	Whole Wheat, unspecified		Unit					
	09 04	Rye - light		21					
	09.05	Rye - dark, Pumpernickel		21					
Do you usually add BUTTER or MARGARINE? (Y N)		Other?		21					
If you usually add JAM, JELLY, HONEY or other SWEET SPREAD? (Y N)									
	09 33	Dinner Rolls, Buns - White		22					
	09.34	- Whole Wheat		22					
	09 35	Hamburger Buns		Unit					
	09 36	Hot Dog Buns		Unit					
	09.37	Bagels		Unit					
	09.38	Croissants (Butter Roll)		Unit					
		Other?							

QUESTION	CODE	ITEM	✓	MODEL	NO. OF SERVINGS DWM	SERVING SIZE	FAT	SWEET SPREAD	COMMENTS
* SHOWCARD 13 From this list which did you eat over the last 4 months? A (D/W/M) B. Biscuits Scones Servings-Corn Bread Slices-Fruit Bread Muffins Servings-Pancakes Servings-Waffles Slices-French Toast Breadsticks Melba Toast Crackers C. (Pancake) Using this ruler, please show me the diameter of your usual serving of pancakes. (Waffle) Using this ruler, please show me the dimensions of your usual serving of waffles. Did you usually add BUTTER or MARGARINE? (Y/N) Did you usually add JAM, JELLY, SYRUP or other SWEET SPREAD? (Y/N)	10.01	Quick Breads - Biscuits, Scones		Unit					
	10.02	- Corn Bread		23					
	10.03	- Fruit & Nut Bread		21					
	10.04	- Other?							
	10.05	Muffins - Bran		24					
		- Other?		24					
	10.10	Pancakes		Ruler					
	10.11	Waffles		Ruler					
	10.12	French Toast		21					
	10.33	Breadsticks		Unit					
	10.34	Melba Toast		Unit					
	10.35	Soda Crackers		Unit					
		Other Crackers?		Unit					
RICE - FRIED - STEAMED A. (D/W/M) B Servings C. Did you usually add BUTTER MARGARINE or GRAVY? (Y/N)	10.66	Rice - Fried		25					
	10.67	- Steamed		25					

QUESTION	CODE	ITEM	✓	MODEL	NO OF SERVINGS DWM	SERVING SIZE	FAT	COMMENTS
I will now be discussing 4 groups of VEGETABLES First let's talk about POTATOES. POTATOES can be prepared in many different ways I would like to talk about each of them separately • SHOWCARD 14 Which types of POTATOES on this list did you eat over the last 4 months? A (D/W/M) B Baked Potatoes Boiled Potatoes Servings - Other Did you usually add GRAVY, SAUCE, BUTTER or SOUR CREAM?		VEGETABLES A						
		POTATOES						
	11 01	Baked		26				
	11 02	Boiled		26				
	11.03	Mashed		27				
	11.04	French Fried		28				
	11 05	Pan Fried or Hash Browns		28				
	11 06	Scalloped-with cheese		28				
	11 07	- without cheese		28				
	11.08	Salad		27				
		Other?		•				

QUESTION	CODE	ITEM	✓	MODEL	NO. OF SERVINGS DWM	SERVING SIZE	FAT	SAUCE	COMMENTS
* SHOWCARD 15 Now let's discuss other VEGETABLES which are eaten as individual vegetables, rather than in mixed dishes such as stews or salads. Could you please tell me which of the vegetables on this list you have eaten either raw, cooked, or canned. A (D/W/M) * B Carrots Cobs of Corn Servings - Other C Was FAT added in preparation or at the table? (Y/N) Was sauce (such as CHEESE SAUCE or WHITE SAUCE) added to the vegetables? (Y/N)		VEGETABLES B							
	12.01	Sweet Potatoes - regular		26					
	12.02	- candied		½C					
	12 03	- diet pack		½C					
	12 04	Yams		26					
	12 05	Turnips		27					
	12.06	Beets		27					
	12.07	Carrots - raw		Unit					
	12.08	- Candied		½C					
	12.09	- Cooked, Canned		½C					
	12.10	Corn - on cob		Unit					
	12.11	- Other		29					
	12 12	Onions		½C					
	12 13	Green/Yellow Bean		29					
	12 14	Bean/Alfalfa Sprouts		½C					
	12.15	Green Peas		29					
	12.16	Mixed Vegetables		29					
	12.17	Lima Beans		½C					
		Other Beans, Peas, Lentils		½C					

QUESTION	CODE	ITEM	✓	MODEL	NO OF SERVINGS DWM	SERVING SIZE	FAT	SAUCE	COMMENTS
* SHOWCARD 16		VEGETABLES C							
From this list of VEGETABLES, could you please tell me which you have eaten	13 01	Beet Greens		29					
	13.02	Broccoli		29					
	13.03	Brussels Sprouts		29					
(D W/M)	13.04	Cabbage		29					
Servings-Beet Greens to Squash -Spears of Asparagus Stalks of Celery Whole Green Peppers Whole Tomatoes Avacados	13.05	Cauliflower		29					
	13 06	Spinach		29					
	13 07	Zucchini		29					
	13 08	Squash (other types)		29					
	13 09	Asparagus		Unit					
Was FAT added in preparation or at the table? (Y/N)	13 10	Celery		Unit					
Was sauce (such as CHEESE or WHITE SAUCE) added? (Y/N)	13.11	Green Pepper		Unit					
	13 12	Tomato		Unit					
** Alternative Questions Instead of talking about the use of each individual vegetable let's consider these vegs as a group. Could you please tell me how often you have eaten vegetables from this group?	13 13	Avocado		Unit					
		Other?							
	13 99	**TOTAL VEGETABLES							
How many servings did you have per ?									

QUESTION	CODE	ITEM	✓	MODEL	NO. OF SERVINGS DWM	SERVING SIZE	Cal. Red.	Regular	COMMENTS
* SHOWCARD 17 From this list of SALADS PICKLES and OLIVES which did you eat over the last 4 months? A (D/W/M) B. Servings C Did you usually add CALORIE REDUCED DRESSING to your (Chef's, Combination, Spinach) salad?(Y/N) Did you usually add REGULAR SALAD DRESSING to your (Chef's, Combination, Spinach) salad?(Y/N)		VEGETABLES D							
		SALADS							
	14.01	Bean		½C					
	14.02	Carrot & Raisin		½C					
	14.03	Chef's (with cheese, meat eggs)		2C					
	14.04	Coleslaw (Cabbage)		½C					
	14.05	Combination/Lettuce & Tossed, Tomato)		1C					
	14.06	Spinach		1C					
		Other?		*					
	14.33	PICKLES-Sweet pickles		*					
	14.34	-Sweet pickle relish		tbsp					
	14.35	Olives -black, large		Unit					
	14.36	-green, medium		Unit					
* SHOWCARD 18 From the list of SOUPS which did you eat? A. (D/W/M) B Bowls C. Were there any other types of soup that you had? If "yes", REPEAT A.B.C.		SOUPS							
	14 66	Bean, Pea, Lentil		Bowl					
	14.67	Clear Soups		Bowl					
	14.68	Cream Soups-made with water		Bowl					
	14.69	-made with milk		Bowl					
	14.70	Chunky Soups		Bowl					
		Other?							

QUESTION	CODE	ITEM	✓	MODEL	NO. OF SERVINGS DWM	SERVING SIZE	COMMENTS
• SHOWCARD 19 Here is a list of some STEWS and CASSEROLES Could you please tell me which you have eaten? A (D/W/M) B Serving/Bowls C (length x diameter of Cabbage Roll) Are there any other types of STEWS/CASSEROLES you have eaten that we have not discussed? If "yes" record name of dish A (D/W/M) B Servings C May we please discuss details of the recipe at the end of the questionnaire?		MIXED DISHES					
		STEWS					
	15 01	Beef and Vegetable Stew		Bowl			
	15.02	Chicken Stew		Bowl			
	15 03	Chili Con Carne with Beans		Bowl			
	15.04	Chili Con Carne without Beans		Bowl			
	15 05	Fish Stew		Bowl			
	15 06	Irish Stew		Bowl			
		Other?		Bowl			
		CASSEROLES					
	15 33	Beef & Kidney Pie		32			
	15 34	Cabbage Roll		rule			
	15 35	Chicken Pie		32			
	15.36	Shepherd's Pie		32			
	15 37	Tourtiere (Pork Pie)		32			
		Other?		•			

QUESTION	CODE	ITEM	✓	MODEL	NO. OF SERVINGS DWM	SERVING SIZE	COMMENTS
• SHOWCARD 20		PASTA					
From this list of PASTA and PIZZA which did you eat during the past 4 months?	16 01	Spaghetti - Tomato Sauce		1C			
	16.02	- Tomato Sauce and Cheese		1C			
	16.03	- Italian Meat Sauce		1C			
A. (D/W/M)	16.04	- Meatballs and Sauce		1C			
B. Servings	16.05	Macaroni and Cheese		1C			
C.	16.06	Macaroni Salad		1C			
	16.07	Egg Noodles - Fat added		1C			
	16.08	- Meat added		1C			
	16.09	Lasagna		23			
		Other Pasta?		1C			
							L M S
	16.33	PIZZA - without meat		Unit			
A. (D/W/M)	16.34	- with meat		Unit			
B. Fraction of a whole Pizza (¼, ½, etc)							
Was the PIZZA you usually had large, medium or small (L/M/S)							

QUESTION	CODE	ITEM	✓	MODEL	NO. OF SERVINGS D W M	SERVING SIZE	CANNED PACKAGE	TOPPING	SUGAR	COMMENTS
* SHOWCARD 21 Now let's talk about FRUIT. In discussing the fruit you have eaten, please include fruit eaten alone, whether raw, cooked or canned, and fruit used on cereal. We are also interested in whether the canned fruit you eat is packed in syrup. A. (D/W/M) B. Units - how many Servings - ½ cup Fraction of Whole - Melon C Was your CANNED FRUIT packed in syrup? (Y,N) Did you add a TOPPING such as WHIPPED CREAM, ICE CREAM, or other TOPPING? (Y/N) Did you add SUGAR or HONEY to your fruit? (Y/N)		FRUIT								
	17.01	Apples - fresh		Unit						
	17.02	Applesauce, cooked apples - unsweetened		½C						
	17.03	- sweetened with sugar or honey		½C						
	17.04	Bananas - fresh		Unit						
	17.05	Cherries - fresh		½C						
	17.06	- canned		½C						
	17.07	Grapefruit - fresh		Unit						
	17.08	- canned		½C						
	17.09	Grapes - fresh		½C						
	17.10	- Canned		½C						
	17.11	Oranges - fresh		Unit						
	17.12	- Canned		½C						
	17 13	Peaches - fresh		Unit						
	17 14	- Canned		½C						
	17 15	Pears - fresh		Unit						
	17.16	- Canned		½C						
	17 17	Pineapple - fresh		½C						
	17.18	- Can		½C						
	17.19	Plums - fresh		Unit						
	17.20	- Canned		½C						

FRUIT (cont'd..)

SHOWCARD 21A

A. (D/W/M)

B. Units - how many Servings - ½ cup

Fraction of Whole - Melon

C. Was your CANNED FRUIT packed in syrup? (Y/N)

Did you add a TOPPING such as WHIPPED CREAM, ICE CREAM, or other TOPPING? (Y/N)

Did you add SUGAR or HONEY to your fruit? (Y/N)

CODE	ITEM	✓	MODEL	NO. OF SERVINGS (DWM)	SERVING SIZE	CANNED PACKAGE	TOPPING	SUGAR	COMMENTS
17.21	Prunes - dried, uncooked		½C						
17.22	- cooked		½C						
17.23	Strawberries - fresh		½C						
17.24	- frozen		½C						
17.25	Tangerine - fresh		Unit						
17.26	Fruit Cocktail - canned		½C						
17.27	Fruit Salad - fresh		½C						
	- canned		½C						
17.28	Honeydew Melon		½U						
17.29	Other Melon		½U						
	Other fruit?								

QUESTION	CODE	ITEM	✓	MODEL	NO. OF SERVINGS DWM	SERVING SIZE	COMMENTS
• SHOWCARD 22 From this list of JUICES, which did you drink over the last 1 months? Please include any juices which you have used as mixes with alcoholic beverages A (D/W/M) B Glasses C		FRUIT JUICES					
	18 01	Orange Juice		4oz			
	18 02	Apple Juice		4oz			
	18.03	Grapefruit Juice - - unsweetened		4oz			
	18 04	- sweetened		4oz			
	18.05	Cranberry Juice Cocktail		4oz			
	18 06	Grape Juice - canned bottled		4oz			
	18 07	- frozen		4oz			
	18 08	Apricot Nectar		4oz			
	18 09	Lemonade, Limeade		4oz			
	18 10	Pineapple Juice		4oz			
	18 11	Prune Juice		4oz			
		Other?		4oz			
		FRUIT DRINKS					
	18 11	e.g. Tang, Quench		4oz			
		VEGETABLE JUICE					
	18 66	Tomato Juice		4oz			
	18 67	Mixed Vegetable Juice e.g. Garden Cocktail		4oz			
		Other?		4oz			

QUESTION	CODE	ITEM	✓	MODEL	NO OF SERVINGS DWM	SERVING SIZE	SUGAR	HONEY	COMMENTS
There are some other beverages that I would like to ask about. There is no card for these, so we will discuss each individually	19 01	COFFEE Decaffeinated		30					
A. (D/W/M/)	19.02	Ordinary		30					
B Cups	19.03	Milk - Whole							
	19.04	- 2%							
C	19.05	- Skim							
D. What did you usually use in your coffee? Whole Milk, 2% Milk, Skim Milk, Cream, Powdered Whitner, Liquid Whitner	19.06	Cream							
	19.07	Whitener - Powder							
	19.08	- Liquid							
E. (If more than one of the above) In what percent of the total cups of coffee did you use each of these?	19.33	TEA Ordinary Black		30					
F Did you usually use sugar in your coffee? (Y/N)	19.34	Other Types		30					
	19.35	Milk - Whole							
REPEAT A,B,C,D,E,F, for Tea	19.36	- 2%							
G. (Tea only) Did you usually use honey in your tea? (Y/N)	19.37	- Skim							
	19.38	Cream							
	19.39	Whitner - Powder							
		- Liquid							

QUESTION	CODE	ITEM	√	MODEL	NO. OF SERVINGS DWM	SERVING SIZE	COMMENTS
I would now like to discuss ALCOHOLIC BEVERAGES		ALCOHOLIC BEVERAGES					
A (D W M)	20 01	Beer - regular		1Btl			
B Bottles or Mugs-Beer Glasses-Wine Drinks-Distilled Beverages	20 02	- light		1Btl			
	20 01	- unspecified		1Btl			
	20 03	Wine - regular		4oz			
	20 04	- light		4oz			
	20 03	- unspecified		4oz			
	20.05	Sherry, Port, Vermo[illegible]		2oz			
	20 06	Distilled Beverages e.g. Whiskey, Vodka, Gin		1.50 oz			
		Other?		•			
Now let's discuss SOFT DRINKS. I am interested [illegible] DIET [illegible] Please include soft drinks used as mixes [illegible]		SOFT DRINKS		11			
A [illegible] W M		Regular					
B [illegible]	[illegible]	Colas, root beer [illegible] 7-UP		11			
	20 34	Ginger ale		11			
	20 35	Fruit fla[illegible]		11			
		Other?		11			
[illegible]		OTHER BEVERAGES					
	[illegible]	[illegible]		[illegible]			
	[illegible]	Instant [illegible]		[illegible]			
		Other					

QUESTION	CODE	ITEM	✓	MODEL	NO. OF SERVINGS DWM	SERVING SIZE	TOPPING	COMMENTS
Finally let's discuss DESSERTS and SNACK FOODS. * SHOWCARD 23 From this list of MILK DESSERTS which did you eat during the past 4 months? A. (D/W/M) B. Scoops-Ice Cream to Sherbert Servings-Puddings Custards C. Did you usually have a TOPPING such as CHOCOLATE or a WHIPPED TOPPING?		MILK DESSERTS						
	21.01	Ice Cream - Regular		1Scp				
	21.02	- Rich		1Scp				
	21.03	- Soft		1M Cone				
	21.01	- unspecified		1Scp				
	21.04	Ice Milk		1Scp				
	21 05	Frozen Yogurt		1Scp				
	21.06	Sherbert		1Scp				
	21.07	Milkshake		11				
		Puddings						
	21.08	- made from home recipe - chocolate		½C				
	21.09	- made from - other home recipe (specify)		½C				
	21.10	- made from mix - regular		½C				
	21 12	- ready to serve		½C				
	21.10	- unspecified		½C				
	21 13	Baked Custard		½C				
	21.14	Other Milk/Cheese Desserts		*				
		(Specify) _____						

QUESTION	CODE	ITEM	✓	MODEL	NO. OF SERVINGS DWM	SERVING SIZE	TOPPING	COMMENTS
SHOWCARD 24		SWEET DESSERTS						
From this list of SWEET ES ERTS which types did you eat? D W M'	21 33	Cakes - Fruit		23				
	21 34	- Plain, Chocolat		34				
slices (Pound cake nly.) Using this ruler please show me the dimensions of your serving of pound cake	21 35	- Pound		ruler				
	21 36	- Sponge		34				
	21 37	- Cheese cake		34				
Brownies		- Other						
Cookies								
Do you usually have a TOPPING such as WHIPPED CREAM ICING?								
Please tell me what types of COOKIES you usually ate Brand names or general e g jar Cookies homemade?	21 66	Brownies		23				
		Cookies		unit				
Please put date of it								

QUESTION	CODE	ITEM	✓	MODEL	NO. OF SERVINGS DWM	SERVING SIZE	TOPPING	COMMENTS
* SHOWCARD 25 A (D/W/M) B Date Squares Donuts Slices - Pie Pastries Gelatins Chocolates Chocolate Bars Candies Servings-Popcorn Potato Chips Nuts Seeds Seed & nut mixes eg. Trail mix C PIES only Did you usually add a CREAM or CHEESE TOPPING? (Y/N) Gelatins only Did you usually add a TOPPING such as ICE CREAM or a WHIPPED CREAM TOPPING? (Y/N)	22.01	Date Squares		23				
	22.02	Donuts - regular		Unit				
	22.03	- fancy		Unit				
	22.04	- filled		Unit				
	22.05	Pies - Apple		31				
	22.06	- Blueberry		31				
	22.07	- Mincemeat, Raisin		31				
	22.08	- Custard		31				
	22.09	- Lemon meringue		31				
	22 10	- Cherry, Peach		31				
	22.11	- Pumpkin		31				
		- Other? (Specify)		31				
	22.33	Sweet Pastries- Danish		Unit				
	22.34	- Eclaire		Unit				
	22.35	- Other (Specify)		Unit				
		Gelatins						
	22 37	- regular, plain		½C				
	22.38	- regular with fruit		½C				
	22.39	- Other e.g. Mousse (Specify)		½C				
		Other Sweet Desserts		*				

QUESTION	CODE	ITEM	✓	MODEL	NO. OF SERVINGS DWM	SERVING SIZE	COMMENTS
* SHOWCARD 26 From this list of SNACKS which did you eat? A (D,W M) * CHOCOLATE BARS Please tell me the brand names and estimate the size 'CANDIES' What kinds of candies did you eat?		SNACKS					
	22 66	Chocolate Bars		Unit			
	22 67	Chocolates		Unit			
	22 68	Candies					
	22 75	Popcorn - plain		IC			
	22 76	- with butter		IC			
	22 77	Potato Chips		IC			
	22 78	Nuts - all kinds		IC			
	22 80	Seeds - all kinds		IC			
	22 81	Seed & Nut Mixes - Trail Mix		IC			
	22 82	Pretzels		IC			
		Other (Specify)					

SOCIO-ECONOMIC FACTORS I would be very grateful if you would provide me with some final background information.

46. From this list (Showcard B), please tell me the highest certificate, degree or diploma you have obtained. (Record relevant number)

_____

47 Would you please tell me which of the jobs on this card most closely reflects your usual or most recent occupation? (Showcard C)

1 ☐ Business manager/owner
2 ☐ Farmer
3 ☐ Government official
4 ☐ Manual worker
5 ☐ Professional person
6 ☐ Salesperson/Buyer
7 ☐ Secretary/clerical worker
10 ☐ Skilled tradesperson
11 ☐ Teacher/professor
12 ☐ Other (please specify) _____

48. What is your present marital status?

1 ☐ Married, living common-law
2 ☐ Widowed
3 ☐ Divorced
4 ☐ Separated
5 ☐ Single
6 ☐ Refused

49. What number on this card corresponds to your total family income from all sources before taxes? (Showcard D)

_____

8 ☐ DK
9 ☐ Refused

TIME INTERVIEW ENDED. _____ a.m.
p.m.

Now I would like to return to the CASSEROLES and other MIXED DISHES you mentioned earlier. These were: (read names from your side list)

Could you please give me the recipe for this/these?

If YES: I would like a list and the amount of all the ingredients, in addition to the proportion of the total recipe you ate.

If NO: Would you mind if I contact the person who normally prepares this/these dish(es)?

If YES: The person's name _____

Telephone Number _____

Most convenient time to call _____

RECIPE SPECIFICATION FORM

Recipe Name or Description	Ingredient Description	Household Measure	Yield	Portion Consumed		

1) Respondent's cooperation and interest was
   Very Good = 1
   Good = 2
   Fair = 3
   Poor = 4
   Very Poor = 5

2) Reliability of information as assessed by interviewer was
   Very Good = 1
   Good = 2
   Fair = 3
   Poor = 4
   Very Poor = 5

3) Please record impressions of interview, including any distractions or contributions from other respondents.

Time Finished
a m.
p.m

APPENDIX B

BREAST DISEASES STUDY

SUBJECT ID

MEDICAL, SMOKING AND REPRODUCTIVE HISTORY QUESTIONNAIRE

To begin, I would like to ask you some questions about your childbearing history, smoking habits, occurrence of certain illnesses, as well as get some general background information. Please keep in mind that the information you give will be strictly confidential.

Let's begin with questions on some general background information.

Participant _____

Study representative _____

Date _____

Time interview began _____ a.m. p.m.

1. How tall are you?

_____ feet ____ inches
_____ cm

2. What weight were you six months ago say in _____ (month)?
Jan Feb Mar Apr May Jun) 6 months
Jul Aug Sep Oct Nov Dec) apart

_____ lbs
_____ kg

3. What did you weigh at age 20?

_____ lbs
_____ kg
8 ☐ don't know Were you ☐ lighter) than you
☐ heavier) were 6
☐ the same) months ago

4. What is your date of birth?

____/____/____
year month day

5. Where were you born?

Province (if in Canada) _____ Go to question 7
Country (if outside Canada) _____

6. IF BORN OUTSIDE CANADA
a. How old were you when you came to live in Canada?

_____ years old

b. To which ethnic or cultural group did you belong on first coming to North America? (eg. French, German, etc)

_____

GO TO QUESTION 8

7. IF BORN IN CANADA
a. To which ethnic or cultural group did your paternal ancestors belong on first coming to North America? (eg. English, French, Scottish, Russian...)

_____

b. To which ethnic or cultural group did your maternal ancestors belong on first coming to North America?

_____

8. What was your religion at birth?

_____

MENSTRUAL HISTORY

I would now like to ask you some questions about your menstrual periods.

9. When did your periods start? Year: _____ Month: _____
   PROBE: Do you remember how old you were and what month it was?

10. Between the ages of 20 to 40, how many days were there usually between the start of one menstrual period and the start of the next period?
    Comments. _____

    _____ Days
    77 ☐ irregular: describe: _____
    88 ☐ DK
    99 ☐ NA

11. What was the date of your last menstrual period? ___/___/___ (year / month / day) If less than 6 months ago, go to Q.13

    PROBE Was it more than one year ago?

    IF LAST PERIOD WAS MORE THAN ONE YEAR AGO, ASK.
    How many years ago was it? _____ years ago
    OR
    Do you remember how old you were and what month it was? _____ age _____ month

    IF LAST PERIOD WAS LESS THAN ONE YEAR AGO, ASK
    How many months ago was your last period? _____ months ago
    If less than 6 months ago, go to Q.13

12. When your periods stopped, did this occur naturally, or because you had an operation or radiation treatments?

    Comments: _____

    1 ☐ naturally
    2 ☐ operation
    3 ☐ radiation (x-ray, radium)
    4 ☐ both operation and radiation
    8 ☐ DK
    9 ☐ NA

PREGNANCY AND BIRTH HISTORY

3.

Now that you have told me about your menstrual history, I would like to know about your pregnancies, including all livebirths (babies), stillbirths, miscarriages and terminated pregnancies.

13. Have you ever been pregnant?

1 ☐ Yes
2 ☐ No)
8 ☐ DK) Go to question 17
9 ☐ NA)

14. How many times have you been pregnant? _____ pregnancies

First, let's talk about the babies (or livebirths) you have had.

15. How many babies did you have? _____ number of babies If woman had no babies (no.=0) go to question 16.

a) When was your first baby born? (Date: Year/Month)

b) For how many weeks (or months) were you pregnant? (Note: full term=9 months=40 weeks)

c) Was this a baby boy or girl?

d) Did you breast-feed (nurse) this baby? ("No" = 0 duration)
If "Yes":

For how many weeks did you nurse?

Now let's talk about your (2nd, 3rd,...) baby.

PROBE: Were there any others?

Birth order	Date (Year/Month)	Duration of pregnancy(weeks)	Sex of baby	Duration of nursing(weeks)

16. Did you have any

	1 Yes	2 No	8 DK	9 NA
a) miscarriages?				
b) stillbirths?				
c) terminated pregnancies?				

If "No/DK/NA" to all, go to question 17

If "Yes" to any of a), b), c):

d) How many miscarriages/stillbirths/terminated pregnancies did you have?

_____ miscarriages
_____ stillbirths
_____ terminated pregnancies

e) When did you have your (1st, 2nd,...) miscarriage/stillbirth/terminated pregnancy? (Date)

f) For how many weeks were you pregnant?

PROBE: Were there any others?

Outcome Miss/Still/Term.preg			Date Year/Month		Duration of pregnancy (weeks)

FAMILY HISTORY

5. ☐☐☐☐☐☐

Now I would like to ask you some questions about your family's size and health. These questions refer to your female blood relatives; that is those people, such as your mother, grandmothers, sisters and aunts who are actually related to you.

17. Is your mother still alive?

1 ☐ Yes - alive
2 ☐ No - dead
8 ☐ Don't know

☐

18. If "Yes - alive": How old is she?

If "No - dead": How old was she at the time of her death?

Age _____ ☐☐

19. How old was your maternal grandmother (your mother's mother) at the time of her death?

Age _____ ☐☐

20. How old was your paternal grandmother (your father's mother) at the time of her death?

Age _____ ☐☐

Let's talk about your biological sisters. By "biological" I mean those sisters whose both birth parents are the same as yours.

21. How many sisters do you have?

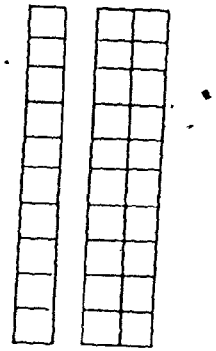
_____ sisters (if answer=0, go to question 23) ☐☐

22. Let's start with your oldest sister.

(a) Is she still alive?
(b) If alive. How old is she?
If dead: How old was she at the time of her death?

	(a) alive/dead	(b) age
1		
2		
3		
4		
5		
6		
7		
8		
9		
10		

Now, the next oldest sister...



Now let's talk about your aunts, that is, your mother's and father's sisters.

23. How many maternal aunts do you have?
(ie., how many sisters did your mother have?) _____ maternal aunts (if answer=0, go to question 25)

24. Again, starting with the oldest.

(a) Is she still alive?
(b) If alive: How old is she?
If dead: How old was she at the time of her death?

Now, the next oldest...

	(a) alive/dead	(b) age
1		
2		
3		
4		
5		
6		
7		
8		
9		
10		

25. How many paternal aunts do you have?
(ie., how many sisters did your father have?) _____ paternal aunts (if answer=0, go to question 27)

26. Again, starting with the oldest

(a) Is she still alive?
(b) If alive: How old is she?
If dead: How old was she at the time of her death?

Now, the next oldest...

	(a) alive/dead	(b) age
1		
2		
3		
4		
5		
6		
7		
8		
9		
10		

FAMILY HISTORY

Now I would like to ask you some questions about your family's health. These questions refer to your female blood relatives we've been discussing - that is, your mother, grandmothers, sisters and aunts.

27. Did any of the blood relatives we've talked about ever have breast cancer?

1 ☐ Yes
2 ☐ No)
8 ☐ DK) Go to question 29.
9 ☐ NA)

28. If "YES"

a. Could you please tell me which relative it was? (PROBE: what was her relationship to you? be specific: ie maternal aunt)

b. How old was she when the breast cancer was diagnosed?
(PROBE in decades: was she in her 30's, 40's, 50's ..)

c. At the time of diagnosis, were one or both breasts affected?

(a) Relative	(b) Age	(c) One breast	Two breasts	DK

PROBE: Was there anyone else?

8.

MEDICAL HISTORY Now I would like to ask you some questions about your general health and your use of certain medications.

First let's talk about some particular operations you may have had

a. Have you ever had _____?

b. When did you have this (Year/month) Probe: Do you remember the year? OR How old were you at the time? (interviewers, change to date)

OPERATION	a) 1 Yes	2 No	8 DK	9 NA	b) DATE (year/month)
29. A hysterectomy					
30. Both ovaries removed at the same time					
31. Only one ovary removed					
32. A surgical biopsy for a lump in your breast					1 _____ 2 _____ 3 _____ 4 _____

Other surgeries: _____

_____

Comments: _____

_____

9.

33. Have you ever had THYROID DISEASE

1 ☐ Yes
2 ☐ No
8 ☐ DK
9 ☐ NA

34. Have you ever had RADIATION THERAPY?

1 ☐ Yes
2 ☐ No)
8 ☐ DK) Go to question 35
9 ☐ NA)

If "YES"

a. What part of your body was treated?

b. For what reason did you have the radiation therapy?

c. for each site: When did you have this? (year/month)
PROBE: how old were you at the time? (interviewers, change to date)

a. SITES	DK	NA	b. REASON	DK	NA	c. DATE (year/month)	DK	NA
1. _____			1. _____			1. _____		
2. _____			2. _____			2. _____		
3. _____			3. _____			3. _____		

35. Have you ever had CHEMOTHERAPY?

1 ☐ Yes
2 ☐ No)
8 ☐ DK) Go to question 36
9 ☐ NA)

If "YES"
When did you have this? (year/month)
PROBE Do you remember how old you were? (interviewers, change to date)
DATE (year/month)

		DK	NA
1.			
2.			
3.			

36. Have you ever had ANTI-ESTROGEN THERAPY
For example, Tamoxifen or Nolvadex

1 ☐ Yes
2 ☐ No)
8 ☐ DK) Go to question 37
9 ☐ NA)

If "YES"
When did you have this? (year/month)
PROBE Do you remember how old you were? (interviewers, change to date)
DATE (year/month)

		DK	NA
1.			
2.			
3.			

COMMENTS _____

OTHER ILLNESSES: _____

From this list of medications (Showcard A) could you please tell me which you have taken or are presently taking. (If the woman stops in the middle of the list PROBE: have you taken the other?)

37. Oral Contraceptives (Birth Control Pills)

1 ☐ Yes
2 ☐ No
8 ☐ DK
9 ☐ NA

☐

If "YES":

(a) What type(s) did you take?

TYPE(S): DK NA

1. _____
2. _____
3. _____
4. _____

(b) What date did you start taking the (1st...) type? PROBE: Do you remember how old you were?

YEAR/MONTH DK NA

1. _____
2. _____
3. _____
4. _____

(c) When did you stop? PROBE: Do you remember how old you were?

YEAR/MONTH DK NA

1. _____
2. _____
3. _____
4. _____

a b c

38. Estrogens or Hormones

1 ☐ Yes
2 ☐ No
8 ☐ DK
9 ☐ NA

If "YES":

(a) What type(s) did you take?

TYPE(S). DK NA

1. _____
2. _____
3. _____
4. _____

(b) What date did you start taking the (1st...) type? PROBE: Do you remember how old you were?

YEAR/MONTH DK NA

1. _____
2. _____
3. _____
4. _____

(c) Are you still taking them now? If "NO" →

YES NO DK NA

1.
2.
3.
4.

(d) When did you stop? PROBE: Do you remember how old you were?

YEAR/MONTH DK NA a b c d

1. _____
2. _____
3. _____
4. _____

SMOKING HISTORY Now I would like to ask you some questions about your smoking habits

39. Do you smoke cigarettes now?

1 ☐ Yes
2 ☐ No)
8 ☐ DK) Go to question 42
9 ☐ NA)

40. Current Smoker:

On the average, how many cigarettes do you smoke each day?

_____ cigarettes/day
or _____ packs/day - How many cigarettes are in the package you usually smoke?
☐ large = 25 ☐ small = 20

41. For how many years have you been smoking? _____ number of years smoked (1 decimal place)

GO TO END

42. Have you ever smoked cigarettes

1 ☐ Yes
2 ☐ No)
8 ☐ DK) Go to END
9 ☐ NA)

43. Ex-Smoker

What was the maximum number of cigarettes per day that you smoked for at least one year?

_____ cigarettes per day
or _____ packs/day - How many cigarettes were in the package you usually smoked?
☐ large = 25 ☐ small = 20

44. How many years (or months) has it been since you have stopped smoking?
PROBE: When did you stop smoking?

_____ number of years since stopped smoking (1 decimal place)

45. For how many years did you smoke?

_____ number of years smoked (1 decimal place)

Time this questionnaire finished. _____ a.m. / p.m

Appendix C: Chart information

This form of record, in three pages, was included in the protocol dated 1984.09. For the shortened form of record used in the research proper, see Annex VI.

CHART INFORMATION

APPENDIX C

Study ID number: ☐☐☐☐☐
Sequence number: ☐☐☐☐☐
Study representative: _____ ☐☐

Name of patient: _____
last name/first name

Date of birth: ____/____/____ ☐☐☐☐☐☐
year month day

Hospital number: _____ Hospital: _____ ☐☐

Name of surgeon: _____ ☐☐

Date of surgery: ____/____/____ ☐☐☐☐☐☐
year month day

Type of surgery:

1 ☐ modified radical mastectomy
2 ☐ simple (total) mastectomy
3 ☐ subcutaneous mastectomy
4 ☐ lumpectomy (tylectomy, segmental resection, wedge resection, excisional biopsy, wide excision, partial mastectomy)
5 ☐ incisional biopsy
6 ☐ needle biopsy
7 ☐ other (specify): _____
8 ☐ DK
9 ☐ NA ☐

Location of tumour:

1 ☐ upper-inner (superior mesial) quadrant
2 ☐ upper-outer (superior distal) quadrant
3 ☐ lower-outer (inferior distal) quadrant
4 ☐ lower-inner (inferior mesial) quadrant
5 ☐ nipple
6 ☐ central portion
7 ☐ axillary tail
10 ☐ other (specify)
88 ☐ DK
99 ☐ NA ☐☐

Laterality of tumour. 2.

1 ☐ left
2 ☐ right
3 ☐ bilateral
8 ☐ DK
9 ☐ NA

Tumour size:

_____ length (longest) X _____ width (cm) (2 dec. places)

8 ☐ DK
9 ☐ NA

Pathological diagnosis:

INVASIVE

1 ☐ ductal carcinoma
2 ☐ lobular carcinoma
3 ☐ medullary carcinoma
4 ☐ tubular carcinoma
5 ☐ inflammatory carcinoma
6 ☐ papillary carcinoma
7 ☐ mucinous carcinoma
10 ☐ multifocal carcinoma

NON INVASIVE

11 ☐ lobular carcinoma in situ
12 ☐ ductal carcinoma in situ

13 ☐ other (specify): _____
88 ☐ DK
99 ☐ NA

Were the nodes dissected? 3.

1 Yes
2 No
8 DK
9 NA

If "Yes"

Total number of nodes examined: _____
Number of nodes positive for malignancy: _____

Grade of tumour. duct differentiation

1 Yes
2 No
8 DK
9 NA

If "Yes", describe: _____

Stage:

1 I
2 II
3 III
4 IV
5 Other: specify:
8 DK
9 NA

TNM: T_____ N_____ M_____

Are metastases present?

1 Yes
2 No
8 DK
9 NA

Site of metastases:
(check appropriate boxes)

1 nodes
2 bone
3 brain
4 liver
5 lungs
6 other (specify): _____
8 DK
9 NA

Appendix D: Letter of invitation given to patient when visited in hospital

If the subject was visited in hospital by a study representative, she was given this letter, along with a copy of the pamphlet (Appendix E). The use of University of Toronto stationery was to avoid the word "cancer" in the LICR letterhead.

Patients visited at Hospital A, however, were given instead the letter (1) in Annex III.

Department of Preventive Medicine and Biostatistics
Faculty of Medicine, University of Toronto
Toronto, Ontario, M5S 1A8

The University of Toronto, in conjunction with several Toronto hospitals and the Ludwig Institute for Cancer Research, Toronto Branch, is conducting a study of breast diseases in women. In order to do this we would like to collect information on a variety of factors from a large number of women throughout the Toronto area, and your doctor has agreed that we could ask you for your help.

Enclosed you will find a pamphlet which describes the study. Women who are over 50 years of age, able to speak English and have recently had breast surgery may be eligible. Within the next two weeks an interviewer may be telephoning you to see if you are eligible and wish to consider participating in the study. If you are willing, an interview will be arranged at your convenience. During this interview you will be asked questions about your health, smoking habits, diet and child-bearing history. All information which you give will be kept strictly confidential. Your data will be combined with that from many other women and the total results studied so that no single woman can be identified.

Your help is vital to identifying the causes of this important issue of women's health, and your participating would be greatly appreciated.

Gail Eyssen, Ph.D.
Associate Professor.

GE/mjs

Appendix E: Breast diseases pamphlet

This sheet reproduces the two sides of a folded card which formed the pamphlet given and/or sent to all patients who were approached to participate in the investigation. Patients visited in hospital were given a copy of this document, and another copy was enclosed with the letter mailed to their homes (Appendix F or Annex III).

The gap after the block "A study by" has been created by obliterating the names of the participating hospitals. This is to preserve anonymity as far as possible; the lists of cooperating surgeons have been omitted for the same reason.

BREAST DISEASES WHAT CAUSES THEM?

A STUDY BY

LUDWIG INSTITUTE FOR CANCER RESEARCH

UNIVERSITY OF TORONTO

BREAST DISEASES are a major health concern for Canadian women Approximately half of the women living in this country will, at some time in their lives, develop breast lumps or thickenings large enough to require an operation

WHAT CAUSES BREAST DISEASES?

No one knows exactly what causes breast problems but research is adding to our knowledge every year. In countries where people live differently than Canadians, breast disease is not so common This indicates that one or more factors of our lifestyle may be important in the development of breast problems As it is still not possible to identify these factors, more information is needed That is why we are asking women like yourself for assistance You can help to get a better understanding of the causes of breast diseases by participating in this study of breast problems

IF YOU DECIDE TO JOIN THE STUDY...

You would be asked to provide information about your health, smoking habits, diet and childbearing history This would be done in an interview which takes about one hour and which would be arranged at a time and place convenient to you We will be contacting a selection of Toronto women over 50 years of age who have had breast surgery at one of the participating hospitals, and we may be calling you soon to see if you wish to consider joining the study Meanwhile, if you would like further information, please contact our research coordinator Mrs Iris Rogers at 923-1505

Information from this study should help to identify factors which may be relevant in the occurrence of breast problems.

WE HOPE YOU WILL JOIN US.

PLEASE NOTE All information you give during the study will be strictly confidential Your data will be combined for analysis with information from many other women so that no one person can be identified

Appendix F: Mailed letter of invitation

This letter was mailed to all patients invited to participate in the study (with the exception of those subjects from Hospital A who were not visited in hospital - see letter (2) in Annex III). The letter was on University of Toronto letterhead, for the reason given in the title page of Appendix D. Each letter was addressed to the recipient, the surgeon's name was in the first paragraph, and the letter was signed personally by Dr. Eyssen.

Department of Preventive Medicine and Biostatistics
Faculty of Medicine, University of Toronto
Toronto, Ontario, M5S 1A8

Dear :

The University of Toronto, in conjunction with several Toronto hospitals and the Ludwig Institute for Cancer Research, Toronto Branch, is conducting a study of breast diseases in women. In order to do this we would like to collect information on a variety of factors from a large number of women throughout the Toronto area, and Dr. has agreed that we could ask you for your help.

Enclosed you will find a pamphlet which describes the study. Within the next week a member of the research team will be telephoning you to see if you wish to consider participating in the study. If you are willing, an interview will be arranged at your convenience. This interview will include questions about your health, smoking habits, diet and child-bearing history. All information which you give will be kept strictly confidential. Your data will be combined with that from many other women and the total results studied so that no single woman can be identified.

The success of the research depends upon the participation of women such as yourself. We will be very grateful if you are able to help.

Yours sincerely,

Gail Eyssen, Ph.D.
Associate Professor.

GE/md

Appendix G: Consent form

This form was offered to the patient for her signature when she was interviewed in her home. The original contained the names of the participating hospitals and surgeons, which have been omitted here to preserve anonymity.

All subjects agreed willingly, although a few delayed the signing until after the interview was completed.

STUDY OF BREAST DISEASES

CONSENT FORM

I _____ agree to participate in a study of breast diseases of women. I understand that my participation includes providing information on my general health, smoking habits, diet and childbearing and menstrual history in an interview lasting about two hours. In addition, I permit the principle investigators of this study and their representatives to contact my physician(s) and to review my medical records for details of my medical history.

All information I give will be STRICTLY CONFIDENTIAL and will be combined with that of other participants so that no one person can be identified. I understand that I may withdraw from the study at any time.

SIGNATURE _____

DATE _____

WITNESSED BY _____

Appendix H: Letter of thanks for participation

This letter was mailed to patients after they were interviewed, thanking them again for their cooperation. Each patient's name and address were entered in typescript, and the letters were signed personally by Dr. Eyssen.

Department of Preventive Medicine and Biostatistics
Faculty of Medicine, University of Toronto
Toronto, Ontario, M5S 1A8

Dear :

Thank you very much for participating in the study of breast diseases of women. The information you provided will be most useful in helping to identify factors which may be related to the development of these problems.

Thank you again for your help.

Yours sincerely,

Gail Eyssen, Ph.D.,
Associate Professor

GE/mjs

Annex III

ADDITIONAL LETTERS

This Annex contains three letters which were found necessary after the research had started.

At Hospital A, each patient had to be approached in writing by her physician: Letter (1) was handed to the subject if she was visited in hospital (this replaced the letter in Annex II Appendix D); Letter (2) was mailed to the patient if she was not contacted in hospital (instead of letter in Annex II Appendix F). Both these letters, (1) and (2), were prepared on the surgeon's letterhead; Letter (2) was signed personally by the surgeon.

Letter (3) was mailed to all subjects who had been visited in hospital but who could not be included in the investigation because their receptor levels were "intermediate". This letter was on University of Toronto letterhead, and was signed by the author.

Letter (1)

I am taking part in a study of breast diseases in women in collaboration with the University of Toronto, several Toronto hospitals and the Ludwig Institute for Cancer Research. We would like to collect information on a variety of factors from a large number of women throughout the Toronto area and we would be very grateful for your help.

Attached you will find a pamphlet which describes the study. Within the next two weeks an interviewer may be telephoning you to see if you wish to consider participating in the study. If you are willing, an interview will be arranged at your convenience. This interview will include questions about your health, smoking habits, diet and child-bearing history. The information which you give will be kept strictly confidential and combined with that from many other women so that no single person can be identified.

If you have any questions regarding the nature of the study or the interview, please feel free to call the study co-ordinator, Ms. Iris Rogers, at Tel: 923-1505.

Your help is vital to identifying the causes of this important issue of women's health, and your participation would be greatly appreciated.

Letter (2)

I am taking part in a study of breast diseases in women in collaboration with the University of Toronto, several Toronto hospitals and the Ludwig Institute for Cancer Research. We would like to collect information on a variety of factors from a large number of women throughout the Toronto area and we would be very grateful for your help.

Enclosed you will find a pamphlet which describes the study. Within the next week a member of the research team, Mrs. Virginia Hunter, will be telephoning you to see if you wish to consider participating in the study. If you are willing, an interview will be arranged at your convenience. This interview will include questions about your health, smoking habits, diet and child-bearing history. The information which you give will be kept strictly confidential and combined with that from many other women so that no single person can be identified.

If you have any questions regarding the nature of the study or the interview, please feel free to call the study co-ordinator Mrs. Iris Rogers, at 923-1505.

Your help is vital to identifying the causes of this important issue of women's health, and your participation would be greatly appreciated.

Yours sincerely,

Letter (3)

Department of Preventive Medicine and Biostatistics
Faculty of Medicine, University of Toronto
Toronto, Ontario, M5S 1A8

Dear :

As you may remember, following your recent breast surgery you were introduced to a study of breast diseases in women being conducted by the University of Toronto, several Toronto hospitals, and the Ludwig Institute for Cancer Research.

We have been pleased by the overwhelming response of many women to help us in this research. Unfortunately however, it is not possible to arrange interviews with all the women who have expressed an interest in participating. We are very grateful to you for your offer of assistance, and we are sorry that we will not be able to include you in this study.

Thank you again for your kind offer of help.

Yours sincerely,

Iris Rogers
Research Co-ordinator

IR/mjs

Letter (3)

Department of Preventive Medicine and Biostatistics
Faculty of Medicine, University of Toronto
Toronto Ontario M5S 1A8

Dear :

As you may remember, following your recent breast surgery you were introduced to a study of breast diseases in women being conducted by the University of Toronto, several Toronto hospitals, and the Ludwig Institute for Cancer Research.

We have been pleased by the overwhelming response of many women to help us in this research. Unfortunately however, it is not possible to arrange interviews with all the women who have expressed an interest in participating. We are very grateful to you for your offer of assistance, and we are sorry that we will not be able to include you in this study.

Thank you again for your kind offer of help.

Yours sincerely,

Iris Rogers
Research Co-ordinator

IR/mjs

Annex IV

EXAMPLE OF THE CANDAT PROCESS

This Annex illustrates the workings of the CANDAT system by means of a highly simplified fictitious example. The Annex is in four parts, each with a correspondingly numbered table.

Part IV.1 Details of the reported dietary consumption of the example

Part IV.2 The Questionnaire Table

Part IV.3 The Model Table

Part IV.4 Details of the calculation of certain nutrient intakes

Part IV.1

In the example, a woman is assumed to have reported the consumption of:

- 1 small carton of 2% milk each day
- 1 litre of 2% chocolate milk twice a week
- homemade hamburger, made with lean beef, 1/4 lb. each, twice a week, one time with a slice of cheese, always with a bun
- whole wheat bread (don't know what kind), 2 slices per day, 5 days a week
- chili con carne with beans, twice as much as the "standard" bowl, once a month
- macaroni and cheese, (half a box of Kraft Dinner), once a week

The entries on the food frequency questionnaire as would be recorded by the interviewer, for this example, are shown in the five parts, (a) to (e), of Table IV.1.

TABLE IV.1: Illustrative example of entries on the food frequency questionnaire

(a) Page 1 (Dairy products)

QUESTION	CODE	ITEM	✓	MODEL	NO OF SERVINGS DWM				SERVING SIZE			COMMENTS
*SHOWCARD 1		DAIRY PRODUCTS										
Please look at the list of types of milk on the first CARD. During the last 3 months what type, of MILK did you drink, not including milk used in coffee, tea or on cereal?	01.01	White milk - Whole		11								
	01 02	- 1%	✓	11	D	0	1	0	1	0	0	1 small carton/day
	01 03	- Skim		11								
	01 04	- Buttermilk		11								
A Did you drink milk daily, weekly or monthly? (D/W/M)	01 02	- Unspecified		11								
B How many glasses of did you drink per ?		Chocolate Milk										
		(commercial or home made with mixes and milk)										
C Compared to this model how large was your usual serving?	01.11	- Whole		11								
	01 12	- 2%	✓	11	W	0	2	0	4	0	0	1 litre 2 x/week
	01 13	- Skim		11								
	01 12	- Unspecified		11								
Hot chocolate Was your hot chocolate mix sugar-free? (Y/N)	01 15	Hot chocolate		10								
		(made from mix and hot water)										
		Other?		11								

TABLE IV.1: Illustrative example of entries on the food frequency questionnaire

(b) Page 3 (Meat - beef)

QUESTION	CODE	ITEM	✓	MODEL	NO. OF SERVINGS D W M	SERVING SIZE	CHEESE Y N	COMMENTS
Now I would like to ask you about MEAT At this point, I only want to discuss individual portion of meat. We will talk about mixed dishes such as casseroles later		MEAT						
		BEEF						
		Ground Beef						
		Hamburgers						
*SHOWCARD 1 Here is a list of types of BEEF. During the last four month which did you eat?	03 01	- Commercial - Regular (McDonald)		Unit				
	03 02	- (Big Mac, Whopper) Large		Unit				
A (D/W/M) B Hamburgers C Did you usually have these with CHEESE? (Y/N) Did you usually add MAYONNAISE? (Y/N)								
Were the HOMEMADE HAMBURGERS usually prepared with Regular or Lean Beef? A (D/W/M) B Homemade Hamburgers C Did you usually have these with CHEESE? (Y/N) Did you usually add MAYONNAISE? (Y/N)	03 33	- Homemade - Regular Beef		15				
	03 34	- Lean Beef	✓	15	W 0 2 0	1 3 3 5		½ lb meat 1 slice cheese 1 week
	03 33	- Undecided		15				

TABLE IV.1: Illustrative example of entries on the food frequency questionnaire

(c) Page 9 (Bread, buns, rolls)

QUESTION	CODE	ITEM	✓	MODEL	NO OF SERVINGS DWM	SERVING SIZE	FAT	SWEET SPREAD	COMMENTS
SHOWCARD 12		BREADS, BUNS, ROLLS							
From this list of BREADS, BUNS and ROLLS which did you eat over the last 4 month?	09 01	White		Unit					
	09 02	Whole Wheat (10-60%)		Unit					
A (D/W/M)	09 03	Whole Wheat, (100%)		Unit					
B Slice - Bread Rolls Buns Bagels Croissants	09 02	Whole Wheat, unspecified	✓	Unit	W 1 0 0	1 0 0	1.00	N	2 slices/day, 5 days a week = 10 slices/week
	09 04	Rye - light		21					
	09 05	Rye - dark, Pumpernickel		21					
Did you usually add BUTTER or MARGARINE? (Y/N)		Other?		21					
Did you usually add JAM, JELLY, HONEY or other SWEET SPREAD? (Y/N)									
	09.33	Dinner Rolls, Buns - White		22					
	09 34	- Whole Wheat		22					
	09.35	Hamburger Buns	✓	Unit	W 0 2 0	1 0 0	N	1/	with 3.34
	09 36	Hot Dog Buns		Unit					
	09.37	Bagels		Unit					
	09.38	Croissants (Butter Rolls)		Unit					
		Other?							

TABLE IV.1: Illustrative example of entries on the food frequency questionnaire

(d) Page 15 (Mixed dishes)

QUESTION	CODE	ITEM	✓	MODEL	NO OF SERVINGS DWM	SERVING SIZE	COMMENTS
* SHOWCARD 19		MIXED DISHES					
Here is a list of some STEWS and CASSEROLES Could you please tell me which you have eaten?		STEWS					
	15 01	Beef and Vegetable Stew		Bowl			
A (D/W/M)	15 02	Chicken Stew		Bowl			
B Serving/Bowls	15 03	Chili Con Carne with Bean	✓	Bowl	M 0 1 0	2 0 0	
C (length x diameter of Cabbage Roll)	15 04	Chili Con Carne without Beans		Bowl			
Are there any other types of STEWS/CASSEROLES you have eaten that we have not discussed?	15 05	Fish Stew		Bowl			
	15 06	Irish Stew		Bowl			
If "yes" record name of dish		Other?		Bowl			
A (D/W/M)							
B Servings		CASSEROLES					
C	15 33	Beef & Kidney Pie		32			
May we please discuss details of the recipe at the end of the questionnaire?	15 34	Cabbage Roll		Roll			
	15 35	Chicken Pie		32			
	15 36	Shepherd's Pie		32			
	15 37	Tourtiere (Pork Pie)		32			
		Other?					

TABLE IV.1: Illustrative example of entries on the food frequency questionnarie

(e) Page 16 (Pasta)

QUESTION	CODE	ITEM	✓	MODEL	NO OF SERVINGS DWM	SERVING SIZE	COMMENTS
• HOWARD - 0		PASTA					
From this list of PASTA and PIZZA which did you eat during the past 4 months?	16 01	Spaghetti - Tomato Sauce		1C			
	16 02	- Tomato Sauce and Cheese		1C			
	16 03	- Italian Meat Sauce		1C			
A (D/W/M)	16 04	- Meatballs and Sauce		1C			
B Servings	16 05	Macaroni and Cheese	✓	1C	W 0 1 0	1 5 Q	½ box Kraft Dinner = 1½C
	16 06	Macaroni Salad		1C			
	16 07	Egg Noodles - Fat added		1C			
	16 08	- Meat added		1C			
	16 09	Lasagna		23			
		Other Pasta?		1C			
							L M S
	16 33	PIZZA - without meat		Unit			
A (D/W/M)	16 34	- with meat		Unit			
B Fraction of a whole Pizza (¼, ½, etc) Was the PIZZA you usually had large, medium or small							

Part IV.2

For each item code, the CANDAT program selects, from the Questionnaire Table, the relevant food code and model code not only for the food item but also for any "add-ons" included on the questionnaire. Table IV.2 gives the small selection of entries needed for the illustrative example.

TABLE IV.2: Selected entries in the Questionnaire Table

Item code	Food code	Model code	Add-ons* Fat code	Fat model	Sugar code	Sugar model
01.02	100790	1100	-	-	-	-
01.12	101030	1101	-	-	-	-
03.34	3680	1500	120420	21	-	-
09.02	4705	25	431320	7	[11480	10]
09.35	19020	40	[431320	7]	[11480	10]
15.03	7560	269	-	-	-	-
16.05	13040	211	-	-	-	-

* In these entries, only at most two "add-ons" were appropriate, but there are many food items where three "add-ons" are allowed. Further, there are many classes of "add-ons" in addition to fat and sugar.

The "codes" for "add-ons" are exactly equivalent to food codes; in these instances the following translations apply:-

120420	cheese
431320	margarine
11480	jam

[ ] indicates an entry in the Questionnaires Table not made use of in the example of this Annex.

Part IV.3

The program next seeks in the Model Table the weight (in grams) of a standard portion of every food included in Table IV.2. The small selection of lines from the Model Table, necessary for the example, is shown in Table IV.3.

TABLE IV.3: Selected entries in the Model Table

model code	mls.	gms.	For item code *
1100	250	258	01.02
1101	250	264	01.12
1500	-	85	03.34
21	-	21	03.34
25	-	25	.02
7	7.5	7	09.02
[10	7.5	10	09.02]
40	-	40	09.35
269	250	269	15.03
211	250	211	16.05

* This column has been added in this Annex solely to ease cross-reference.

The entry in square brackets is included, because the model code 10 appears for item codes 09.02 and 09.35, although this "add-on" was not reported as consumed.

Part IV.4

Table IV.4 shows, for the example, how the intakes are calculated.

The suffices i (representing here the example) and f (the food) are omitted in this section.

The derivations of columns (4), (6), (10), (11), (12) and (13) are as follows:

If column (2) = D, column (4) = column (3)

If column (2) = W, column (4) = [column (3)]/7

If column (2) = M, column (4) = [column (3)]/30

Column (6) = column (4) x column (5)

Column (10) = column (8) x [column (7)]/100

Column (11) = column (9) x [column (7)]/100

Column (12) = column (6) x column (10)

Column (14) = column (6) x column (11)

All calculations have been rounded to the number of decimal places quoted in Table IV.4.

TABLE IV.1: Calculation of daily intakes of fat and fibre

Food code	D/W/M	No. of times				g/standard portion	nutrient /kg		nutrient per standard portion		Intakes (g) daily	
							fat	fibre	fat	fibre	fat	fibre
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)
100790	D	1	1.00	1	1.00	258	1.92	0	4.95	0	4.95	0
101030	W	2	.29	4	1.16	264	2.00	0.06	5.28	0.16	6.12	0.19
* 3660	W	2	.29	1.33	.39	85	11.30	0.30	9.61	0.26	3.75	0.10
* 120420	W	1	.14	1	.14	21	31.25	0	6.56	0	0.92	0
4705	W	10	1.43	1	1.43	25	3.33	5.00	0.83	1.25	1.19	1.79
451320	W	10	1.43	1	1.43	7	80.5	0	5.64	0	8.07	0
* 19020	W	2	.29	1	.29	40	5.60	2.90	2.24	1.16	0.65	0.34
7560	M	1	.03	2	.06	269	6.10	2.87	16.41	7.72	0.98	0.46
16040	W	1	.14	1.5	.21	411	11.10	0.69	25.42	1.46	4.92	0.31
											31.59	3.14

* Food in hamburger

Details are followed through for hamburger: each recorded component has to be considered separately, i.e. the beef (item code 3.34, food code 3680), cheese (add-on, food code 120420), and bun (item code 9.35, food code 19020). Each of the relevant lines of Table 3.4 is marked with an asterix.

To obtain the daily value of t, the number of servings (per day, week or month), as it appears on the questionnaire, is divided by 1, 7, or 30 (by means of an adjustment incorporated into CANDAT). [For the beef t = 2/7; for cheese t = 1/7; and for bun t = 2/7.] The serving size, s, requires no adjustment [1.33 (beef), 1.00 (cheese), 1.00 (bun)], and the products t x s are in column (6) [0.39 (beef), 0.14 (cheese) and 0.29 (bun)].

The weights of standard portions [85 g (beef), 21 g (cheese), and 40 g (bun)], derived from the Questionnaire and Model Tables, are in column (7), and the values q (nutrient per hg) are obtained from the food component files [11.30 g of fat and 0.30 g of fibre (beef); 31.25 g of fat and 0 g of fibre (cheese); 5.60 g of fat and 2.90 g of fibre (bun)]. The nutrients per standard portion are found by multiplication [i.e. (11.30 x 85)/100 = 9.61 and (0.30 x 85)/100 = 0.25 (beef); (31.25 x 21)/100 = 6.56 and (0 x 21)/100 = 0 (cheese); and (5.60 x 40)/100 = 2.24 and (2.90 x 40)/100 = 1.16 (bun)] - see columns (10) and (11).

Thus, the intakes of fat and fibre from hamburger are obtainable:-

	fat (g/day) [col (12)] = [col (6) x col (10)]	fibre (g/day) [col (13)] = [col (6) x col (11)]
beef:	0.39 x 9.61 = 3.75	0.39 x 0.25 = 0.10
cheese:	0.14 x 6.56 = 0.92	0.14 x 0.00 = 0.00
bun:	0.29 x 2.24 = 0.65	0.29 x 1.16 = 0.34
Total for hamburger:	5.32	0.44

Annex V

INTERVIEWER MANUAL

This manual was prepared by the author for use with the questionnaire in Annex II, Appendix B.

INTERVIEWER MANUAL

Background information:

5. If the woman cannot remember her weight at age 20, probe with a question of whether she was lighter, heavier or the same weight at age 20 compared to six months ago

6-7. "Ethnic or cultural group" refers to the country and/or ethnic background to which the woman and her family belonged (i.e. Russian Jews)

8. Try to determine the woman's family's religion at the time of her birth. Record answers of "atheist" or "agnostic" and they will be coded accordingly (not as 99=no answer or 88=don't know).

Menstrual history:

9. Code 88 88=don't know/remember, 99 99=never had a menstrual period. If a woman never menstruated, omit all questions about menstruation. When probing for the age and month when periods started remember to record and then convert the age to year.

10. If a range of days is given (i.e. 26-28) record as such. By "irregular" is meant that the number of days between periods was not constant (i.e. not always the same). Record in the space for comments any information on the present state of menstruation (e.g. not really regular, only every three months).

11. If a woman cannot remember at least the year of her last period, probe with the question of whether the last period was more than one year ago or not. If the last period was more than one year ago, try to determine how many years ago it was (if necessary probe: <2 years, 2-5 years, 6-10 years, 11-20 years, >20 years). If the year only is given and/or the last period is within the preceeding twelve months, probe to ascertain the number of months since the last period. Once a month and year have been determined (for periods within the last year) or a year (periods more than one year ago) record in the date space.

12. Record in the space for comments any information about the cessation of periods (e.g. regular periods until age 45, period every four months for 3 years). Remember that a woman who has had a hysterectomy may have had a natural menopause before the surgery.

Pregnancy and birth history:

13. If the answer isn't a definite "no", then treat as "yes" and continue with questions until a negative answer is received.

14. If necessary, say "Again, this would include the number of babies you have had, as well as any other pregnancies such as miscarriages, stillbirths or terminated pregnancies".

15-16. When there are too many pregnancies to record in the space allotted, always record at least the first and last occurrence of each outcome, particularly first full term pregnancy, first birth and any events occurring before the first birth.

15(d). No breast-feeding is recorded as duration=0.

16. A miscarriage will be defined as a pregnancy of less than or equal to 20 weeks duration. A stillbirth will be defined as a pregnancy of more than 20 weeks duration. A terminated pregnancy (abortion) will be defined as surgical or other intervention resulting in the termination of the pregnancy.

Family history:

21-22. Sisters will only be considered related if they have the same two biological parents.

23-26. We are only interested in relatives related by birth, not adoption or marriage. If age or age at death is not known, probe in decades (40's, 50's,...) or even larger age groupings if necessary (<40,..).

27-28. When recording which relative had breast cancer make sure the woman understands that you are only interested in the female relatives you have been discussing with her. Probe for the specific relative, e.g. "maternal aunt".

Medical history:

29-32. If the woman cannot remember the date of the operation or illness (at least the year and season), probe for her age at that time; record and calculate the year.

29. A hysterectomy is defined as the removal of the uterus.

30-31. Removal of both ovaries but not at the same time: record only one removed at the date when the first was removed, and both removed with the date of the last removed.

32. Obtain information on all breast biopsies, and if the woman offers the outcome of the biopsy (i.e. benign, malignant) record this information under comments with the date. If a woman has had more than four biopsies, record the number she has had, and information on the first two nad the two most recent.

34-36. Multiple occurrence of a procedure: always record at least the first occurrence and the most recent.

Other major surgery and illness: Do not ask for any information, but if the woman wishes to describe any other conditions, record in this space in order to appear interested (this will not be coded).

Medication use:

37-38. If the woman cannot remember the time or duration of use, probe with her age at the time of starting and stopping, record and calculate the year. For more than one use of a

drug, always record at least the first use and the most recent uses.

37. For oral contraceptives: It is most important to probe for and record uses before the first full term pregnancy and the first birth, as well as the first and most recent use.

38. For estrogens/hormones: It is most important to record use at the time of menopause and recent use.

Record information on use of other medications only to appear interested (as per other surgeries/illnesses).

Socio-economic factors:

47. (Page 22B FFQ) If the woman gives her occupation as "homemaker", probe to determine whether this has been so for the majority of her life. If so, record under "other", and specify. If the woman says she is retired, try to determine what her usual occupation was.

Annex VI

MEDICAL CHART INFORMATION FORM

The following two page form was used for collecting information from the patients' hospital charts. It is a revised version of Annex II, Appendix C.

CHART INFORMATION

Date: _____ Study ID number: ☐☐☐

Study representative: _____

Name of patient: _____
(last name/first name)

Hospital number: _____ Hospital: _____

Surgeon: _____ Date of surgery: _____

1. Bilateral tumours: [1] Yes [2] No ☐

   Laterality of tumour: [1] left [2] right ☐
   [8] DK

2. Type of surgery:

   [1] modified radical mastectomy
   [2] simple (total) mastectomy
   [3] subcutaneous mastectomy
   [4] partial mast. (segmental/wedge resection, lumpectomy)
   [5] partial mastectomy plus axillary dissection
   [6] biopsy
   [7] other: _____
   [8] no info. (DK)
   [9] NA

   ☐

3. Location of tumour:

   [1] upper-inner quadrant
   [2] upper-outer quadrant
   [3] lower-outer quadrant
   [4] lower-inner quadrant
   [5] nipple
   [6] central portion
   [7] axillary tail
   [10] other: _____
   [88] no information (DK)
   [99] NA

   ☐☐

4. Tumour size:

   _____length (longest) X _____ width (cm)

   [1] one size recorded
   [2] > one size recorded
   [8] no information (DK)
   [9] NA

   ☐

5. Pathological diagnosis:

   Invasive:
   [1] ductal ca
   [2] adenocarcinoma
   [3] lobular ca
   [4] medullary ca
   [5] tubular ca
   [6] inflammatory ca
   [7] papillary ca
   [10] mucinous ca
   [11] multi-focal ca

   Non-invasive:
   [12] ductal ca in situ
   [13] lobular ca in situ

   [14] other: _____
   [88] no info. (DK)
   [99] NA

   ☐☐

study ID number: ☐☐☐☐

6. Were the nodes (axillary) dissected?

[1] Yes
[2] No
[8] no information (DK)
[9] NA

☐

If "Yes": Total number of nodes examined: _____ ☐☐

No. of nodes positive for malignancy: _____ ☐☐

7. Grade of tumour: duct differentiation

[1] well differentiated
[2] moderately diff.
[3] poorly diff.
[4] other: _____
[8] no information (DK)
[9] NA

☐

8. Stage:

[1] I
[2] II
[3] III
[4] IV
[5] other: _____
[8] no information (DK)
[9] NA

☐

9. Are distant metastases present?

[1] Yes
[2] No
[8] no information (DK)
[9] NA

☐

Site of metastases:

[1] chest wall
[2] bone
[3] brain
[4] liver
[5] lungs
[6] other: _____
[8] DK
[9] NA

☐☐☐☐☐☐