

THE EPIDEMIOLOGY OF GALLSTONES  
IN WOMEN



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## Introduction and Acknowledgements

There have been many studies that have investigated the epidemiology of gallbladder disease yet the results of these studies have been inconclusive and myth still pervades the understanding and teaching of the pathogenesis of gallstones. Accurate data about even the most basic of epidemiological aspects of gallbladder disease - its prevalence - is not available. However, evidence suggests that well over a tenth of women living in western communities have gallstones and about half that rate in men and the rate appears to be increasing. The aetiological factors that may influence the development of gallstones need also to be carefully investigated as there is still great uncertainty and controversy surrounding the importance of a number of possible factors such as pregnancy and diet.

This thesis describes the two studies which were carried out in an attempt to obtain some accurate data on the epidemiology of gallstones. Preceding the study descriptions is a literature review of the relevant literature and I hope that this review conveys to the reader the large gaps that exist in our knowledge of the epidemiology of gallstones.

There are many people to thank for their unstinting help throughout the course of my thesis but I cannot mention anyone without first thanking Dr Jim Mann whose initial ideas, subsequent advice and constant help and encouragement contributed a great deal to the enjoyment with which I carried out this work. His wide-ranging experience was invaluable and he

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The vegetarian participants were recruited from all over the country and this was only made possible by the support of the Vegetarian Society (United Kingdom) and specifically the help of Dr Conrad Latto, Dr Gordon Latto, Dr Alan Long and the Bradford, Gloucester, Guildford, Nottingham, Oxford and Reading vegetarian societies.

The nonvegetarian participants were recruited from two Oxford general practices and I would like to thank the general practitioners and staff at 27 Beaumont St and the Botley Health Centre for their willingness to help with my every request.

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### Abstract

A survey of 652 nonvegetarian and 130 vegetarian women aged 40-70 years using real-time ultrasonography was carried out to determine the prevalence of gallstones. 24.6% of nonvegetarian women were found to have gallstones compared with 11.5% of vegetarian women ( $p < 0.05$ ). Each participant completed a postal questionnaire on general health aspects and dietary habits. Aetiological factors shown to have a strong influence on the development of gallstones included increasing age, obesity and positive family history. The prevalence of gallstones increased steadily with age ( $p < 0.01$ ) and obesity ( $p < 0.001$ ). Women with gallstones were much more likely to have a first degree relative with a history of gallstones ( $p < 0.01$ ). The vegetarian participants were younger and less likely to be obese than the nonvegetarian participants but, even after correction for these confounding influences, gallstones were significantly less prevalent in vegetarian women ( $p < 0.05$ ). Other aetiological factors that have been purported to be risk factors in the pathogenesis of gallstones but were not shown to be so in this study were parity, exogenous oestrogen intake and a history of smoking.

In order to further study the effect of diet in the pathogenesis of gallstones, an age-matched case-control study was carried out using the women with gallstones identified in the prevalence survey as cases and women proven to be free of gallstones as the controls. 107 nonvegetarian and 12 vegetarian pairs completed a four-day dietary diary and were subsequently interviewed about their dietary habits, exogenous oestrogen intake and upper gastrointestinal symptomatology. There were no

significant differences in energy intake, total protein and animal and vegetable protein, fat and cholesterol, carbohydrate, simple sugars and fibre intake in the diet consumed by cases compared with controls. Vegetarian women, however, ate a very different diet to the nonvegetarian women suggesting that diet does play an important role in the pathogenesis of gallstones. Failure to demonstrate a dietary association in the case-control study may be due to the fact that the overwhelming majority of people in western communities consume a diet that is too high in fat, animal protein and simple sugar content and too low in unrefined starches to be able to detect any dietary differences between cases and controls - the threshold effect.

Another important aspect of gallstone disease revealed by the study was the proportion of cases shown to be asymptomatic. Over 70% of all cases had not been previously diagnosed. Moreover, the case-control study showed that cases were no more likely to suffer from "biliary" symptoms than controls except for those symptoms associated with acute cholecystitis or common bile duct obstruction. These data provide persuasive support for the conservative management of asymptomatic gallstones.

## Explanatory Notes

Several aspects of terminology require clarification. The term "gallstone disease" is used interchangeably in this thesis with the term "gallbladder disease". The latter is less specific as it covers disease entities such as gallbladder carcinoma and acalculous cholecystitis but the great majority of gallbladder disorders are associated with the presence of gallstones. Considering that the gallbladder's role is that of storing, concentrating and then expelling bile secreted by the liver, the importance of gallstones in its disease spectrum is not surprising.

There does not seem to be any consensus concerning the grammatical structure of the words gallbladder and gallstone. Perhaps it reflects the general confusion and controversy that still surrounds gallstones as a disease entity. Stedman's Medical Dictionary gives both as single words. The British Medical Journal now appears to favour a two word format for both (viz gall stone and gall bladder) but has used the hyphenated form and the single word form in the past. The Lancet used the hyphenated form until the 1970's but converted to the single word thereafter. A similar trend is apparent in most other journals and since the single word is now the most common form it will be used in this thesis.

Epidemiology may be defined as the study of the determinants of the incidence and prevalence of disease. The methods of studying these determinants can be classified according to the basic unit of population studied. Descriptive

epidemiology is the description of disease frequency according to the characteristics of populations, particularly geographic location, era of observation and the personal characteristics of the population sub-groups. The term "ecological studies" is sometimes used as a substitute for descriptive epidemiology when disease frequency is correlated with some measure of aggregate exposure to a supposed agent of disease in a population. Analytical epidemiological studies are usually set up to examine hypotheses suggested by descriptive studies. They differ from descriptive studies primarily in that data are collected for individuals rather than groups or populations. Analytic approaches include case-control studies, cohort studies and intervention studies. Metabolic studies may belong to either descriptive surveys or analytic studies according to the size of the population being examined.

## Section I. A Review of the Literature

### Chapter 1. Gallstones up to the Twentieth Century

Much of the recent interest in gallstones stems from the inclusion of this condition in the list of "Western Diseases" (Burkitt & Trowell, 1975). This label implies that gallstones are a consequence of industrialization and therefore a modern phenomenon. In fact, gallstones have been written about in medical manuscripts for many centuries and paleopathological studies of mummies have revealed gallstones in at least one of the specimens (A&E Cockburn, 1983).

According to Siegel's translation of Galen's work, "A System of Physiology and Medicine", neither Galen nor any other physician of this period, (130-200 AD), ever mentioned the occurrence of gallstones although they were quite familiar with stones of the urinary tract (Siegel, 1968). Galen did write about common bile duct obstruction and consequent jaundice as had Erasistratus some centuries earlier but the diagnosis of gallstones did not become established until the time of the Renaissance. Siegel suggests that physicians of Galen's time would not have overlooked the disorder if it was as frequent then as it is now. He argues plausibly that the Greeks and Romans of antiquity ate a diet similar to underdeveloped nations now with more cereals and vegetables and very few animal products and that they rarely survived even to middle age so gallstones may well have been most uncommon.

Human gallstones began to appear in mediaeval literature.

Foligno carried out an autopsy of a woman in Padua in 1341 and discovered a greenish stone in her gallbladder (Mani, 1959). Later, according to Muleur (1884), Benevenius made the first connection between gallstones and clinical symptoms when he carried out an autopsy on a woman in 1506 and retrieved a "chestnut-sized, black stone". Benevenius wrote, "In these last days there died a noble woman by the name of Diamantes who was struck down by the pain of a calculus. But as never previously had she experienced the slightest illness, it appeared well to the physicians to open the body after death". It is interesting to note that Benevenius wrote of this case in his book "On Hidden Causes of Diseases". Many other notable authors (Vesalius, Fernel, Estienne, Fallopius) began to write of stones in the gallbladder around this time. Coiter in 1573 was the first to connect jaundice with the presence of gallstones, however in 1658 Wepfer established that stones in the gallbladder or cystic duct only produce jaundice with obstruction of the common bile duct (Muleur, 1884).

Epidemiological associations first began to be noted at this time. Fabricius reported that gallstones were more frequently observed than stones of the urinary bladder. As early as 1755 Haller observed in his "Opuscula Pathologica" that gallstones occurred more frequently in some countries (Morgagni, 1761). Estienne had asserted by then that they were found chiefly in older women while Hoffman had said they were rare in young men, more frequent in old men and more frequent still in women (Morgagni, 1761). Morgagni had collected information on at least 200 cases of gallstones and had about equal observations in both

sexes but he did concur with the effect of increasing age. Haller found stones commonly in prisoners who had been imprisoned for long periods and ascribed this to physical inactivity (Morgagni, 1761). Obesity was mentioned by Morgagni as a possible cause but as his personal series contained some very thin cases as well as some enormously fat ones, he was not impressed by this association. By the time of Crisp's address to the Medical Society of London in 1841 the following associations were regarded as being important : sex (female to male ratio of 3:1), melancholic temperament and mental disquietude, sedentary habits and good living, especially eating (Crisp, 1841). On one major issue Crisp disagreed with other authors at that time, claiming that, "fat people are not more subject to the disease than those of spare habit". He thought that stones were most common between the ages of 30 and 60 and that alcohol was not a contributing factor.

Physicians in the 16th-18th centuries lacked the means of determining the constituents of gallstones and how they developed but observations on gallstone colour, consistency and inflammability were routine. Morgagni collected and analysed a number of the earlier reports and concluded that, "...these gall-stones are not found for the most part of a black or brown colour ... they most frequently incline to yellowness". From these observations, it can be assumed that gallstones were usually cholesterol in type. Haller had earlier divided gallstones into two classes, the first being large, egg-shaped yellowish stones that were often solitary with a radiate cut-surface appearance and combustible (Mani, 1959). The stones of the second class were small, black and very numerous, often found in the bile ducts and

also combustible. Speculation on stone pathogenesis then extended to the belief that bile became thicker with age and that, associated with a less brisk agitation of bile in older people, led to an increased likelihood of stones. Van Swieten had shown that bile that was left to stand in a glass vessel formed a "calculus coagula in the bottom of the vessel" (Morgagni, 1761). By Crisp's time, it was believed that the constituents of bile were altered in cases of gallstone formation and that "an abundance of fatty matter (cholesterine) in the bile is the chief cause of their formation". Some authors at this time attributed gallstone formation to infection in the form of a "putrescent condition". This theory gained support throughout the 19th century as will be seen in a later section.

The natural history of gallstones was not considered in great detail until surgery became an option in the treatment of gallstones. The first cholecystostomy was performed in 1867 by Stough Hobbs and the first cholecystectomy was carried out by Langenbuch in 1882 in Berlin. It was successful. Prior to then writers were initially concerned solely with their presence in the body and then later on in determining their more exact signs and symptoms. Morgagni wrote at length on possible signs that could be used to diagnose gallstones and eventually concluded that he agreed with Fernel who stated, "...no manifest marks by which the existence of these stones may certainly and easily be known". Symptomatology was considered similarly but Morgagni recognised that gallstones could lie latent within the gallbladder for many years. Not all authors believed this and Robertson (1945) cites Guidetti as having an opposite and more modern view in 1725 when

he stated that biliary calculi could not exist without giving rise to symptoms that were, more often than not, severe. Coe, in one of the earliest English monographs on gallstones in 1757, thought that gallstones may sometimes remain in the bladder without causing symptoms, but he felt that most stones did eventually cause severe symptoms (Robertson, 1945). Baillie felt that gallstones were quiescent unless they passed into the cystic duct (Robertson, 1945) while Crisp believed that calculi could exist without producing any inconvenience (1841).

In summary, towards the end of the nineteenth century, the epidemiological associations of gallstones were regarded as sex, an overindulgent lifestyle with inactivity and good eating, stress and depression and perhaps obesity. In contrast, knowledge of the underlying causes of gallstone formation was extremely limited. Subsequent research will be considered under the appropriate headings.

## **Chapter 2. The Prevalence of Gallstones**

### **2.1. Method of Detection**

Gallstones can be detected in a number of ways: at autopsy, by investigation of a group of symptomatic patients or by screening an asymptomatic population. All three methods may be expected to produce different prevalence rates resulting from the inherent strengths and weaknesses of each.

#### **2.1.1 Autopsy Studies**

The vast majority of studies, especially those of the first half of this century, attempting to determine the prevalence of gallstones have been based on data obtained post mortem. Any autopsy survey must present certain difficulties when used to estimate the frequency of the disease in the general population. Spurious results may arise as most autopsies are carried out on hospital patients who differ from the general population in several important respects. Age and sex are the most important factors known to affect prevalence. Hospital patients tend to be older and more men come to autopsy than women (Bateson & Bouchier 1975, Opit & Greenhill, 1974). The studies must contain large numbers of autopsies to be useful and are therefore carried out over a number of years so time trends may be obscured (Opit & Greenhill, 1974). Previous cholecystectomy and cholecystostomy patients are sometimes difficult to discern and many autopsy studies therefore ignored this pool of cases (Opit & Greenhill, 1974). Another problem which plays a more

important role in determining clinical associations with gallstones is that of the bias of associated disease as it has been shown that the occurrence of two disorders in the same person gives an increased possibility of hospital admissions (Mainland, 1953). The major advantage of the autopsy method is that it detects both symptomatic and asymptomatic stones. As a result prevalence figures in these studies appear greater than in other studies.

### **2.1.2 Clinical Studies**

Clinical studies are much fewer in number than autopsy studies and have been carried out relatively recently. Cases are either identified as symptomatic cases having been diagnosed by oral cholecystography or ultrasonography or as surgical patients undergoing cholecystectomy. The most obvious criticism that can be levelled at such studies is that prevalence rates are underestimated by the exclusion of asymptomatic stones. Evidence of this is provided by the comparison of the results of these studies with autopsy studies in similar countries. Friedman et al in Boston found a prevalence rate of 8.2% in 1966 compared with the rate of 24.3% quoted in an autopsy study of New York (Newman & Northup, 1959). Clinical rates tend to be about half those of autopsy rates. The individual techniques can be criticised further. For example, the Framingham study (Friedman et al, 1966) used anecdotal evidence of gallstone disease as well as accurate hospital records of cholecystectomy or positive oral cholecystogram to identify their cases. Diagnostic

Table I.1 - The Autopsy Prevalence of Gallstones in Western Communities

Place	Author	Year	Age	Prevalence		
				Male %	Female %	Total %
London	Bouchier	1969	> 20	9.5	19.2	15.5
London	Cooke	1941-50	> 25	6.2	12.1	8.6
Malmo	Lindstrom	1971	> 20	26.5	46.8	36.2
Oslo	Torvik	1952-57	> 20	13.5	28.6	20.1
New York	Newman	1959	> 20	16.0	32.5	24.3
Melbourne	Joske	1945-59	all	11.3	20.3	14.9
Am. Indian	Reichenbach	1962	> 15	16.6	40.0	29.9
Santiago	Marinovic	1960-71	> 20	20.5	50.0	35.2

criteria in clinical studies are liable to such difficulties.

Cholecystectomy rates are also used to determine the prevalence of gallstones as at least 98% of all cholecystectomies are carried out for gallstones (Anderson et al, 1971). Biases are an even greater problem in these studies though because many factors apart from the prevalence of a disorder influence surgery rates. Supply variables are crucial factors in the production of surgical rate variations (McPherson et al, 1981). A marked difference was found in cholecystectomy rates between North America and England & Wales which was not accountable for by different disease prevalence (McPherson et al, 1981). Such studies are therefore more useful in detecting changes in prevalence within an area as was done by Holland & Heaton in Bristol (1972) rather than differences between areas, although surgical supply variables can change within an area.

### **2.1.3 Prevalence Surveys**

Prevalence surveys are even fewer than clinical studies and most have studied selected populations known to be at high risk of developing gallstones such as American Indian tribes (Sampliner et al, 1970). The first study that screened an unselected population was carried out by Bainton et al (1976) in a South Wales industrial town using oral cholecystography. The response rate was low in this study (68.3% for men and 61.2% for women). A more recent study was carried out in Rome on female office workers and used, as the screening technique, real-time ultrasonography (GREPCO,

1984). This method was also used in a prevalence survey of a Tyrolean village (Rhomberg et al, 1984). These surveys are less likely to be biased as they study unselected populations and allow inclusion of both symptomatic and asymptomatic cases. A common problem is that of poor response rates.

Comparison of prevalence data from studies using different methodologies is not appropriate.

## **2.2 International Differences**

Important geographical and racial variations in the prevalence of gallstones have been observed by several workers. Brett & Barker in 1976 summarized all published autopsy data to determine the world distribution of gallstones. Their results should be interpreted with caution as the included studies varied in sex and age distributions which in some cases were not even known. Nevertheless they calculated the mean prevalence of gallstones in Europe after 1940 to be 18.5%. Rates varied within Europe from Ireland (the lowest at 5%) to Sweden (the highest at 38%). Other western nations such as the United States and Australia showed prevalence rates similar to those of Great Britain and several other European countries whose rates lay between the two extremes. See table I.1 for a summary of the international differences described both above and on following pages.

International variation becomes much more marked when African rates are compared to those in western nations. Gallstones are so uncommon in Africa that in some areas they are virtually never

Table I.2 - The Autopsy Prevalence of Gallstones in African and Asian Communities

Place	Author	Year	Age	Prevalence		Total %
				Male %	Female %	
Ibadan	Parnis	1964	all			0.8
Kampala	Owor	1950-62	all			0.9
Johannesbg	Becker	1936-50	all	w 10.0	19.5	14.7
				b 1.0	3.8	2.4
Japan	Maki	1955-61	all			4.4
Singapore	Hwang	1962-66	>20	6.4	7.9	6.6

(w = white population, b = black population)

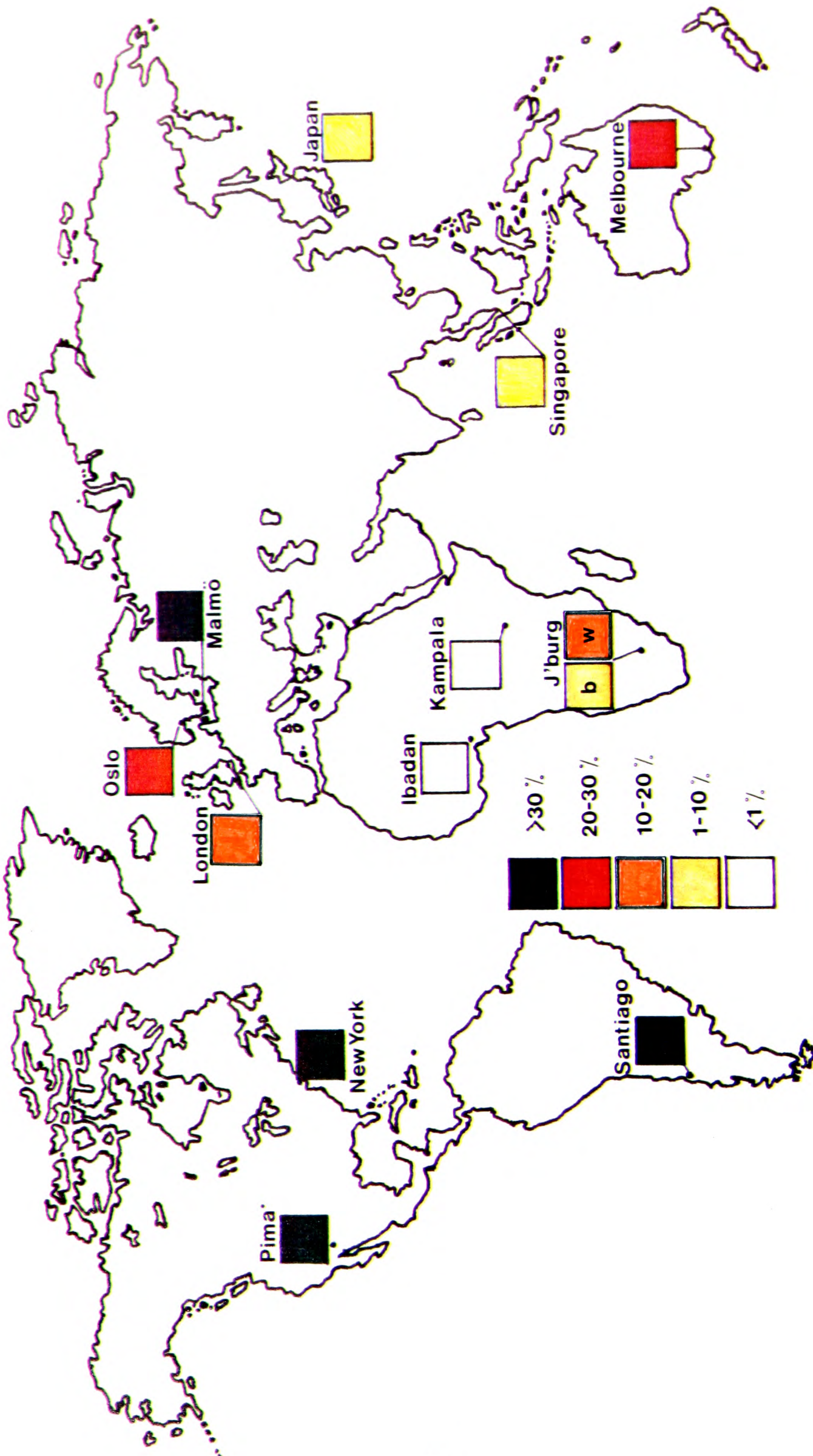
seen (Biss et al, 1971) and most recorded rates are less than 1% (Burkitt & Tunstall, 1975, Owor, 1964, Parnis, 1964, Trowell, 1960)(see Table I.2). The degree of urbanization appears to be very important in disease patterns in Africa. Studies of urbanized blacks in Johannesburg show that western influences result in an increased risk of gallstones but these rates are still well below those of the white population living in Johannesburg (Becker & Chatgidakis, 1952). Similar changes have been noted in Canadian eskimos. Influences apart from lifestyle factors are also important in determining racial variations as differences continue to exist when lifestyles converge. This was demonstrated by a study carried out in Alabama where gallstones were found to be four times as prevalent in whites than blacks (Cunningham & Hardenbergh, 1956). A study in Panama also showed that whites were at twice the risk of developing stones than the blacks (Hall, 1963). According to some researchers, this risk differential between blacks and whites in similar communities may not be persisting. Trotman & Soloway reported a comparable prevalence in black and white populations living in Philadelphia in 1973.

Not many studies have been carried out in Asia but in those that have, low prevalences are found except in Singapore (Hwang, 1970)(see Table I.2). Japanese studies present an interesting epidemiological feature. In western civilization most gallstones are composed mainly of crystalline cholesterol (Sutor & Wooley, 1971) while Asian stones are almost always pigment. Japan occupies an intermediate position in that both types of stones occur. Nakayama & Miyake confirmed that the composition of gallstones in Japan is gradually changing from the once predominant pigment

stone (55% in 1927) to cholesterol (85% in 1968). The prevalence of gallstone disease has increased over a similar period from 1.7% to 6.7%. The increasing urbanisation and changing food habits that had taken place in Japan since the second world war were blamed for the changes in character of gallstone disease (Nakayama & Miyake, 1970).

It has been convincingly demonstrated for the last 35 years that the American Indian is particularly prone to developing cholesterol stones. Clinical evidence suggested this in earlier studies (Lam, 1954, Sievers & Marquis, 1962) but a prevalence survey using oral cholecystography confirmed the relationship in 1971 (Sampliner et al, 1970). They found that the Pima Indian tribe of Arizona had an overall prevalence of gallbladder disease of 48.6% and, even more strikingly, in females the rate rose rapidly to 73% in the group aged 25-34 years and remained around this figure in the older age groups. As more epidemiological research is being carried out in South America, it is becoming evident that cholesterol gallstones are very common there also. A Chilean study in 1960-71 showed that 35.2% of people over 20 in Santiago had gallstones (Marinovic et al, 1972 ).

Immigration effects have rarely been reported except for those already mentioned in terms of black Americans. One other study warrants a mention despite being somewhat poorly designed. Women who had migrated to Melbourne, Australia from mediterranean countries - Italy, Greece, Malta and Cyprus - had an prevalence of gallbladder disease that was higher than both their mediterranean countrywomen and Australian women (Loftus Hills, 1971). The mediterranean figures were obtained from the Instituto



**Fig I.1 The Autopsy Prevalence of Gallstones in Women**

Centrale de Statistica, Rome, for patients both male and female discharged from Italian hospitals with the diagnosis of cholelithiasis. These figures should not have been extrapolated to a different study design and for a multi-racial population. The findings confirmed an earlier study from the same group (Wheeler et al, 1970) and the increased prevalence was attributed to dietary changes on emigration.

Figure I.1 illustrates on a world map the international differences mentioned above.

As well as noting international variations in the prevalence of gallstones, within-nation variations have been shown to occur. Malhotra showed that railway workers from the north of India had a seven times greater risk of developing gallstones than did their counterparts in southern India. This risk was blamed on the diet of the northern population as they ate much more fat, much of it being animal in origin. Barker et al in their autopsy survey of nine towns in England and Wales showed wide regional variations in the prevalence of gallstones (1979). The towns were selected to cover three different socio-economic groups in three different geographic regions of England and Wales divided according to latitude i.e. York was the northern town with the better socio-economic conditions, Derby was the town from the middle region with intermediate conditions and Newport was the southern town with the worst conditions. The variations in gallstone prevalence did not follow those in all-cause mortality which shows a trend towards the highest mortalities occurring in the north and west of Great Britain. If anything, the prevalence of gallstones appeared to be negatively correlated with the prevalence of

Table I.3 - Western Trends in the Autopsy Prevalence of Gallstones

Place	Author	Year	Age	Prevalence		
				Male %	Female %	Total %
Copenhagen	Scheel	1911	>20	10.1	21.7	14.8
Oslo	Torvik	1952-57	>20	13.5	28.6	20.1
Stockholm	Martensson	1925-34	all	12.0	27.0	19.6
Malmo	Lindstrom	1971	>20	26.5	46.8	36.2
London	3 studies	1911-12	?			4.1
London	Bouchier	1969	>20	9.5	19.2	15.5
New York	2 studies	1903-12	?			7.4
New York	Newman	1959	>20	16.0	32.5	24.3
Adelaide	Cleland	1920-48	>20	10.1	19.4	13.3
Melbourne	Joske	1945-59	all	11.3	20.3	14.9

ischaemic heart disease despite the two disorders both being classified as western diseases. The significance of these variations have been disputed on the basis that the study looked at too few autopsies over a very short period of time. Regional variation has also been reported when different British studies have been compared. The autopsy prevalence of stones in women in Leeds before 1929 was about twice that in Birmingham despite the fact that the study in Birmingham was carried out some 25 years later (Gross, 1929, Horn, 1956). Moreover, a national autopsy survey showed that cholelithiasis was twice as common in elderly people in Glasgow compared with those in London (Watkinson, 1966). Thus, gallstones may be more prevalent in the northern regions of Great Britain but there is insufficient data to confirm this.

### **2.3 Western Trends**

The prevalence of gallstones appears to have risen quite markedly in the western world during the past century. Ehrstrom in 1942 reported a rise in autopsy findings of gallstones in Finland from 0.2% in 1879 to 4.0% in 1930-39 (cited by Burkitt & Tunstall, 1975), Dessau (1943) reported an increase in Boston from 18.9% in 1900-22 to 22.2% in 1923-42 and Cleland (1953) showed an increase of 10-15% in Adelaide, Australia between 1920 and 1948. The increase is even more clearly evident if early autopsy surveys are compared with recent ones as was done by Brett & Barker in 1976. Comparisons between studies that are not standardized for age, sex or race can lead to difficulties (Lieber, 1952, Zahor et al, 1974) but, looking at table I.3 which has adapted the two tables given by Brett & Barker (1976), an increase can be seen in the gallstone

prevalence of western nations. Holland & Heaton gave support to this impression when they reported that gallbladder operations had become significantly more common in Bristol. This increase was particularly large in the younger age groups and appeared to be essentially a post-war phenomenon (Holland & Heaton, 1972). Similar increases had been found by workers in Sweden (cited by Holland & Heaton) and by Plant et al (1973) who showed that cholecystectomy rates had doubled in Canada, France and England between 1961 and 1971. Such increases cannot be fully explained by better diagnostic or surgical techniques or by service availability (Holland & Heaton, 1972).

Unfortunately, studies based on autopsy figures and surgical statistics can be confounded by influences such as the age structure of the population being studied (Zahor et al, 1974). Bateson & Bouchier published a serial analysis of autopsies carried out in Dundee in 1975. By standardizing the populations according to age, they determined that there was no evidence of a rise in the true prevalence of gallstones in Dundee between 1902-9 and 1953-73. The crude increase in prevalence was attributed to the increasing age of the population coming to autopsy in Dundee but their figures were based on small numbers for the periods 1902-9 and 1936-8. Bateson, in an analysis of the 1974-83 autopsies in Dundee, detected an increase in the age standardized rates compared with both the 1953-73 figures and sequentially from 1974 to 1983. He also showed that cholecystectomy rates had trebled in Dundee between 1961 and 1981 but he remains uncertain as to whether this subsequent study reveals a true or a spurious increase in prevalence (Bateson, 1984a).

Less confusion surrounds the nature of increases reported in non-western areas such as Japan and Africa. The documented increase in gallstone prevalence in these areas is attributed to lifestyle changes, specifically those associated with western cultures (Nakayama & Miyake, 1970, Burkitt, 1973). It is not unreasonable to extrapolate these observations to our own communities which have also undergone enormous lifestyle and dietary changes, especially with industrialization (Trowell, 1977). The influence of lifestyle and diet on increasing the prevalence of gallstone disease in western nations may well have taken effect earlier than the more accurate but recent attempts to quantify these changes.

In summary, the commonest method of detection of gallstone prevalence has been the autopsy method but problems arise with the selected nature of the population samples studied. Prevalence surveys using screening procedures are based on representative population samples and the prevalence rates and epidemiological studies of aetiology based on them are more accurate. The prevalence of gallstones has been determined in many different areas in the world and striking international differences have been shown. Gallstones are far more common in the western countries and appear to have increased in prevalence during the course of this century. Most gallstones in industrialised nations are cholesterol in composition while those of Asia tend to be of pigment. Furthermore, countries undergoing the processes of industrialisation show a change in the prevalence and type of gallstone. Japanese studies report an increase in the prevalence of gallstones and the predominant type is now cholesterol.

## Chapter 3. The Aetiology of Cholesterol Gallstones

### 3.1 Physicochemical Aspects

#### 3.1.1 Historical Review

Several hypotheses were postulated by early researchers to explain gallstone formation but the first real attempt at a logical explanation was made by Naunyn in his "A Treatise on Cholelithiasis" in 1896. Prior to then cholesterol had been recognised as a component of gallstones for over a century and, according to Thudichum (1863), a deficiency of bile salts had been implicated in the causation of gallstones as early as 1806 by Thenard.

Naunyn believed that the initiating and essential step in the development of gallstones was infection. The source of the infection was thought to be from the intestines, these "ferments" gaining access by direct biliary spread following stagnation of the bile. Gallstones formed as a result of the harmful action of the infecting agents on the mucous membrane of the gallbladder producing a "pultaceous mass" of epithelial debris and bacteria. Cholesterol crystal formation was considered to be very much a secondary procedure caused by "cholesterinization" of the previously formed stones. Cholesterinization was a term coined by Naunyn to describe the formation of cholesterol in gallstones, a process he wrote of in these terms, "...gradually extensive transformation takes place in the concretions which takes the form, in most cases, of a continuously progressive infiltration with cholesterin and the deposit within these concretions of this substance in crystalline form". The cholesterol was believed to be

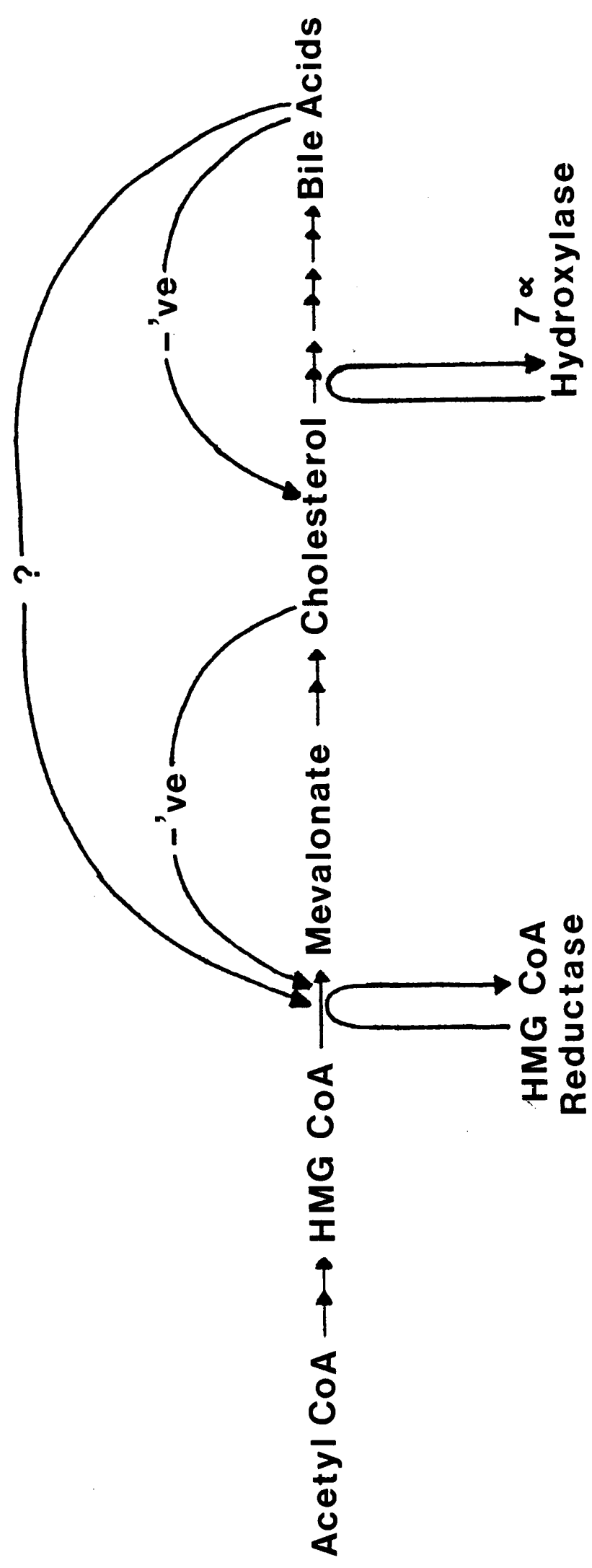
derived from the mucous membranes of the bile passages and was held in solution through the action of bile salts, salts and fats. He also believed that the solubilization of cholesterol in bile was not influenced by its concentration and that precipitation of cholesterol did not result from a deficiency of its solvents. His theory was accepted widely and further refined. Osler, in the third edition of "Principles and Practices of Medicine", stated that cholesterol and calcium salts were secreted mainly by the mucous membranes of the biliary tree when they were "in a state of catarrhal inflammation excited by the presence of microbes". These secretions were thought to alter the chemical constitution of bile and lead to the deposition of calcium salts and bilirubin around epithelial debris and bacteria. Indeed, infection did play a larger role in earlier days. The typhoid bacillus has a predilection for the gallbladder and was often identified in bile cultures and found at the centre of gallstones. Cushing (1898) and Moynihan (1928) blamed this bacterium for many cases of gallstones.

Aschoff and Bachmeister challenged the bacterial causation theory in 1909, suggesting that metabolic factors, in addition to bile stasis and infection, were important in gallstones formation. With preliminary biliary stasis they claimed that the formation of gallstones was always under aseptic conditions. Cholesterol precipitated from the neck of the gallbladder and formed a solitary, obstructive, cholesterol calculus which then led to a secondary infection. When infection supervened multiple faceted calculi ensued. However, medical opinion was still in favour of the primary influence being infection. William Mayo (1911) supported the bacterial causation theory but believed the source

of the infection to be the portal circulation while Moynihan (1913) thought that both direct intestinal spread and blood spread from the portal circulation were contributors. Moynihan also attributed the formation of some stones, albeit a very small minority - the solitary cholesterol stones, to aseptic metabolic causes. Blood-borne infection from the systemic circulation was also thought by Rosenow (cited by LaMorte, 1979) to be a cause.

While Aschoff and Bachmeister were developing their theory, a third theory on the pathogenesis of gallstones was put forward by Boysen and by Rovsing (cited by LaMorte, 1979). These workers believed that gallstones were formed initially as pigment calculi in the intrahepatic canaliculi as a result of liver disease and after the stones reached the gallbladder cholesterol was added to them in layers to form much larger stones.

The metabolic argument gained more support throughout the course of this century as the importance of physicochemical aspects in the formation of gallstones was realised. Phospholipids were recognised as bile constituents in 1939 (Johnson et al, 1939) and in 1968 Admirand and Small published their work on the ability of bile to solubilize cholesterol. The inability of bile to solubilize cholesterol for whatever reasons was regarded as the critical step in gallstone formation. Their approach revolutionised thinking about gallstone pathogenesis and led to a great deal of research on the generation of bile that was supersaturated with respect to cholesterol. So much so that attention was directed away from other areas that are perhaps equally vital such as crystal nucleation and growth in supersaturated bile. Recently more research has been carried out



**Fig. 1.2 The Metabolism of Cholesterol**

on these areas and the importance of such factors as infection and stasis is again being realised. Despite this, the validity of much of the work by Admirand and Small still stands and the current concepts of the pathogenesis of gallstones as outlined below concentrate on the metabolic factors.

### 3.1.2 Cholesterol Metabolism

Gallstones have been classified in a variety of different groups according to their physical appearance and chemical composition. Haller spoke of two groups; the large, egg-shaped, yellowish stones that were often solitary but if multiple they became faceted through friction and the small, dark, multi-faceted stones (cited by Mani, 1959). Naunyn (1896) divided them further into six groupings according to their composition. Recent opinion classifies them as either predominantly cholesterol or bile pigment in composition. Cholesterol is the major component of most gallstones in western communities (Sutor & Wooley, 1971) and this review will discuss cholesterol stones almost exclusively.

The major site of cholesterol production is the liver although small bowel synthesis is significant (Shaffer & Small, 1976). Hepatic synthesis begins with the substance acetyl co-enzyme A (acetyl CoA) and is outlined very briefly in figure I.2. The rate-limiting step in cholesterologenesis is the conversion of hydroxy-methyl-glutaryl co-enzyme A (HMG CoA) to mevalonate by the action of HMG CoA reductase and this process is regulated by several mechanisms including dietary and endogenous cholesterol and the enterohepatic circulation of bile salts, although lymph

cholesterol may be more important than bile salt circulation in the regulation (Heaton, 1972). A diet high in cholesterol markedly inhibits cholesterologenesis (see fig. I.2).

Bile acids are the major end-product of cholesterol metabolism following a process of at least 16 reactions but the first step is the rate limiting one (Heaton, 1972). Bile acids are synthesized at a rate of about 0.5-0.7g daily to make up losses from the enterohepatic circulation which has a total bile salt pool of about 2-4g. Human bile consists of three main bile acids: cholic acid, chenodeoxycholic acid and deoxycholic acid, and a minor fourth one called lithocholic acid. Deoxycholic acid and lithocholic acid are secondary bile acids produced by bacterial dehydroxylation of the primary, liver-synthesized bile acids, cholic acid and chenodeoxycholic acid (Heaton, 1972).

Cholesterol and bile acids form the major constituents of bile along with phospholipids of which the main one is lecithin. The insoluble cholesterol is excreted by the liver into the aqueous bile and is solubilized by the detergent action of the bile salts. They are aided in this process by lecithin which is also insoluble but somewhat polar so aligns with the bile salts to form a larger detergent unit. The detergent unit with its load of solubilized cholesterol is known as a mixed micelle and appears to be disc-shaped. Each unit stacks with other discs to form long, cylindrical complexes (Heaton, 1972). The optimal physical state is one where all three components are in proportions that allow all insoluble molecules to be held in solution within micelles. These proportions can be represented on a triangular phase diagram where any mixture can be plotted as a single point within the

triangle according to its relative percents of the three major components. This technique was first devised by Admirand and Small (1968) and later modified by Carey and Small (1978) who determined, at physiological water contents of 80-95%, which mixtures should be and are fully soluble.

A close correlation between hepatic bile salt, phospholipid and cholesterol secretion has been documented in man: at low rates of bile salt secretion there is relatively little secretion of lecithin and cholesterol but with increasing bile salt flux through the liver the secretion of the other components of bile increases similarly. The relationship is not a fixed one in that lecithin secretion and cholesterol secretion plateau at higher bile salt secretion rates (Shaffer & Small, 1976). Thus, the bile salt secretion rate is the predominant determinant of biliary lipid composition and as it decreases the relative amount of cholesterol increases. Cholesterol gallstones form when cholesterol is present in the bile at a concentration that is beyond the capacity of the detergent molecules and has crystallized out in the form of cholesterol monohydrate crystals (Craven, 1976, Sedaghat & Grundy, 1980).

### **3.1.3 Gallstone Pathogenesis**

The pathogenesis of cholesterol gallstones can be looked at in terms of three main phases.

#### **1. Cholesterol Supersaturation**

Cholesterol supersaturation is the first and necessary step in the process of gallstone formation. Cholesterol gallstone patients have been shown to have

supersaturated gallbladder and hepatic bile without exception (Carey & Small, 1978). There are a number of ways to form abnormal bile. Many normal people will produce supersaturated hepatic bile during their overnight fast as fasting reduces bile salt secretion rates and increases the concentration of cholesterol accordingly (Capron et al, 1981, Holzbach et al, 1973, Northfield & Hofmann, 1975). This supersaturated bile mixes with large amounts of unsaturated gallbladder bile in normal people to give an unsaturated mean composition. Pathophysiological mechanisms that may produce abnormal bile include:

**(a).** Excessive bile salt loss which may occur with diseases that affect the terminal ileum where most of the bile salts are actively reabsorbed from the intestines. Such diseases include ileectomy, extensive Crohn's disease and ileal bypass surgery (Heaton & Read, 1969, Cohen et al, 1971, Hill et al, 1975, Marks et al, 1977, Wise & Stein, 1978). Losses can be greater than hepatic synthetic capacity resulting in low bile salt secretion rates and subsequent cholesterol supersaturation. Drugs such as cholestyramine and lignin can cause excessive loss by chelating bile salts in the gut and preventing reabsorption but they only tend to cause severe losses when given in association with terminal ileal disease (Schaffer & Small, 1976).

**(b).** Oversensitive bile salt feedback appears to be a possible mechanism where certain patients have a small

bile salt pool but hepatic synthesis does not increase to compensate. Vlahcevic et al (1970) have shown that patients with gallstones have significantly smaller bile salt pools than normal controls. This overactivity of the normal feedback inhibition (see fig. I.2) results in depression of bile acid synthesis by low rates of bile salt return (Shaffer & Small, 1976). Alternatively, a small bile salt pool can be sustained by increased enterohepatic cycling (Bennion & Grundy, 1978). Increased cycling augments bile salt flux through the liver (Low-Beer & Pomare, 1973). In this case bile acid synthesis is normal but there is increased intestinal loss. However, Vlahcevic et al (1970) reported that the reduced bile salt pools were not due to increased losses as the half-life of cholic acid in their subjects with gallstones was the same as the controls. Northfield and Hofmann (1975) suggested that an underlying, extrahepatic defect that is not yet understood may be responsible for increased enterohepatic cycling of bile salts while Schoenfield et al (1981) believed that alteration of feedback inhibition was a more important influence and that this alteration was likely to be hepatic in origin.

(c). Inadequate bile acid synthesis, per se, is probably very uncommon but is found in the rare disease cerebrotendinous xanthomatosis which has, as one of its features, a greatly increased risk of cholesterol cholelithiasis (Bennion & Grundy, 1978).

**(d).** Excessive cholesterol secretion has been shown to be the cause of bile supersaturation in the face of normal or even increased bile salt pools. Bennion and Grundy (1975) have reported that obesity is associated with an increase in the biliary secretion of cholesterol. This observation has been confirmed by Mabee et al (1976). In their comprehensive investigation, Bennion and Grundy showed that weight reduction in obese patients resulted in a significant reduction of cholesterol output but not of bile salt or phospholipid output. During the actual period of weight loss bile saturation increased as a result of the relatively greater decrease in bile salt and phospholipid secretion that occurs with chronic caloric restriction. The exact mechanisms responsible for increased cholesterol synthesis in obesity are not known but Bennion and Grundy have hypothesized that HMG CoA reductase activity is increased, perhaps by insulin stimulation as insulin levels are chronically elevated in the obese.

**(e).** Phospholipid synthesis reduction is another possibility but little is known about it except for one study that has shown that gallstone patients have a greatly diminished phospholipid secretion (Swell et al, 1971). This diminution is thought to be a result of the relationship where phospholipid secretion is directly linked to the availability of bile salts in the enterohepatic circulation.

(f). It seems likely that the basis of lithogenic bile formation is a reduced bile salt pool size in many but not all cases (Heaton, 1972). A combination of reduced bile salt pools and increased cholesterol secretion has been documented in the American Indians of the Southwestern areas (Grundy et al, 1972). The authors postulated that a defect in the conversion of cholesterol to bile acids may be present curtailing the production of bile acids and allowing unmetabolized cholesterol to build up.

(g). Other factors than the relative concentrations of the three components of mixed micelles can influence the capacity of bile to hold cholesterol in secretion. These include the water content and ionic strength of bile. Dilute bile is less able to solubilize cholesterol than concentrated bile of the same relative lipid proportions (Carey & Small, 1978).

The liver is mainly responsible for the production of abnormal bile while the gallbladder is the reservoir that stores, concentrates and expels bile. Thus, it plays no part in the essential first step of gallstone pathogenesis. Indeed, until recently it was widely considered to be the innocent victim of gallstone formation but recent research into the subsequent phases of gallstone pathogenesis has revealed their importance and it is during these next two steps that the gallbladder makes its contribution.

## 2. Cholesterol Crystal Nucleation

Nucleation of cholesterol can occur by homogeneous nucleation if the cholesterol concentration is high enough to allow spontaneous coalescence of the cholesterol molecules. However, it is most uncommon to have gallbladder cholesterol concentrations within this range. Heterogeneous nucleation can occur at lower cholesterol concentrations as precipitation starts around some other substance such as mucus, calcium bilirubinate, bacterial fragments or epithelial slough from the gallbladder wall (Bennion & Grundy, 1978). Differential microanalyses of gallstones have shown regional differences in stone composition. The centre is very commonly pigmented. This suggests that gallstone formation could be initiated at the time of a disturbance in the bile salt circulation (Shaffer & Small, 1976). Bile that is supersaturated to the extent that heterogeneous nucleation is possible in the presence of appropriate nucleating agents is said to be "metastable" while bile that allows homogeneous nucleation is called "labile". The boundary between the two concentration zones has been labelled the "metastable/labile" limit (Carey & Small, 1978).

It is well recognised that many people secrete supersaturated bile yet do not go on to form gallstones (Holzbach et al, 1973). Sedaghat and Grundy (1980) showed that the supersaturated bile of people without gallstones did not contain cholesterol monohydrate

crystals while patients with gallstones almost always had cholesterol crystals. Moreover, these crystals were shown to have formed de novo in the bile rather than arising from crystal shedding from the gallstone surface. Cholesterol within gallstones is in the form of clumps of cholesterol monohydrate crystals (Craven, 1976, Sutor & Wooley, 1971) and cholesterol monohydrate precipitation seems to be an essential prerequisite of gallstone formation. This suggests that gallstone patients are either lacking a factor in their bile that normally prevents cholesterol crystallization or they have factors present in their bile that promote crystal nucleation (Bennion & Grundy, 1978). Recent research has provided substantial evidence for the existence of a potent nucleating factor in the bile of gallstone patients. Whiting and Watts (1984) demonstrated that supersaturated bile from obese subjects without gallstones would not form cholesterol crystals in vitro unless small seed crystals of cholesterol monohydrate were added. In the absence of seed crystals the bile of patients with gallstones showed a much greater tendency to form cholesterol crystals in vitro although biliary cholesterol saturation levels were similar in both groups. Another study has been carried out to specifically determine whether the rapid nucleation time of gallbladder bile obtained from gallstone patients is due to the presence of a nucleating factor or the absence of a protective factor. Burnstein et al (1983)

mixed the bile of normal controls with that of gallstone patients and showed that the mixtures had rapid nucleation times, similar to those of the gallbladder bile from gallstone patients. This indicated the presence of a nucleating factor in the abnormal bile and further investigations using decreasing amounts of abnormal bile in the mixtures revealed that the factor was extremely potent. Microfiltration at a level that would be expected to remove microcrystals of cholesterol did not eliminate the nucleating potency of the abnormal bile. Thus, some other nucleating factor that promotes the initial nucleation of cholesterol seems to be present in the bile of people who have gallstones. High cholesterol levels in the bile may also initiate excess secretion of gallbladder mucus which, in turn, acts as a nucleating agent for cholesterol gallstone formation (Lee et al, 1981).

### **3. Gallstone Growth**

The microscopic crystals need time to grow to macroscopic size and people without gallstones occasionally have been shown to have microliths in their bile (Bennion & Grundy, 1978). Sluggish gallbladder contraction and stratification of the bile within the gallbladder influence gallstone growth (Lamorte et al, 1979, Nakayama & Van der Linden, 1975). The concentration of counter ions such as calcium is also thought to be important in determining the aggregate growth of crystals (Evans & Cussler, 1974). Pregnancy

and diabetes mellitus have been associated with impaired gallbladder contraction. Braverman et al (1980) showed that there was incomplete emptying of the gallbladder with a large residual volume in late pregnancy. However, they were not able to show any effect of the oral contraceptive pill on gallbladder emptying. Gallstone patients have been shown by Shaffer et al (1984) to have gallbladder stasis. In contrast however, it has been suggested that gallbladder atony may also lead to an increase in the bile salt pool size by decreasing the enterohepatic circulation of bile salts and thereby decreasing intestinal losses and negative feedback inhibition (Low-Beer & Pomare, 1973). This would theoretically reduce the risk of developing gallstones and therefore counteract the direct effect of gallbladder stasis on gallstone growth.

Thus, each of the three stages is a necessary step in the evolution of a gallstone. The first stage was believed to be the most important single step and certainly seems to be subject to the greatest number of influences that can alter the normal metabolism. The importance of gallstone nucleating and growth factors has only recently been realised and physicochemical research into gallstone formation has ironically been brought through something of a full circle as the role of infection and stasis is again being considered as it was in Naunyn's time.

Table I.4 - The Strasbourg Autopsy Series

Age	Number Autopsied	Number with Gallstones	%
0-20	82	2	2.4
21-30	188	6	3.2
31-40	209	24	11.5
41-50	252	28	11.1
51-60	161	16	9.9
60+	258	65	25.2
Total	1150	141	12.3

## 3.2 Associated Risk Factors

### 3.2.1 Historical Review

Well before theories were evolved on the physicochemical aspects of gallstone formation, opinions were formed on the type of person most likely to harbour gallstones. By Naunyn's time a number of autopsy series had already been published enabling him to evaluate possible risk factors (see Table I.4). He claimed that the prevalence results in the different series were liable to error because gallstones were less frequent in younger populations (2-3% in people less than 30 years) than older populations (25% in "old" people). This was one of the first attempts at quantifying the effects of aging. He then looked more closely at Schroder's analysis of Von Recklinghausen's autopsies carried out between 1880-87 and pointed not only to the increase in prevalence with age but also the preponderance in women - 20.6% of women had gallstones compared with 4.4% of men. These two influences had been implicated by Morgagni. The effects of age were thought to be mediated through two influences : increasing age was believed to cause increased epithelial disintegration of the gallbladder with formation of cholesterol in the epithelial debris and Charcot had demonstrated smooth muscle atrophy of the biliary passages which was thought to lead to biliary stasis and subsequent infection. The sex difference also was attributed to a combination of effects. 90% of the affected women were parous so parity was thought to play an important role despite the fact that cases were never compared with controls to see if 90% of the normal population of women were also parous. Women were also thought to be at risk of developing gallstones because of their style of

dress. Naunyn believed that gallstone formation was greatly influenced by any condition that interfered with and retarded the flow of bile and the tight corsets that women wore then certainly restricted abdominal movement and often left rib markings on the liver. A sedentary lifestyle was also blamed as a risk factor as it involved decreased body activity and consequently decreased biliary flow but Naunyn did not believe that obesity or diet were at all important as possible risk factors as can be seen in the following statement, "There is no firmer foundation for the views that obesity and slowness of metabolism, luxurious mode of life and alcoholism favour the production of concretions. Nothing of the kind has been proved or even rendered probable....The impression I have formed is that gallstones apparently occur with equal frequency in people of all constitutions and degrees of nutrition". Similarly, he discounted beliefs that gallstones were much commoner in certain areas of the world and that these were perhaps due to dietary differences because he could show from the list of previous studies (see Table I.5) that Dresden, Vienna and Basle with their very different soil conditions and climate, had similar prevalences. Disease associations with gallstones that were thought to be positive around the turn of the century were ischaemic heart disease and diabetes although several authors quoted by Naunyn (Ord, Kraus and Hull) believed that diabetes was a consequence of gallstones.

Osler in 1901 mentioned the associated risk factors as being age, with nearly 50% of all cases occurring in people over 50 years old, sex, parity and any condition which favoured the stagnation of bile in the gallbladder such as "corset-wearing,

occupations requiring a leaning-forward position, lack of exercise, sedentary occupations, an overindulgence in food, constipation and depressing mental emotions".

Naunyn's dismissal of the importance of diet in the aetiology of gallstones is interesting when viewed in the context of the general body of thought about diet at that time. Thudichum (1863) acknowledged the lack of agreement about the importance of diet but he believed that meat predisposed least to gallstones while vegetable foodstuffs provided the greatest risk. Other authors believed that animal foodstuffs and alcohol were to blame for inducing gallstones.

The opinions outlined above show that the understanding of associated risk factors in the aetiology of gallstones had not developed much further in the sixty years since Crisp's address to the Medical Society in London (1841). This state of affairs continued well into the twentieth century. Littler and Ellis' review of 1952 quotes writers as recently as 1944 blaming sedentary habits and tightly fitting garments for gallstones. By this time, though, an hereditary predisposition had been recognised. Even with the sudden increase in epidemiological studies of gallstones in the last 30 years or so, only a few risk factors have been established as confirmed influences and many more are disputed. Yet the well worn aphorism of the "fair, fat, fertile, forty year old female who is flatulent" being the most likely sufferer of gallbladder disease is still commonly being taught in our medical schools.

A great number of clinical associations with gallstones have been made, many of which have only been mentioned by only one or

two studies such as the importance of epilepsy in the aetiology of gallstones (Bateson & Bouchier, 1975). This review will focus mainly on the more important or controversial associations with gallstones and only mention briefly any other possible associations.

### 3.2.2. Age

The frequency of gallstones increases with age as has been shown by the overwhelming majority of epidemiological surveys (Crump, 1931, Dessau, 1943, Friedman et al, 1966, GREPCO, 1984, Gross, 1929, Hamilton, 1932, Horn, 1956, Joske et al, 1954, Kozoll et al, 1959, Lieber, 1952, Ludlow, 1937, Martensson, 1937, Opit & Greenhill, 1974, Torvik & Hoivik, 1960, Zahor et al, 1974). The study in South Wales was one of the very few that was unable to show any age effect (Bainton et al, 1976). Studies do vary, though, in the constancy of the increase with age. A few reported a decrease in prevalence after the age of about sixty (Hamilton, 1932, Horn, 1956) but the numbers of autopsies carried out on people aged seventy and over are very small.

Metabolic studies investigating the underlying causes of this increased prevalence with age are very uncommon but there is one of interest, a study carried out on two groups of Chilean women - 12 young and 12 elderly women with recent normal oral cholecystograms - showed that the older group had a higher biliary saturation index than the younger group (Valdivieso et al, 1978). This was believed to be due to an increase in the canalicular secretion of cholesterol as the study also showed that the bile

acid pool sizes and the metabolism of cholic acid were the same in the two groups.

The increase is due, in part at least, to the cumulative effect of age but there are no studies of incident cases categorised by age to allow analysis of the different risks faced by each age group of developing gallstones. A prospective survey would be necessary to reveal such information.

### 3.2.3 Sex

Gallstones are also very clearly associated with sex as has been demonstrated for centuries. The exact strength of the relationship varies from study to study but most give a ratio of about 2 or 2.5 : 1, female : male (Bainton et al, 1976, Bateson & Bouchier, 1975, Cleland, 1953, Cunningham & Hardenbergh, 1956, Dessau, 1943, Friedman et al, 1966, Gross, 1929, Horn, 1956, Holland & Heaton, 1972, Joske et al, 1954, Lieber, 1952, Ludlow, 1937, Martensson, 1937, Nakayama & Miyake, 1970, Parnis, 1964, Sampliner et al, 1970, Torvik & Hoivik, 1960, Wheeler et al, 1970, Zahor et al, 1974). However, a few studies have disputed this sex effect but these were early studies (Hamilton, 1932, Mitchell, 1918). Several surveys have shown that the disparity between women and men diminished in the older age groups (Bateson, 1984, Gross, 1929, Horn, 1956) and have related this to a relatively reduced risk in postmenopausal women.

Specific metabolic studies designed to investigate physicochemical differences in the bile of women compared with men have shown that both the bile salt pool size and the total amount of chenodeoxycholic acid are reduced in women (Bennion et al,

1978a). The total bile salt pool and chenodeoxycholic acid pool size decreases in Pima Indian girls at the menarche. This is accompanied by a slight rise in the biliary cholesterol saturation (Bennion et al, 1978b). Puberty in the Pima Indian boys is also associated with an increase in their biliary cholesterol concentration but to a lesser degree than the girls (Bennion et al, 1979). Bennion also looked at changes in the bile of a woman undergoing surgical menopause and demonstrated an expansion of her bile salt pool size with a simultaneous decrease in her biliary cholesterol saturation (Bennion, 1977).

The exact mechanism behind the contraction of the bile salt pool size in women of reproductive years is not known but it seems logical that endogenous female sex hormones mediate some change in the pathway of cholesterol metabolism.

#### **3.2.4 Obesity**

Obesity is the other condition that has been clearly associated with gallstones. Numerous epidemiological investigations have confirmed the clinical impression that gallstones are more frequent in obese people (Friedman et al, 1966, GREPCO, 1984, Gross, 1929, Layde et al, 1982, Littler & Ellis, 1952, Rhomberg et al, 1984, Sievers & Marquis, 1962, Smith & Gee, 1979, Sturdevant et al, 1973, Van der Linden, 1961, Wheeler et al, 1970, Zahor et al, 1974) although findings in a few studies were limited to the younger age groups (Bernstein et al, 1973, Horn, 1956, Scragg et al, 1984a).

In support of these descriptive surveys, metabolic studies have demonstrated that bile is lithogenic in overweight individuals

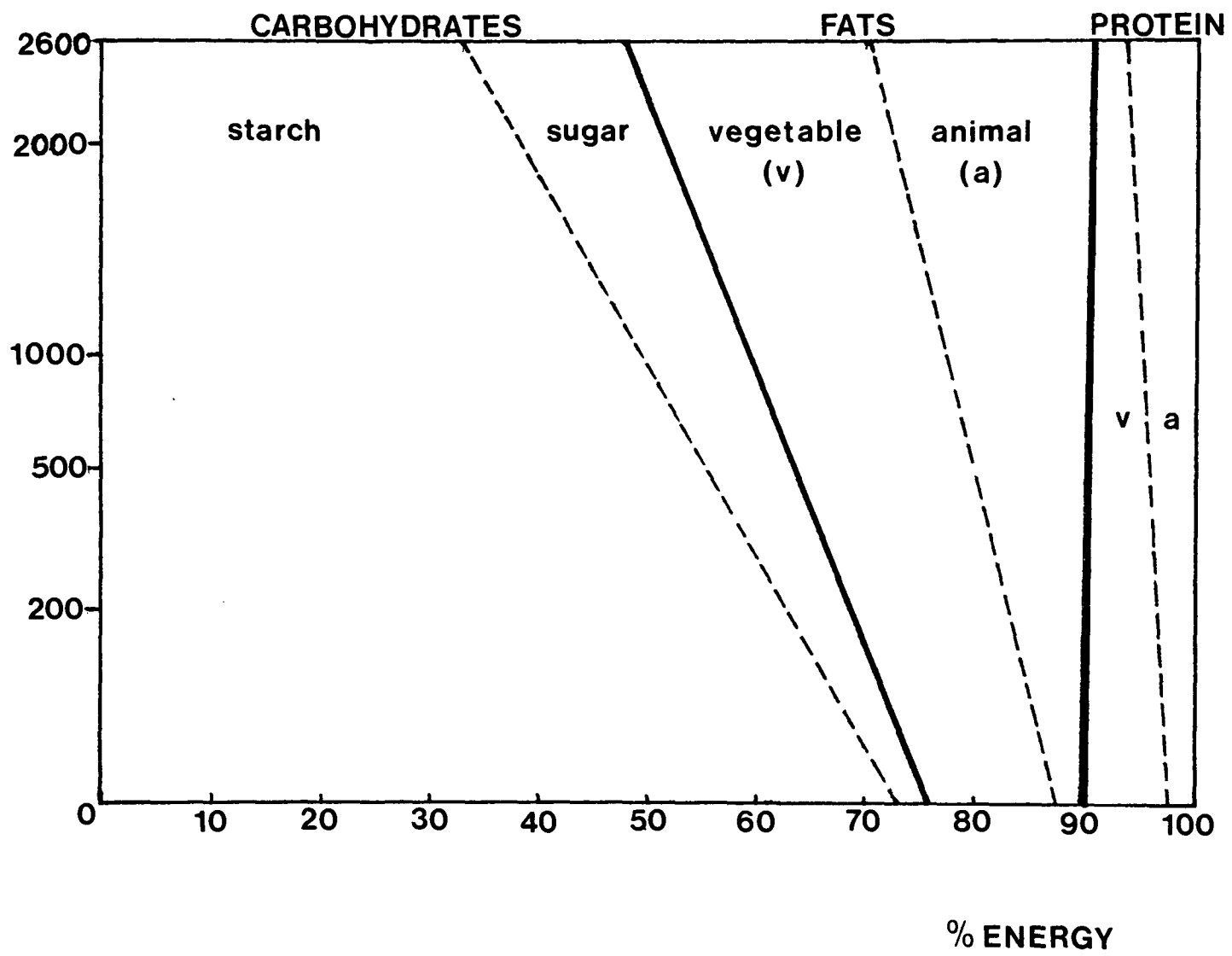
(Bennion & Grundy, 1975, Mabee et al, 1976, Shaffer & Small, 1977). Excess hepatic secretion of cholesterol appears to be the basic mechanism in this lithogenicity and is almost certainly a consequence of increased hepatic synthesis of cholesterol as bile salt and lecithin output are unchanged or even increased in obesity (Bennion & Grundy, 1975, Mabee et al, 1976, Shaffer & Small, 1977). It has been hypothesized that the underlying cause is increased activity of the enzyme, HMG CoA reductase (see fig. I.2)(Bennion & Grundy, 1975). Weight reduction resulted in a significant lessening of cholesterol output in the subjects studied by Bennion and Grundy (1975) with no change in the output of the other biliary constituents. During the actual period of weight loss the lithogenic index did not decrease.

Obesity may be a stronger influence in younger women. Those overweight women who develop gallstones could be considered as being more susceptible than women of normal weight and therefore liable to developing them early on in their adult life (Heaton, 1984).

### **3.2.5 Diet**

The question of dietary influence on gallstone pathogenesis is a much more difficult one to evaluate than the preceding factors. Descriptive epidemiological studies have emphasized that factors related to lifestyle, and more specifically diet, are involved in the causation of gallstones. The wide international variations, especially between developed and developing nations, and the increase in prevalence within developed countries throughout the course of the last hundred years or more support a role for

Gross Domestic  
Product/Head/Year  
US\$ (log scale)



**Fig. I.3 Dietary Changes with Industrialization**  
(from Trowell, 1977)

lifestyle and diet in the aetiology of gallstones. Trowell has summarised the changes in dietary energy sources accompanying economic development in fig. I.3. The major changes are, quite clearly, an increase in animal protein consumption with no change in overall protein intake, a great reduction in complex starch intake and therefore in fibre intake with an increase in simple sugars and a large increase in the intake of fat, this increase occurring in animal fats only (Trowell, 1977). The greatest alterations in diet associated with a change from rural to urban life in South Africa are an increase in sugar and meat consumption with a five-fold decrease in the consumption of fibre (Burkitt, 1973). Further African studies are pertinent from another, quite different angle. Beef from British cattle contains ten times as much lipid as Ugandan wild buffalo and only 2% of British beef fatty acids are polyunsaturated. By contrast, 30% of meat fatty acids from woodland buffalo and 10% of those in grassland buffalo are unsaturated. Turning from oil-rich woodland to water-rich grassland feeding, therefore, may be detrimental from a nutritional point of view (Epstein, 1971). The changing nature of gallstone disease in Japan has been attributed to dietary changes occurring since the second world war (Nakayama & Miyake, 1970). Observations such as these plus the increasing prevalence of a number of other diseases typical of western countries including ischaemic heart disease, diabetes mellitus, diverticular disease and cancer of the large bowel led Cleave, Burkitt, Painter, Trowell and others to the hypothesis that many of the diseases of western civilization were due to the consumption of highly refined and fibre depleted carbohydrate diets (Cleave, 1974, Burkitt &

Trowell, 1975). Much earlier, authors had also incriminated "good living" and "dietary indiscretion" (Crisp, 1841, Osler, 1901, Weiss, 1944). However, descriptive studies cannot give any specific details about dietary components which might be causative as they provide information about the total population, not diseased individuals within that population. They generate useful hypotheses that are subsequently more carefully examined using clinical metabolic studies and analytical epidemiological studies.

Metabolic and analytic investigations are, therefore, the major group of studies that have implicated specific dietary components in the aetiology of gallstones. Unfortunately a number of methodologic difficulties arise with these techniques and, although some of the difficulties are better described in later sections on methodology, a brief critique will be listed here :

1. All the dietary nutrients that have been implicated in gallstone disease - excess total calories, excess fat and/or cholesterol, excess highly refined carbohydrates and simple sugars and lack of dietary fibre - are highly correlated in western diets and it is extremely difficult to separate out their individual effects.
  2. A source of error that is particularly liable to occur in case-control studies is the effect the disease has on the usual dietary intake of a case. Symptomatic persons will often spontaneously alter their diet to prevent recurrence of pain and other unpleasant symptoms while doctors will commonly advise their symptomatic patients to avoid many foods, particularly fatty foods.
- Sarles et al (1969) showed that gallstone cases may

lower their daily caloric intake after the onset of symptoms and again after cholecystectomy. Thus, the diet of the asymptomatic individual can be quite different to that of the symptomatic person yet the case-control studies that have been carried out so far have looked only at predominantly symptomatic populations. The changes in dietary intake after symptom onset and treatment may diminish with time. Sarles et al (1969) went on to show that the caloric intake of symptomatic people had increased again prior to their cholecystectomy. Thus, the time at which dietary interviews and questions are administered is also an important consideration.

3. Other confounding influences can also affect results if the cases differ from the controls in important respects such as body mass index. Smith and Gee (1979) carried out a dietary survey to determine the influence of diet on gallstones and showed that gallstone cases were much more overweight than their controls and yet consumed a diet lower in total energy, protein, fat and crude fibre. When corrected for any weight-reducing diets that the participants were on, this difference decreased considerably. Moreover, it has been shown that overweight people tend to minimise food intake on recall while underweight people maximise it (Madden et al, 1976).

4. The number of study participants should be large enough to minimise statistical errors associated with

small numbers yet dietary surveys often include barely adequate numbers for results to be significant. This is particularly true of metabolic studies where individuals investigated are often fewer than ten in number because of the specialised and time-consuming methods employed (DenBesten et al, 1973, Pomare & Heaton, 1973, Sarles et al, 1970b). Case-control studies include larger numbers of participants than metabolic investigations yet, until Scragg et al published the results of 267 cases this year (1984a), the highest number previously studied were 101 case-control pairs (Sarles et al, 1969).

5. The controls selected as a comparative group for the cases in case-control studies should be representative of the general disease-free population from which the cases were derived. If a study were then to select their control group from the asymptomatic general population, unless they specifically investigate these controls to exclude the presence of asymptomatic gallstones, the control group will possibly contain quite a number of people with asymptomatic stones who should actually belong to the case group. Most studies, though, select their controls from a more easily accessible group such as hospital patients admitted at the same time as symptomatic gallstone patients but suffering from a different ailment. To ensure that the controls do not have gallstones, most have already undergone tests to rule out gallstones as a possible cause of their symptomatology. This means that, commonly, the controls

were admitted with some form of upper gastrointestinal tract symptomatology. These controls may well have altered their diet as a consequence of their symptoms or disease. Hospital patients and particularly those with some ailment of the upper gastrointestinal tract cannot be considered as representative of the disease-free population and their dietary consumption may be biased either up or down with respect to various nutrients. Thus, the researchers eliminate one form of bias from the study design but introduce another and the latter form of bias is more dangerous than the misclassification of asymptomatic cases which is only likely to minimise real differences.

6. The dietary methodology employed by the various case-control studies varies widely with some employing the food-frequency method, some the recall method and others the record technique. Each technique has its own advantages and problems with respect to detecting dietary differences in the presence of gallstones disease. These will be discussed more fully in the section on dietary methodology. Scragg et al (1984a) claim that the food-frequency method of measuring usual intake in the past is the most appropriate and their team has work in support of this claim (Baghurst & Baghurst, 1981). Yet this method has only rarely been employed (Friedman et al, 1966, Reid et al, 1971, Scragg et al, 1984a). Recall methods have been more commonly used (Hauton, 1966, Sarles et al, 1957, 1969, Smith &

Gee, 1979, Wheeler et al, 1970) and the period of recall is commonly the week prior to the interview. This may not reveal a typical picture and does not allow determination of intakes of earlier years. The record or diary method (Williams & Johnston, 1980) is similarly unable to give any idea of previous dietary habits.

7. An important problem in the investigation of dietary influences in the aetiology of gallstones is the possibility of a threshold effect (Heaton, 1984). When a disease becomes very common within a population associations with risk factors may well become more difficult to demonstrate. By only studying people who consume a western diet, investigators are already looking at a highly selected population and dietary influences can be obscured. It has been postulated that most people in western communities already eat a diet that is above the threshold for fat intake and fibre lack (Scragg et al, 1984a). There is a need to study groups of people within western communities who do not adhere to the usual dietary practices of their communities.

8. Ideally, to determine the effect of diet on the development of gallstones, a prospective study of gallstone-free people is needed. That would allow investigators to accurately determine the usual dietary intake of people in the process of producing lithogenic bile rather than building up a picture of the usual intakes of people who have already developed the end

product. Retrospective studies that attempt to determine the usual dietary intake of people some years previously in an attempt to discover practices which may have led to the development of a condition are usually very inaccurate (Block, 1982, Moller Jensen et al, 1984).

Despite these listed problems and more, a number of studies have provided useful information regarding specific dietary factors and are described below.

### **1. Total Calories**

As a result of the association between obesity and gallstone risk, work has been carried out on the effect of total calorie intake both on the composition of bile in metabolic studies and on gallstone prevalence in epidemiological studies. Much of this work has been carried out by one team in France. In 1957 Sarles and his group first showed, in a small case-control study of 54 pairs, that cases had a significantly higher total caloric intake than the controls. The controls were a mixture of patients admitted with dyspepsia yet not found to have any gallstones on oral cholecystogram and patients admitted with non-gastrointestinal complaints and, therefore, had not had gallstones excluded by X-Ray. The same group carried out a similar survey in 1963-65 although, this time, stones were excluded in the control group by oral cholecystography (Hauton, 1966). The results supported their original observations. The percentage intake of protein, carbohydrates and fats was the same in cases and controls but cases had a greater overall intake. A third and larger study was carried out in 1967-68 (Sarles et al, 1969) with numbers almost

doubled to 101 female pairs. The controls were selected for the first time from a non-hospital population but asymptomatic disease was not excluded from this group. The investigators used the same dietary information collection technique in all the studies; the dietary intake of the week prior to the onset of symptoms for cases and prior to admission or interview for controls was recorded. In the larger study the patients with gallstones had a significantly increased intake of calories ( $p < 0.001$ ), irrespective of dietary composition. There was no difference in body weight and exercise participation between the two groups.

A study in Nova Scotia reported similar results although this was a very small study of Caucasian women living in a rural community (20 cases and 77 controls). Dietary data was collected by a four day dietary diary of current consumption but most cases (75%) were post-cholecystectomy. The investigators found a significantly increased caloric intake in cases ( $p < 0.001$ ) (Williams & Johnston, 1980). Sarles et al (1978a) then carried out a descriptive survey in seven different countries. The autopsy prevalence of gallstones was compared to the diets in these countries : France, India, Japan, Portugal, South Africa, Sweden and Uganda. A positive correlation between caloric intake and gallstone prevalence was found in those countries with an intake of less than 3,000 Kcal per head. In countries with high calorie, high protein and high lipid intakes, such a correlation was not found. In yet another study by the same team, this time using a completely different design where 214 women from a population of 1,045 underwent oral cholecystography, different results were obtained. 11 cases were found and their diet did not differ

significantly from that of the controls (Sarles et al, 1978b). From these two studies, despite the design of the former being somewhat suspect, the authors postulated that dietary factors had become less important than in the period following the second world war deprivations in France.

The earlier results above have not been confirmed by other work. In fact, another Canadian study of post-cholecystectomy patients reported a significant decrease ( $p < 0.05$ ) in the total daily calorie intake of the female cases (Smith & Gee, 1979). A 48 hour dietary recall method was used for data collection and further information on crude fibre intake was gained by asking the usual weekly intake of foods with an appreciable fibre content. The controls had had a past gynaecological admission and had not suffered from any recent illness. A major flaw in this study was their assumption that diets did not change after cholecystectomy and interviews were sometimes conducted as soon as three months after surgery. Another problem was that of observer variation as the interviews were conducted by 21 different dietitians or dietetic interns. In a clinical and dietary survey of gallstones in Australia (Wheeler et al, 1970), no significant difference was found in the mean daily calorie intake of cases and controls of either sex. The cases (71) in this study were positively diagnosed yet it is not stated whether or not they had already had surgery. The control group (72) was made up of random admissions to the same hospital. Data was collected on the person's "average" weekly diet for the week before symptoms occurred (i.e. dietary recall). Seasonal variation was also taken into account.

Scragg et al (1984a) found an association between increased

calorie intake and gallstones only in subjects less than 50 years of age. The risk for older people actually decreased with increased calorie intake. This study was based on a very large series with a combination of both hospital and community controls to overcome the problems associated with each. The age effect seen in their results has not been reported previously and the authors suggest that the discrepancy can be explained in terms of susceptibility to high energy intakes. People, and particularly women, who are susceptible to high calorie intakes will tend to develop their gallstones at an early age. Their data relating to obesity support this hypothesis as they found that obesity was a positive risk for gallstones only in cases under the age of 50 years. The earlier studies that found a positive association with high energy intake were carried out on relatively young populations with women aged 20-55 years (Sarles et al, 1969) and women aged 15-50 years (Williams & Johnston, 1980). However, the mean age of cases in Smith and Gee's study was only 38 years. Both Scragg et al (1984a) and Heaton (1984) point to the possibility of a threshold effect preventing any differences being detected in the total calorie intake of gallstone cases and controls in western communities. The survey of dietary intake and gallstone prevalence in seven countries provides support for this argument as a positive effect was only seen in those countries with a low total calorie intake (Sarles et al, 1978a).

Metabolic studies lend weight to the argument for a positive effect of high energy intake on the aetiology of gallstones. Sarles et al (1970a) studied the T-tube bile of cholecystectomised

patients for a period of 3-37 days post-operatively. Dietary intakes of protein, carbohydrate, fat and total calories were varied during this time and the biliary cholesterol concentration correlated positively with mean caloric intakes for cases but not for controls. The biliary cholesterol level also tended to reflect the caloric intake of the previous day. In a study of obesity and biliary lipid metabolism, Bennion and Grundy (1975) showed that a hypercaloric diet ingested by non-obese subjects led to an increase in the biliary cholesterol output. The subjects did put weight on during the hypercaloric diet period but not to an extent which would account for such an increase in their biliary lipid levels.

A low total caloric intake has also been implicated as a risk factor in the aetiology of gallstones. Bennion and Grundy (1975) studied the effects of caloric restriction on bile lithogenicity. Subjects lost weight on the regime and ultimately lowered their biliary cholesterol saturation once they had stabilised at a lower weight. During the period of weight loss, though, bile acid and lecithin output were reduced even more than the cholesterol output and the biliary saturation increased in six out of 10 subjects. In contrast, Sarles et al (1971) investigated the biliary effects of a low caloric intake in four post-operative gallstone patients and showed a less frequent saturation of bile.

In summary then, no definite association between total caloric intake and gallstone prevalence can be assumed from the studies so far reported. However, several cross-cultural studies do implicate caloric intake as a risk factor in the aetiology of gallstones. Failure to confirm these findings in studies designed to test this

hypothesis may result from the fact that western communities may now consume an average total energy intake that is greater than the threshold level at which differences can be detected. Another difficulty experienced in these studies is that it is extremely difficult, if not impossible, to accurately measure total energy intake.

## **2. Dietary Fats**

Dietary fat intake is closely correlated with the total calorie intake so is not easily analysed as a separate risk factor in the aetiology of gallstones. It is more usual to discuss fats in terms of specific items such as the polyunsaturated to saturated fat ratio (P/S ratio) and cholesterol intake. However, some studies have reported analyses of total fat intake. These are nearly all descriptive or clinical epidemiological surveys as opposed to metabolic investigations.

The descriptive studies tend to implicate a high fat intake as being a positive risk factor. In two Australian studies, mediterranean immigrants were shown to have an increased risk of developing gallstones in their adopted country (Wheeler et al, 1970, Loftus Hills, 1971). The only major changes in lifestyle that could be associated with this increased risk were dietary with an increase in the immigrants' fat intake as a result of meat forming a much larger part of their diet and a decrease in carbohydrate intake with less pasta and bread. A survey of railway workers in India reported a seven times greater risk for the development of gallstones in workers from the north of the country compared with those from the south (Malhotra, 1968). The author

attributed this finding to dietary differences, particularly fat, as northern workers consumed eight to nineteen times more fat than the southern workers. Finally, Richardson et al (1973), in a clinical epidemiological study of gallstones in an Appalachian community in the United States, found a higher prevalence of gallstones in that community compared with the Framingham study. The age-adjusted prevalence rates were given as 16.7% and 3.9% respectively although it is not clearly shown how the figures were achieved for the Framingham study whose original prevalence rate was given as 8.2%. The authors noted that residents of this Appalachian community ate excessive amounts of fats, meat and cholesterol. These descriptive studies are difficult to analyse as they are based on general rather than specific dietary information taken from selected community groups.

Clinical epidemiological surveys are less supportive of the effect of high fat intake in the prevalence of gallstones. No significant difference was found in the fat intake of cases compared with controls in the Framingham study (Friedman et al, 1966). The cases (67) were not individually matched to the controls (812) and the method of collection of dietary data was not reported in detail. Similarly, Sarles et al (1957, 1969) were not able to show any significant difference in the total fat intake of cases compared with controls. However, Smith and Gee (1979) showed a significant reduction in the amount of dietary fat consumed by the cases compared with the controls ( $p < 0.05$ ).

Metabolic studies have yielded inconsistent results also. Hyperlipidaemic patients did not show any change in their biliary cholesterol saturation when fat intake was changed from 5% to 40%

of total calories (Grundy & Metzger, 1972). The hepatic secretion of all three biliary lipids increased equally. Sarles et al (1970) reported similar results when the proportion of dietary fat was altered in their study of dietary effects on T-tube bile composition. However, in another study of hyperlipoproteinaemic patients, a change from a fat-rich diet to a carbohydrate-rich diet was found to decrease the biliary cholesterol saturation by increasing the formation of primary bile salts (Andersen & Hellstrom, 1980). Most metabolic studies alter dietary carbohydrate intake in order to alter dietary fat intake and the carbohydrate addition is usually in the form of simple sugars. As a result, it is not possible to determine which of the two nutrients is responsible for any biliary changes. Van der Linden and Nakayama (1976) infused 16 gallstone patients with a fat emulsion in the pre-operative period whereupon bile lithogenicity was increased and cholesterol crystals were later demonstrated in the bile.

Experimental studies on animals have induced gallstones in a number of different species using a variety of different diets. An entirely fat-free diet will produce cholesterol gallstones in hamsters within six or seven weeks (Dam, 1971). Similar diets do not have such an effect on mice and chicks while other animals cannot even be induced to eat a fat-free diet. It is also extremely unlikely that the kind of diet used by Dam is ever consumed by man.

It can be seen, therefore, that the importance of total dietary fat in the aetiology of gallstones is not clearly known. The fact that no study has been conclusive in its support of either a

positive or a negative risk suggests that dietary fat, per se, is probably not an important factor. More interest has been shown in specific aspects of dietary fat intake and these are discussed below.

### **Polyunsaturated/Saturated Fat Ratio**

The observation that polyunsaturated fats in the diet caused a lowering of the serum cholesterol concentration led to further research into the nature of the mechanisms behind the lowering of the cholesterol. One postulated mechanism was an increase in the rate of excretion of cholesterol and its metabolites in bile and earlier studies did indeed show an increase in faecal bile acids when sunflower seed oil was given in place of coconut oil in the diet, indicating increased biliary excretion of cholesterol metabolites (Gordon et al, 1957a, Gordon et al, 1957b, Lewis, 1958). However, these studies may well have used techniques that were not specific enough (Heaton, 1972, Spritz et al, 1965) and some later studies reported different results. Spritz et al (1965) were unable to show any increase in five studies of faecal steroids when polyunsaturated fats were exchanged for saturated fats. However, in order to avoid inaccuracies in faecal steroid estimations, sterol free fats were used in three of the patients. The two subjects with sterols in their diets did show a significantly greater excretion of bile acids compared with the three who were on sterol-free diets. In contrast, Connor et al (1969) found that corn oil (unsaturated) caused greater faecal bile acid excretion than cocoa butter (saturated) in normal men. They attributed these results to study design as Spritz and his

co-workers had studied hyperlipidaemic patients and may not have examined the faecal steroids for a long enough period to detect any changes. Connor et al also used cocoa butter which contains long-chain saturated fats unlike butter and coconut oil which have short chain fats. Grundy and Ahrens (1970) found a slight increase in the bile acid secretion of only three out of 11 patients with various types of hyperlipoproteinaemias given unsaturated fats. Grundy (1975) then studied a group of hypertriglyceridaemic patients and this time he reported an increased excretion of bile acids and neutral steroids on a diet high in polyunsaturates. In doing so he had identified a subset of hypertriglyceridaemic patients who increased their biliary cholesterol saturation by increasing their cholesterol excretion at the expense of bile acids. Earlier studies had suggested that unsaturated fats actually lowered the cholesterol saturation of bile by either reducing cholesterol excretion and raising bile acid output (Lewis, 1958) or raising lecithin output (Watanabe et al, 1962). Sarles and his team (1970a) were not able to show any change in the lipid concentrations of T-tube bile in patients on diets of different fat saturations. The numbers of patients investigated in these studies, however, were very small. Lewis based his results on just three patients. Problems also arise with spontaneous variations in the excretion of bile acids and cholesterol (Sarles et al, 1970a) and with inaccuracies in the techniques of determination of biliary lipid concentrations, especially in earlier studies (Whiting et al, 1981).

Animal studies have not been useful in helping to determine the importance of the type of dietary fat on biliary lipid composition

as there is a paucity of animal models which form gallstones in an analogous way to the human disease (Brenneman et al, 1972). However, Redinger et al (1971) improved the cholesterol saturation of bile in rhesus monkeys by feeding them safflower oil. Dam's work with hamsters showed that polyunsaturated fats protected against the development of gallstones and the fat-free diet that he developed to induce gallstone formation in hamsters was developed mainly to exclude all polyunsaturated fats (Dam, 1971).

Clinical studies have not been able to resolve the situation either. The P/S ratio has not been analysed carefully enough in case-control studies for any useful conclusions to be drawn. In the survey of Indian railway workers, the gallstone-prone residents from the northern areas of India were reported to eat nineteen times the amount of fat compared with workers from the southern regions (Malhotra, 1968). The type of fat that they consumed tended to be animal in origin and short-chained while people in the south ate long-chain, unsaturated vegetable fats. Interesting results were obtained in a study of men fed a diet high in polyunsaturates as a cholesterol lowering measure (Sturdevant et al, 1973). These men were found to have more gallstones at autopsy than the control group on a typical American diet. They reported a dose effect with the greatest risk of gallstones occurring in the group of men who had consumed the greatest proportion of experimental meals over the years. These results were not confirmed by a later study in Finland (Miettinen et al, 1976). In this study, people on a cholesterol-lowering, high P/S ratio diet were no more likely to develop gallstones as detected at autopsy than people on a normal diet. Numbers were

small in both studies.

If a diet that is high in polyunsaturates lowers serum cholesterol by increasing the biliary lipid excretion then the lithogenic effect of the diet is dependent on the ratio of the changes of the three biliary lipids. Grundy (1975) demonstrated an increase in the biliary concentration of cholesterol with either no change or just a slight increase in the size of the bile acid pool in subjects fed polyunsaturated fats. This may explain the underlying mechanism behind the observation that the prevalence of gallstones was increased in men who were fed, over a long period of time, a diet that was high in polyunsaturated fats (Sturdevant et al, 1973). However, the P/S ratio in that study was extremely high, being close to 2.0. There are no known free-living populations that have a P/S ratio that is greater than 1.0. The above studies do not show any consistent results for the effects of a diet high in polyunsaturates on bile. Many of the earlier studies should probably be discounted on the basis of inaccurate or insensitive techniques but, even so, the later studies do not show any consensus and were usually based on short-term dietary interventions only. The prevalent view is that a high P/S ratio does lead to increased gallstone formation but evidence for this view is based on one study in which the P/S ratio was more than double that of the highest ratio achieved by free-living populations (Sturdevant et al, 1973).

### **Cholesterol**

Total body cholesterol is derived mainly from liver synthesis

although small bowel synthesis makes a significant contribution. Dietary cholesterol also contributes to total body pools but to a lesser extent (Consensus Conference, 1985). Homeostasis of cholesterol involves a combination of control of hepatic cholesterol synthesis, biliary excretion of cholesterol and its metabolites and intestinal absorption of dietary cholesterol (Wilson & Lindsey, 1965). The individual importance of the three mechanisms is not certain. Wilson and Lindsey (1965) claimed that control of intestinal cholesterol absorption was the most important mechanism. However, Quintao et al (1971) and Nestel and Poyser (1976) demonstrated that homeostasis, in the face of dietary cholesterol loading, was maintained by a combination of decreased hepatic synthesis and increased hepatic secretion. There was great individual variation in the importance of each of the mechanisms shown in these two studies. The effectiveness of the mechanisms varied also. Plasma cholesterol levels increased slightly if at all in the eight subjects in the one study (Quintao et al, 1971) while it increased markedly in three of the eight subjects in the other study (Nestel & Poyser, 1976). The subjects who showed an increase in their cholesterol levels were the ones who relied more heavily on increased biliary excretion of cholesterol to maintain homeostasis. Lin and Connor (1980) confirmed the importance of the control of hepatic synthesis and biliary excretion of cholesterol as opposed to intestinal absorption in cholesterol homeostasis. Genetic influences may determine an individual's response to dietary cholesterol. The Masai tribespeople of East Africa consume a diet that is extremely high in cholesterol yet their plasma and biliary cholesterol

levels are uniformly low and it has been suggested that they have a more efficient negative feedback control of hepatic cholesterol synthesis (Biss et al, 1971).

Similar to studies of total energy and fat intake, studies of the effect of high dietary cholesterol intakes on biliary cholesterol levels have conflicting results. Sarles et al (1970b) fed a sixty year old woman, who had just undergone a cholecystectomy, with varying amounts of cholesterol for six days and found no change in her biliary cholesterol levels. However, a study of only one subject over just six days with a major intervention technique such as a T-tube to sample bile cannot be usefully extrapolated. The cholesterol saturation index of bile shows a considerable amount of biological variation even on a constant diet (Whiting et al, 1981). Dam et al (1971) fed nine healthy young women a diet loaded with 1-2g of cholesterol daily in the form of egg yolks and reported a variable biliary cholesterol response with both increases and decreases in the saturation index. Changes took up to three weeks to occur. Similar results were reported in another study although the subjects included hypertriglyceridaemic patients as well as normolipidaemic people (Andersen & Hellstrom, 1979). In contrast, DenBesten et al (1973) fed ten healthy young men 750mg of cholesterol daily in the form of egg yolks and demonstrated a significant increase in the biliary cholesterol saturation of all subjects. In the same study a hypercholesterolaemic subject was fed a diet of increasing cholesterol content and her biliary cholesterol concentration increased accordingly. After three months on a diet of 1g of cholesterol daily cholesterol crystals were detected in her bile.

In another study 15 patients showed a decrease in their biliary cholesterol saturation index after consuming a diet of 100mg of cholesterol daily for one week (Maudgal et al, 1978). Seven patients were then investigated further and their daily cholesterol was reduced from 600mg to 100mg and their biliary cholesterol saturation decreased accordingly by a significant amount.

Animal studies generally support a positive effect of dietary cholesterol loading on biliary cholesterol levels. Brenneman et al (1972) fed 24 prairie dogs with a cholesterol-rich, egg yolk diet and within 2-6 months induced the formation of gallstones in all the dogs. DenBesten et al (1974), in a larger experiment, showed similar results and some dogs had begun to develop gallstones within 14 days of commencing the diet.

Case-control studies do not provide any further data to help determine the effect of dietary cholesterol on biliary cholesterol concentration. Two studies (Friedman et al, 1966, Reid et al, 1971) showed that the cholesterol intakes of cases and controls were not significantly different and Scragg et al (1984a) demonstrated a significant increase in dietary cholesterol intake for female cases compared with the community controls only. No significant difference was shown between female cases and their hospital controls and male cases and either of their control groups.

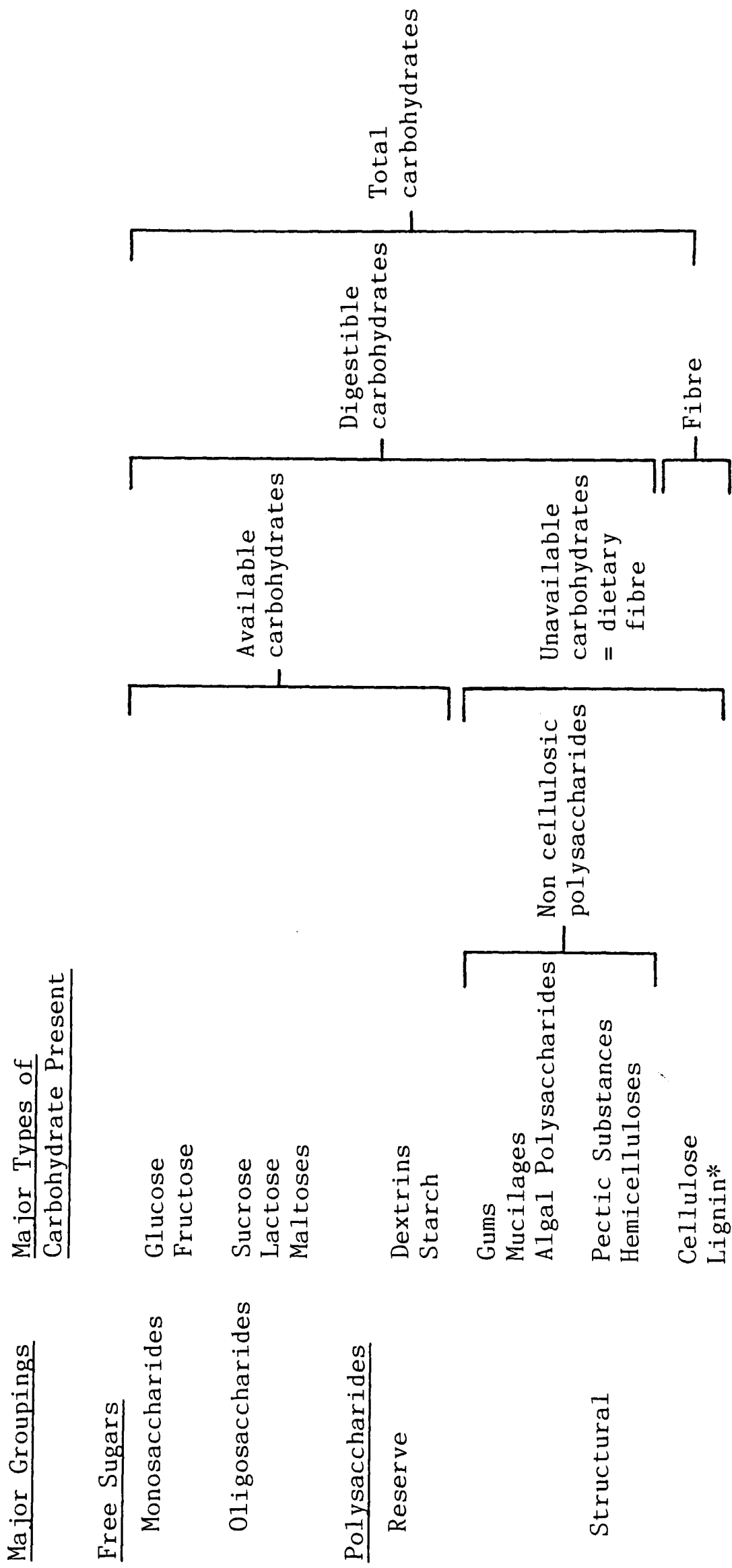
The few studies that have demonstrated a positive effect of dietary cholesterol on biliary saturation have only shown modest changes even with considerable dietary loading. This suggests that the effects of excess dietary cholesterol on bile composition are

not as great as those influences which directly affect hepatic cholesterol synthesis such as obesity.

### **3. Fibre**

There is considerable controversy surrounding the definition and terminology of the word fibre. Crude fibre has been defined as the residue of plant food left after sequential extraction with solvent, dilute acid and dilute alkali. This method of analysis is obviously inadequate yet, until recently, crude fibre levels were still listed in most food tables. The term dietary fibre was recommended by Trowell (1974) because it was clear that the physiological effects of dietary fibre on the digestive system related to all the indigestible components of the plant cell in the diet. The conventional crude fibre analysis only measures some of these components. For example, whole wheatmeal has a dietary fibre of about 11% and a crude fibre of about 2%. Trowell defined dietary fibre in physiological terms as the remnants of plant cells resistant to hydrolysis by the alimentary enzymes of man.

Dietary fibre is a biological unit and the mixture of polymers should be regarded as a whole (Eastwood & Passmore, 1983). The polymers are derived predominantly from the cell wall and consist of fibrillar polysaccharides such as cellulose, matrix polysaccharides such as pectins, hemicelluloses and glycoproteins and encrusting substances such as lignin. Other fibre groups include gums from plant exudates and mucilages from seeds and seaweeds. All are carbohydrates except lignin (Southgate et al, 1976). The chemistry of dietary fibre varies from plant to plant and is affected by the growing conditions and age of the plant. (Eastwood



\* Not a carbohydrate but an aromatic polymer

Fig. I.4 - Nutritional Classification of the Carbohydrates in the Diet (from Southgate et al, 1976)

& Passmore, 1983). Southgate has categorised the nutritional carbohydrates and lignin in figure I.4. The physicochemical actions of dietary fibre include an ability to hold water in quantities much greater than the original weight of the fibre. Vegetable fibres have a much greater water-holding capacity than bran. A second important capacity is that of cation exchange as fibre can act as a weak cation-exchange resin (Eastwood, 1977).

The physiological actions of dietary fibre are still being investigated but high-fibre diets have been shown to increase stool bulk, decrease large bowel transit time and reduce the luminal pressure in the sigmoid colon (Burkitt et al, 1972, Findlay et al, 1974). The increased stool mass is due to a combination of increased stool water content, a greater bacterial mass within the stools and the weight of the fibre itself. Fibre may actually slow down the passage of food in the stomach and result in slower transmission of gastric contents to the small bowel (Holt et al, 1979). Pectins and gums increase the viscosity of intestinal contents so will slow down the absorption of nutrients in the small bowel (Royal College of Physicians Report, 1982). However, fibre decreases the overall intestinal transit time as a result of its effect on the large bowel. Virtually all the dietary fibre reaches the caecum unchanged and lignin and most of the cellulose passes through the large bowel unaltered. The remaining cellulose and most of the hemicelluloses and pectins are broken down by bacterial fermentation in the colon. Some of the breakdown products are then absorbed (Eastwood & Passmore, 1983).

The importance of fibre in so-called western diseases was realised initially by Cleave (1941) during his war service as a

ship's surgeon when he reported that miller's bran was effective in relieving the constipation of men at sea. Later he, Burkitt, Trowell, Painter, Walker and others, with their experience in developing nations, developed their hypothesis that many diseases seen almost exclusively in western communities : diabetes mellitus, ischaemic heart disease, obesity, colonic disorders such as diverticular disease and cancer of the large bowel, varicose veins and other venous disorders and gallbladder disease; were due to the fibre-depleted diets in these communities (Cleave, 1974, Burkitt, 1969, Walker, 1974, Burkitt & Trowell, 1975). There are two inter-related aspects to the fibre hypothesis. As diets become fibre-depleted as a result of refining cereals, increasing amounts of refined sugars, especially sucrose, are consumed and both these changes may be important in contributing to the prevalence of western diseases. With increasing affluence, dietary changes common to all industrialized countries can be summarised as an increase in the consumption of animal products and a decrease in the consumption of complex starches (see fig. 1.3). Dietary fibre consumption from cereals fell in Britain from 12g daily in 1880 to 8g in 1970 although the total dietary fibre intake did not change significantly because the cereal fibre decrease was offset by an increase in the fibre from vegetables and fruits (Robertson, 1972, Southgate et al, 1978). The changes were predominantly those of a marked decrease in wheat flour and potato intake (Trowell, 1976), with an increase in the refinement of flour (Robertson, 1972). In America, the total intake of crude fibre has declined by 28% from 1909 to 1975 because the vegetable and fruit intake did not increase to compensate for the reductions in cereal and potato

intake (Heller & Hackler, 1978). According to Cleave (1974) though, most of the cereal fibre in the diet of the United Kingdom was lost by the year 1800 when white bread had finally reached all but the poorest homes. The situation was different in the United States where maize meal constituted a large part of the usual fibre intake and its consumption did not fall off markedly until some 100 years later (Cleave, 1974).

During the second world war, food rationing in Britain resulted in considerable dietary changes. Cereal fibre intake with compulsory National flour doubled during the war and remained higher in post-war years compared with both pre-war intake and intake in the years following removal of National flour in 1953 (Southgate et al, 1978, Trowell, 1976). Sugar and fat supplies fell during the war and immediate post-war years (Cleave, 1974). Trowell (1974b) demonstrated a negative correlation between cereal crude fibre supplies and deaths due to diabetes mellitus during the war years. The death rates rose again with the phasing out of National flour. Painter (1969) demonstrated similar trends with diverticular disease mortality.

Thus, there is considerable circumstantial evidence of an association between fibre-depleted diets and the prevalence of gallstones and other western diseases. However, specific metabolic and dietary intervention studies looking at the effect of dietary fibre on bile composition are inconclusive. Studies of the faecal output of bile acids and plasma cholesterol concentration in relation to amounts of dietary fibre have given inconsistent results. One epidemiological survey reported that faecal steroid excretion and especially bile acid excretion was lower in

Africans, Indians and Japanese than Britons and Americans (Hill et al, 1971) yet another reported that bile acid excretion was similar in rural Finns and urban Danes despite large differences in dietary fibre intake and stool weight (IARCIMG, 1977). Adding wheat bran to a normal western diet did not increase faecal bile acids in six studies (Eastwood et al, 1973, Findlay et al, 1974, Jenkins et al, 1975, Pomare et al, 1976, Raymond et al, 1977, Walters et al, 1975) yet increased them by 40% in a study by Cummings et al, (1976).

Changes in biliary lipid concentrations after the addition of extra fibre to the diet have also been reported. Pomare et al (1976) fed six subjects (2 with radiolucent stones) an average of 57g of wheat bran daily for four to six weeks and demonstrated a modest decrease in molar percentage of cholesterol and the saturation index. Few other studies have been carried out to either substantiate or refute the above findings.

Pomare and Heaton (1973) reported changes in the proportions of bile acids in five subjects fed bran for six to 10 weeks. The amount of deoxycholic acid decreased by 50% and this was compensated for by an increase in the chenodeoxycholic acid levels so that the total bile acid pool size remained unchanged. These findings were confirmed in a later study by the same group (Pomare et al, 1976) and by Wicks et al (1978). However, Watts et al (1978) and MacDougall et al (1978) were unable to demonstrate any increase in chenodeoxycholic acid levels to accompany the decrease

in deoxycholic acid.

Oral chenodeoxycholic acid feeding results in a decrease in the saturation index of bile (Thistle & Schoenfield, 1971) and is, therefore, used in the medical treatment of gallstones. Deoxycholic acid feeding results in an increase in bile lithogenicity as a result of its negative feedback action on bile acid synthesis from cholesterol (Low-Beer & Pomare, 1975). If the relative proportions of the bile acid pool were altered in favour of an increase in chenodeoxycholic acid then a decrease in the lithogenicity of bile would be expected to occur. Deoxycholic acid is formed in the colon by bacterial dehydroxylation of any cholic acid that has not been reabsorbed in the terminal ileum. The deoxycholic acid is absorbed in the colon and enters the bile acid pool. If dietary fibre alters the bacterial metabolism of cholic acid by either adsorbing it and preventing bacterial breakdown or adsorbing deoxycholic acid and preventing its absorption, then fibre may protect against the formation of gallstones. Low-Beer and Nutter (1978) decreased the anaerobic bacterial flora by administering metronidazole to 11 men. A concomitant decrease in biliary cholesterol saturation was demonstrated. The proportion of deoxycholic acid in the bile acid pool decreased from 24% to 7% while chenodeoxycholic acid increased from 33% to 46%.

The colonic action of fibre may be mediated by its effect on the pH of the colon. Bile acid dehydroxylation is inhibited at low pH values and in a study of lactulose administration, which results in a lowering of colonic pH, chenodeoxycholic acid proportions rose while deoxycholic acid proportions fell (Thornton

& Heaton, 1981). The authors claimed that the effects of lactulose resemble those of wheat bran as both are partially metabolised by colonic bacteria to acids. However, in a later study Thornton et al (1983) were unable to show any differences in the rates of primary bile acid synthesis and the total and individual bile acid amounts in 13 gallstone subjects when they were fed a refined carbohydrate diet and an unrefined carbohydrate diet for six weeks each. The cholesterol saturation index, though, was significantly reduced in all but one subject when on the unrefined diet. The alteration in the saturation index could only be explained in terms of a decrease in the secretion of cholesterol.

The mechanism by which fibre reduces the saturation index of bile, if it does so at all, is uncertain. Most studies have indicated that there are changes in the total amounts and/or proportions of bile acids in the bile acid pool but the particular changes reported are inconsistent. One of the most recent studies (Thornton et al, 1983) indicated that fibre may not affect the bile acids at all and that the reduction of biliary saturation index by unrefined diets may be by a decrease in cholesterol secretion. This study differed from previous studies, though, in that it did not supplement the diets with a particular form of fibre but compared the effects of an unrefined carbohydrate diet (wholegrain products and unlimited fruit and vegetables) with those of a refined carbohydrate diet (refined sugar, white flour and white rice). Subjects, therefore, not only ate substantially more fibre but substantially less sugar and less total energy on the unrefined diet. The observed difference in the saturation index could have been due to the different intakes of total

calories or refined sugar. Weight loss occurred on the unrefined diet but not to an extent that it could account for the decrease in the saturation index.

Thus, metabolic studies seem to provide some support, if inconsistent, for the beneficial effect of fibre that descriptive studies have indicated. The mechanism of this beneficial effect is even less certain. Case-control studies do not add any more information to that which is already known. Very few investigators even measured the fibre intake of the participants. Smith and Gee (1979) reported that crude fibre intake was significantly lower in female cases compared with the controls and Sarles et al (1969) noted that fruit and vegetable consumption was very much lower in the cases than the controls. Fibre was negatively associated with the development of gallstones in both sexes in the recent study by Scragg et al (1984a) but this association was not significant after multiple logistic regression analysis.

In summary, the depletion of complex starches and fibre in the western diets of the last 100-200 years may have been an important influence in the increasing prevalence of gallstones in developed nations. Perhaps it is not possible, however, to separate the effects of fibre depletion from increased simple sugar consumption on the composition of bile and it might be more useful to consider the two elements as one factor. The inconsistency of the metabolic study results and the lack of useful data from the case-control studies would appear to support this view.

#### **4. Refined Carbohydrate**

Just as total calorie intake is closely correlated with total

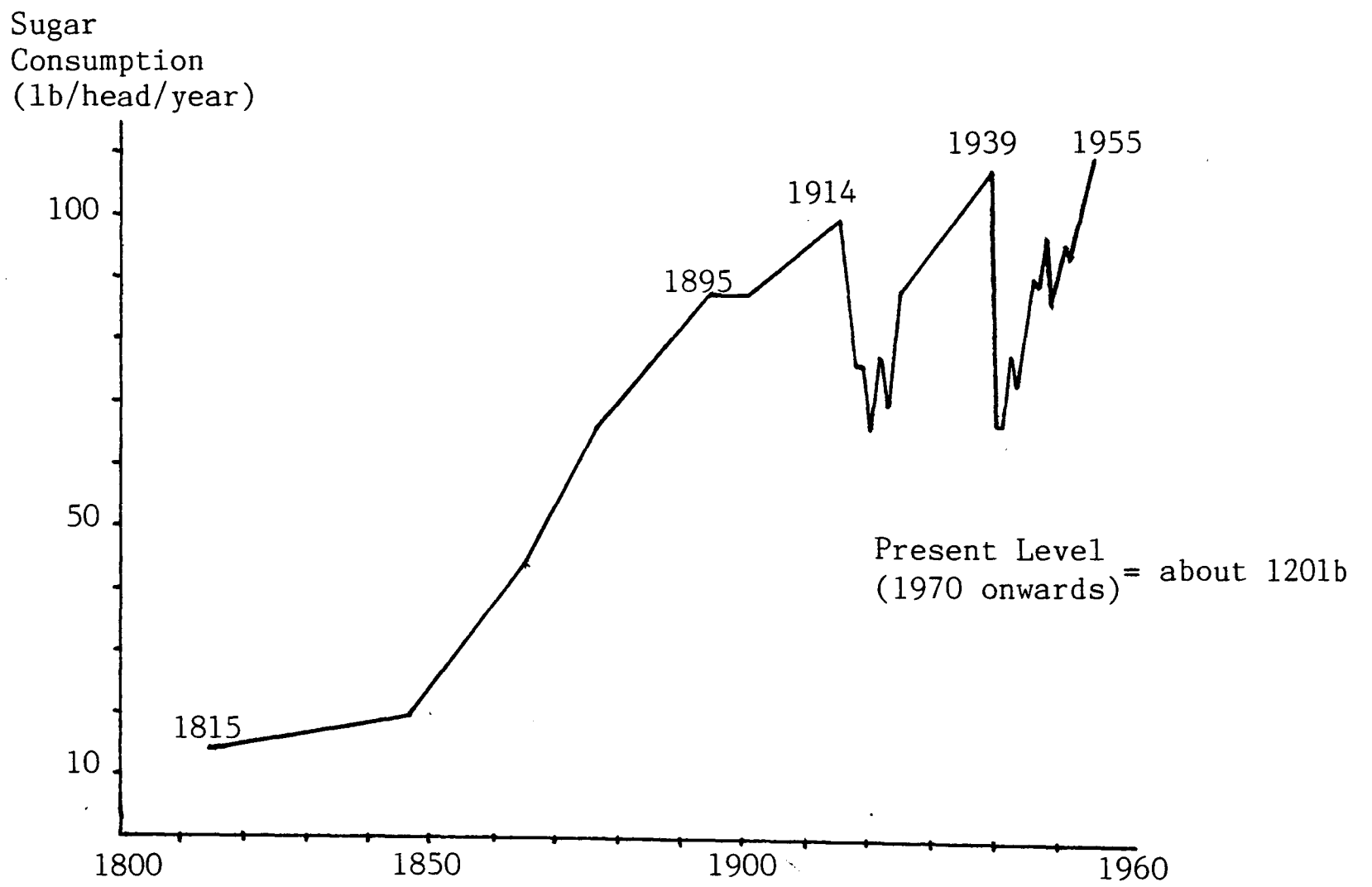


Fig. I.5 Sugar Consumption in the United Kingdom  
(from Cleave, 1974, page 7)

fat intake, fibre intake is very closely negatively correlated with refined carbohydrate intake, particularly sucrose (Thornton et al, 1983). Fibre may indeed have an effect on the large bowel, preventing bacterial metabolism of primary bile acids or subsequent absorption of the less active secondary bile acids but the refined carbohydrates may have a significant influence also. The rise in sugar consumption in the United Kingdom has been massive over the last 150 years (Cleave, 1974). This rise is illustrated in figure I.5.

The proposed mechanism of action of refined carbohydrates on bile was put forward initially in relation to the aetiology of diabetes mellitus (Cleave, 1974). Refined carbohydrate is quickly available for absorption as little molecular breakdown is necessary before disaccharides are produced and ready for absorption. Rapid absorption results in relatively higher blood glucose peaks and this stimulates the pancreas to secrete relatively more insulin. Diets with sucrose levels increased at the expense of complex carbohydrates result in increased plasma insulin levels (Reiser et al, 1981). Bennion and Grundy (1977) showed that gallbladder bile was significantly more saturated in Pima Indian diabetic subjects when they were on regular insulin therapy than when they had uncontrolled hyperglycaemia. Bile acid production was decreased during the insulin therapy period. Hyperinsulinaemia may, therefore, be associated with an increase in biliary cholesterol saturation.

Case-control studies have yielded the usual inconsistent results for the importance of refined carbohydrates and specifically sucrose in the pathogenesis of gallstones. Reid et al

(1971) reported a slightly lower intake of sucrose and starch by cases compared with controls but these differences were not significant. Italian immigrant women decreased their total carbohydrate intake in their adoptive country, Australia, but increased their simple sugar intake in the form of soft drinks, biscuits and canned fruit (Loftus Hills, 1971). These women showed a four-fold increase in the prevalence of gallstones after emigration. The most definite evidence implicating sugar as a positive risk factor has been reported in another Australian study (Scragg et al, 1984a). In this study sugar in drinks and sweets was associated with an increased risk of gallstones in both men and women. The same group has also demonstrated a positive association between fasting plasma insulin levels and gallstones (Scragg et al, 1984c). Animal studies lend support to the possible association between high refined carbohydrate intakes and gallstones. A fat-free, high glucose diet produced gallstones in young hamsters (Dam, 1971). If rice starch was substituted for glucose as the carbohydrate source the ability of the hamsters to form gallstones was almost completely lost.

In a recent study Werner et al (1984) investigated the effects of dietary sucrose on the bile of 12 subjects with gallstones. Earlier, the same group had evaluated the effects of an unrefined carbohydrate diet compared with a refined carbohydrate diet in biliary lipid composition (Thornton et al, 1983). The later study compared diets containing 112g and 16g respectively of refined sucrose in the form of a patty, with free access to other foods being permitted. Energy intake was 24% higher on the high sucrose diet but the bile composition with

respect to bile acid, cholesterol and phospholipid proportions was unchanged over the six week dietary period. Whereas the earlier study implicated simple sugars rather more than fibre depletion in the pathogenesis of supersaturated bile, the later study showed that a high sugar content did not adversely affect the saturation index of bile after six weeks. The subjects did put weight on during the high sucrose period supporting the theory that sucrose is important in the causation of overnutrition and obesity (Cleave, 1974).

The importance of dietary carbohydrate and its relative degree of refinement in the formation of gallstones remains unresolved. The effects of the two inter-related factors, an increased sucrose intake together with a decreased fibre intake, are very difficult to separate. The apparently conflicting results of the two metabolic studies above, Thornton et al (1983) and Werner et al (1984), may indicate the importance of investigating unrefined and refined carbohydrates in combination rather than separately.

## **5. Alcohol**

Unlike other nutrients, the results for alcohol intake are much more consistent. The consumption of alcohol has been associated with a decreased risk of developing gallstones in a number of studies.

In case-control studies the association was first reported by Friedman et al in 1966 when it was noted that people in the Framingham study who consumed little or no alcohol ran a higher risk of subsequent gallbladder disease than moderate drinkers.

Wheeler et al (1970) supported this finding. Sarles et al were unable to show any significant difference in the alcohol intake of cases compared with the controls in their first study (1957) but showed a protective effect in their later and larger study (1969). Scragg et al (1984a) reported a substantially reduced risk associated with a low alcohol intake compared with total abstinence. An inverse relationship between alcohol use and hospitalizations for cholelithiasis has been demonstrated (Klatsky et al, 1981).

The protective effect of alcohol was predicted on a metabolic basis by Thornton et al (1981) when they demonstrated an inverse correlation between plasma high density lipoprotein cholesterol concentration and biliary cholesterol saturation. Petitti et al (1981) had earlier reported a decreased risk of gallbladder disease in association with increased levels of high density lipoprotein cholesterol and alcohol has been shown to increase serum high density lipoprotein cholesterol levels (Yano et al, 1980). Studies of the direct metabolic effect of alcohol further support its protective effect. The basal collection by duodenal aspiration of bile salts was significantly higher in alcoholic patients than in normal controls suggesting increased formation of bile acids in response to chronic alcohol ingestion (Marin et al, 1973). In a sterol balance study the total sterol excretion in two out of three hyperlipidaemic patients was similar when taking ethanol to the period of abstinence but the proportion of bile acids to neutral steroids in faecal steroids was increased in all three when on ethanol (Nestel et al, 1976). This difference could either result from a decrease in bile acid reabsorption or from an

increased conversion of cholesterol to bile acids and the second alternative would explain the reduction in the saturation index of bile. In an experiment involving mature pigs ethanol substituted for sucrose did not alter the total faecal steroids but both bile acids and the ratio of bile acids to neutral steroids were increased (Topping et al, 1982). The most persuasive evidence so far for the protective effect of alcohol has been published recently (Thornton et al, 1984). In this study 12 healthy volunteers who normally drank very little alcohol were investigated for biliary lipid changes when on a six week period of a daily intake of half a bottle of wine followed by a six week period of complete abstinence. The biliary saturation index fell significantly during the period of alcohol consumption and rose again during abstinence. The high density lipoprotein cholesterol concentration was inversely related to the biliary cholesterol saturation. The authors suggested that the most likely mechanism of action of alcohol is hepatic enzyme induction.

All the above studies are positive for moderate alcohol intakes only and similar effects may not occur with higher intakes. In the case-control study by Scragg et al (1984a) the substantial decrease in relative risk associated with a low alcohol intake became much smaller with higher alcohol intakes.

Thus, alcohol in moderate quantities does seem to exert a protective effect on the development of gallstones by reducing the saturation index of bile. The mechanism of this decrease is not known but hepatic enzyme induction may be involved.

In summary then, the individual effects of dietary nutrients

in the formation of gallstones are ill understood despite a large number of studies, both metabolic and clinical, that have set out to investigate specific nutrients and their metabolic effects. Diet does appear to play an extremely important role in the pathogenesis of gallstones as evidenced by the wide international differences in gallstone prevalence particularly between developed and developing nations. The descriptive surveys which have demonstrated these variations have led to the formulation of specific dietary hypotheses such as the refined carbohydrate hypothesis of Cleave, Burkitt and Heaton which outline very plausible mechanisms for the increased formation of gallstones secondary to dietary changes. Unfortunately, metabolic and case-control studies provide little in the way of confirmatory evidence for the hypotheses. In western communities one of the major problems that researchers face in trying to determine the importance of dietary changes in gallstone pathogenesis is the threshold effect. Western societies, as a result of their affluence, consume a diet that is very high in fats (particularly animal fats), refined carbohydrates and total calories. This is true of most individuals within the societies and it may be impossible now to demonstrate any differences between the intakes of diseased individuals and healthy individuals. Virtually the entire population may be above the threshold at which differences could still be detected. For this reason it is important to study groups of individuals within western societies who differ in their dietary habits such as vegetarians. Perhaps these groups do not consume fats and refined carbohydrates above the threshold levels.

### 3.2.6 Pregnancy

When Naunyn published his work on gallstones in 1896 he relied heavily on the findings of his student, Schroder, who had analysed the autopsy series of Von Recklinghausen. In the analysis, Schroder had calculated the number of women with gallstones who had had at least one pregnancy and found it to be exactly 90% (99 out of 110 women). This was the first analysis of its kind and was accepted without criticism by Naunyn despite the fact that no attempt had been made to determine the proportion of gallstone-free women who were also parous. Osler adopted this figure for the later editions of his text as did William Mayo (1911) and many other authors throughout the first half of this century. Thus it was a well accepted risk factor associated with the development of gallstones without ever having been statistically proven in the first place. A few autopsy surveys carried out during this period looked carefully for evidence of a positive association of pregnancy with gallstones and were unable to find one. Gross (1929) reported that 89.8% of women with gallstones in her survey in Leeds were married compared with 86.6% without gallstones. Robertson and Dochat, in a meticulous review of pregnancy and gallstones in 1944, came to the conclusion that pregnancy could not account to any great extent for the increased prevalence of gallstones in women compared with men. In a combined series of autopsy studies, where they grouped the results of their autopsy survey with those of a number of other authors, 79.6% of women with gallstones were parous and they estimated that about 79.2% of the population of women become pregnant at some stage. Unfortunately the compiled series was not separated according to

race and a large proportion were black and more likely to be parous. As the negro race is less likely to have gallstones (Ludlow, 1937, Lieber, 1952, Cunningham & Hardenbergh, 1956) the results could be quite inaccurate.

The studies carried out more recently do not provide any confirmatory evidence either despite the fact that parity is widely believed to be a risk factor. Major textbooks claim that gallstones are commoner in multiparous women than nulliparous women (Sherlock, 1981). There are a number of studies which have found no evidence to support this claim. Dessau (1943) showed a marked rise in the prevalence of gallstones after the age of fifty and claimed that this did not support the view that pregnancy was a risk factor. Likewise, Joske et al (1954) reported that parity had little influence on the development of gallstones. Smith & Gee found no association of pregnancy with gallstones in their case-control study (1979). There are more studies which report a positive association of gallstones with pregnancy. These studies include the Framingham study (Friedman et al, 1966) which showed that women with clinical gallbladder disease had had more pregnancies than women without evidence of clinical disease. They also showed a modest overall trend of increasing risk with increasing pregnancies. The statistical significance of this trend, though, was not very strong. In clinical survey by Wheeler et al (1970), parous women were more likely to have presented to hospital with symptomatic disease than nulliparous women. A highly statistically significant association ( $p < 0.005$ ) of parity with clinical gallbladder disease was reported in an Appalachian community (Richardson et al, 1973). In the large study

by Layde et al (1982), a trend was reported for increasing parity and surgically confirmed gallbladder disease but this was not significant when standardised for other risk factors.

Two important factors need to be taken into consideration when considering the effect of parity on the development of gallstones. Firstly, the age of the study population may influence the significance of the results. Horn reported in 1956 that there appeared to be a transitional age when the effect of parity changed. In his autopsy series, gallstones occurred more commonly in parous women up to the age of 50 after which nulliparous women had more gallstones. In the clinical study by Bernstein et al (1973), women with a history of gallbladder disease had a significantly higher average number of live births than women without such a history. This result was found in each age group from 30 to 62 but the relationship was strongest in the youngest age group. In Chippewa Indian women, pregnancy was associated with an increased risk of gallstones, especially in women less than 30 years old (Thistle et al, 1971). In a recent case-control study (Scragg et al, 1984b), the risk of developing gallstones was shown to increase in association with increasing parity, particularly among younger women. The risk was also negatively correlated to the age at first pregnancy, independent of parity. These results suggest that there may be a group of women who are susceptible to the early formation of gallstones and are selected out. The effect of parity, therefore, cannot be detected in the older, less susceptible age groups.

The other influence that appears to be even more important than that of age is the difference between asymptomatic and symptomatic

gallbladder disease. Virtually all the studies that found a positive relationship between gallbladder disease and parity were clinical studies of people who had either undergone surgery for symptomatic gallstones or had had some diagnostic procedure to confirm the presence of gallstones after complaining of symptoms. Autopsy surveys provide conflicting but usually negative evidence for the effect of parity on the development of gallstones. In their review, Robertson and Dochat (1944) quote several authors who concluded that, although gallstone colic may first be noted during or shortly after pregnancy, it did not prove that pregnancy predisposed to the development of gallstones. In a careful autopsy study and review by Van der Linden in 1961, his results showed a positive association between parity and symptomatic gallbladder disease. This association was not seen in women with atypical symptoms of gallstones and asymptomatic stones and the author concluded that parity did not influence the development of gallstones, only the development of symptoms associated with gallstones. Studies of American Indians illustrate this effect well. In 1962 Sievers and Marquis reported that early and frequent childbearing was associated with an increased risk of developing gallstones in Indian women of the southwestern areas of the United States although no figures were given in evidence of this claim. Their cases were derived from both surgical and autopsy data so included some asymptomatic cases. A clinical study of the Pima Indians supported the association (Comess et al, 1967) but a later prevalence survey of the same tribe using oral cholecystography failed to show any association (Sampliner et al, 1970). It is evident that prevalence surveys are necessary to be able to

determine the nature of the association of parity with gallstone risk as asymptomatic cases will also be included. As these studies are still few in number it is difficult to draw any conclusions from their results. In the ultrasonographic study of female office workers in Rome (GREPCO, 1984), the mean age-corrected number of pregnancies was significantly higher ( $p < 0.001$ ) in women with gallstones. There was a slight dose-response effect seen as women with three or more pregnancies had a higher risk than women with one or two pregnancies. A smaller ultrasonographic survey of people aged 55-69 in a Tyrolean village showed no effect of parity on the prevalence of gallstones.

Metabolic studies do not provide consistent results either. One study has shown that there is incomplete emptying of the gallbladder after eating in the second and third trimester of pregnancy (Braverman et al, 1980). Gallbladder stasis may result and could influence the growth of gallstones provided that supersaturation and crystal nucleation has already occurred. Increased bile saturation during pregnancy has been recently reported associated with a decrease in the amount of chenodeoxycholic acid in the bile (Everson et al, 1982).

Thus, epidemiologic and experimental evidence for an association between pregnancy and the new formation of gallstones is weak. More information on the changes in biliary lipid composition and gallbladder function during pregnancy is needed to determine possible causality as well as prevalence surveys of asymptomatic populations to determine the strength of the relationship.

### **3.2.7 Drugs**

Gallstones can be labelled an iatrogenic illness when they develop as a consequence of drug treatment. Some drugs have been fairly convincingly determined as playing a causative role in the formation of gallstones while the situation is much less clear for others. Certain drugs such as the oral contraceptive pill are taken by enormous numbers of people so it is important to determine the risk associated with the drug while others are prescribed for uncommon conditions so are not as important in risk calculations. The more important drug groups will be discussed below.

#### **1. Oral Contraceptives and Exogenous Oestrogens**

The first report to identify an association between oral contraceptive use and gallstones was published in 1973 (Boston Collaborative Drug Surveillance Programme, BCDSP, 1973). 31% of women with symptomatic gallstones or a history of cholecystectomy compared with 20% of the controls had used a form of oral contraceptive pill in the three months prior to hospital admission. The relative risk of developing gallstones for women who used the oral contraceptive pill was calculated to be 2.0. In the following year, similar findings for the effects of hormone replacement therapy were published by the same group (BCDSP, 1974). The relative risk estimate for conjugated oestrogen therapy was 2.5. In both studies the result was highly statistically significant and was not confounded by other risk factors such as age or obesity. The oral contraceptive users appeared to have a greater risk of developing symptoms after six to 12 months of use

compared with longer term use although the risk was still increased for long-term users compared with controls. Another study carried out around the same time reported that 32% of women undergoing biliary tract surgery had used the oral contraceptive pill compared with 19% of the controls (Stolley et al, 1975). It also yielded a relative risk of 2.0.

These earlier studies were supported further by the evidence available from metabolic studies. 22 healthy women were studied during the routine use of oral contraceptives and also when on no medication (Bennion et al, 1976). Gallbladder bile was significantly more saturated with cholesterol during oral contraceptive treatment than during normal menstrual cycling. The proportion of chenodeoxycholic acid in the bile acid pool decreased in favour of cholic acid during the treatment period. Lynn et al (1973) reported that oestriol administration to female rhesus monkeys decreased the secretion of bile acids while Nestel et al (1965) showed that ethinyl oestradiol increased the cholesterol turnover in humans which implies an increase in cholesterol excretion into bile. The findings of decreased daily synthesis of bile acids along with an increase in biliary cholesterol levels suggests that both mechanisms may be important in the possible causation of gallstones by the oral contraceptive pill.

The well known observation that gallstones are about twice as common in women as men fits in well with the above results as the possible adverse biliary effects of oestrogen would explain the difference in prevalence between the sexes. Oestrogen was administered to a proportion of men who had had a myocardial

infarction in the Coronary Drug Project (The Coronary Drug Project Research Group, 1977). Men who were treated with oestrogen had a significantly greater incidence of gallstones than either the control group or the placebo group. However, more recent studies do not necessarily confirm the above findings. In 1974, a preliminary report of the Royal College of General Practitioners' Oral Contraception Study showed that current pill-users were 1.32 times more likely to develop symptomatic gallstone disease than controls (Royal College of General Practitioners, RCGP, 1974). The result was not statistically significant but was used to support the earlier studies' findings. This association was even less significant when the results of the full study were published in 1982 (RCGP, 1982) and the relative risk was calculated as 1.12. When the figures were analysed for duration of contraceptive use it was evident that there was an initial rise in incidence during the first four years of use followed by a decline over the next six years to a rate below that of the controls. The authors concluded that there was a positive association between oral contraceptive use and gallbladder disease that diminished with long-term use. They also pointed out that if this was the case then oral contraceptives do not probably cause the formation of new gallstones but encourage the development of symptoms associated with previously asymptomatic gallstones. Another study, which had earlier published evidence of a positive effect of oral contraceptives, reported similar findings to the RCGP study (Layde et al, 1982). In this study the use of oral contraceptives had little overall effect on the risk of surgically confirmed gallbladder disease. After standardisation, ever-users of the oral

contraceptive pill had a 26% greater incidence of gallbladder disease than never-users but this difference was not significant. Analysis of duration of use in this study was not possible because of the small numbers of women who had used oral contraceptives for short periods but long-term use was not associated with any deleterious effects. Their earlier findings of a positive effect, though, support the hypothesis that oral contraceptive use may select out susceptible people early on in the course of their oral contraceptive use. Long-term users would, therefore, not have any increased risk above that of the never-users. Scragg et al (1984b) reported an age dependent variation in the risk associated with oral contraceptive use. The risk was greatest for women aged 29 years or less and decreased to below unity for all other age groups. However, their numbers were very small in these groups and were not significant yet the authors conclude that there is a subpopulation of women who are susceptible to the formation of gallstones and who are likely to develop stones soon after exposure to oral contraceptives.

It is important to note that the above clinical studies analysed the effects of oral contraceptives on the prevalence of clinically apparent gallbladder disease. More accurate analysis is possible from data on asymptomatic populations that have been screened for evidence of gallstones. The Roman study (GREPCO, 1984) reported no significant difference between the percentage of women using the oral contraceptive pill aged between 20 and 49 years with gallbladder disease (39.5%) and those without (36.2%). No data was given on the risks of short-term use compared with long-term use. Autopsy studies are not as useful as screening

surveys in determining the importance of oestrogens in the causation of gallstones but they have the advantage of including asymptomatic cases. In an autopsy study of men who had previously had oestrogen (diethylstilboestrol) treatment for carcinoma of the prostate, gallstones occurred with equal frequency regardless of oestrogen treatment yet cholecystectomy was about nine times as likely to have occurred in men who had had oestrogen therapy as opposed to orchidectomy or placebo (Everson et al, 1982). These results provide evidence that oestrogen may increase the likelihood of gallstones causing symptoms as opposed to actually causing the development of stones.

Metabolic studies continue to supply evidence that oral contraceptive preparations increase the lithogenicity of bile and affect gallbladder function but agreement about what is the cause of the increased bile lithogenicity or the importance of gallbladder function in the possible effect of oral contraceptives is lacking. Eight healthy young women, who had taken an oral contraceptive pill for some time before the investigation, showed significantly higher levels of biliary saturation index compared with the controls (Kern et al, 1982). The rate of cholesterol secretion was 50% greater in the oral contraceptive group and the rate of bile acid secretion was reduced. In an attempt to separate the effects of the two components of oral contraceptives, Down et al (1983) studied the effects of ethinyloestradiol individually and with norgestrel in combined preparations on the degree of biliary cholesterol saturation. No change was noted with preparations of ethinyloestradiol alone or ethinyloestradiol with norethisterone but a significant increase in the saturation index

was observed in combined preparations of norgestrel with ethinyloestradiol. The authors concluded that the progestagen component of oral contraceptives was responsible for biliary lipid changes not the oestrogen component. Exogenous progestin significantly impaired both gallbladder filling and emptying in a recent study by Shaffer et al (1984) and the authors felt that this could predispose to the formation of gallstones. Their findings did not agree with those of Braverman et al (1980) who found that contraceptive steroids did not affect the rate of gallbladder emptying and residual volume but the latter study used ultrasonography to measure gallbladder function and this technique may not be as accurate as the cholescintigraphy technique employed by the former.

In summary, the effects of the oral contraceptive pill on biliary lipids and gallstone prevalence is not certain. Metabolic studies show that biliary cholesterol concentration appears to be increased by the administration of contraceptive steroids but which component causes this increase and the mechanism by which it does so is not clear. Studies of gallstone prevalence are even less definite but researchers seem to have come to the conclusion that oral contraceptives may increase the risk of developing gallstones in those who are susceptible to their development anyway. These women will develop the stones soon after exposure so studies will show an effect in either short-term users or younger women. As the affected women would probably have developed stones at some later time anyway, the long-term overall effect of oral contraceptives will be negative. The effects of oestrogen replacement therapy have rarely been studied and cannot be

usefully summarised.

## **2. Lipid-Lowering Drugs**

Clofibrate (Atromid) is used widely in the treatment of hypercholesterolaemia as it decreases the concentration of serum cholesterol by increasing hepatic cholesterol excretion in hyperlipidaemic patients (Grundy et al, 1972). The net effect is a mobilization of cholesterol from tissue stores and, as the excretory pathway, bile becomes relatively more lithogenic. The biliary effects were shown in a study of seven patients given clofibrate where the biliary cholesterol levels were raised and the bile acid levels were reduced (Pertsemlidis et al, 1974). The authors predicted an increased incidence of cholesterol gallstones in patients on long-term clofibrate treatment. This prediction prompted other researchers to investigate the effects of clofibrate on large populations of people treated with the drug over long periods. The Coronary Drug Project Research Group (1977) were able to study 8341 men who had been recruited to the Coronary Drug Project. Participants were either treated with clofibrate, oestrogen, thyroxine, nicotinic acid or a placebo in an evaluation of post-myocardial infarction treatment. Incident cases of gallstones were determined from clinical reports of symptomatic disease and found to be significantly ( $p < 0.05$ ) higher in the clofibrate group compared with the placebo group. The prevalence of surgically confirmed gallbladder disease in another large trial was also increased in the clofibrate treatment group compared with controls (Cooper et al, 1975). The authors in both studies admitted to the possibility that clofibrate may have induced

symptoms from previously formed stones rather than inducing gallstone formation. These findings suggest that clofibrate may increase biliary cholesterol secretion by mobilizing peripheral body stores and perhaps lead to increased gallstone formation.

Bile acid sequestrants such as cholestyramine and colestipol are used as lipid-lowering agents because they are anion-exchange resins and bind bile salts in the intestines, preventing reabsorption of the bile salts. The reduced bile acid pool leads to enhanced conversion of cholesterol to bile acids in the liver (Shaffer & Small, 1976). Dietary cholesterol absorption is also decreased as a result of decreased bile acid availability in the gut. Theoretically this depletion of bile acids can lead to increased cholesterol saturation of bile but, in practice, this rarely happens (Sarles et al, 1970b, Wood et al, 1972), presumably because the compensatory hepatic synthesis of bile acids is always sufficient to prevent cholesterol supersaturation of bile.

### **3. Other Drugs**

Phenobarbitone has been suggested as a protective factor against the formation of gallstones by either reducing the saturation index of bile or dissolving gallstones (Coyne et al, 1975). Their study of chenodeoxycholic acid and phenobarbital treatment, individually and in combination, in 36 patients with asymptomatic radiolucent stones showed that phenobarbital significantly reduced biliary cholesterol saturation. However, gallstone size was not shown to decrease in those patients treated with phenobarbital alone. The protective effect of phenobarbital has been disputed by Bateson and Bouchier (1975) as analysis of

their autopsy series showed a greater number of epileptics with gallstones was found than expected. Four of the 10 cases had been treated with long-term phenobarbitone.

Thiazide diuretics have been associated with an increased risk of acute cholecystitis (Rosenberg et al, 1980, Van der Linden et al, 1984). Rosenberg et al identified a possible risk in a case-control study of lifetime drug histories when subjects who had taken thiazide diuretics in the month before admission were found to have twice the incidence of acute cholecystitis. Their findings were not confirmed in a similar study (Porter et al, 1981). Van der Linden et al estimated a relative risk of 2.1 for developing acute cholecystitis in patients who had purchased thiazides in the year before admission compared with those who had not. No significant risk was associated with past purchase of thiazides and Rosenberg et al (1980) who found that the relative risk dropped to unity only one month after thiazides had been stopped. The finding of such a quick reversion to normality after thiazide cessation implies that gallstones themselves are not induced by thiazide therapy but that thiazides increase the risk of acute cholecystitis developing in a patient with gallstones. No association between thiazide use and surgically treated gallbladder disease had been noted in the Boston Collaborative Drug Surveillance Programme (Porter et al, 1981).

It is conceivable that other drugs may be associated with the development of gallstones as any drugs that alter the normal hepatic cholesterol and bile acid synthetic pathways or gallbladder function may increase the tendency to form gallstones.

### 3.2.8 Associated Clinical Conditions

A large number of clinical conditions have been associated with gallstones at one time or another. Some conditions have been thought to be causative while others were considered to be complications of gallstones. The same confusion and controversy surrounds clinical associations with gallstones as surrounds dietary and drug associations. There are some conditions which are undoubtedly caused by or which predispose to gallstones but these confirmed risks or complications are in the minority. Failure to allow for age, sex parity, race, diet and obesity combined with observations being made on selected populations at autopsy have led to a wide variety of claims for associations with gallstones, many being inaccurate and ill-founded (Kaye & Kern, 1971). These claims include tuberculosis (Naunyn, 1896), pernicious anaemia as a protective condition (Lieber, 1952) and goitre (Kozoll et al, 1959). The discovery of the clinical co-existence of multiple gastrointestinal diseases is often due to a thorough gastrointestinal examination which reveals otherwise clinically silent disorders. The more definite associations along with several tenuous ones will be discussed below.

#### 1. Ileal Disorders

Bile salts undergo most of their active reabsorption in the terminal section of the ileum and any disruption to this portion of the small bowel will affect the enterohepatic circulation of bile salts. Disruption can decrease the bile acid pool to a level that hepatic compensation is unable to cope with and the bile becomes supersaturated with cholesterol. Significant bile salt

malabsorption can occur with extensive small bowel loss which is a consequence of a number of conditions. In 1969 Heaton and Read reported on the gallstone findings of 72 patients with ileal disorders. All had had a surgical resection of at least 12 cm of the terminal ileum after long-standing disease, usually Crohn's disease, and gallstones were found in 31.9% which was four to five times higher than the prevalence found in an autopsy survey carried out in Norway a few years previously (Torvik & Hoivik, 1960). The prevalence also increased with increasing duration of the disorder. Their findings were supported by a later study. Patients with regional enteritis were found to have a high prevalence of gallstones (34%) in a study by Cohen et al (1971). Confirmatory metabolic evidence of the disruptive effect of ileal resection was published in the following year when nine out of ten patients with ileal dysfunction were shown to have bile that was supersaturated with cholesterol (Dowling et al, 1972). The report does not say if the one subject who did not have supersaturated bile was also the only subject with ileal dysfunction who had not had an ileal resection. At this stage researchers were not certain that the increased risk of developing gallstones in Crohn's disease was secondary to resective surgical treatment or whether Crohn's disease, per se, was associated with an increased risk. In one study of patients with Crohn's disease, only those patients who had had an ileal resection showed cholesterol supersaturation of the bile (Smith et al, 1973). In a later study Marks et al (1977) confirmed the finding of increased biliary cholesterol saturation in patients with Crohn's disease and ileal resection but they also found a similar increase in Crohn's patients without

an ileal resection. No correlation between biliary cholesterol saturation and duration or extent of disease was found in this study but the numbers of patients studied were small. An earlier study had shown a correlation between prevalence of gallstones in patients with Crohn's disease and the length of resected ileum (Hill et al, 1975). In this study Crohn's disease was also associated with an increased prevalence of gallstones when less than 10 cm of ileum had been resected. Thus, it appears that people with Crohn's disease suffer from an increased risk of gallstones not only as a result of resective surgery but also due to some intrinsic cause associated with the disease itself. The actual mechanism is not known but hepatic dysfunction occurs in Crohn's disease and this may lead to an alteration in biliary lipid composition.

Interestingly, Marks et al (1977) also studied seven patients with ulcerative colitis and no significant difference was shown in the biliary cholesterol saturation between these patients and the control group. If the patients had undergone ileal resection, though, their risk increased accordingly.

Finally, ileal bypass surgery for morbid obesity has been associated with an increased risk of gallstones (Wise & Stein, 1978). 101 patients with a small bowel bypass were followed up for over two years and of the 69.3% who did not have evidence of gallstones at the time of the operation, 12.9% had developed gallstones. This represented a yearly incidence rate of 5.2%.

However, recent research into the composition of gallstones induced by ileal dysfunction has implied altered bilirubin metabolism as the underlying mechanism (Pitt et al, 1984). They

have shown that the majority of stones found in patients with ileal disease are pigment gallstones. Further studies of the composition of these stones is needed.

## **2. Peptic Ulceration and Surgery for Peptic Ulcers**

It is not clear from the literature exactly when peptic ulceration was first associated with gallstones. More literature is available on the possible risk of developing gallstones following surgery for peptic ulceration. Consequently, it is difficult to determine whether or not ulceration or surgery for the ulceration, individually or together, are important in the aetiology of gallstones. Some autopsy surveys have reported an association between gallstones and peptic ulceration while others have not been able to do so. Lieber (1952) demonstrated an increased incidence of both gastric and duodenal ulceration in women with gallstones and a mild increase in the incidence of duodenal ulceration in men with gallstones. In another autopsy survey gastrointestinal lesions were found more commonly in patients with gallstones than those without (Kozoll et al, 1959). However, the lesions included gastrointestinal carcinomas as well as peptic ulcers. In contrast, Gross (1929) was unable to show any difference in the incidence of either gastric or duodenal ulcers in patients with gallstones compared with controls. Similarly, no association was found between gallstones and peptic ulcers by Newman and Northup (1959) and by Zahor et al (1974). An inverse correlation between gallstones and peptic ulceration has been reported by Malhotra (1968) where northern railway workers were much more likely to have gallstones and much less likely to have

peptic ulcers than railway workers in the south of India.

Peptic ulcer surgery was first associated with a possible risk of gallstones in 1947 (Majoor & Suren, 1947). They described six cases where biliary symptoms occurred soon after a Billroth II gastrectomy and in each case they claimed to be reasonably certain that the gallbladder had been normal at the time of the gastric resection. The authors believed that the surgery had led to the development of the gallstones. Further studies followed and described a positive association with peptic ulcer surgery and gallstones but all these studies were uncontrolled (Anderson et al, 1980). The implicated surgical techniques included Billroth I operations and truncal vagotomy as well as Billroth II gastrectomy. A prospective survey of 118 patients was undertaken to determine the true incidence of gallbladder disease and, although results were not conclusive, there was a suggestion that cholelithiasis was increased up to three years after surgery (Anderson et al, 1980). Three of the 59 surgically treated patients developed gallstones compared with none in the medically treated group.

As long as there is doubt about the effect of peptic ulceration, per se, on the formation of gallstones, the role of surgery will be difficult to evaluate. Mechanisms underlying possible changes are also unclear.

### **3. Cystic Fibrosis**

Children with cystic fibrosis have an increased risk of developing gallstones and abnormal mucous was thought to possibly interfere with bile flow or allow gallstone nucleation (Bennion &

Grundy, 1978). Roy et al (1977) have also shown that cystic fibrosis is associated with increased biliary cholesterol saturation secondary to bile acid malabsorption. Pancreatic enzyme replacement therapy returns the bile saturation to normal.

#### **4. Diabetes Mellitus**

Autopsy studies have suggested an association between gallstones and diabetes mellitus for a number of years. Naunyn mentioned in his treatise on gallstones (1896) that several authors as well as himself had noted an association between gallstones and diabetes (Ord, Kraus, Loeb, Hull). The opinion at that time was that biliary colic led to the development of diabetes. Gross (1929) reported that there was an association between diabetes and gallstones in her autopsy series. 25.7% of the diabetic cases had gallstones compared with 15.6% of the controls. The risk was thought to be a result of the hypercholesterolaemia found in diabetes together with the high-cholesterol diet recommended for diabetics at that time. 3.8% of gallstone cases had diabetes in another early study (Hamilton, 1932) but this finding was not compared with any control group. In a later autopsy survey (Lieber, 1952), gallstones were found in 30.2% of diabetic cases. In cases over the age of fifty in this study, gallstones were present in approximately 50% of white female diabetics and 20% of white male diabetics. Newman and Northup (1959) and Kozoll et al (1959) also found an association between diabetes and gallstones but not as strongly as had been shown by Lieber (1956). Other autopsy surveys have not been able to demonstrate such an association. Zahor et al (1974) found no

association in Swedish and Czechoslovakian autopsy populations.

Clinical studies of living populations have not provided any conclusive evidence on the relationship of gallstones and diabetes. There were too few diabetics in the Framingham study to justify an examination of the possibility of a relationship (Friedman et al, 1966). Diabetes was reported as a pathogenic factor for gallstones in an early study of American Indians (Sievers & Marquis, 1962). Pima Indian studies have been contradictory in their findings. In the initial survey of clinically identified cases, there was no association found between gallstones and diabetes in women but there was in men, particularly the older men (Comess et al, 1967). The screening survey of the Pima Indians failed to show any significant association between gallstones and diabetes (Sampliner et al, 1970). However, there was a suggestion of an association in subjects over the age of 54 years but numbers were too small for the result to be significant.

Metabolic studies have been carried out in an attempt to clarify this relationship. Bennion and Grundy (1977) demonstrated that gallbladder bile was significantly more saturated with cholesterol during insulin treatment than during uncontrolled hyperglycaemia in six Pima Indians with maturity-onset diabetes. In a study that is more useful for extrapolation to caucasian populations, the biliary lipid composition and bile acid pool size were measured in eight juvenile and 16 maturity-onset diabetics (Ponz de Leon, et al, 1978). The saturation index of bile was significantly raised in the maturity-onset group while the juvenile diabetic group did not differ from the controls. There

were no differences in either the proportion of the individual bile acids or the size of the bile acid pool between the three groups.

Thus, an empirical basis has been provided for the frequent association between gallstones and diabetes. The metabolic abnormality was only associated with maturity-onset diabetes so more accurate figures on the strength of the relationship may be provided by studies of maturity-onset cases only. The mechanism behind the cholesterol supersaturation of bile in these diabetics is uncertain but may be, in part, secondary to an autonomic neuropathy causing gallbladder dysfunction (Gitelson et al, 1963).

## **5. Pancreatitis**

There is no doubt that gallstones are more common in patients with pancreatitis (Carter, 1983, Joske, 1955, Robbins, 1974). The mechanism underlying the association is disputed with some authors believing that gallstones lead to pancreatitis while others argue that pancreatitis causes gallstones (Kaye & Kern, 1971).

## **6. Hepatic Cirrhosis**

Bouchier (1969) reported that cirrhotics coming to autopsy had an increased frequency of gallstone disease (29.4%) compared with controls (12.8%). Thus, the risk was more than doubled for cirrhotics. These findings were not in complete agreement with those of Lieber (1952) who recorded frequencies in cirrhotics that were only slightly higher than those in the control group or with those of Newman and Northup (1959) who found no consistent association of gallstones with cirrhosis. In addition, Bouchier found an association with all types of cirrhosis while Lieber

reported an increased risk only with alcoholic cirrhosis. Bouchier also found that pigment stones accounted for a much higher proportion of stones in cirrhotics than in the normal population.

The possible underlying mechanism of the increased risk of forming gallstones in hepatic cirrhosis is uncertain. In a metabolic study of 12 cirrhotic patients, Vlahcevic et al (1972) showed that there was impairment of cholesterol conversion to cholic acid in the liver with consequent reduction in the total bile acid pool size. The question of why a reduction in the amount of bile acids caused the development of pigment gallstones and not cholesterol stones led to a further study (Vlahcevic et al, 1973). They reported that cholesterol secretion in the bile was reduced along with the bile acid secretion and, therefore, the increased risk of gallstones was related to some disruption in the bilirubin metabolism.

## **7. Carcinoma of the Gallbladder**

An association between gallstones and carcinoma of the gallbladder is well established. Numerous autopsy studies have found that gallstones are much commoner in people with gallbladder cancer than in the general population (Bateson & Bouchier, 1975, Black et al, 1977, Cooke et al, 1953, Crump, 1927, Gross, 1929, Hamilton, 1932, Hart et al, 1971, Kozoll et al, 1959, Lieber, 1952, Maram et al, 1979, Zahor et al, 1974). Gallstones are present in 60-95% of gallbladder cancer cases although gallbladder cancer itself is a rare tumour found in less than one percent of gallstone cases (Comfort et al, 1948, Lund, 1960, Wenckert & Robertson, 1966). The main controversy surrounding the association

between gallstones and gallbladder cancer has been the nature of the association in terms of causality. The idea that gallstones may play a causal role in the pathogenesis of carcinoma of the gallbladder is supported by a number of observations. Gallstones are present in the vast majority of patients with gallbladder cancer and the female to male ratio of the cancer is similar to that of gallstone disease although gallstones are found in a similar percentage of men and women with cancer of the gallbladder. However, direct evidence of any causal link is not available and indirect evidence such as that given above implies only that the two conditions may have common causes.

Since only a small fraction of people with gallstones develop gallbladder cancer (<1%) and approximately 20% of cancer cases do not have associated gallstones, even if gallstones are causal, it would have importance only in subpopulations of people identified as having a high risk of developing gallbladder cancer. Subpopulations such as these have been identified: American Indians, Mexicans and Mexican-Americans have been shown to have an incidence of carcinomatous change of 4-5% in gallbladders removed for stones (Weiss & Hanis, 1984). Otherwise gallbladder cancer is a rare malignancy that occurs only in elderly people.

## **8. Hyperlipidaemia**

An increased risk of developing gallstones has been reported in certain types of hyperlipoproteinaemias. While the WHO classification of hyperlipidaemic states is not based on separate, metabolically distinct disorders, it is a useful classification

in terms of lipoprotein abnormalities (Lewis, 1983). The two types of abnormal lipoprotein patterns most commonly encountered are type II and type IV (Shaffer & Small, 1976). They differ in that type II is associated with hypercholesterolaemia, probably as a result of reduced hepatic conversion of cholesterol to cholic acid. The cholesterol-rich low density lipoprotein is increased in this disorder. Type IV hyperlipoproteinaemia is associated with an increase in triglyceride-rich very low density lipoprotein levels and an increase in bile acid pool size (Andersen & Hellstrom, 1979).

Once again, the results of studies determining the importance of hyperlipoproteinaemias in the aetiology of gallstones are conflicting. A study in 1975 reported that the overall incidence of gallstones in patients with type IIa hyperlipoproteinaemia did not differ from that of the normal population while the incidence in type IV was abnormally high (13% in men and 22% in women with type IIa disease and 41% in men and 68% in women with type IV). Their findings were disputed in a later study which showed that hyperlipidaemias were not associated with an increase in gallstone risk once patients on clofibrate therapy were excluded (Bateson et al, 1978). However, numbers in this study were very small especially with the exclusion of the clofibrate group. In a more recent study 210 patients with primary hyperlipoproteinaemia were screened for evidence of gallbladder disease and the results supported the findings of the first-mentioned study (Ahlberg et al, 1979). The prevalence of gallbladder disease was 8%, 18% and 42% in males with type IIa, IIb and IV hyperlipoproteinaemia respectively and 22%, 48% and 72% in the corresponding groups of

females. The occurrence of gallbladder disease was, therefore, within normal limits in type IIa and abnormally high in type IV hyperlipoproteinaemia. People with type IV disease tend to be overweight which would confound results but the prevalence was also shown to be increased in a subgroup of men with type IV disease who were close to normal weight (Ahlberg et al, 1979). This study showed an increase, although not significant, of gallstones in women with type IIb disease. Type IIb is associated with higher levels of triglyceride-rich very low density lipoprotein than type IIa disease and this is in accordance with the observation that serum triglyceride levels appear to be higher in patients with gallstones (Bell et al, 1973, Scragg et al, 1984c). In contrast, serum cholesterol concentration is not raised in gallstone patients compared with controls (Van der Linden, 1961, Friedman et al, 1966, Sampliner et al, 1970) and was even decreased in cases compared with controls in one study (Scragg et al, 1984c). It is interesting that, despite the abnormal biliary lipid metabolism seen in type II hypercholesterolaemia, there is not an increased tendency to form gallstones, yet, in the face of increased bile acid pools in type IV disease, gallstones are more likely to occur. Serum triglyceride appears to be more important in the determination of biliary lipid levels than serum cholesterol.

Plasma high density lipoprotein levels have been little studied in relation to the aetiology of gallstones. Petitti et al (1981) reported a decreased risk of gallstones in women with high levels of this lipoprotein and Thornton et al (1981) demonstrated that biliary cholesterol concentration was inversely related to serum

high density lipoprotein levels. An inverse relationship between high density lipoprotein concentration and risk of gallstone formation that was confounded by triglyceride and insulin levels was reported by Scragg et al (1984c). Further work is needed on this potentially important serum lipid.

## **9. Ischaemic Heart Disease**

The problem of spurious associations with gallbladder disease becomes an even more complex one when an association with ischaemic heart disease is considered. Both conditions are extremely common in western communities so it is not surprising that the two have been considered to be related. Many papers have been published on the question of an association between gallstones and ischaemic heart disease but no consensus has been agreed upon. Some autopsy studies have found a positive association between the two conditions (Gross, 1929, Hamilton, 1932, Kozoll et al, 1959, Naunyn, 1896) while others have not been able to demonstrate such a relationship (Bateson & Bouchier, 1975, Cleland, 1953). Newman and Northup (1959) described several other autopsy surveys as well as their own that were unable to demonstrate a significant correlation between arteriosclerotic heart disease and gallbladder disease once Yule's correction for the degree of association between two diseases had been applied. In a regional study of gallstones and ischaemic heart disease, Barker et al (1979) found that gallstone prevalence was negatively correlated with standardised mortality rates for ischaemic heart disease for the nine towns studied throughout England and Wales. Moreover, American Indians, with their extremely high prevalence

of gallstones, have a low prevalence of ischaemic heart disease (Sampliner et al, 1970, Sievers & Marquis, 1962).

In summary then, many factors and diseases have been claimed to be associated with increased frequency of gallstone formation but very few of the claims actually stand up to critical examination. Many of the tenuous associations were made in studies which were uncontrolled or confounded by important factors such as age, sex and race. Gallbladder cancer and pancreatitis are two conditions which are definitely related to cholelithiasis but the mechanisms underlying the relationships are not understood. Ileal disease probably belongs in the definite category also and the mechanism is related to bile salt malabsorption. The association for diabetes mellitus and hepatic cirrhosis is less certain while the evidence for the many other associations is either inadequate or contradictory or both.

### **3.2.9 Genetic and Ethnic Factors**

The evidence for genetic factors being important in the aetiology of gallstones is quite strong. The American Indian tribes, particularly those in the Southwest such as the Pimas, show extremely high prevalence rates of cholesterol gallstones (Comess et al, 1967, Sampliner et al, 1970, Sievers & Marquis, 1962). Other areas of the world also have high rates of gallstones such as Sweden (Lindstrom, 1977) and South America (Marinovic et al, 1972) while gallstones very rarely develop in some ethnic groups such as the Masai (Biss et al, 1971). These ethnic variations correlate roughly with the biliary cholesterol

concentration of the different races (Redinger & Small, 1972). Studies of the Pima Indians have shown that they have a dual genetic defect leading to the formation of supersaturated bile. They secrete excessive amounts of cholesterol into the bile and also secrete deficient amounts of bile acids (Grundy et al, 1972).

Further evidence for genetic influences is given by reports of familial aggregations of gallbladder disease. A positive family history of gallstones is commoner in people with gallstones compared with controls (Richardson et al, 1973, Wheeler et al, 1970). Moreover, in a study of sisters of women with cholesterol gallstones, their bile was more highly saturated with cholesterol than the controls (Danzinger et al, 1972).

It appears, therefore, that a genetic and probably polygenic predisposition to the formation of cholesterol gallstones exists.

### **3.2.10 Miscellaneous Associations**

Social class analysis is fraught with difficulties. Many researchers consider that the occupationally-based Registrar General's method used commonly by the epidemiologists is inappropriate and inadequate (Jones & Cameron, 1984). A number of reports which mention social class gradients in gallstone prevalence do not use any system of classification at all and the evidence is largely anecdotal. However, these anecdotal reports deserve a brief mention. Around the turn of the century, gallstones were considered to be commoner in those with sedentary occupations and those who indulged in "good living" (Naunyn, 1896, Osler, 1898). These influences were still considered important until well into this century (Weiss, 1944) but any positive

association with these influences, if they ever existed have been largely obscured in western communities recently. In Africa, the rare cases of gallstones seen in black Africans are usually seen in the more affluent ones who have adopted western lifestyles (Burkitt & Tunstall, 1975). Malhotra (1968) in his study of railway workers in India claimed that gallstones were much commoner in the workers of the two highest social classes compared with those of grades III and IV.

Smoking has also been considered as a possible influence in the formation of gallstones. In the Framingham study, smoking seemed to offer a slight protective effect in the aetiology of gallstones (Friedman et al, 1966) while the relative risk of gallbladder disease increased with current cigarette smoking in another study (Petitti et al, 1981).

## Chapter 4. The Symptomatology and Natural History of Gallstones

### 4.1 Gallbladder "Dyspepsia"

An outline of the early thoughts on the ability of gallstones to give rise to a variety of symptoms was given in the first section of the literature review. It was some time after the first discovery of gallstones at autopsy that physicians even realised that they caused any symptoms at all. It was not until this century that a more exact description of the symptoms and signs of gallbladder disease was described when Moynihan wrote of the "inaugural symptoms" of gallstones (1913). These included epigastric fullness and discomfort, flatulence, nausea and vomiting and heartburn, with or without acid regurgitation, all being brought on by greasy foods in particular. These symptoms were said to precede by months or even years the sudden onset of acute pain. This pain or biliary colic was also thought to be quite characteristic. It was, and still is, described as epigastric but predominantly right sided, radiating through or around to the back and often associated with shoulder-tip pain, nausea and vomiting. This description was accepted by most physicians and surgeons and was not actively questioned in any clinical study for 50 years. In an attempt to determine the significance of "gallbladder dyspepsia", Price (1963) investigated 142 women aged 50-70 years for the presence of gallstones and/or symptoms. He found that complaints of abdominal pain, fullness or discomfort, nausea and vomiting, heartburn, acid regurgitation and intolerance of particular foods occurred in 50% of women with gallstones and 53% of women who had none. In the prevalence survey

of gallstones in women in South Wales similar findings in a population of both men and women were reported (Bainton et al, 1976). Thus, it can be seen that the symptoms of so-called gallbladder dyspepsia alone discriminate poorly between people with gallstones and those without yet the syndrome of gallbladder dyspepsia is still part of medical doctrine.

#### **4.2 The Natural History of Gallstones**

Uncertainty not only surrounds the significance of symptoms in gallbladder disease. The question of whether or not most gallstones eventually go on to cause symptoms is equally perplexing if not more so. The early writers such as Benevenius (Mani, 1959) were mainly concerned with their presence and, as most stones were discovered purely by accident, no symptoms were attributed to their presence. Morgagni (1761) believed that symptoms of gallbladder disease were uncommon and did not appear to follow any specific pattern. By this time, though, several writers began to claim that gallstones rarely existed without giving rise to some form of discomfort (Robertson, 1945). The argument continued throughout the course of the last century but it was largely academic until successful surgical removal of either the gallstones or the gallbladder became established. Once a successful form of treatment was available the controversy inevitably deepened and polarised. The surgeons believed strongly that symptomless gallstones were very uncommon. Mayo (1911) stated that it was not the gallstones that were "innocent" but the doctor who believed them to be. Moynihan (1913) echoed his views when he claimed that gallstones caused symptoms as soon as they had formed

and that surgery was always required once the diagnosis had been made. The physicians were more cautious in recommending surgical treatment and Osler (1898) thought that cholecystectomy or cholecystotomy should only be considered if the patient was suffering from quite severe symptoms or had developed one of the complications such as obstructive jaundice.

With the introduction, in 1924, of contrast media and X-Ray techniques to reveal the contents of the gallbladder the situation became more complex. Prior to then most cases of gallstones that came to the attention of the doctors were symptomatic. Asymptomatic cases were usually discovered at autopsy or during the occasional laparotomy so did not constitute a large clinical problem. Oral cholecystography allowed the detection of large numbers of previously undiagnosed and often asymptomatic gallstones. There was widespread agreement that surgery was indicated when symptoms became severe or complications developed (Comfort et al, 1948) but agreement on whether or not silent stones required treatment was lacking. Initially the argument centred around whether or not silent stones inevitably went on to cause symptoms. A new factor was introduced in 1943 when Watson, in Cecil's "A Textbook of Medicine", stated that the important association between gallstones and gallbladder cancer must be taken into account. The proponents of a conservative approach argued that symptomless stones should be left in situ because the morbidity and mortality of surgery outweighed the risks of the stones eventually becoming symptomatic. The aggressive, surgical approach was advocated by surgeons in particular as they believed that a significant proportion of silent stones would eventually

Table I.6 - The Outcome of Non-operated Cases of Gallstones

Author	Year	Place	Number of Cases	% with Symptoms
Comfort	1948	Mayo Clinic	112	46
Lund	1960	Copenhagen	526	50 women 30 men
Wenckert	1966	Malmo	781	51
Gracie	1982	Ann Arbor	123	18

cause problems and the mortality rates associated with elective procedures in younger patients were much lower than those seen in the older patients suffering from complicated gallbladder disease. The crucial but missing factor in both these arguments was a knowledge of the natural history of asymptomatic gallstones.

Several studies have attempted to address this problem since (see Table I.6). One of the first yet also one of the better studies in this area was carried out by Comfort et al (1948) at the Mayo Clinic. They followed up 112 people who had had previously unsuspected gallstones discovered during a laparotomy between 11-15 years earlier. Patients operated on originally for cancer or upper gastrointestinal tract problems were excluded from the study because of follow-up difficulties and indeterminate symptoms respectively. Thus, of the 998 original cases, only 184 were considered suitable. 115 of these replied to the communication. They were all asked whether or not they had had any symptoms of indigestion or colic prior to the first laparotomy and only three were excluded on this basis. However, it is difficult to believe that only three people out of 115 had experienced symptoms of indigestion before their gallstones had been diagnosed. Nevertheless, they claimed that 112 people had been asymptomatic at the time of the discovery of the gallstones. After 11-15 years 51 people (45.5%) had gone on to develop symptoms ranging from low-grade indigestion to biliary colic and/or jaundice with a 6.5% mortality rate. On this basis the authors concluded that cholecystectomy could be advised but not urged for people with silent gallstones. The figure of 45.5% of people with silent stones going on to experience morbidity is high on two

counts. Firstly, it was probably incorrect to label 112 of the 115 suitable patients as truly asymptomatic in the first place and secondly, 30 (27%) people went on to experience indigestion only and, as was shown in the preceding section, the symptoms of indigestion are not necessarily indicative of biliary problems. Only 19% developed severe symptoms or complications.

Two more studies of the natural history of gallstones followed but neither can be legitimately labelled as follow-up studies of asymptomatic cases. Lund (1960) observed 526 non-operated gallstone patients for five to 20 years after they were admitted to the Copenhagen County Hospital suffering from gallstones. The hospital's policy for elective cholecystectomy at that time was not stated in the study but most of the 526 patients followed up had suffered from mild to severe symptoms prior to the diagnosis. Very few cases were truly asymptomatic having had their gallstones discovered accidentally during laparotomy and they were included in the mildly symptomatic group which comprised only 18% of the total number of cases. After follow-up, irrespective of the original degree of symptom severity, at least 50% of the women and 30% of the men had subsequently developed severe symptoms. In a similar study of non-operated cases, Wenckert and Robertson (1966) traced 781 patients who had had gallstones diagnosed by oral cholecystogram after a symptomatic presentation. After 11 years 49% were asymptomatic or had mild symptoms, 33% had developed colicky pain and 18% had gone on to develop complications. The limitations of these two studies are obvious. They cannot be regarded as investigations into the natural history of the silent gallstone as very few such cases were actually included in either

of these studies. Yet these studies have become the most widely quoted by doctors arguing for the necessity of carrying out prophylactic cholecystectomies for asymptomatic gallstones (Br Med J Editorial, 1975, Glenn, 1974, Method et al, 1962, Sato & Matsushiro, 1974).

A more recent study of the natural history of silent gallstones reported on the outcome of truly asymptomatic stones (Gracie & Ransahoff, 1982). 110 men and 13 women, as staff of the University of Michigan medical faculty, had been discovered to have silent gallstones on routine oral cholecystography for insurance purposes. After 15-20 years only 18% had gone on to develop biliary pain or complications. Unfortunately, the numbers of people, especially women, investigated in this study were very small. In another study of men undergoing routine cholecystography for insurance purposes, 70% of executives found to have asymptomatic stones were free of symptoms up until 10 years after the investigation (Wilbur & Bolt, 1958).

An interesting observation was made in the study by Gracie and Ransahoff (1982). The cumulative probability of a case developing symptoms was 10% at five years, 15% at 10 years and 18% at 20 years. Lund (1960) hinted at a similar phenomenon in his study as 90% of the people who went on to develop symptoms did so within five years of the original admission. This may possibly explain the early clinical impression that gallstones occurred most often in the middle-aged. People who harbour gallstones, if they are going to develop symptoms, are likely to develop them sooner rather than later in the course of their condition. In support of this argument, a study which compared the clinical

features of patients with silent gallstones with those suffering from symptomatic stones, reported that the age distribution of the patients with silent stones was older than that of the symptomatic cases (Sato & Matsushiro, 1974). Recently, the National Co-operative Gallstone Study provided further evidence to support this possibility (Thistle et al, 1984). In a two year follow-up of people with gallstones who were given a placebo instead of chenodeoxycholic acid, those aged less than 55 years were significantly more likely to suffer biliary symptoms than those aged 55 or more years. This may reflect the removal of a subpopulation of patients with a more symptomatic course leading to cholecystectomy, leaving patients with a more quiescent course to grow older with their gallstones.

The risk of developing gallbladder cancer in association with gallstones must always be considered but it is a very uncommon cancer which occurs only in elderly people and in less than 1% of people harbouring gallstones. No cases of gallbladder cancer complicated the course of cases in either the study by Comfort et al (1948) or that by Gracie and Ransahoff (1982) while three cases each occurred in the studies by Lund (1960) and Wenckert et al (1966). Another consideration has been put forward recently. An association between previous cholecystectomy and colo-rectal cancer was shown in a retrospective study by Vernick et al (1980). A gradient was found in the incidence of cancer in different sites of the large bowel: ascending colon cancer (10.5%) decreasing to rectal cancer (2.1%). Bile acids have been implicated in the pathogenesis of large bowel cancer (Reddy & Winder, 1977) and cholecystectomy results in a continuous flow of bile acids through

the intestines rather than the interrupted flow that a functioning gallbladder produces. Cholecystectomy also alters bile acid metabolism and composition of the bile acid pool with an increase in secondary bile acids (Reddy & Wynder, 1977). These studies have resulted in the hypothesis that cholecystectomy predisposes to cancer of the large bowel. Linos et al (1981) provided further support for the hypothesis when they followed up 1681 post-cholecystectomy patients and found a higher than expected number of cases with carcinoma of the colon. The risk was significant in women with a relative risk of 1.7 and even stronger for right-sided carcinoma of the colon (2.1). Other studies have also produced evidence of a risk of carcinoma of the colon following cholecystectomy and this is an area that will need serious research and consideration as it is an important factor in the argument about treatment options for asymptomatic gallstones.

There are, therefore, two strategies for the treatment of silent gallstones : prophylactic cholecystectomy or expectant management. Two studies have attempted to compare the benefits and risks of each strategy on a theoretical basis. Fitzpatrick et al (1977) based their comparison on a cost-benefit analysis of mortality only as they were unable to classify morbidity in such a way. They used the figure reported by Wenckert and Robinson that 51% of people with silent stones were likely to go on to develop symptoms or complications, and operative mortality figures were obtained from the National Halothane Study of 1969. The decision analysis, using Bayes theorem of probability, was striking in that losses and gains associated with the different treatments were

small. Younger, healthy subjects stood to lose two weeks of life if managed expectantly while elderly or poor risk patients stood to gain one month by the same treatment. Obviously the equation for Bayes theorem is influenced by two important figures. The age-specific operative mortality rates will vary but can be determined with some accuracy but the proportion of silent stones that will eventually go on to cause problems can only be estimated and Fitzpatrick et al used a figure from a study of the natural history of gallstones that had serious flaws. The figure of 51% is very likely to be overestimated. Ransahoff et al (1983) carried out a decisional analysis based on the figures obtained from their follow-up study of silent gallstones. This figure of 18% was very much lower than the 51% used in the earlier study so their finding that prophylactic cholecystectomy slightly decreases survival is hardly surprising. Once again, though, a sensitivity analysis showed that the differences between the two treatment options remained small over a broad range of probability values for both sexes.

In summary, it is clear that very little is known about the natural history of the gallstone. There are no known clinical clues which allow identification of stones that are more likely to give rise to symptoms and the actual proportion of asymptomatic stones that go on to cause symptoms is unknown. A prospective study of cases identified during an initial screening survey is needed to clarify the situation. In the meantime, then, it is not possible to determine whether or not prophylactic surgery should be advocated for asymptomatic gallstones. However, the results of the study by Gracie and Ransahoff (1982) would appear to support

the conservative approach. Further such studies of much larger numbers are needed before informed decisions can be made. Important considerations such as the risk of developing carcinoma of the gallbladder with gallstones in situ and the risk of developing carcinoma of the colon after cholecystectomy also need further investigation. The results of the two decisional analyses, despite being based on inaccurate figures, show that the differences in outcome associated with the two treatments are very small. The importance attached to this problem may be ill-founded and the ultimate arbiter should perhaps be the individual faced with possible surgery.

## **SECTION II - Methodology**

### **Chapter 5. Dietary Methodology**

#### **5.1 Introduction**

In recent years medical researchers have become increasingly interested in the effects that diet may have on health and disease. However, there is uncertainty about the role of diet in the aetiology of many chronic diseases and this is due, to a great extent, to the problems inherent in the accurate measurement of dietary intake.

Epidemiological analyses of the relationship between diet and disease can be considered under two general headings. The first relates to analyses of the dietary intakes of groups of people while the second involves the assessment of the food intake of individuals.

##### **5.1.1 Population Surveys - Using National Dietary Data**

In large scale surveys attempting to compare disease prevalence in populations and relate differences to nutrient intakes, national dietary data rather than individual data are required. Food balance sheets on a national basis deal with total food turnover and are calculated from figures of food produced and available for consumption such as that produced, imported or brought back from storage less the food produced yet used for other purposes such as that exported, fed to animals, converted to other goods or put into storage. However, there is usually no account taken of the losses incurred during storage, distribution

and consumption of food. This can mean an overestimation of actual consumption by as much as 30% (Ministry of Agriculture, Fisheries and Food, 1953-1980). Yet Food Balance Sheets are commonly employed in cross-cultural studies. A problem common to all world-wide studies is that the use of different areas and societies with different food habits will result in the incorporation of environmental and cultural factors into the analyses. Moreover, the reliability of the information collected varies from nation to nation. Regional differences are obscured so methods which look at regional intake rather than national intake are required for studies which want to investigate these differences.

Group and population surveys also include household observations which are undertaken by some countries to monitor the purchasing practices of families. There are several approaches to household observations. The British National Food Survey employs one such method to monitor the diets of certain groups within the population as well as look at habits of the nation as a whole. The survey was begun in 1940 by the Ministry of Food to monitor the diets of the urban working class families and provide an assessment of the effectiveness of wartime food policy. A random sample of the population was originally selected from electoral registers but local authority districts are now being used (Derry & Buss, 1984). Specially trained interviewers recruit households and one person within each household is asked to keep a record for seven days of the description, quantity and cost of all food items entering the home for human consumption. The only foods excluded are sweets, chocolates and alcohol. Members of the household and visitors are noted at each meal along with the meal served. The

principal limitations of the National Food Survey are described below. It is confined to food that is brought home so all meals eaten out are excluded and it records food bought rather than food consumed so does not account for wastage. Alcohol and sweets are not estimated and individual intakes cannot be determined from household intakes. The main strength of the survey is that it provides continuous information on national intakes over 30 years and also allows closer looks at smaller subgroups within the population and regional analysis. This has been done recently by Bull and Barber (1984). They were able to identify vegetarian households from the National Food Survey and compared the intakes of these households with nonvegetarian households. Vegetarians consumed most nutrients in amounts lower than the national average, particularly fats and simple sugars. They also tended to have a much higher polyunsaturated to saturated fat ratio.

#### **5.1.2 Individual Surveys - Using Individual Dietary Data**

Epidemiological studies most commonly attempt to determine the nutrient intake of individuals. However, according to Marr (1971) there is no generally accepted method of measuring the dietary intake of "free-living" individuals which may explain the plethora of methods that can be found in the literature. These methods can usually be classified under one of the following four groups of dietary methodologies :

1. Diet record
2. Dietary recall
3. Diet history
4. Food frequency questionnaire.

There have been a large number of refinements and variations introduced by different researchers and the methods overlap to some degree and are often used in combination. The original diet history method pioneered by Burke (1947) included a three-day diet record, an assessment of usual consumption and a food frequency questionnaire as a cross check. The first two methods attempt to define actual food intake during a specific period and the values so obtained are assumed to be representative of the usual diet. The last two methods attempt to measure usual intake directly.

It is beyond the brief of this study to give a comprehensive review of dietary methodology and I refer the reader to several excellent reviews: Baghurst & Baghurst (1981), Block (1982), Marr (1971) and the report from the Medical Research Council's Nutritional Meeting in Southampton (1983). A short description of the techniques is given below.

1. Diet record - there are numerous approaches to the dietary record or diary. The length of time that the diary is recorded for can vary from 24 hours to 21 days and the method of recording can involve careful weighing of all foods, accurate use of household measures or just estimations of food portion sizes. The method of analysis of the foods eaten can also affect the accuracy. Aliquot sampling and chemical analysis of food will be more accurate than reference to food tables as food tables are based on analyses of a few samples of any one food. They do not take into account the variability of food content. For example, the content of an apple will vary according to degree of ripeness, from season to season and depending on the soil in which it was grown. Thus, the accuracy of food tables for flour and milk is better than for

cakes and stews and better for macronutrients than for micronutrients. The longer period for which a diary is recorded the more accurately it will reflect usual intake but participant compliance is also an important aspect. A dietary record kept for any period of time requires a high degree of cooperation on the part of the subjects and accuracy will decrease if they cannot be bothered to complete the diary fully after several days. Similarly the method of recording the food will affect participant compliance. Weighing every article of food eaten is the most accurate method but is very time-consuming and disruptive. Participants may therefore modify their intake. Moreover, it is not possible to weigh food that is eaten out. Therefore, the method of choice is usually a compromise between the highly accurate but tedious measures such as weighing over the longest time possible, and participant compliance, permissible expense and the experience and training of personnel involved in the study.

2. Dietary recall - the term dietary recall is used in the context of actual foods eaten during the recent past. The 24 hour recall is one of the most commonly used methods of dietary intake measurement because it is relatively inexpensive and can be carried out rapidly at a minimum of inconvenience to the participant. The interviewer does not need a great deal of training and experience and the technique is reasonably accurate provided only the last 24 hours is assessed. The major problem is that of underestimation of actual intake because the method relies on memory. Acheson et al (1980) compared the actual food records kept of the previous day with 24 hour dietary recalls and the subjects underestimated their energy intake by 21% using a printed

questionnaire and by 34% using a blank sheet. The underestimation was usually due to the omission of a single item or underestimation of a portion size. This "underestimation" effect has been reported in a number of other studies also (Block, 1982, Madden et al, 1976) and if problems with loss of memory occur for 24 hours then a longer period of assessment of recall should not be contemplated. The other major problem associated with the 24 hour recall method is that an atypical intake and seasonal variation cannot be accounted for so it should not be used in the determination of individual intakes but only for that of large groups (Madden et al, 1976). Heady (1961) had 116 male bank clerks record their intake for seven days and he found that, while the mean intake of the group was not significantly different from day to day, there were large daily individual variations. Thus, it seems clear that a single 24 hour recall is not an appropriate tool for assessing the usual diet of an individual and a very large number of recalls may be needed to represent the usual intake of an individual.

3. Diet history - the use of the diet history was originally developed by Burke (1947) and has been modified by many investigators since. The original method tried to establish the usual pattern of dietary intake directly and actually incorporated other methods by including a three day record and a food frequency check list. Household measures were used to determine portion sizes. The overall eating pattern was established in the context of the social, economic and personal background of the subject by means of a structured interview. This method is still one of the most accurate available but is time-consuming, taking

approximately one hour and requires a highly trained interviewer and a cooperative subject. Burke emphasized that the method, even in its strictest form, should only be used to place subjects in relative categories for nutrient intake. The reliability of the method based on repeated administrations has been found by most investigators to be very good (Block, 1982). However, its use in large scale epidemiological studies is limited because of the time and expense required to assess each participant.

4. Food frequency questionnaire - these questionnaires enquire about the usual intake in terms of frequency of consumption of various food items. The respondent is asked about the frequency of consumption of specific items per day, week or month. Originally, the method was devised for descriptive purposes only and did not attempt to quantitate intake in terms of portion size. For this reason the method has usually been employed to determine if there is any association between dietary factors and specific conditions such as cancer of the large bowel (Graham et al, 1978). More accurate methods are subsequently applied for specific testing of any diet-disease associations. Thus, the food frequency method is a very quick and relatively inexpensive way to collect dietary data on large numbers of people either by post or interview. It is a qualitative frequency approach and is very useful as a screening procedure.

However, researchers have recently been working on adapting this technique to allow quantification of nutrient intake. The questionnaire design is a crucial aspect of this adaptation. It is important to include as many items as needed to get an accurate idea of food intake without including so many items as to fatigue

the participant. Portion size must also be incorporated in some way as well as some way of recording seasonal variation. A food frequency questionnaire incorporating these principles has been developed (Baghurst & Baghurst, 1981) and successfully used in a large case-control survey recently (Scragg et al, 1984a). A short dietary questionnaire designed for self-administration in studies of ischaemic heart disease has also recently been developed (Yarnell et al, 1983).

### **5.1.3 Validation of Dietary Methods**

Regardless of what method of dietary intake measurement is undertaken it is important that the method be validated for use with the group concerned. However, validity in terms of dietary methodology is very difficult if not impossible to achieve. In most studies of the validity of a method, the researchers have "validated" their method against some other method which has greater acceptance but which nevertheless has not been validated itself. Moreover, repeatability is often considered rather than validity. Some researchers have attempted to demonstrate a method that elicits a usual intake by showing that it can produce similar results on two different occasions. True validation in terms of accuracy is almost impossible without extensive studies involving enormous costs and expertise. The approach of relative validation may have to be accepted using, as a reference criterion, a method which has greater accepted validity. For a comprehensive review of the studies which have attempted to validate particular methodologies, I refer the reader once again to the reviews listed earlier.

Most of the methodologies described above appear to have some

degree of validity when measured against one another. While any single approach may be inconclusive, the entire pattern of agreement between different methods, repeatability of methods in different populations and lack of repeatability when there has been a change in diet suggests that several of these methods are valuable. Rather than requiring precise and accurate figures of nutrients, it may be more important to produce and evaluate a method which can place individuals into broad categories of intake. The method chosen for any particular study should be appropriate for the number of individuals in the study, their degree of intelligence and literacy and the type of information required for analysis. This thesis describes two studies which attempt to investigate epidemiological aspects of gallbladder disease. Both studies involved some degree of dietary assessment and their individual methodologies are described below.

## **5.2 Postal Questionnaire**

In the initial study carried out for this thesis (see Section III) a postal questionnaire was completed by all participants (see Appendix A). It was a self-administered questionnaire that, in its original form, had been developed by Gear (1978) for use in his study on the prevalence of diverticular disease and its dietary aspects. The first 17 questions collected information on personal details and the general health of the respondents while the rest of the questionnaire consisted of dietary questions.

The questionnaire described by Gear was devised as an economical method of categorising individuals according to their dietary fibre consumption provided they consumed a diet of

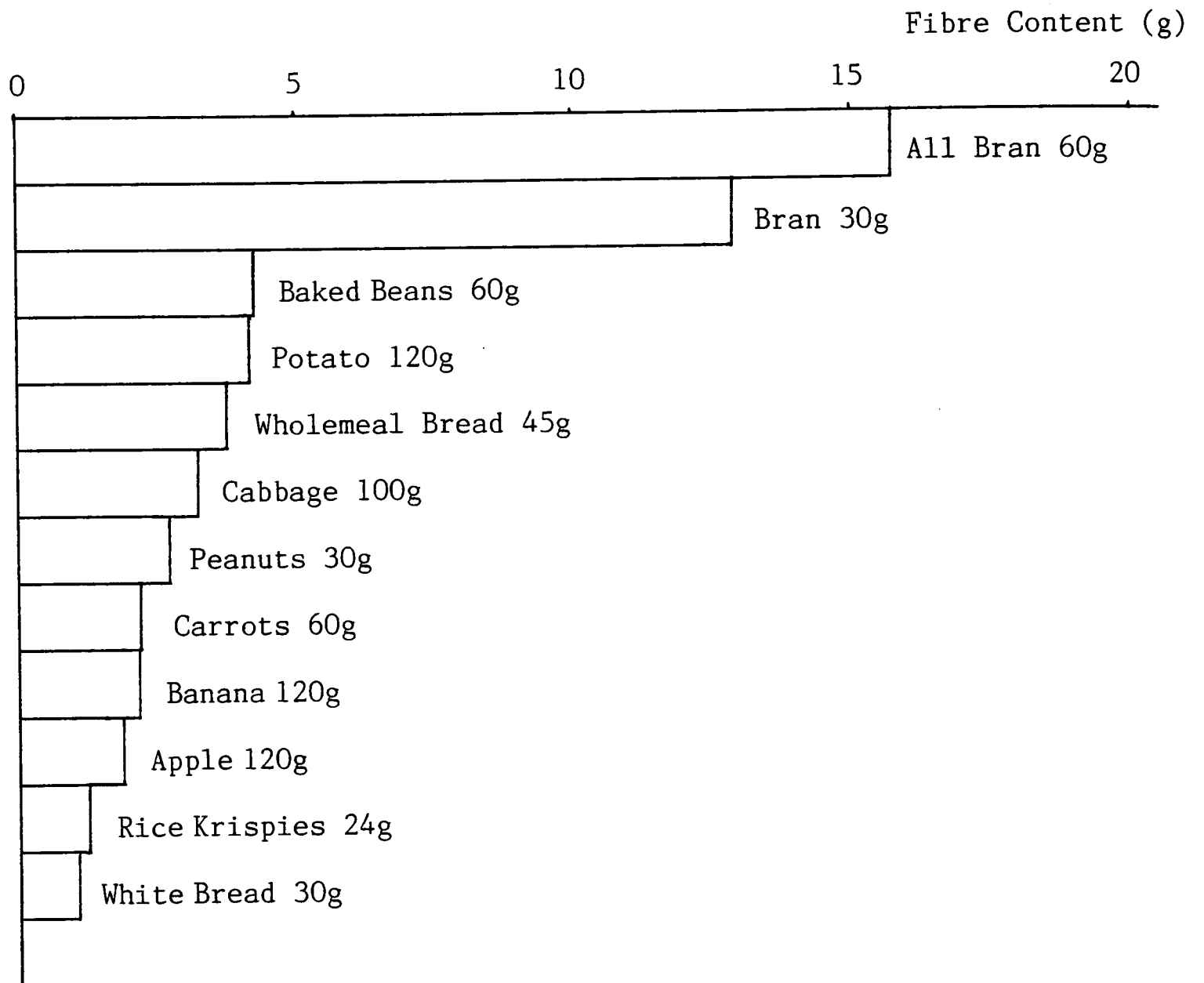


Fig. II.1 Total Dietary Fibre Content of "Average" Portions of Some Common Foods

Table II.1 - Fibre Values of Foods (Based on Southgate et al, 1976)

Item	Average Portion(g)	Fibre Value/ Portion	Fibre Value/ 100g
Bread			
White	30	0.82	2.72
Hovis	30	1.36	4.54
Granary/Plain	30	1.53	5.11
Rye/Wholemeal	45	3.83	8.50
Biscuits			
Digestive	25	0.90	3.60
Crispbread	20	1.66	8.28
Other	30	0.60	1.99
Cakes	60	0.63	1.05
Breakfast Cereals			
Corn Flakes	30	0.00	0.00
Rice Krispies	24	1.07	4.47
Porridge	30	2.10	7.00
Weetabix	32	4.07	12.72
Shredded Wheat	32	4.07	12.72
Sugar Puffs	32	4.07	12.72
Muesli	60	4.45	7.41
30% Bran	60	4.45	7.41
Sultana Bran	60	4.45	7.41
Bran Flakes	60	4.45	7.41
All Bran	60	16.02	26.60
Bran	24	10.56	44.00
Other Cereal			
Sweet Corn	60	3.88	6.48
Vegetables			
Peas	60	3.77	6.28
Greens	100	2.83	2.83
Baked Beans	60	3.88	6.48
Other Beans	60	6.07	10.12
Potatos	100	4.21	3.51
Other Roots	60	2.22	3.70
Fruit			
Fresh	120	1.98	1.65
Tinned	120	1.52	1.27
Raisins, Sultanas	30	1.32	4.40
Nuts	30	2.55	8.52

foodstuffs readily available in the United Kingdom. The questions refer to the frequency of food consumption in a "usual" week of food items that contain considerable amounts of fibre. Important fibre-containing foods commonly consumed by English vegetarians were also included in the format. Each food was allocated a dietary fibre value based on the size of an arbitrary "average" portion of that food (see Table II.1, fig. II.1) and a semi-quantitative analysis of fibre intake was carried out by multiplying the frequency of intake of each item by its fibre value. Respondents were asked to choose the frequency of consumption that most accurately corresponded to their usual weekly consumption of each food item. The frequency categories were "never", "less than once a week", "1-4 times/week", "5-9 times/week" and "10 or more times/week". The frequency factors for these categories were derived from the midpoint of each and were 0, 0, 2.5, 7 and 12 respectively. Bread fibre consumption was similarly calculated except that the frequency of consumption was classified in slices of the various types of bread eaten weekly. The multiplication factors for the bread consumption frequencies were 0, 7, 22, 37 and 52 for the categories of "never", "1-14 slices/week", "15-29 slices/week", "30-44 slices/week" and "45 or more slices/week" respectively. By this method results were obtained for total fibre, cereal fibre, vegetable fibre and fruit fibre intakes for each participant in the study.

More accurate information was obtained from a group of volunteers within the study who completed a 21 day dietary diary. All food and drink except for water that was consumed during the day was recorded for 21 days by 14 participants. The results of

the calculated fibre intakes from both dietary techniques were compared and the respective Pearson correlation coefficients for total fibre, cereal fibre, vegetable fibre and fruit fibre were 0.71, 0.62, 0.54 and 0.73 respectively. Thus, although the questionnaire data was not as accurate as the data obtained from the diaries, it allowed the placement of respondents into broad groups of fibre consumption and the comparison of the fibre consumption of vegetarians with that of nonvegetarians.

The dietary fibre questions in the postal questionnaire were almost identical to those used by Gear and consisted of 21 questions. A further nine questions asked about details of meat, poultry, fish, eggs and dairy product consumption, mainly to permit classification of respondents into vegan, vegetarian and nonvegetarian groups. The vegetarians were also asked an additional question about the length of time they had been a vegetarian. The final group of eight questions attempted, in a semi-quantitative fashion, to determine the fat intake of the respondents by asking about the quantities of fats consumed both in cooking and for spreads. Respondents were asked to determine their weekly purchases of solid fats and oils and then to assess how much of these they would personally have consumed. The categories were "never", "less than 1/4 lb/week", "1/4-1/2 lb/week", "1/2-1 lb/week" and "1lb or more/week" for solid fats and "never", "0-150 mls/week", "150-300 mls/week", "300-500 mls/week" and "500mls or more/week" for liquid oils.

The vegetarian group were included in the study because they provide a contrast group with respect to diet for the usual, nonvegetarian members of the community. A number of researchers

believe that it may be impossible now to show any differences in dietary intakes between diseased and normal individuals in western societies (Heaton, 1973, Sarles, 1978b, Scragg et al, 1984a). They have postulated the presence of a threshold level of nutrients above which certain diseases are more likely to occur and it may be that individuals within western societies may be above the threshold levels for most, if not all, nutrients. It is, therefore, important to consider groups of individuals within western societies who do not adhere to the usual dietary regimens of the societies. The vegetarians are one such group and they have been shown to have increased fibre intakes (Gear, 1978, Hardinge et al, 1958), decreased fat intakes and an increased polyunsaturated to saturated fat ratio (Bull & Barber, 1984) as compared to nonvegetarians. In order to increase the possibility of finding dietary differences between women with gallstones and those without, a group of vegetarian women were recruited to this study. A woman was considered to be eligible as a vegetarian if she ate meat less than once a week and had done so for five or more years.

### **5.3 Dietary Diary**

The second study of this thesis was a case-control study investigating the risk factors associated with the development of gallstones and is fully described in Section IV. In this study an attempt was made to examine more fully the role of diet, among other factors, in the aetiology of gallstones. Each participant was sent a four day dietary diary to complete and return (see Appendix B). The diary was designed for use in the Oxford

Vegetarian Study and consisted of a very careful explanation about how to fill in the record followed by an completed example diary page and the four pages in which to write the record. Participants were asked to record each food item as eaten and not to change their intake as a result. They were encouraged to use quantifiable measures such as a cup, a tablespoon or to record the size of the packet or can. As an aid to estimating portion sizes when household measures were not appropriate, photographs of graduated quantities of specific food items such as chicken, quiche and trifle were included at the end of the diary. The last page allowed room for a description of any dietary changes that have occurred in the previous five years. Four days were chosen as the shortest period possible that would allow the collection of reliable dietary information while achieving the best cooperation. Marr (1971) argues that the minimum period required to collect reliable dietary information is seven days but several studies have shown that the dietary intake calculated over three to four days agrees closely with the average for seven days as weekday intake does not vary a great deal (Cellier & Hankin, 1963, Heady, 1961). Thus, the diary covered a period that included two week days and both a Saturday and a Sunday.

A pilot study was carried out to evaluate the accuracy of this new postal technique and the diary was sent to 76 vegetarians participating in the original Oxford Vegetarian Study. The response rate after one reminder was 83% and the standard of the completed diaries was excellent. An analysis of individual nutrients was possible in each case. To test the reliability of the information contained in the diaries, the nutritionist who

Table II.3 - Dietary Diary Validation Study: Correlation of Daily Dietary Intake by Postal and Interview Techniques

Correlation of Energy (Kcal)	0.80
Correlation of Protein (g)	0.76
Correlation of Carbohydrate (g)	0.73
Correlation of Fat (g)	0.81
Correlation of Fibre (g)	0.88

Table II.2 - Analysis of Dietary Intake by Postal and Interview Techniques

ID N <sup>o</sup>	Postal Method					Interview Method					
	Energy (Kcal)	Protein (g)	Fat (g)	CHO (g)	Fibre (g)	Energy (Kcal)	Protein (g)	Fat (g)	CHO (g)	Fibre (g)	
0110	1249	38	12	57	41	142	43	23	126	45	13
0332	2001	57	11	101	46	230	43	42	177	38	28
0569	1491	46	12	56	34	174	44	31	241	42	34
0831	2128	60	11	80	34	287	51	49	259	48	55
2092	2841	76	11	132	42	361	48	31	398	51	40
2785	1996	102	20	107	48	168	32	20	153	34	20
33941	2891	86	12	139	43	347	45	43	188	36	28
34211	2092	66	13	93	30	247	44	27	210	35	36
34212	1813	63	14	94	47	175	36	22	224	41	48
3536	894	48	22	23	23	132	56	15	142	60	10
3591	1881	60	13	99	47	186	37	28	151	40	17
3667	1164	36	12	58	45	133	43	24	140	44	25
0295	1807	44	10	64	32	278	58	30	252	45	30
0569	1526	46	12	61	36	174	43	30	241	42	34
30161	1823	52	11	58	29	291	60	41	397	58	55
30162	2274	76	13	85	34	321	53	62	395	52	75
2689	2672	73	11	123	41	336	47	44	361	48	45
5223	2101	65	12	83	36	238	42	40	338	52	43
5397	2013	50	10	88	39	272	51	20	305	53	41
3257	1807	50	11	70	35	248	51	27	216	41	28
0275	1944	57	12	88	41	243	47	30	223	36	23
2760	1220	41	13	55	41	149	46	27	139	41	18
2593	3559	90	10	165	42	336	35	19	346	40	26
1578	2971	89	12	162	49	306	39	33	212	31	25
0265	1701	42	10	62	33	262	58	41	208	42	35
4864	1663	49	12	63	34	228	51	22	186	52	15
Mean	1982	60	12	87	39	241	46	32	2037	44	33

designed the diary visited 26 of the respondents and obtained a diet history combining a 24 hour recall with a detailed cross check list from them. She also tested the accuracy of estimations of portion size using the photographs by weighing actual samples produced by the participants. The results of these two methods of dietary assessment were then compared (see Table II.2) and the correlations of the individual nutrients were very good (see Table II.3). Thus, it was shown that the postal dietary diaries could be used to collect accurate dietary data.

The diary was used in its piloted form for the case-control study and, to ensure further accuracy, each respondent was interviewed by one of two interviewers after the diary had been returned. The interviews followed a semi-structured format and questions were asked about general health aspects and gastrointestinal symptomatology as well as dietary aspects (see Appendix C). The interview was used to determine the intake of an "average" day and the usual daily intake of items such as milk and sugar. Other information collected included the amount and types of particular fats that the respondent favoured for cooking and using as a spread and the frequency that sweets, cakes and biscuits were eaten. The final questions asked about the respondent's smoking and alcohol habits. The interview was particularly useful for clarifying any illegible or incomplete entries in a diary particularly for the type of fats and milk consumed and for finding out ingredients in specific dishes for which there was no immediate food analysis. However, much of the information gained at the interview was not used in the analysis.

A comprehensive computer database programme was compiled for

Table II.4 - An Example of Dietary Diary Computer Analysis (Diary N<sup>o</sup> 002).

Diet Component		Weekdays	Sat/Sun	Total	
Total Amt	g	656.2	349.6	1005.8	
	%Tot*	65.2	34.8		
Energy Value	kcal	1412.2	606.1	2018.3	
	%Tot	70.0	30.0		
Protein	g	43.9	22.5	66.5	
	Cal	175.8	90.1	265.9	
	%Tot	66.1	33.9		
Fat	g	68.5	26.2	94.8	
	Cal	616.6	236.1	852.8	
	%Tot	72.3	27.7		
Carbohydrate	g	165.5	71.9	237.4	
	Cal	620.7	269.6	890.3	
	%Tot	69.7	30.3		
Simple Sugars	g	59.2	22.8	82.0	
	Cal	221.9	85.7	307.6	
	%Tot	72.2	27.8		
Dietary Fibre	g	12.2	6.8	18.9	
	%Tot	64.3	35.7		
Cholesterol	g	171.6	62.6	234.3	
	%Tot	73.3	26.7		
Prot/Energy	%	12.4	14.9	13.2	} 100% - alcohol
Fat/Energy	%	43.7	39.0	42.2	
CHO/Energy	%	44.0	44.5	44.1	
Sugar/Energy*	%	35.8	31.8	34.6	

\*%Tot = % of total weekly intake.

\*Sugar/Energy = that portion of carbohydrate intake that is highly refined. It does not contribute to the total calorie intake separately.

the analysis of the dietary diaries by a computer expert and a nutritionist. The programme is labelled, "Food Table Database Interrogation System" or FTDBIS and is a system comprising a macro which organizes the operation, a data base containing the currently accessible dietary information and a source module which performs the required use interrogation. It was designed to remove the majority of textual reference and manual computation from routine dietary analysis. Each individual food item and its quantity was coded in every diary and the code reference numbers for items and quantities were supplied as the basic data. The database consisted of two files; a direct access master file containing all the relevant information for the food code references available, and a catalogue file containing pointers to specific food codes and whether or not information about specific nutrients were contained in the database. The master file contained one "record" for each food code represented. This record held all the relevant information available for the composition of 100g of that particular food type such as total calorie value, total fat value, value for simple sugars, amino acid composition and fatty acid composition. The database included the analyses from "The Composition of Foods" by McCance and Widdowson (1978), some 600 food items kindly provided by the Dunn Nutrition Laboratory and a further 600 or more collected from other sources including a number of dishes favoured by vegetarians. In addition, there was a series of data collected for proprietary brand foods and containing restricted information. An example of the programme analysis of one dietary diary is given in Table II.4.

## Chapter 6. Ultrasonographic Methodology

### 6.1 Introduction

Oral cholecystography has been accepted as one of the most accurate diagnostic tests in radiology. It was first described in 1924 (Graham & Cole, 1924) and rapidly became incorporated into usual medical practice in the differentiation and diagnosis of causes of upper gastrointestinal symptoms. However, the technique does have problems with accuracy and diagnosis. Mujahed et al (1974) published a retrospective review of 5,000 patients seen over a period of three years. They found that non-visualization of the gallbladder on single-dose examination, requiring a double-dose re-examination, occurred in 25% of their cases. Repeated examination was adequate in 67% so a diagnostic oral cholecystogram was possible in 92% of the 5,000 patients. If extrinsic causes of non-opacification of the gallbladder were excluded, the remainder all had evidence of disease with or without stones or cancer of the gallbladder. Another problem encountered in oral cholecystography is the patient with symptoms suggestive of biliary disease and a normal oral cholecystogram, even after two or more examinations (Gough, 1977). Reid and Rogers (1975) described 17 patients with a strong clinical history of gallbladder disease but normal oral cholecystography. At operation all had evidence of gallbladder disease and nine had gallstones. In another study three patients with a strong history of biliary disease and a normal oral cholecystogram were examined by ultrasonography and found to have gallstones (deGraaff et al,

1978). Problems also arise with patients who are unable to undergo oral cholecystography because of jaundice, pregnancy or vomiting and diarrhoea.

Ultrasonic cholecystography is an alternative method of imaging the gallbladder. Early studies of this procedure reported disappointing results. Fifty patients who had already been examined by oral cholecystography had ultrasonic B-mode examinations performed and the gallbladder was demonstrated in 92% with a diagnostic accuracy for gallstones in those patients whose gallbladders were seen of over 80% (Doust & Maklad, 1974). Goldberg et al (1974) studied the accuracy of ultrasonography in 195 patients compared with oral cholecystography and concluded that oral cholecystography was still the procedure of choice in detecting gallstones. However, these studies used fairly primitive scanners such as bi-stable machines. Recent technical improvements in the scanners and better understanding and interpretation of ultrasonograms has led to a much improved diagnostic accuracy associated with ultrasonography of the gallbladder. Bartrum et al (1977) studied 208 randomly selected patients with possible gallstones. The subjects underwent both an oral cholecystogram and a gray-scale ultrasound examination with the results interpreted in blind fashion. The overall accuracy of the ultrasound was 93% with a false-negative rate of 11% compared with the oral cholecystography results. The authors concluded that the initial investigation of choice was single-dose oral cholecystography with immediate ultrasonography if the gallbladder was not visualised. Techniques have improved further since the above study. The development of high-resolution real-time ultrasound scanners meant

that a thorough examination of the gallbladder and related anatomy was possible in less than five minutes. Moreover, the technique is relatively easy to learn and perform (Cooperberg & Burhenne, 1980). In a study of the accuracy of real-time ultrasonography, calculi were correctly diagnosed in 256 patients and correctly excluded in 43, an accuracy of 96% (Cooperberg & Burhenne, 1980). The false-negative rate was 1.6% and the false-positive rate was less than one percent. A further investigation of the accuracy of ultrasound was carried out in the same study. 124 patients underwent both ultrasonography and oral cholecystography and in five patients, stones were found on ultrasonography after normal oral cholecystography and two went on to be confirmed at surgery. The other three cases were not operated upon. Of the eight patients in whom the gallbladder was poorly visualised on oral cholecystography, ultrasound revealed stones in three. The relative accuracy of the two procedures in this study could not be determined because only 21 cases went on to cholecystectomy. However, the authors concluded that real-time ultrasonography should replace oral cholecystography as the technique of choice in the evaluation of the patient with suspected gallbladder disease. A recent study of the relative merits of the two techniques has been carried out (De Lacey et al, 1984). Their findings were that both procedures were found to be highly accurate in detecting calculi with false-negative rates of less than one percent and that, if a clinician wishes solely to diagnose the presence or absence of stones, then the investigations are equally accurate. As some calculi were missed by either technique, the authors recommended that all gallbladders considered normal by one

technique should be re-examined using the other technique.

Thus, the relative merits of the two main techniques available for the diagnosis of gallstones do not permit an obvious choice for the preferred technique. In a recent review article on diagnostic radiology in biliary disease, Frommhold and Wolff (1983) stated that ultrasonography should be the first differential diagnostic procedure undertaken after the initial history, examination and laboratory tests if biliary pathology is suspected. Unquestionable advantages listed by the authors included the rapid location of the gallbladder, the demonstration of its form and size and the reliable detection of gallstones more than 3-5mm in diameter. For research purposes in particular, ultrasonography is perhaps the better tool for a number of reasons. It is a technique that can be carried out in less than five minutes and does not require the administration of oral contrast agents the evening before the examination. Fasting need only be carried out for five to six hours prior to the procedure. These factors are very important when considering the recruitment of volunteers from the general population as inconvenience will affect response rates. Ultrasound is ethically more acceptable as a screening technique since it does not involve the application of X-Rays and there are no documented reports of harmful effects of ultrasonography in adult humans. Ultrasonography is also a much less expensive technique than oral cholecystography and, while requiring a reasonable level of skill from the operators, is a technique that is not too difficult to learn (Cooperberg & Burhenne, 1980). Its ease and quickness of execution permit large numbers of volunteers to be examined.

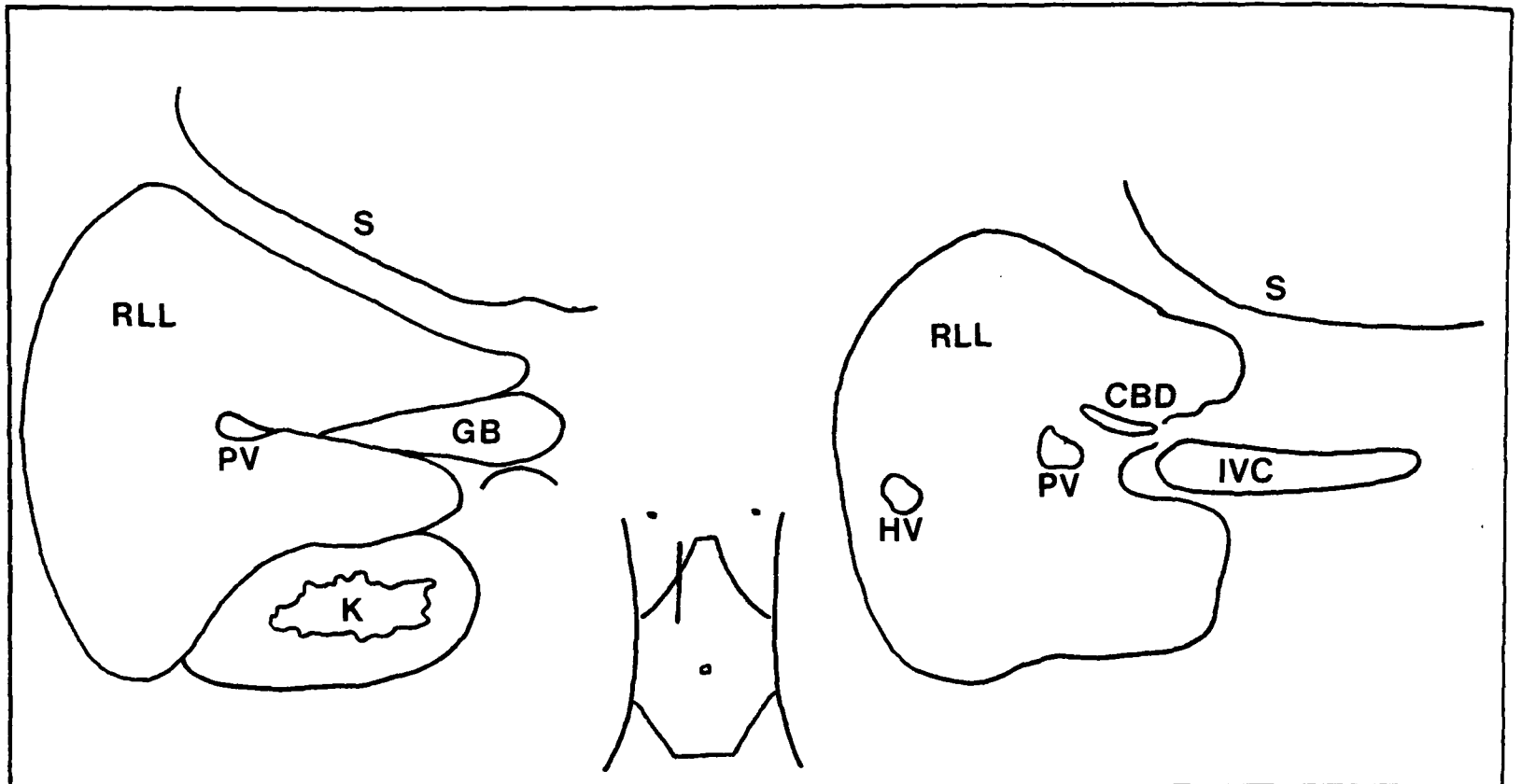
## 6.2 Ultrasound Technique

The machine used in this study was a Toshiba Sonolayer SAL 30A real-time 3.5 MHz linear array ultrasound apparatus. On the one occasion when that particular machine was unavailable for use when visiting a vegetarian centre some distance from Oxford, an identical machine was used at the hospital visited. A 3.5 MHz transducer allows an axial resolution of up to 2mm or better and a linear array of crystals results in a composite picture being built up immediately as opposed to the carrying out of a series of scans in order to produce a composite picture. B-mode scanners require a series of scans in order to produce a composite picture. A review of ultrasonography, its different modalities and their underlying principles has been written by McDicken (1981).

All patients were requested to fast for five to six hours prior to the examination and only liquids were allowed to be consumed during this time. Metreweli (1978) recommended that patients be fasted for 12 hours prior to the examination but it was felt that a fasting period of this length would affect response rates particularly with the examinations being carried out in the late afternoon. A fasting period of five to six hours allowed the participants to have a light breakfast and lengthy fasting periods are not advised routinely.

The procedure used to examine the participants was essentially that described by Cosgrove and McCready (1982) and Metrewelli (1978). The examinations were performed with the subject lying supine and the probe applied in a transverse position initially. Metreweli recommended that a longitudinal scan should be carried out first followed by a transverse scan to allow

**Fig. II.2 Ultrasonogram: Line Drawing of a Normal Gallbladder**



**(a). Longitudinal Scans:**

**S - Skin**

**RLL - Right Lobe of Liver**

**PV - Portal Vein**

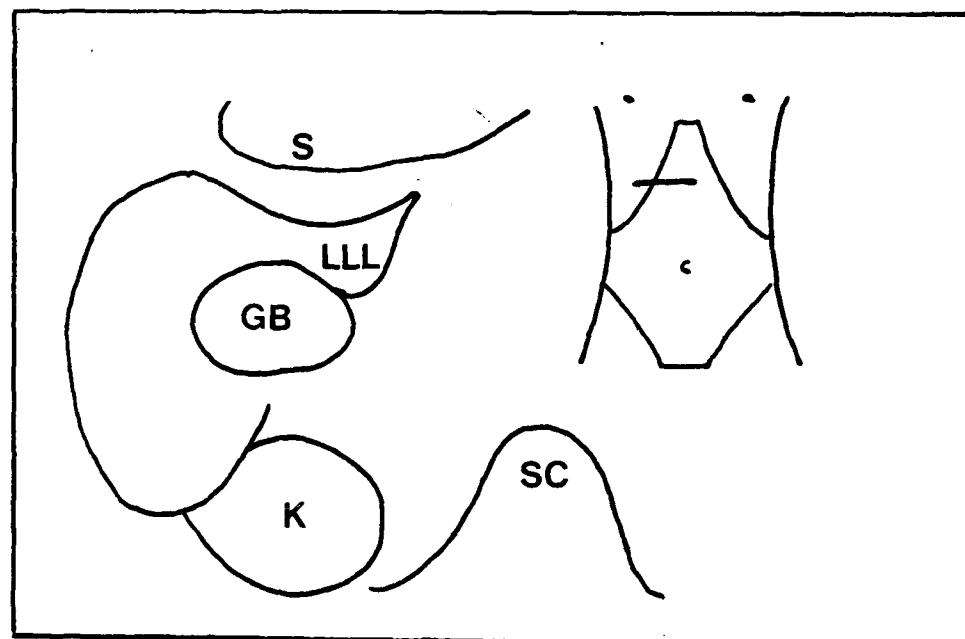
**GB - Gallbladder**

**K - Kidney**

**CBD - Common Bile Duct**

**IVC - Inferior Vena Cava**

**HV - Hepatic Vein**

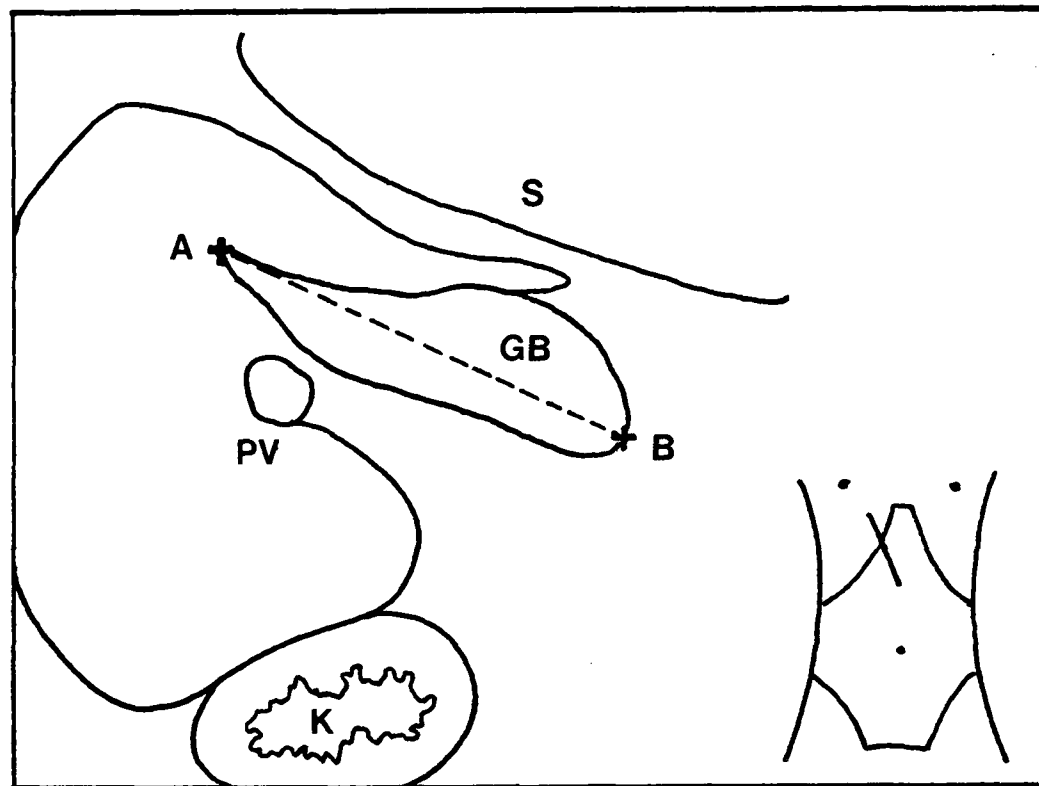


**(b). Transverse Scan:**

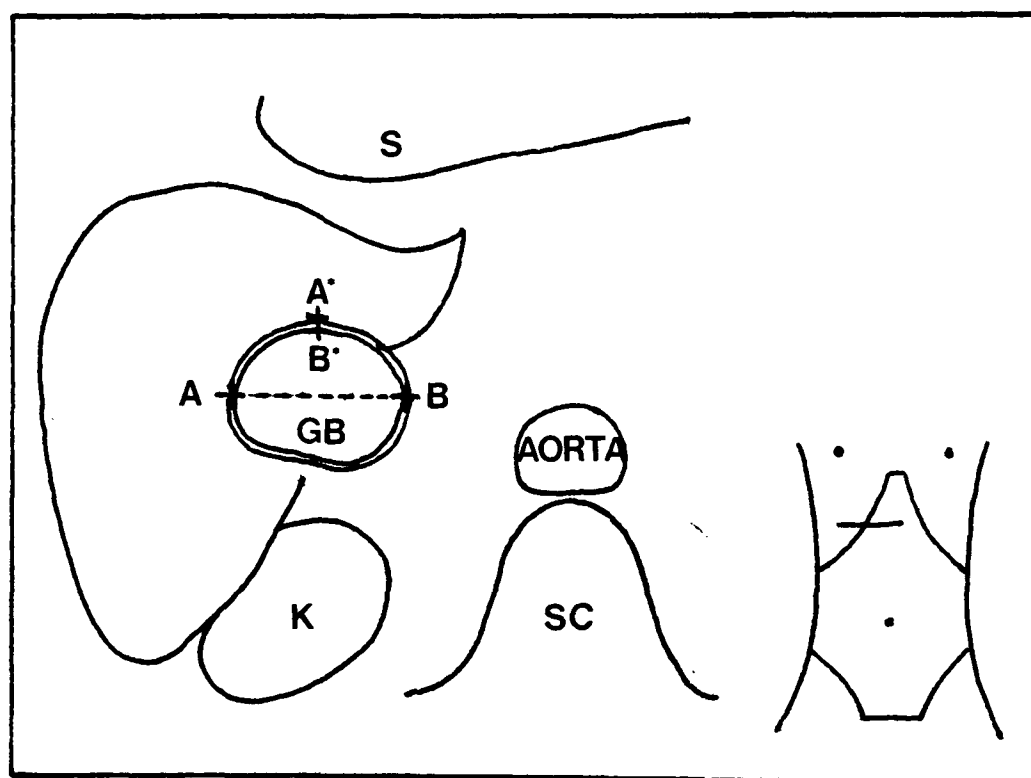
**LLL - Left Lobe of Liver**

**SC - Spinal Column**

**Fig. II.3 Gallbladder Measurements**



**(a). Oblique Scan:  $A-B = \text{Length}$**



**(b). Transverse Scan:  $A-B = \text{Width}$   
 $A'-B' = \text{Wall Thickness}$**

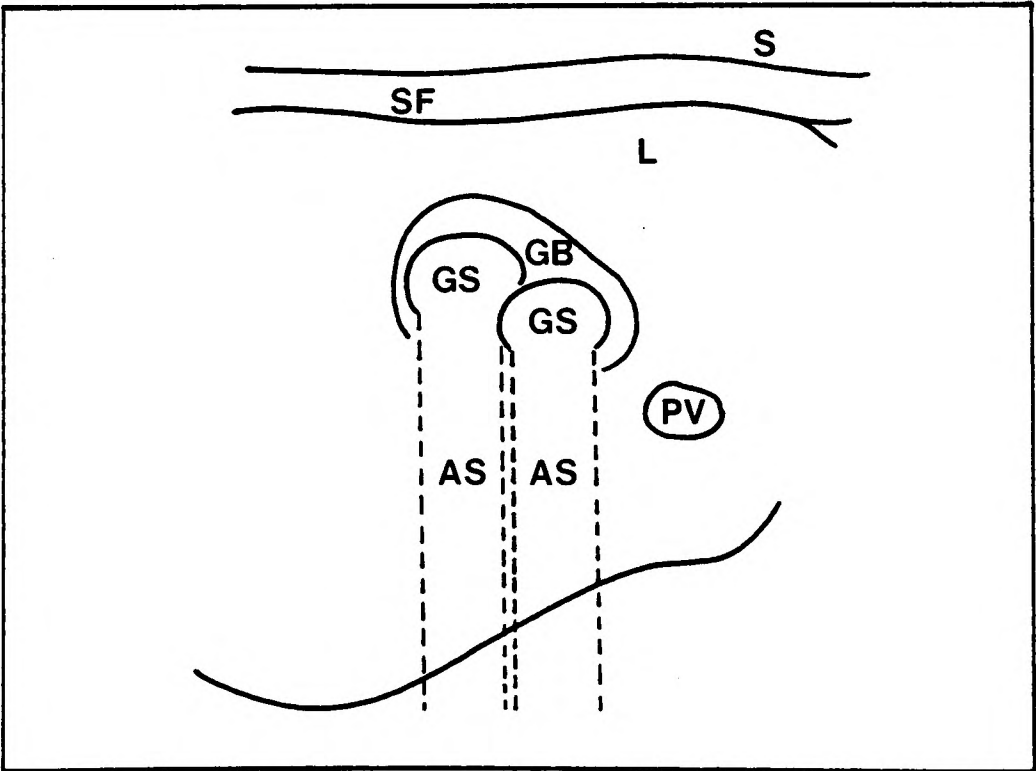
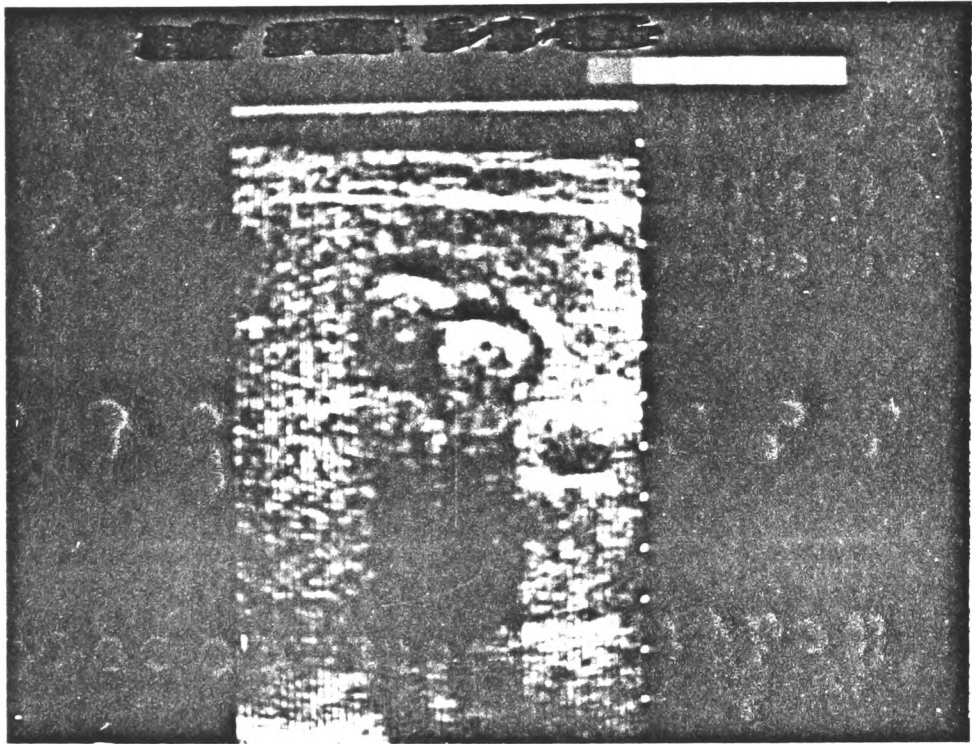
However, Raskin (1980) claimed that a transverse scan should precede a longitudinal scan because a good view at this angle rules out the need for a longitudinal scan. In most patients, the gallbladder was only visible on full, arrested deep inspiration. Once the gallbladder was located in the transverse plane and its anatomical relations determined, the probe was angled obliquely to provide the best delineation of the long axis of the gallbladder (see fig.II.2(a) & (b)). This was measured using the electronic calipers contained in the apparatus (see fig. II.3(a)). The transverse diameter of the gallbladder was then measured from a transverse axial scan and the gallbladder wall thickness was taken from the anterior wall (see fig.II.3(b)). The common duct (uncertainty about whether the duct is the common bile duct or the common hepatic duct is usually present) was measured medially to the gallbladder in the transverse plane, usually at some point where the common duct was above the portal vein (see fig. II.2(a)).

The same two investigators, myself and Dr David Wilson, a consultant radiologist, were present for virtually all of the examinations and, except for an initial period when I was gaining experience and therefore watching Dr Wilson, equal numbers of examinations were carried out by each of us alternatively. Agreement by both investigators on the gallstone status of each subject was obtained at the first examination if possible and any disagreement resulted in careful re-examination of the subject. The most common problem preventing easy visualisation of the gallbladder was that of over-laying bowel gas. This was often dealt with by rolling the subject onto her left side causing the

Table II.5 - Criteria for Diagnosing the Presence of a Gallstone

1. Echogenic region within the gallbladder
2. Acoustic shadowing
3. Movement of the stone on changing posture of the patient (postural Movement)

**Fig. II.4 Ultrasonogram: Gallstones**



GS - Gallstone  
AS - Acoustic Shadow

SF - Subcutaneous Fat  
L - Liver

dealt with by rolling the subject onto her left side causing the bowel to fall away to the left also. If further difficulty was experienced in visualising the contents of the gallbladder the subject was asked to return at a later date if possible for a further examination. This only rarely proved to be necessary.

Table II.5 lists the ultrasonographic criteria for diagnosing the presence of a gallstone as given by Cosgrove and McCready (1982). On ultrasonography gallstones appear as echogenic foci within the gallbladder and they typically cast a strong acoustic shadow and sink to the most dependent position of the gallbladder as they are denser than bile (see fig. II.4). Postural movement of stones may be demonstrated by rotating the subject or asking them to sit up. The acoustic shadow cast by a gallstone is due to the high absorption of the stone and the shadow is devoid of acoustic information. This allows differentiation from shadowing formed by gas-filled viscera as these shadows frequently contain reverberation echoes. If all three criteria are found then the accuracy of ultrasonography is very high (98%) with only a few small stones escaping detection and scarcely any over-diagnosis. These criteria were searched for when the presence of a gallstone was suspected in any examination carried out in this study.

The ultrasound appointment was also used to clarify any uncertain answers found in the returned questionnaire. This was very useful as the questionnaires were not usually completed fully and clearly by the respondents.

### **2.3 Validation of Ultrasonography**

As the accuracy of ultrasonography in the diagnosis of

Table II.6 Ultrasound Validation Study Results

Subject	Ultrasound Report	Oral Cholecystogram Report
VH ♀	Normal	Normal
RH ♀	Normal	Normal
RP ♀	Normal	Normal
JE ♀	Normal	Normal
BD ♀	Normal	Normal
KW ♀	Normal	Normal
JL ♀	Normal	Normal
GS ♂	Normal	Normal
SL ♂	Normal	Normal
LH ♀	Normal	Normal
KMc ♀	Normal	Normal
MA ♂	Normal	Normal
AE ♂	Normal	Poor opacification of the gallbladder, no lesion seen
HS ♂	Several calculi	Non-opacification of gallbladder
BE ♀	Single small calc.	Non-opacification of gallbladder
CG ♂	Probable calculi	Non-opacification of gallbladder
HH ♂	Several large calc.	Faint opacification of gallbladder, stones cannot be excluded
MB ♀	Multiple calculi + sludge	Poor opacification of gallbladder, little contraction after fatty meal, no calculi identified
MHo ♀	Multiple calculi in g/bladder neck	Multiple calculi
MHi ♀	Several small calc.	Normal
ES ♀	Several small calc.	Normal
AB ♂	Large calculus imbedded in wall	Poor opacification of gallbladder yet no lesion seen
SB ♂	Normal	Non-opacification of gallbladder

gallstones is highly dependent on the skill of the investigator, it was thought desirable to carry out a validation study. The accuracy of gallstone diagnosis by the two investigators using real-time ultrasonography was compared to that of oral cholecystography. For one month all patients referred to the John Radcliffe Hospital by their general practitioners for oral cholecystography were asked to also undergo an ultrasound examination (see Appendix D). The ultrasound examinations were carried out by the two study investigators immediately prior to the oral cholecystograms which were performed by radiologists who had no connection with the study and who did not know the outcome of the ultrasound examinations. A total of 23 patients (15 women and 8 men) were so examined and the results are illustrated in Table II.6. Both techniques showed no evidence of stones in 13 cases although, in case A.E., the oral cholecystogram showed poor opacification. Agreement on the presence of a gallbladder abnormality was found in six cases. Gallstones were demonstrated by ultrasonography in three cases where there was nonopacification of the gallbladder under oral cholecystography (H.S., B.E. & C.G.), in one case where there was faint opacification of the gallbladder with probable stones (H.H.) and in one case where there was poor opacification of the gallbladder and little contraction after the fatty meal yet no evidence of any stones (M.B.). In only one case were there stones shown clearly by both investigations (M.Ho.). Ultrasonography demonstrated small calculi in two cases where the oral cholecystogram was normal (M.Hi., E.S.) and in one case ultrasonography demonstrated a large calculus that may have been imbedded in the gallbladder wall while the oral

cholecystogram revealed poor opacification yet no evidence of any stones (A.B.). There was only one case where the ultrasound examination was normal yet the oral cholecystogram revealed a nonopacifying gallbladder (S.B.).

In summary, the results of the validation study showed that complete agreement on gallbladder status was reached in 82.6% and abnormalities were found by one investigation only in 17.4%. In only one case was an abnormality found by oral cholecystography and not by ultrasonography while the reverse was true in three cases. It is interesting to note that the Radiology Department of the John Radcliffe Hospital changed their policy concerning the diagnosis of gallstones once this validation was completed. Ultrasonography is now the initial investigation of choice in cases of suspected gallstones with oral cholecystography being used to clarify uncertain outcomes only.

## **SECTION III - Cholesterol Gallstones: Prevalence and Some Risk Factors**

### **Chapter 7. Methodology of the Prevalence Survey**

#### **7.1 Introduction**

The literature review has attempted to demonstrate that, despite the volume of literature on the prevalence of gallstones and their associated risk factors, there remains a great deal of conjecture and little substantiated fact with respect to gallbladder disease. The true prevalence of gallstones in any community cannot be determined by clinical studies of symptomatic cases only or autopsy surveys of atypical hospital populations. It is necessary to also screen asymptomatic members of the population to reveal asymptomatic cases. Very few prevalence surveys using screening procedures have so far been carried out. An oral cholecystographic survey of the Pima Indian tribe, a group with an extremely high prevalence of gallstones (Comess et al, 1967), was carried out by Sampliner et al in 1970 but their results could not be extrapolated to the American population as a whole. An oral cholecystographic survey of an industrial community in South Wales was carried out by Bainton et al in 1976. Prevalence rates of 12.1% for women aged 45-69 years and 6.2% for men of the same age were reported. These rates were surprisingly low compared with prevalence rates reported in autopsy studies of British populations. The rates may well have been underestimates due to the use of single-dose oral cholecystography (Mujahed et al, 1974).

With the advent of real-time ultrasonography a very useful research tool has become available for radiological epidemiology. This technique is at least as accurate as oral cholecystography (Cooperberg & Burhenne, 1980, De Lacey et al, 1984) but more acceptable as a screening procedure as it does not involve the use of X-Rays, does not require the administration of oral contrast agents beforehand, only takes between five and 10 minutes to carry out and is a very inexpensive technique. A large ultrasonographic survey of female office workers in Rome was reported recently (GREPCO, 1984) and the prevalence of gallstones in these women aged 20-64 years was 9.4%. It is important to note, however, that in this study 25% of all ultrasound examinations were not conclusive. The prevalence of gallstones in a female British population has only been determined by a somewhat inadequate oral cholecystographic survey and it was therefore thought desirable to undertake an investigation of the true prevalence of gallstones in such a population using real-time ultrasonography. Certain associated risk factors could also be investigated in this population by administering a postal questionnaire at the time of initial written contact with the participants.

## **7.2 Methods Used in the Postal Survey**

### **7.2.1 The Population**

It was decided at the outset of the study that only women aged 40-70 years would be investigated as it has been convincingly shown by a large number of earlier studies that the prevalence of gallstones in men is about half that in women (see section I.2.3).

The age restrictions were imposed for similar reasons. The prevalence of gallstones in women increases steadily with age from the menarche but does not become common until late in the fourth decade (Dessau, 1943, Friedman et al, 1966, Horn, 1956, Torvik & Hoivik, 1960, Zahor et al, 1974).

In order to have the best possible chance of detecting dietary differences between women with gallstones and those without, two groups were investigated; nonvegetarian women and vegetarian women. Using the sample size tables of Fleiss (1973) and setting the significance level at 0.05 and the power  $1-\beta$  at 0.80, it was shown that 800 nonvegetarian women and 200 vegetarian women would need to be investigated to enable a significant difference in prevalence between the groups to be demonstrated if an assumption that vegetarian women were two thirds as likely to have gallstones was made. The two groups were recruited by slightly different techniques as outlined below;

(a). The nonvegetarian group was recruited from two general practices in Oxford. Each woman of the correct age group on the age-sex register of a central Oxford general practice was considered as a potential participant and when it was realised that there were not enough women in this age group a second general practice in the Oxford area was sought. As the study progressed, it became evident that the prevalence rates of gallstones in the vegetarian and nonvegetarian women were greater than predicted and the number of nonvegetarian women required in the study was reduced to 650. The required number of nonvegetarian women was reached when about half the women in the second general practice had been contacted.

(b). The vegetarian women could not be recruited from the Oxford area alone as their numbers were too small. The names of all the women participating in the Oxford Vegetarian Study who lived in the Oxford area were collected and those of the correct age group numbered just over 20. With the help of the Vegetarian Society of the United Kingdom active vegetarian communities in towns elsewhere within England were identified. The local societies in these specific areas were subsequently contacted and asked whether it would be feasible for someone within their society to prepare a list of possible participants in that area. The centres approached that were able to participate included every centre contacted except one and were the societies in Bradford, Gloucester, Guildford, Nottingham, Oxford and Reading. This enabled a total of 100 vegetarian women to be investigated and a final 30 women were recruited at the annual conference of the Vegetarian Society of the United Kingdom held in Chester in 1984.

### **7.2.2 Postal Survey**

All eligible women in the nonvegetarian and vegetarian groups were contacted by post. The first letter (see Appendices E & F) contained an explanation about the study and was countersigned by the main participating general practitioner in each practice. Accompanying the letter was a dietary and general health questionnaire (see Appendix A) which was to be completed and returned by post.

The postal questionnaire was based on the format used successfully by Gear in his thesis (1978) and which was modified for use in the Oxford Vegetarian Study. It was self-administered

and the general health questions enabled determination of social class for both the respondent and her husband, body mass index, parity and age at first pregnancy, medical and surgical gastrointestinal history, drug history, family history of gallstones and smoking history. The rest of the questionnaire was devoted to dietary information (see Section II.5.2).

For the nonvegetarian women an appointment for the ultrasound examination was included with the initial letter. This was only a provisional appointment but avoided the problem of subsequent correspondence being required for every volunteer (see Appendix G). The respondents were assured of the flexibility of arrangements and were given the choice of changing the appointment to a more suitable date. All ultrasound investigations on nonvegetarian women were carried out in either the John Radcliffe Hospital in the case of the first general practice or the Radcliffe Infirmary for participants from the second general practice. They had to be done out of normal working hours which meant that the only feasible times were between 5.00 and 6.00 pm on working days.

The vegetarian women presented a special problem because they were, for the most part, recruited from areas distant from Oxford and therefore could not be expected to travel to Oxford for their ultrasound examination. A different technique was used in these cases. Once the list of possible participants had been compiled by the respective local vegetarian societies, they would all be contacted by a letter very similar to the one posted to the women in the general practices (see Appendix H). In this letter a set of dates would be proposed when the ultrasound machine could be brought to the hospital in their area and the date most suitable

to the majority of the women would be selected. The only facilities required in the local hospitals were a darkened room with a trolley and a 13 amp plug.

In the manner described above, 652 nonvegetarian women and 130 vegetarian women were recruited to the study. It was obviously not possible to keep the vegetarian status of most participants hidden from the investigators due to the travel involved for the vegetarian participants. The fact that the study design was not blind may have introduced, therefore, a source of bias.

### **7.2.3 The Problem of Non-Responders**

The nonvegetarian women were first contacted five weeks before their provisional appointment. If no reply had been received two weeks later a second letter was sent. This letter (see Appendix I) also contained a copy of the postal questionnaire and a reminder of the ultrasound appointment. After a further two weeks, if a reply had still not been received, a third letter, identical to the second letter was sent. A final posting (see Appendix J) was sent to all those who had not replied by the time their provisional appointment date had passed. When the study commenced there was a large percentage of nonresponders and a number of letters were returned by the post office. It was evident that the age-sex register of the first general practice was very inaccurate. When the addresses of the patients were checked against the Oxford City Council electoral rolls for 1981 over 15% were found to be incorrect. Silman (1984) found similar problems when updating the age-sex registers of two east London practices. 16% were found to be incorrect according to the return by the post

Table III.1 - Nonvegetarian Reply and Acceptance Rates

Reply	First Post	Second Post	Third Post	Final Post	Total
Acceptances	387	173	126	1	687
Refusals	41	83	115	2	241
No Reply	612	356	115	112	112
Total	1040	612	356	115	1040
% Replies	46.1	27.6	26.0	0.3	-
% Acceptances	56.3	25.2	18.3	0.2	-

office and by checking the addresses against telephone directories. Nonresponders for whom a new address was not found were visited at home and the eventual true address error rate was found to be 26% which was substantially greater than that calculated from returned letters. The fact that the population under study in the above investigation was an inner city population and therefore highly mobile would tend to increase the error. However, it is important to note that nonresponders for whom a new address is not found may still not live at the address on the age-sex register. The second general practice in this study did not have such a high rate of error in the age-sex register and the fact that it was a practice on the outskirts of Oxford may have influenced this.

The reply rates of the nonvegetarian women after the first, second, third and fourth letters can be seen in Table III.1. The overall reply rate which included women who were unwilling to participate in the study was 89.2% (excluding the women who were found to have moved from the address given in the age/sex register). 1040 women aged 40-70 were contacted and 928 replied. Of those women who replied, 687 women agreed to participate in the study which gives an overall acceptance rate of 66.1%. This rate, despite being slightly better than that achieved by Bainton et al (1976) in their cholecystographic survey, was nonetheless disappointing. There may be several reasons why the response rate was poor. The ultrasound examinations were carried out at a time of day that many women would have found inconvenient. Working women often found that they were unable to make any appointment between 5.00 and 6.00 pm because they would not have been able to

leave work in time. Parking was also difficult to find at the Radcliffe Infirmary at these times and travel to the John Radcliffe Hospital was often considered too difficult especially for women of the older age groups. A very common reason given for inability to participate was that meals were usually prepared for the family at this time and children had come home from school.

However, the extra letters sent to initial nonresponders proved useful (see Table III.1). Only 46.1% of all those women who replied did so after the first letter, while 27.6% replied after the first reminder and 26.0% needed a second reminder. Only 0.3% replied after the final letter. Of the 687 women who agreed to come for an ultrasound examination only 652 were eventually screened or considered eligible. 35 were excluded because either the age-sex registers had recorded the incorrect date of birth (22 cases) or the women were unable to keep their confirmed appointments (13 cases).

It could be argued that, with such a poor response rate, there is a danger of bias entering the study as the respondents will be, to some degree, self-selected. For instance, all women who had had a previous cholecystectomy may all agree to participate and therefore bias the prevalence results towards a higher rate. For this reason a study of the nonresponders and women who had refused was carried out.

All the nonresponders and women who had refused to participate in the second general practice were contacted, either by telephone or by post and asked just two questions: their date of birth and whether they had ever suffered from any gallbladder problems. A total of 169 women were thus contacted. 34 of the 46

(73.9%) written to replied and 99 of the 123 (80.5%) with telephones were contacted. 121 of these 133 (91.0%) women agreed to participate in the non-response survey whilst 5 had moved out of Oxford and only 6 refused to help.

Vegetarians tend to be a well motivated group and their willingness to participate in dietary studies has been noted in earlier papers (Hardinge et al, 1954, Mann et al, 1984). In this study, of 141 vegetarian women contacted through their various vegetarian societies, 130 (92%) agreed to participate. Three were vegans. A follow-up study of the non-responders was not carried out in the vegetarian group.

## Chapter 8. Results of the Prevalence Survey

### 8.1 Prevalence of Gallstones

A total of 652 nonvegetarian women and 130 vegetarian women were eventually recruited to the study and found to be eligible according to the age criteria. A few of these women did not have an ultrasound performed during the course of the study. 20 of the 47 women who had previously undergone a cholecystectomy declined to have an ultrasound examination but the history of gallbladder removal was confirmed from the general practice notes in each case. Four women had had gallbladder pathology excluded by either oral cholecystography or ultrasonography in the preceding twelve months during investigations for abdominal symptoms and another three had had gallstones diagnosed by an oral cholecystogram prior to being asked to participate in this study. These women were not investigated further in the study as their results were confirmed from medical records. Of the 130 vegetarian women recruited to the study only two had had previous cholecystectomies and the remaining 128 were all examined by ultrasonography during the course of the study. Therefore 754 ultrasound examinations of eligible women were carried out by the investigators and there were only two inconclusive results. One subject weighed over 125Kg and it was not possible to visualise her gallbladder distinctly while the gallbladder of the other subject was completely obscured by bowel gas and she was unable to return for a second examination. In other cases where bowel gas prevented the gallbladder from being clearly seen the subject was able to return after a day or two for another examination.

Table III.2 - The Prevalence of Gallstones

Group	Number Examined	Number $\bar{c}$ Gallstones	Prevalence %
General Practice 1	235	62	26.4
General Practice 2	417	98	23.5
Nonvegetarians	652	160	24.6
Vegetarians	130	15	11.5

Amongst the 650 nonvegetarians who were successfully examined, 113 (17.4%) were found to have asymptomatic stones and 47 (7.2%) had previously undergone cholecystectomy, giving an overall prevalence rate of 24.6%. The results of the two general practices were slightly different; 62 of the 235 women (26.4%) were found to have gallstones or to have had a previous cholecystectomy in the first general practice compared with 98 of the 417 participants from the second practice (23.5%). Vegetarian women had a significantly lower overall prevalence rate of 11.5% ( $p < 0.01$ ); 13 of the 130 women (10.0%) had asymptomatic stones and two had already undergone cholecystectomy (1.5%). These results are summarised in Table III.2. The odds ratio of developing gallstones in nonvegetarians compared with vegetarians was 2.5:1. 2.5% of the nonresponders were found to have had cholecystectomies. However, it cannot be assumed that the proportion of asymptomatic stones is the same for this group as the larger study group, as postcholecystectomy cases would have been more likely to have agreed to participate in the study. This reasoning is supported by the fact that women who had had cholecystectomies were much more likely to reply to the first posting (62.2%) than women with asymptomatic stones (43.4%).

## **8.2 Gallbladder Dimension Measurements**

During the ultrasound examination, measurements of the gallbladder dimensions were taken (see Section II.6). The length and width of the gallbladder was measured along with gallbladder wall thickness and the patency of the common bile duct. The dimensions varied widely despite that fact that nearly all the participants

Table III.4 - Prevalence of Gallstones by Age\* and Diet Group

Age	Nonvegetarian		Vegetarian	
	Nº	%	Nº	%
40-44	21	15.6	1	3.8
45-49	21	22.8	2	7.7
50-54	30	26.1	3	11.5
55-59	22	19.1	5	22.7
60-64	32	31.4	3	17.6
65-69	30	37.5	1	9.1
Total	156	24.4	15	11.7

\*excluding cases aged 70 years

Table III.5 - Age Distributions of the General Practices and the Vegetarian Group

Age	Practice 1 %	Practice 2 %	Vegetarian %
40-44	22.9	19.5	20.0
45-49	10.8	16.5	20.0
50-54	14.3	19.5	20.0
55-59	20.3	15.1	16.9
60-64	15.2	15.9	13.1
65-69	13.0	12.0	8.5
70	3.5	1.7	1.5

Table III.3 - Gallbladder Dimension Measurements (in mm)

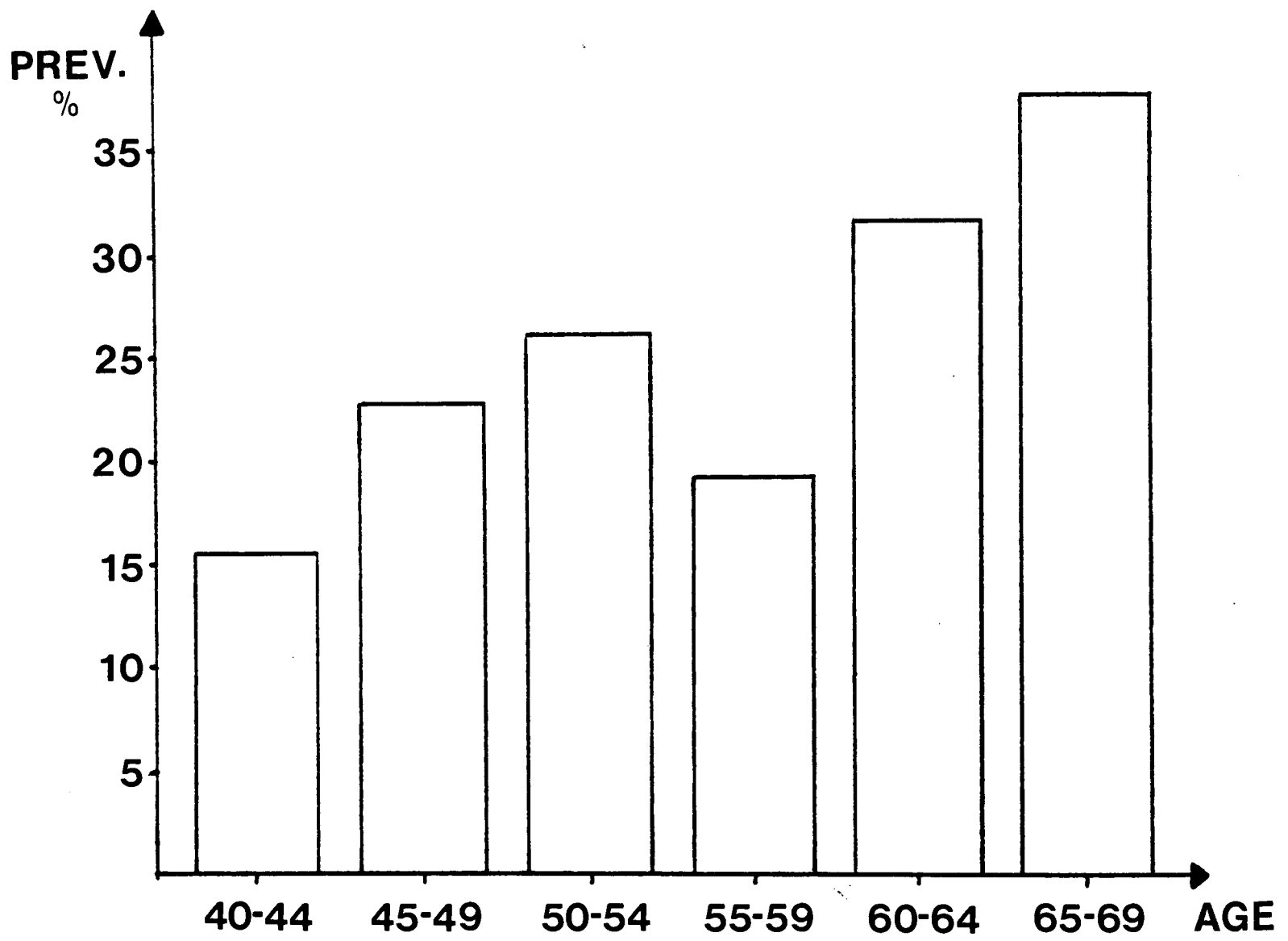
Gallbladder Status	Length		Width		W.Thickness		Common Duct	
	Mean	Rnge	Mean	Rnge	Mean	Rnge	Mean	Rnge
All Participants	56.9	24-89	21.4	5-49	2.16	1-5	2.32	1-7
Normal Gallbladder	57.1	25-86	21.6	6-42	2.14	1-5	2.23	1-5
Gallstones	56.4	24-89	20.7	5-49	2.23	1-3	2.63	1-7

complied with the request to fast for five to six hours. The range for gallbladder length was 24-89 mm. Table III.3 summarises these results and allows comparison between normal gallbladders and those containing stones. It is clear that there is no evidence for any change in gallbladder dimensions following the development of stones. There is no significant difference between the mean measurements of normal gallbladders and those with gallstones.

### **8.3 Associated Risk Factors**

#### **8.3.1 Age**

The ages of the individuals participating were taken at the time of the ultrasound examinations. The women were then allocated to five-year age groups and, for the purposes of even group widths, women who were 70 years old were excluded from the age analyses. The frequency of gallstones tended to increase with age in both nonvegetarians and vegetarians (see Table III.4). The trend was significant at the 1% level ( $\chi^2 = 12.564$ ) in the non-vegetarian group using the Chi-square test for trend (Armitage, 1971). The numbers of vegetarian women in the older age groups are too small to comment on the apparent reduction in frequency after the age of 60 years ( $\chi^2$  for trend = 2.28, NS) but exclusion of the oldest age group from the analysis gives a significant result for trend ( $p < 0.05$ ,  $\chi^2 = 4.046$ ). The nonvegetarian group is of sufficient size to analyse further. The increase of gallstone prevalence with age is a steady one throughout the age distribution except for one age group. There is a noticeable decrease in the prevalence of gallstones in the 55-59 year age group (19.1%)



**Fig III.1 Prevalence of Gallstones x Age**

compared with the 50-54 year group (26.1%). The trend of increasing prevalence with age is well illustrated in fig. III.1 and the discrepancy at the 55-59 year age group is clearly seen. Table III.5 displays the age distributions of the two general practices and the vegetarian group separately and it is evident that the first general practice has an older age distribution than the second practice. This probably explains the difference in gallstone prevalence between the two practices (Table III.2). It can also be seen that the age distribution of the vegetarian group is similar to that of the second general practice so age can not be a confounding factor in the decreased prevalence of gallstones in vegetarian women.

### **8.3.2 Obesity**

All participants were asked their height and weight as part of the postal questionnaire. The accuracy of the replies was not checked by formal measurement at the time of their ultrasound examination and it is appreciated that some inaccuracies will have occurred. Khosla and Lowe (1967) confirmed that Quetelet's index;  $Wt (g) / [Ht (cm)]^2$ , best satisfied the criteria necessary for an epidemiological index of obesity in that it is highly correlated with body weight yet is independent of height. The index was grouped in this study into the divisions of 1.50-1.99, 2.00-2.49, 2.50-2.99, 3.00-3.49 and equal to or greater than 3.50. A Quetelet's index of less than 2.50 suggests that the subject is of low to normal weight, 2.50-2.99 indicates overweight and an index of over 3.00 indicates obesity.

The frequency of gallstones increased with body mass index

Table III.6 - Prevalence of Gallstones by Body Mass Index and Diet Group

Body Mass Index	All Participants			Nonvegetarian			Vegetarian		
	Nº	Nº $\bar{c}$	% $\bar{c}$	Nº	Nº $\bar{c}$	% $\bar{c}$	Nº	Nº $\bar{c}$	% $\bar{c}$
	stones stones			stones stones			stones stones		
1.50-1.99	74	15	20.3	52	11	21.2	22	4	18.2
2.00-2.49	436	72	16.5	356	66	18.5	80	6	7.5
2.50-2.99	198	60	34.3	176	56	31.8	22	4	18.2
3.00-3.49	59	24	40.7	53	23	43.4	6	1	16.7
≥3.50	11	4	36.4	11	4	36.4	0	0	0.0
Total	778	175	22.5	648	160	24.7	130	15	11.5

Table III.7 - Body Mass Index by Diet Group

B.M.I.	Nonvegetarian		Vegetarian		Total
	Nº	%	Nº	%	
1.50-1.99	52	8.0	22	16.9	74
2.00-2.49	356	54.9	80	61.5	436
2.50-2.99	176	27.2	22	16.9	198
3.00-3.49	53	8.2	6	4.6	59
≥3.50	11	1.7	0	0.0	11
Total	648		130		

and the strength of the relationship is quite striking (see Table III.6). Using the Chi-square test for trend, a p value of less than 0.001 was obtained ( $\chi^2 = 20.915$ ). This trend was evident for the nonvegetarian group ( $\chi^2 = 17.996$ ,  $p < 0.001$ ) but not for the vegetarian group ( $\chi^2 = 0.055$ , NS) (see Table III.6). Table III.7 illustrates the body mass index distribution of all the non-vegetarian participants compared with the vegetarian participants and it is quite clear that nonvegetarian women are much more likely to be obese than vegetarian women. However, Table III.6 shows that there are fewer vegetarian women who develop gallstones than nonvegetarian women in every Quetelet's index grouping. When the prevalence rates are standardised for body mass index using the Mantel-Haenszel estimate of relative risk (Mantel & Haenszel, 1959), vegetarian women still have significantly fewer gallstones than nonvegetarian women ( $\chi^2 = 6.64$ ,  $p < 0.01$ ).

The Mantel-Haenszel estimate was also used to standardise the body mass index results for age. Age was not shown to influence the strength of the association between obesity and the prevalence of gallstones ( $\chi^2 = 16.304$ ,  $p < 0.001$ ). Moreover, after adjusting for the effect of both age and body mass index, the odds ratio of developing gallstones in nonvegetarians compared with vegetarians was 1.9:1 (confidence limits 1.1-3.3,  $\chi^2 = 5.24$ ,  $p < 0.05$ ).

### 8.3.3 Diet

The postal questionnaire provided adequate information, especially once poorly answered questions were corrected at the ultrasound examination, for the fibre intakes of the participants to be calculated. The questionnaire data were accurate enough to

Table III.8 - Fibre Intake by Vegetarian Status

Fibre Intake (g/day)	Nonvegetarians	Vegetarians
Total	20.09	33.08
Cereal	8.93	14.71
Vegetable	7.96	12.54
Fruit	3.17	5.84

Table III.9 - Fibre Intake by Vegetarian Status and Gallbladder Status

Gallbladder Status Fibre Intake	Nonvegetarians			Vegetarians		
	Normal	Gall-stones	Post-chole.	Normal	Gall-stones	Post- * chole.
Total	20.00	20.49	19.61	33.30	31.29	32.90
Cereal	8.93	9.21	8.41	14.74	14.63	13.40
Vegetable	7.96	7.94	8.00	12.74	10.79	13.47
Fruit	3.11	3.33	3.20	5.83	5.88	12.07

\* This group consisted of only two women = Post-cholecystectomy.

allow comparison of fibre intakes between groups of participants. Table III.8 contains the different fibre scores for the non-vegetarian and vegetarian groups. In this study vegetarians ate over one and a half times the amount of fibre that nonvegetarians ate. All categories of fibre contributed to their greater consumption with a 65% higher cereal fibre intake, 58% higher vegetable fibre intake and 84% higher fruit fibre intake. When the results were looked at in terms of gallbladder status, it was thought desirable to consider those who had already undergone a cholecystectomy as a separate group from those who had had gallstones diagnosed by ultrasonography. Dietary habits cannot be assumed to remain unchanged after such an operation. Table III.9 shows that for all fibre categories and in both the nonvegetarian group and the vegetarian group, there is no significant difference in the intake between women with normal gallbladders and those who have either undergone cholecystectomy or have had gallstones diagnosed.

The questionnaires were not sufficiently well completed by the respondents to allow determination of their fat intakes. Most respondents reported an inability to calculate quantities of fats and oils bought weekly as these items were usually bought in large quantities occasionally. Further difficulties were experienced in trying to calculate the amount of fat eaten by the respondent only as opposed to the household consumption. These problems led to the abandonment of any semi-quantitative fat analysis.

#### **8.3.4 Parity**

Information was sought concerning the number of pregnancies that had gone beyond 20 weeks and the age at first pregnancy.

Table III.11(a) - Prevalence of Gallstones by Age at First Pregnancy and Diet Group

Age at First Preg.	All Participants			Nonvegetarian			Vegetarian		
	Nº	Nº $\bar{c}$	% $\bar{c}$	Nº	Nº $\bar{c}$	% $\bar{c}$	Nº	Nº $\bar{c}$	% $\bar{c}$
		stones	stones		stones	stones		stones	stones
<20	36	14	38.9	32	14	43.8	4	0	0.0
20-24	234	50	21.4	184	48	26.1	31	2	6.5
25-29	229	47	33.3	197	45	22.8	32	2	6.7
30-34	91	24	26.4	75	20	26.7	16	4	25.0
35-39	25	5	20.0	18	3	16.7	7	2	28.5
≥40	8	1	12.5	5	0	0.0	3	1	33.3
Total	623	141	22.6	530	130	24.5	93	11	11.8

Table III.11(b) - Age at First Pregnancy by Diet Group

Age at 1st Pr.	Nonvegetarian		Vegetarian		Total
	Nº	%	Nº	%	
<20	32	6.0	4	4.3	36
20-24	203	38.3	31	33.3	234
25-29	197	37.2	32	34.4	229
30-34	75	14.2	16	17.2	91
35-39	18	3.4	7	7.5	25
≥40	5	0.9	3	3.2	8
Total	530		93		623

Table III.10(a) - Prevalence of Gallstones by Parity and Diet Group

Parity	All Participants			Nonvegetarian			Vegetarian		
	N <sup>o</sup>	N <sup>o</sup> $\bar{c}$	% $\bar{c}$	N <sup>o</sup>	N <sup>o</sup> $\bar{c}$	% $\bar{c}$	N <sup>o</sup>	N <sup>o</sup> $\bar{c}$	% $\bar{c}$
		stones	stones		stones	stones		stones	stones
0	155	34	21.9	118	30	25.4	37	4	10.8
1	124	29	23.4	100	26	26.0	24	3	12.5
2	219	44	25.1	188	41	21.8	31	3	9.7
3	167	35	21.0	148	33	22.3	19	2	10.5
≥4	113	33	29.2	94	30	31.9	19	3	15.8
Total	778	175	22.5	648	160	24.7	130	15	11.5

Table III.10(b) - Parity by Diet Group

Parity	Nonvegetarian		Vegetarian		Total
	N <sup>o</sup>	%	N <sup>o</sup>	%	
0	118	18.2	37	28.5	155
1	100	15.4	24	18.5	124
2	188	29.0	31	23.8	219
3	148	22.8	19	14.6	167
≥4	94	14.5	19	14.6	113
Total	648		130		778

Table III.10(a) summarises the raw data for parity and the prevalence of gallstone disease for all participants. Analysis for the presence of any trend in the parity figures was carried out and there was no evidence that increasing parity influences the presence of gallstones. ( $\chi^2$  for trend for all participants = 0.736, for nonvegetarians = 0.240 and for vegetarians = 0.115). Similarly, no significant difference was found between the prevalence of gallstones in nulliparous and parous women ( $\chi^2$  = 0.0074). However, vegetarian women are significantly less likely to be parous than nonvegetarian women ( $\chi^2$  = 6.505,  $p < 0.05$ ) (see Table III.10(b)).

Table III.11(a) shows similar results for the prevalence of gallstones by age at first pregnancy. There is no evidence that an earlier age at first pregnancy increases the risk of developing gallstones ( $\chi^2$  for trend = 0.416). Vegetarian women differ from nonvegetarian women in this analysis also (see Table III.11(b)). They are significantly more likely to be aged thirty or more at the time of their first pregnancy ( $\chi^2$  = 3.89,  $p < 0.05$ ).

### 8.3.5 Drugs

The postal questionnaire provided information about the intake of four particular drugs; the oral contraceptive pill, other hormones, clofibrate and cholestyramine. The questions were phrased on an "ever/never" basis. Neither clofibrate nor cholestyramine had ever been taken by any participant in the study.

The relative old age of the women investigated in this study meant that only 176 of 778 women had ever taken an oral contraceptive pill (22.6%). In the initial analysis of gallstone

Table III.12 - Prevalence of Gallstones According to History of Oral Contraceptive Intake and Diet Group

OCP History	All Participants			Nonvegetarian			Vegetarian		
	N <sup>o</sup>	N <sup>o</sup> $\bar{c}$	% $\bar{c}$	N <sup>o</sup>	N <sup>o</sup> $\bar{c}$	% $\bar{c}$	N <sup>o</sup>	N <sup>o</sup> $\bar{c}$	% $\bar{c}$
	stones stones			stones stones			stones stones		
Never-Users	601	146	24.3	498	132	26.5	103	14	13.6
Ever-Users	176	28	15.9	149	27	18.1	27	1	3.7
Total	777	174	22.4	647	159	24.6	130	15	11.5

Table III.13 - Prevalence of Gallstones According to History of Hormone Replacement Therapy and Diet Group

HRT History	All Participants			Nonvegetarian			Vegetarian		
	N <sup>o</sup>	N <sup>o</sup> $\bar{c}$	% $\bar{c}$	N <sup>o</sup>	N <sup>o</sup> $\bar{c}$	% $\bar{c}$	N <sup>o</sup>	N <sup>o</sup> $\bar{c}$	% $\bar{c}$
	stones stones			stones stones			stones stones		
Never-Users	692	152	22.0	572	137	24.0	130	15	100.0
Ever-Users	86	23	26.7	76	23	30.3	10	0	0.0
Total	778	175	22.5	648	160	24.7	130	15	11.5

prevalence and oral contraceptive pill intake, a negative association was found ( $\chi^2 = 5.022$ ,  $p < 0.02$ ). However, this association was accounted for by the fact that women with gallstones were older than those without stones and no association was found after age adjustment ( $\chi^2 = 0.324$ , NS). The results are shown in Table III.12. Vegetarian women were just as likely to have taken the oral contraceptive pill as nonvegetarian women.

The postal questionnaire did not differentiate between hormone replacement therapy and steroid therapy such as corticosteroid treatment for asthma or other hormones such as thyroxine in hypothyroidism. In order to determine the influence of hormone replacement therapy on gallstone prevalence, the women were questioned about specific hormone treatment at their ultrasound examination if they had indicated a positive answer to this question. There was no significant difference in hormone replacement therapy rates between women with gallstones and those without stones and any slight trend towards significance for a negative association in the raw data was lost when corrected for age (see Table III.13). The likelihood of vegetarian and nonvegetarian women having been prescribed hormone replacement therapy at some stage was the same.

### **8.3.6 Clinical Conditions**

The clinical conditions specifically asked about in the postal questionnaire were diabetes mellitus, Crohn's disease and coeliac disease. Respondents were also asked to note down any other conditions of the stomach and/or bowel from which they had suffered. Any history of abdominal surgery was also elicited,

Table III.15(a) - History of Appendicectomy by Diet Group

App'dix History	Nonvegetarian		Vegetarian		Total
	Nº	%	Nº	%	
No	499	77.0	106	81.5	605
Yes	149	23.0	24	18.5	173
Total	648		130		778

Table III.15(b) - History of 'Other' Gastrointestinal Illness  
by Diet Group

'Other' Disease	Nonvegetarian		Vegetarian		Total
	Nº	%	Nº	%	
No	565	87.2	117	90.0	682
Yes	83	12.8	13	10.0	96
Total	648		130		778

Table III.14(c) - Prevalence of Gallstones According to History of 'Other' Gastrointestinal Disorders and Diet Group

'Other' Disorders	All Participants			Nonvegetarian			Vegetarian		
	Nº	Nº $\bar{c}$	% $\bar{c}$	Nº	Nº $\bar{c}$	% $\bar{c}$	Nº	Nº $\bar{c}$	% $\bar{c}$
		stones	stones		stones	stones		stones	stones
No	682	148	21.7	565	135	23.9	117	13	11.1
Yes	96	27	28.1	83	25	30.1	13	2	15.4
Total	778	175	22.5	648	160	24.7	130	15	11.5

Table III.14(a) - Prevalence of Gallstones According to History of Peptic Ulcer Disease and Diet Group

Ulcer History	All Participants			Nonvegetarian			Vegetarian		
	N <sup>o</sup>	N <sup>o</sup> $\bar{c}$	% $\bar{c}$	N <sup>o</sup>	N <sup>o</sup> $\bar{c}$	% $\bar{c}$	N <sup>o</sup>	N <sup>o</sup> $\bar{c}$	% $\bar{c}$
		stones	stones		stones	stones		stones	stones
No	756	169	22.4	630	154	24.4	126	15	11.9
Yes	22	6	27.3	18	6	33.3	4	0	0.0
Total	778	175	22.5	648	160	24.7	130	15	11.5

Table III.14(b) - Prevalence of Gallstones According to History of Appendicectomy and Diet Group

App'dix History	All Participants			Nonvegetarian			Vegetarian		
	N <sup>o</sup>	N <sup>o</sup> $\bar{c}$	% $\bar{c}$	N <sup>o</sup>	N <sup>o</sup> $\bar{c}$	% $\bar{c}$	N <sup>o</sup>	N <sup>o</sup> $\bar{c}$	% $\bar{c}$
		stones	stones		stones	stones		stones	stones
No	605	125	20.7	499	116	23.2	106	9	8.5
Yes	173	50	28.9	149	44	29.5	24	6	25.0
Total	778	175	22.5	648	160	24.7	130	15	11.5

specifically with respect to gallbladder surgery, peptic ulcer surgery, appendicectomy and surgery for treatment of Crohn's disease. Information about other abdominal operations was also sought.

244 out of 778 women suffered from at least one gastrointestinal complaint (31.4%). However, over half of these women (128) had included appendicectomy as their only complaint. Very few women reported any history of peptic ulceration and there was no difference between women with gallstones and those without in this respect ( $\chi^2 = 0.0973$ , NS)(see Table III.14(a)). Appendicectomy was the commonest operation performed with 173 women (22.3%) claiming to have undergone it. Table III.14(b) summarises the results of appendicectomy rates by gallstone prevalence and a positive association between appendicectomy and gallstones is seen. This association is significant at the 5% level ( $\chi^2 = 4.83$ ). The results for gallstone prevalence and the presence of other gastrointestinal disorders are shown in Table III.14(c). "Other" disorders included many gastrointestinal complaints but the commonest were haemorrhoids, diverticular disease and hiatus herniae. Many women complained of the irritable bowel syndrome but, in most cases, the diagnosis had been made by the women herself without confirmation by a doctor. Therefore, it was decided not to include these complaints in the analysis. The category of "other" gastrointestinal disorders includes 96 women (12.3%) and there is no difference between women with normal gallbladders and those women who have gallstones.

Separate analyses were undertaken for appendicectomy and "other" disease rates by vegetarian status (see Table III.15(a) &

Table III.17(a) - Prevalence of Gallstones by Current Smoking Status and Type of Diet

Smoker	All Participants			Nonvegetarian			Vegetarian		
	Nº	Nº $\bar{c}$	% $\bar{c}$	Nº	Nº $\bar{c}$	% $\bar{c}$	Nº	Nº $\bar{c}$	% $\bar{c}$
	stones stones			stones stones			stones stones		
No	649	141	21.7	526	127	24.1	123	14	11.4
Yes	129	34	26.4	122	33	20.6	7	1	14.3
Total	778	175	22.5	648	160	24.7	130	15	11.5

Table III.17(b) - Prevalence of Gallstones According to History of Smoking and Diet Group

Smoker	All Participants			Nonvegetarian			Vegetarian		
	Nº	Nº $\bar{c}$	% $\bar{c}$	Nº	Nº $\bar{c}$	% $\bar{c}$	Nº	Nº $\bar{c}$	% $\bar{c}$
	stones stones			stones stones			stones stones		
Never	385	73	19.0	302	64	21.2	83	9	10.8
Ever	393	102	26.0	346	96	27.7	47	6	12.8
Total	778	175	22.5	648	160	24.7	130	15	11.5

Table III.16(a) - Prevalence of Gallstones According to Family History of Gallstone Disease and Diet Group

Family History	All Participants			Nonvegetarian			Vegetarian		
	N <sup>o</sup>	N <sup>o</sup> $\bar{c}$	% $\bar{c}$	N <sup>o</sup>	N <sup>o</sup> $\bar{c}$	% $\bar{c}$	N <sup>o</sup>	N <sup>o</sup> $\bar{c}$	% $\bar{c}$
		stones	stones		stones	stones		stones	stones
None	589	119	20.2	489	110	22.5	100	9	9.0
2nd Deg.	46	11	23.9	37	9	24.3	9	2	22.2
1st Deg.	128	42	32.8	112	39	34.8	16	3	18.8
Total	763	172	22.5	638	158	24.8	125	14	11.2

Table III.16(b) - Family History of Gallstone Disease by Diet Group

Family History	Nonvegetarian		Vegetarian		Total
	N <sup>o</sup>	%	N <sup>o</sup>	%	
None	489	76.6	100	80.0	589
2nd Deg.	37	5.8	9	7.2	46
1st Deg.	112	17.6	16	12.8	128
Total	638		125		

(b)). Vegetarian women were no less likely to have had an appendectomy or to have suffered from some other gastrointestinal complaint than nonvegetarian women ( $\chi^2 = 1.03$ , NS).

Only 10 respondents in the study claimed to be diabetic (1.3%), and all but two had maturity-onset disease that was treated adequately by diet in most cases. Women with gallstones were no more likely to have diabetes than women with normal gallbladders - 1.0% as compared with 1.3%.

### **8.3.7 Family History**

Participants were asked in the postal questionnaire whether there was any family history of gallbladder disease and, if so, whether the affected person was a first or second degree relative. The results are shown in Table III.16 and it can be seen that women with gallstones are much more likely to have a positive family history of the disorder. This association was significant at the 1% level ( $\chi^2$  for trend for all participants = 9.454). The trend was equally significant for the nonvegetarian group ( $\chi^2 = 7.086$ ) but was not significant for the vegetarian group ( $\chi^2 = 1.945$ , NS). Vegetarian women were no less likely to have a family history of gallstone disease than nonvegetarian women ( $\chi^2 = 0.489$ , NS).

### **8.3.8 Smoking History**

Respondents were asked whether or not they currently smoked cigarettes and whether or not they had ever smoked as much as one cigarette a day for at least one year. The results for current smoking rates are given in Table III.17(a) and there is no

Table III.18(a) - Prevalence of Gallstones by Social Class and Diet Group

Social Class	All Participants			Nonvegetarian			Vegetarian		
	Nº	Nº $\bar{c}$	% $\bar{c}$	Nº	Nº $\bar{c}$	% $\bar{c}$	Nº	Nº $\bar{c}$	% $\bar{c}$
	stones stones			stones stones			stones stones		
I	90	16	17.8	70	15	21.4	20	1	5.0
II	156	34	21.8	131	30	22.9	25	4	16.0
IIIN	49	6	12.2	40	5	12.5	9	1	11.1
IIIM	94	24	25.5	90	24	26.7	4	0	0.0
IV	44	6	13.6	42	5	11.9	2	1	50.0
V	10	2	20.0	10	2	20.0	0	0	0.0
NA	335	87	26.0	265	79	29.8	70	8	11.4
Total	778	175	22.5	648	160	24.7	130	15	11.5

NA = Unknown, retired etc.

Table III.18(b) - Social Class by Diet Group

Social Class	Nonvegetarian		Vegetarian		Total
	Nº	%	Nº	%	
I	70	18.3	20	33.3	90
II	131	34.2	25	41.7	156
IIIN	40	10.4	9	15.0	49
IIIM	90	23.5	4	6.7	94
IV	42	11.0	2	3.3	44
V	10	2.6	0	0.0	10
Total	383		60		443

Table III.17(c) - History of Smoking by Diet Group

Smoker	Nonvegetarian		Vegetarian		Total
	Nº	%	Nº	%	
Never	302	46.6	83	63.8	385
Ever	346	53.4	47	36.2	393
Total	648		130		778

significant difference in smoking rates between women with gallstones and women without ( $\chi^2 = 1.080$ , NS). Analysis for smoking history on an "ever/never" basis, however, shows a significant association ( $\chi^2 = 5.061$ ,  $p < 0.05$ ) between gallstone prevalence and a history of smoking (see Table III.17(b)). This association is confounded by the age of the respondents and the significance is lost with age standardization ( $\chi^2 = 3.73$ , NS). Vegetarian women are significantly less likely to smoke than the nonvegetarian respondents ( $\chi^2 = 12.239$ ,  $p < 0.001$ ) (see Table III.17(c)).

### 8.3.9 Social Class

The social class of each participant was determined from the occupation of her husband according to the Registrar General's social class classification. As a result of this and the fact that the population studied was a relatively elderly one, a large proportion of the women were unclassifiable because their husbands had retired. Moreover, a sizeable group of women, particularly in the vegetarian population, were not prepared to divulge information on the occupation of their husbands. Table III.18 illustrates these problems - 265 nonvegetarian women (40.9%) and 70 vegetarian women (53.8%) were not able to be placed in one of the six social class categories listed. Therefore analysis of the remaining figures is likely to be misleading. There is no evidence from the data, however, that social class has any significant effect on the prevalence of gallstones. The figures do show a significant difference between the social class distributions of the nonvegetarian and vegetarian groups. Vegetarian women are much

more likely to belong the highest two social classes ( $\chi^2 = 9.791$ ,  $p < 0.01$ ).

In summary, real-time ultrasonography was used to determine the prevalence of gallstones in women aged 40 to 69 years. These women were divided into two groups: nonvegetarian and vegetarian. 156 (24.6%) of the 632 women recruited from general practice registers and whom ate meat either had gallstones visible on ultrasonography or had previously undergone cholecystectomy. This was significantly greater than the prevalence of gallstones in vegetarian women ( $p < 0.01$ ). 15 vegetarian women of 130 examined had gallstones or had had a cholecystectomy.

The prevalence of gallstones was found to increase with age and body mass index. As vegetarian women tended to be both younger in age and lower in body mass index than their nonvegetarian counterparts, correction for these variables was necessary. The odds ratio of developing gallstones in nonvegetarians compared with vegetarians after adjusting for the effect of age and body mass index simultaneously was 1.9:1 (95% confidence limits 1.1-3.3,  $\chi^2 = 5.24$ ,  $p < 0.05$ ).

Of the 171 women with gallstones, 42 (24.6%) had a positive family history of the condition compared with 86 (14.6%) of the 591 who did not have any gallstones. No association was detected between gallstones and parity, use of oral contraceptives, social class, cigarette smoking or coexisting conditions.

#### **8.4 Cholecystectomy Rates and Asymptomatic Gallstones**

As the study included women who had previously undergone a a

Table III.19 - Cholecystectomy Rates and Prevalence of Asymptomatic Gallstones

Group	Post-Cholecystectomy			Asymptomatic Stones		
	Nº	%cases	%	Nº	%cases	%
Practice 1	16	25.8	6.8	46	74.2	19.6
Practice 2	31	31.6	7.5	67	68.4	16.1
Nonvegetarian	47	29.4	7.2	113	70.6	17.4
Vegetarian	2	13.3	1.5	13	86.7	10.0

cholecystectomy, it was possible to determine cholecystectomy rates from the prevalence results. Table III.19 summarises the cholecystectomy rates for each group and 7.2% of nonvegetarian women aged 40-70 had had their gallbladders removed. This figure was almost five times greater than the cholecystectomy rate for vegetarian women.

The data also allow differentiation of symptomatic cases (i.e. post-cholecystectomy) and asymptomatic cases where there was no known previous history of gallstones. 70.6% of all nonvegetarian women with gallstones and 86.7% of vegetarian cases (75.0% overall) were asymptomatic (see Table III.19). Thus 17.4% of nonvegetarian women in this study had evidence of gallstones that had not previously been diagnosed. The vegetarian women were significantly less likely to have silent gallstones ( $\chi^2 = 3.84$ ,  $p = 0.05$ ).

## 8.5 Discussion

The prevalence of gallstones in women aged 40-70 years was found to be 24.6% in Oxford. The study was the first ultrasonographic prevalence survey to be carried out in Britain and cannot, therefore, be compared directly with other studies which also have attempted to determine the prevalence of gallstone disease in this country. However, a prevalence survey using oral cholecystography was carried out in Barry, South Wales by Bainton et al (1976). The prevalence of gallstones in women aged 45-69 years in that study was 12.1% - less than half the rate reported above. There are several reasons that could explain why the Barry rate was so much less than that in Oxford. Oral cholecystography

is not as accurate at detecting small gallstones and biliary sludge as ultrasonography (Frommhold & Wolff, 1983) and the technique of single-dose oral cholecystography used in the Barry study may not result in an adequate examination. Mujahed et al (1974) reported that 25% of cases undergoing single-dose oral cholecystography required a repeat, double-dose examination to obtain adequate results.

Regional variations in gallstone prevalence have been reported and an inverse relationship between the prevalence of gallstones and the incidence of ischaemic heart disease was demonstrated by Barker et al (1979). Oxford has a low incidence of ischaemic heart disease so might be expected to have a high prevalence of gallstones as opposed to Barry which lies in the industrial area of South Wales and has a moderately high incidence of ischaemic heart disease. However, the existence of regional variations in gallstone prevalence has not been confirmed by other data and the significance of the findings in the Nine Towns survey (Barker et al, 1979) has been questioned because of small numbers and short sampling times (Bateson, 1984). Furthermore, even if regional variations in gallstone prevalence do exist, they could not account for the difference in gallstone prevalence between nonvegetarians and vegetarians. The numbers of women examined in the Barry study were quite small. Only 278 women were seen and with a response rate of 61.2% it is reasonable to suspect that some form of bias may have resulted in an underestimation of the true prevalence.

The results of autopsy surveys of gallstone disease in Britain indicate that 12.1% is an underestimate. Bouchier (1969)

reported a prevalence of 19.2% in women aged over 20 years and if the figures were restricted to women aged over forty years the rate would increase considerably. Earlier autopsy surveys had slightly lower prevalence rates for gallstones in women; Cooke et al (1953) - 12.1%, Gross (1929) - 12.3 and Horn (1956) - 13.2%. However, Gross' results were for women of all ages and the other two studies examined the autopsy records of women aged twenty or more. Also, as was shown in the literature review, there is some evidence that the prevalence of gallstones has increased in Britain during the course of this century. The prevalence rate of 24.6% in women aged 40-70 years reported in this study is therefore consistent with findings in most other studies carried out in Britain and indicates that gallstone disease is as common in Britain as it is in most other western nations. Even if the lower rate of symptomatic cases in the non-responders is taken into account, the overall prevalence rate is only reduced to 22.0%.

A variety of possible risk factors associated with the development of gallstones were investigated as part of the prevalence survey. Age was clearly identified as a definite risk factor and the prevalence of gallstones increased steadily with increasing age so that 65-69 year old women were shown to have a gallstone prevalence of 37.5%. This relationship was not an unexpected one and the great majority of autopsy and clinical studies of gallstones have shown a similar increase in prevalence with age. The fact that no such relationship was reported in the survey by Bainton et al adds further weight to the possibility that the sample was a biased one in that study. Fig. III.1

illustrates the association found between prevalence and age in the Oxford study and there appears to be a clear divergence from the trend in the 55-59 year age group. This may just be due to artefact especially since there were fewer women in this group than in the others. However, the flattening of the prevalence curve at this age group may be real. The women of that age group would have undergone menopausal changes in the previous five years and the diminished oestrogen influence may have meant that fewer gallstones were being formed. A study of Pima Indian girls demonstrated an increase in the prevalence of gallstones following puberty (Bennion et al, 1978) and Bennion (1977) reported a decrease in the saturation index of bile in a woman who had undergone surgical menopause. Several autopsy surveys have reported a decrease in the difference in gallstone prevalence between men and women in the older age groups (Bateson, 1984, Gross, 1929, Horn, 1956). This was thought to be due to a reduced risk of forming gallstones in post-menopausal women. Another possible explanation for the flattening of the prevalence curve is the presence of a cohort effect. The women of the 55-59 year age group would have been entering their reproductive years during the course of the second world war and the post-war rationing years. The diet consumed during this period was markedly higher in fibre content with much less fat intake than the British diet of before and after the war and, therefore, closer to the diet of developing nations. The beneficial effects of this diet may have been maximal in girls who had recently reached the menarche.

Obesity is another well-acknowledged risk factor in the aetiology of gallstones. The association between obesity and

gallstone prevalence in this study was particularly strong. Women with a body mass index of 3.00 or more are regarded as obese and, regardless of age, obese women had a 37.5% gallstone prevalence in this study. These findings add further epidemiological support to the hypothesis that obesity leads to increased hepatic cholesterol synthesis and subsequent gallstone formation.

A positive family history of gallbladder disease is also a strong predictor of risk. 30.8% of women with gallstones had a positive family history of the disease as compared with 20.5% of women without gallstones. The risk was particularly great for women who had one or more first degree relatives with a history of the disease. The strength of this relationship points to the importance of genetic factors in the aetiology of gallstone disease.

The effect of pregnancy on the development of gallstones is far less clear. Evidence for an association between pregnancy and the new formation of gallstones is weak and the results of this study did not support the possibility either. Nulliparous women were just as likely to have evidence of gallstone disease as parous women and there was no trend of increasing gallstone prevalence with increasing parity if parous women only were considered. Similarly the age at first pregnancy did not appear to influence the gallstone prevalence. These findings are at odds with those of Scragg et al (1984(a)) who reported that the risk of developing gallstones increased with increasing parity and with decreasing age at first pregnancy. Their results were more significant for the younger women in the study and the authors suggested that subgroups of women existed who were more

susceptible to the development of gallstones and these women were likely to develop their stones at an early age. The women in this study were possibly too old, therefore, to detect any parity effect if it is only an important influence in younger women. The question of the role that pregnancy plays in the aetiology of gallstones is a very difficult one and the confusion has not been lessened by the results of recent surveys. A strong positive association was reported in the Roman study (GREPCO, 1984) and the researchers in this study also noted a slight dose-response effect with the gallstone risk increasing with multiparity. Another ultrasonographic survey reported no association between gallstones and parity (Rhomberg et al, 1984).

The evidence for oral contraceptive intake being a positive risk factor in the development of gallstones is even less convincing than the evidence for parity. After initial reports that oral contraceptive use predisposed to the development of gallstones (BCDSP, 1973) later studies refuted this claim (Layde et al, 1982, RCGP, 1982) and the feeling is now that oral contraceptive use may result in the formation of gallstones in a subgroup of women who are susceptible to the development of stones but that these women would have gone on to develop gallstones anyway and the eventual prevalence is, if anything, decreased. The results of Scragg et al (1984(a)) support this hypothesis as they found an age-dependent variation in the risk associated with oral contraceptive use. The oral contraceptive pill was not shown, in this study, to have any influence at all on the prevalence of gallstones. The raw data showed a negative association between pill use and the risk of gallstones but this association was lost

when the data were standardized for age. The older women in this study were too old to have been prescribed oral contraceptives in their reproductive years and yet they are more likely to have gallstones. Moreover, due to the relative old age of the population studied, only 22.6% of the women participating in this study had ever taken the oral contraceptive pill. Even fewer participants had ever been prescribed hormone replacement therapy (11.1%) and there was no association with gallstone disease.

Clinical conditions have often been associated with a risk of developing gallstones and the evidence for some of these associations such as terminal ileal disease is quite convincing. However, the fact that none of the implicated illnesses occurred with any frequency suggests that these conditions do not play an important role in determining the prevalence of gallstones in the general population. A history of peptic ulcer disease was elicited from only 22 women in the study and only one woman had coeliac disease. Women with gallstones were no more likely to have had problems with peptic ulcers than women with normal gallbladders. The results were similar if women were looked at in terms of the presence of any other gastrointestinal illness. Women without stones suffered from as many other gastrointestinal problems as the women with gallstones. The operation that was associated with a positive risk of developing gallstones was appendicectomy. 28.6% of women with gallstones had had their appendices removed while 20.4% of women without gallstones had undergone the operation. I have been unable to find any previous studies reporting an association between gallstones and appendicitis but the possibility of a common aetiological factor cannot be discounted.

Dietary influences are considered to play a role in the development of both conditions and there may be a common dietary factor involved. However, the association could be a spurious one. Several women in the study reported that their appendix had been removed at the same time as their gallbladder and histological proof of active appendicitis was not sought for this study.

The smoking status of the participants was one of several other factors analysed in the prevalence survey. No significant difference was shown between the women with gallstones and women with normal gallbladders in their current smoking habits. However, when looked at in terms of the participants ever having smoked, women with gallstones were more likely to have been smokers for at least a year at some time. This positive association was no longer apparent when the data were standardized for age.

The social class findings in this study should be regarded with caution for a number of reasons. The study population was relatively elderly so many husbands had retired, preventing classification of their wives. Furthermore, a number of women were unwilling to fill in the question regarding occupations on the postal questionnaire. As a result, social class information was only available for about half the participants. The data for these women did not reveal any social class trend in the prevalence of gallstones.

The most striking feature of the prevalence rates revealed in this study is the significantly lower gallstone prevalence in the vegetarian women compared with the nonvegetarian women. Only 11.5% of vegetarian women aged 40-70 years had evidence of gallstone disease. Vegetarian women were much less likely to be obese than

nonvegetarian women and tended to be younger than the nonvegetarian women in this study. However, the difference in prevalence rates between the vegetarian and nonvegetarian groups remains significant when standardized for age and body mass index. A family history of gallstone disease is no less likely to be found in the vegetarian group than in the nonvegetarian group so this cannot be considered as a confounding influence. These figures indicate that there is some factor or factors associated with the lifestyle and diet of vegetarianism that protects against the development of gallstones.

A careful search of the literature has not revealed any other study that has attempted to determine the prevalence of gallstones in vegetarian women and compare it with the rate for nonvegetarian women. The above finding is a very important one in that it supports the hypothesis that gallstones are a western disease and have become more common during the past 100 years or more due to changes in the lifestyle and diet of inhabitants of western communities.

Several dietary hypotheses have been put forward by researchers to explain the differences in prevalence between developed and developing nations. Heaton argued that the increasing prevalence of gallstones in western communities was due to an increasing consumption of refined carbohydrates (Heaton, 1973). He discounted the consumption of saturated animal fat as a primary factor because gallstones were rare in some groups of people who consumed very large amounts of animal fat such as the Masai tribespeople (Biss et al, 1971) and because experiments on animals induced gallstone formation if they were fed sugar-rich

diets (Englert et al, 1969) or fat-free, sugar-rich diets (Dam, 1971). Cleave was less specific in his dietary hypothesis that many western diseases were a result of a massive consumption of simple sugars (Cleave, 1974) but both authors, along with Burkitt (1973) pointed to a lack of fibre in western diets associated with an increase in consumption of substances like sugar and white flour as being the underlying cause of an increased prevalence of gallstones. However, analytical evidence for this hypothesis is not convincing despite the findings of a recent case-control study that demonstrated that simple sugars were a positive risk factor in the development of gallstones (Scragg et al, 1984(a)).

A variety of other dietary factors have been implicated in the causation of gallstones but the associations are generally more tenuous than the hypothesis described above. Sarles and his group have carried out a series of case-control studies where they have reported that gallstone subjects are more likely to have an increased total calorie intake than their controls. A major problem associated with analyses of calorie intake is the great difficulty in assessing total calorie intake with any degree of accuracy and the findings of Sarles et al (1957, 1969, 1978(a)) have not been duplicated in other centres. Sturdevant et al (1973) reported an increased prevalence of gallstones associated with a diet high in polyunsaturated fats. However, their findings were not supported by another, similar study (Miettinen et al, 1976) and the P/S ratio in the former study was extraordinarily high and unlikely to be reached by any free-living populations. The effect of dietary cholesterol on gallstone prevalence is even less certain but the only positive evidence for its effect suggests

that cholesterol plays a modest role, if any at all (Dam et al, 1971, DenBesten et al, 1973).

One of the reasons for the lack of evidence for dietary hypotheses may be that dietary studies of western communities can no longer detect differences between people with gallstones and those without because the average nutrient consumption is above the threshold at which differences can be detected (Heaton, 1984, Scragg et al, 1984(a)). One method of avoiding this problem is to investigate people who do not follow a typical western diet.

The vegetarian diet has been shown to contain less saturated fats and more complex starch than nonvegetarian diets in Britain (Bull & Barber, 1984) and is, therefore, closer in content to the diets of developing nations as described by Trowell (see fig. I.3). The postal questionnaire that was part of the prevalence survey was not able to provide very much accurate information about the dietary intakes of the participants in the study but fibre intakes were calculated. Vegetarian women ate more than one and a half times as much dietary fibre as the nonvegetarian women but there was no significant difference shown in the amount of fibre that women with gallstones consumed compared with those who have normal gallbladders in either the vegetarian group or the nonvegetarian group. The results were analysed separately for women who had previously undergone cholecystectomy and women with asymptomatic stones. This was to prevent the dietary changes that may accompany the onset of symptoms or occur after surgery from affecting the data obtained from women who were asymptomatic but no differences in the fibre intake of any group were present.

It was not possible to obtain any further useful dietary

information from the questionnaire. Section IV of this thesis describes the case-control study that followed this prevalence survey and a much more detailed dietary analysis was possible in that study.

One other interesting finding in the prevalence survey warrants a discussion. The presence of asymptomatic gallstones has been recognised for centuries yet their prevalence was unknown. Estimates of about 50% of all gallstones have been made on the basis of autopsy findings and these estimates have then been used in decision analyses on the benefits of prophylactic cholecystectomy for treatment of asymptomatic gallstones (Fitzpatrick et al, 1977). More than 70% of the cases identified in this study as having gallstones were unaware of their condition although not every case of asymptomatic gallstones in this study can be labelled as truly asymptomatic. Some women may well have had some gastrointestinal symptoms as a result of their gallstones. However, only two women of the entire group who had gallstones present upon examination in the study, had ever had symptoms severe enough to persuade them to visit their general practitioner and undergo a diagnostic test. Therefore, the results of this study suggest that asymptomatic gallstones may be a commoner disorder than previously believed and may lead to revision of decision analyses. If over 70% of gallstones are asymptomatic, then previous calculations about the likelihood of gallstones going on to cause symptoms must be altered in favour of a more optimistic outlook. The symptomatology of participants with gallstones in situ was determined in the case-control study described in Section IV.

It was decided at the outset of the study that cases of asymptomatic gallstones diagnosed during the course of the study should be reported to the respective general practitioner. The women themselves were not told of the diagnosis unless they specifically requested it. It was felt that the knowledge of any disorder, regardless of its innocence, was likely to cause distress to some participants and we believed that any decision on that matter was better left to the general practitioner to discuss with his patient.

## 2.6 Summary

The frequency of gallstones has been compared in two groups of women aged 40-70 years using real-time ultrasonography. 652 women were recruited from two general practices in Oxford and 130 vegetarians. 24.6% of the nonvegetarian women and 11.5% of the vegetarian women either had gallstones visible on ultrasonography or had previously undergone cholecystectomy. The odds ratio of developing gallstones in nonvegetarians compared with vegetarians was 2.5:1.

The frequency of gallstones was found to increase with age and body mass index and a family history of gallstone disease was reported more frequently in women with gallstones. However, no association was found between gallstone prevalence and parity, use of exogenous oestrogens, the presence of other gastrointestinal disorders excluding appendicectomy, smoking history and social class gradient.

The prevalence of gallstones in vegetarian women was reduced by their reduced likelihood of obesity but the difference between

their prevalence and that of the nonvegetarians remained significant after correcting for body mass index and no other confounding influence could be identified. The odds ratio of developing gallstones in nonvegetarians compared with vegetarians after adjusting for the effect of age and body mass index was 1.9:1 (95% confidence limits 1.1-3.3,  $\chi^2 = 5.24$ ,  $p < 0.05$ ).

Thus, evidence is offered concerning a protective dietary or lifestyle factor for the common condition of gallstones. Further information about this factor or factors may be obtained from the case-control study that followed this prevalence survey.

## **Section IV - Cholesterol Gallstones: A Case-Control Study**

### **Chapter 9. Methodology of the Case-Control Study**

#### **9.1 Introduction**

The primary aim of a case-control study is to explore the aetiology of a given condition by searching for differences in the prior exposure of the cases and controls to a range of suspect agents or factors. Therefore, a case-control study is based on a hypothesis or set of hypotheses about the aetiology of the condition and the aim is to test these hypotheses (Alderson, 1982). The hypothesis may have been suggested by either descriptive epidemiological surveys or by other analytical studies. The information collected in case-control studies is retrospective and, therefore, subject to problems of validity and recall bias but the advantages of such a design include quickness of execution and recruitment of relatively fewer numbers than prospective studies. Case-control studies are also particularly suitable for the investigation of several hypotheses in the one study (Alderson, 1982).

The literature review cited many examples of case-control studies of gallbladder disease and a lot of valuable information has resulted from these investigations. However, all of the studies are seriously flawed by either one or both of two major problems. Case-control studies examine the differences in exposure to possible risk factors of cases and controls and it is important, therefore, to ensure that the controls do not have the disease being investigated in an asymptomatic form. Many studies

of gallstones did not actively screen the control population to exclude the presence of silent gallstones. Furthermore, almost all analytical studies of gallstones carried out to date have been clinical studies of cases with symptomatic disease. People with asymptomatic stones have been largely ignored despite the fact that these cases are just as important in terms of exposure to risk factors. Biases are likely to arise from analyses of such a selected group as symptomatic cases who are usually recruited in hospital and are often interviewed post-operatively.

The survey of gallstone prevalence which was described in the preceding section of this thesis was able to identify both asymptomatic and symptomatic cases of gallstones. Moreover, disease-free individuals were also identified. A case-control study was set up using the women screened in the prevalence survey and its aim was to examine the influence of some of the possible risk factors on the development of gallstones.

## **9.2 Selection of Cases and Controls**

The prevalence survey screened over 800 women, 782 of whom were suitable for inclusion into the study according to the age restrictions of the study. A total of 175 women with gallstones or a past history of cholecystectomy were identified, 160 nonvegetarian women and 15 vegetarians. Each of the 175 women were regarded as potential cases for the case-control study.

The disease-free individuals formed the population from which the age-matched controls were drawn. Controls were selected by their date of birth which had to be within one year of that of the matching case. The closest matching control was used if there was

more than one possible choice. In almost every pair such close matching of age was possible. However, in a few cases, particularly with the older women where the relative frequency of gallstones combined with fewer participants led to a scarcity of suitable controls, the age difference between the case and control was as large as two years but never greater than this. Initially, each nonvegetarian case was matched with a nonvegetarian control from her specific general practice and the vegetarian cases were matched with vegetarian controls. When the study was almost completed, cases whose designated control did not agree to participate in the study were assigned another control of the correct age. Each case was matched with one control.

### **1.3 Procedure of Investigation**

All the cases and controls were sent an explanatory letter for the case-control study and the wording of this letter was slightly different for the nonvegetarians and vegetarians (see Appendix K(i) & (ii)). The women were not told that the study was of a case-control design but that further detailed dietary information was needed to augment the data obtained from the postal questionnaires. Enclosed with the letter was a copy of the four day dietary diary described in section II.5.3 (see Appendix B). The women were asked to detach the bottom of the letter and return it to inform us of their wish to participate or not in the second part of the study and participants then completed their diaries according to the detailed instructions listed at the beginning of the diary. Contact was made with each participant to arrange a suitable time for an interview. If no reply was received

after the first letter a second letter was sent along with another dietary diary (see Appendix L). Another posting of this second letter was sent if there was still no reply after about one month. A final attempt to recruit the women was made by sending one last letter (see Appendix M).

Within one month of completing the diary, each participant was visited and interviewed by either myself and/or my research assistant, Kath Bunch (SRN). The interview was semi-structured and divided into two main sections (see Appendix II.5.3 for the interview proforma). Initially the dietary diary was carefully checked to enable mistakes to be corrected, handwriting clarified and quantities and recipes accurately assessed and appended. Then, using the proforma, a weight history and any history of gastrointestinal illnesses or surgery were elicited. The presence of any other medical complaints was also asked for. A history of any upper gastrointestinal symptomatology was then elicited with the aid of a checklist and also any history of exogenous oestrogen treatment. The remainder of the interview consisted of a compilation of the participant's usual daily dietary intake and meal routines. A checklist at the end was used to include such items as chocolate, crisps and cakes that may have been forgotten in the description of the "typical" day. The participant was then questioned about usual alcohol intake and whether or not she smoked and for how long and how often.

Each interview took, on average, about 45 minutes and, at the end of it, a 10 ml sample of venous blood was requested. As this request was not mentioned in the initial contact letter to avoid unnecessary refusals, a number of women were not happy to give any

blood at the time of the interview.

#### 9.4 Lipid Analyses

The blood samples collected were not fasting samples because of the decision not to mention the plan to collect blood in the initial letter of contact. Furthermore, the interviews were carried out at varying times of the day and evening and it was felt that the response rate may have been affected if participants were expected to fast for long periods during the day. The only serum lipid measurement that needs to be measured in a fasting state is the triglyceride level so this was not included in the analysis (Simpson et al, 1980). The samples were analysed, therefore, for total serum cholesterol and the various lipoprotein fractions which did not require fasting.

All samples were placed in lithium heparin tubes and separated within 24 hours. The plasma was stored at 4 C and assayed within 48 hours. The technicians performing the analyses were unaware of the gallstone status of the patients.

The cholesterol measurements were made on a Technicon Autoanalyser II by the Liebermann-Burchard reaction using unextracted samples (Huang et al, 1961). Samples for total cholesterol were diluted 1:1 with 0.154-M-NaCl before analysis. High-density lipoprotein (HDL)-cholesterol was measured in the supernatant obtained by precipitation of the low-density lipoprotein (LDL) and very low-density lipoprotein (VLDL) with heparin and manganese chloride (Burstein M et al, 1970). LDL and HDL-cholesterol were measured in the supernatant obtained after precipitation of VLDL by sodium dodecyl sulphate (Ononogba et al,

1976) and the LDL-cholesterol obtained by the difference of this result and the HDL estimation. VLDL levels were determined indirectly by subtracting the sum of the HDL and LDL levels from the total cholesterol estimation.

Blood samples were not obtained from the vegetarian cases and controls as their participation in the case-control study was on a postal basis only. Whilst blood samples could have been returned through the post, venepuncture would have had to be performed by their own general practitioners and contact of these practitioners was not always possible.

### **9.5 Statistical Analyses**

The statistical significance of the results was tested, when appropriate, using a programme called PECAN devised by Storer (1983). PECAN performs regression analysis of conditional likelihood functions which arise in matched or stratified case/control studies. Standard analyses such as the mean and standard deviation of the data groups were also calculated and the means were compared using the t-test.

Table IV.1 - Case-Control Study Response Rates

Reply	First Pract.	Second Pract.	Total Pract.	Vegetarian	Total Partic.
Acceptances	87	141	228	25	253
Refusals	28	45	73	2	75
Exclusions	4	5	9	1	10
No Reply	6	5	11	1	12
Total	125	196	321	29	350

## Chapter 10. Results of the Case-Control Study

### 10.1 Response Rates

In the first general practice, of the 125 women contacted, 119 replied (95.2%) and four women were excluded from further analyses because either they had moved from the Oxford area or, in one case, the woman had died. 87 women agreed to complete the diary (71.9%). 196 women were contacted in the second general practice and 191 replied (97.5%). Five women were excluded from further analyses and 141 agreed to complete the diary (73.8%). Thus, for the nonvegetarian women, a total of 228 women participated in the case-control study, an acceptance rate of 72.8%. One vegetarian case had left for Australia after her ultrasound examination leaving 14 cases and 14 controls to be contacted and 27 replied (96.4%). One case failed to reply and two women refused to participate (coincidentally the same matched pair), resulting in an acceptance rate of 89.3%. Overall, 253 women participated in the case-control study (74.4%). This produced 119 age-matched pairs - 12 vegetarian pairs and 107 nonvegetarian pairs with 31 postcholecystectomy cases. As was mentioned above, the age-matched controls of some cases refused to participate. These cases were matched with controls of the same age (within two years) whose matched cases had also refused to complete the diary. More controls were willing to participate, 134 compared with 119 cases, so 15 control diaries were left out of the final analysis (14 nonvegetarian controls and one vegetarian control). Table IV.1 sets out the response figures for the two general practices and the vegetarian group.

Table IV.2 - Current and Maximum Body Mass Index of Cases and Controls

Mean Body Mass Index	Cases	Controls
Current	2.52 (0.40)*	2.34 (0.34)
Maximum	2.73 (0.47)	2.56 (0.38)

( )\* - Standard Deviation.

## **10.2 Associated Risk Factors**

Several potential risk factors in the pathogenesis of gallstones were investigated in the case-control study. They are discussed individually below.

### **10.2.1 Obesity**

As well as determining each participant's current weight, an attempt was made to sketch a rough "weight history" by asking what their usual weight was and what was their heaviest recorded, non-pregnant weight. Height was also elicited and the current body mass index and maximum body mass index for each woman was then calculated using Quetelet's Index. As expected from the results of the prevalence survey, the cases were significantly heavier than their controls (Z-score = 3.417,  $p < 0.001$ ) using a regression analysis for conditional likelihoods. The mean body mass index of the cases was 2.52 compared with a mean index of 2.34 for the controls (see Table IV.2). The relationship was not quite as strong when maximum body mass index was used instead of current body mass index but still very significant (Z-score = 3.156,  $p < 0.002$ ). Mean maximal body mass indices were greater for both the cases and the controls at 2.73 and 2.56 respectively.

### **10.2.2 Diet**

Using the FTDBIS database programme as described in Section II.5.3 each dietary diary was analysed for intake of total calories, protein, fat, carbohydrate and simple sugars, fibre and cholesterol. The programme was also structured to provide more

Table IV.5 - Nonvegetarian and Vegetarian Nutrient Intake

Nutrient - Mean (S.D.)*	Nonvegetarian	Vegetarian
Total Calories (Kcal)	1774 (451)	1602 (349)
Total Protein (g)	67.0 (15.9)	48.0 (14.0)
Protein/Calories (%)	15.5 (3.36)	12.2 (3.45)
Animal Protein (g)	26.7 (12.3)	00.5 (1.51)
Anim. Product Prot. (g)	20.4 (8.88)	19.1 (10.9)
Non-Animal Protein (g)	19.9 (6.60)	28.4 (10.7)
Total Fat (g)	80.2 (26.7)	67.7 (23.9)
Fat/Calories (%)	40.1 (6.02)	37.3 (7.30)
Total Carbohydrate (g)	194.7 (55.6)	205.8 (44.3)
Carbohydrate/calories (%)	41.3 (6.82)	48.8 (8.46)
Simple Sugars (g)	81.6 (30.4)	91.9 (31.7)
S.Sugars/Calories (%)	17.6 (6.05)	21.8 (7.67)
Fibre (g)	17.0 (6.69)	26.0 (7.73)
Cholesterol (mg)	309 (148)	170 (129)

\* S.D. - Standard Deviation

Table IV.4 - Statistical Summary of Nutrient Intake of Cases and Controls

Nutrient	Z-Score	p Value
Total Calories (Kcal)	-1.707	NS
Total Protein (g)	-2.110	NS
Protein/Calories (%)	-0.165	NS
Animal Protein (g)	-1.293	NS
Anim. Product Prot. (g)	-0.682	NS
Non-Animal Protein (g)	-1.720	NS
Total Fat (g)	-1.786	NS
Fat/Calories (%)	-1.297	NS
Total Carbohydrate (g)	-1.277	NS
Carbohydrate/Calories (%)	0.833	NS
Simple Sugars (g)	-0.786	NS
S.Sugars/Calories (%)	0.906	NS
Fibre (g)	-1.006	NS
Cholesterol (mg)	-1.438	NS

Table IV.3 - Case and Control Nutrient Intake

Nutrient - Mean (S.D.) *	Cases	Controls
Total Calories (Kcal)	1710 (471)	1803 (412)
Total Protein (g)	62.9 (17.2)	67.2 (16.0)
Protein/Calories (%)	15.1 (3.53)	15.2 (3.41)
Animal Protein (g)	23.1 (14.4)	25.0 (13.7)
Anim. Product Prot. (g)	19.9 (9.06)	20.6 (9.12)
Non-Animal Protein (g)	19.9 (7.41)	21.6 (7.59)
Total Fat (g)	76.1 (28.6)	81.8 (24.6)
Fat/Calories (%)	39.4 (6.89)	40.4 (5.42)
Total Carbohydrate (g)	191.2 (54.7)	200.5 (54.4)
Carbohydrate/Calories (%)	42.5 (8.12)	41.7 (6.46)
Simple Sugars (g)	81.1 (30.4)	84.3 (30.9)
S.Sugars/Calories (%)	18.4 (7.05)	17.6 (5.56)
Fibre (g)	17.4 (7.38)	18.4 (7.24)
Cholesterol (mg)	281 (152)	308 (152)

\* S.D. - Standard Deviation

detailed information on protein intake in terms of its specific source. Total protein intake was separated into categories of animal, animal product and non-animal product. The proportion contributed by each major nutrient to the total energy intake was also calculated as a percentage.

There was no significant difference between the number of calories consumed daily by cases compared with controls (Z-score = -1.71, NS)(see Table IV.4). The mean total calorie intake of the cases was slightly less than that of the controls at 1710 and 1803 Kcals respectively (see Table IV.3). Meat-eating participants had a significantly greater total calorie intake (1774 Kcal) than vegetarian participants (1602 Kcal)(T value = 5.57;  $p < 0.00001$ )(see Table IV.5).

Results for total protein intake showed no significant difference between cases and controls (Z-score = -2.11, NS). Similarly, there was no significant difference between the percentage protein intake of the cases and the controls (Z-score = -0.16, NS)(see Table IV.4). Table IV.3 lists the mean and standard deviation values for total protein and percentage protein intake for the cases and controls. More specifically, when protein intake was broken down into its components of animal, animal product and vegetable protein, there were still no differences between cases and controls. Controls ate as much animal and animal product protein as cases (see Table IV.3). Vegetarian women ate significantly less protein than the nonvegetarian women with a mean percentage protein intake of 12.2% compared with 15.5% (T value = 14.4,  $p < 0.00001$ )(see Table IV.5). When looked at in terms of animal and vegetable protein intake vegetarians, not

surprisingly, ate a great deal more vegetable protein than nonvegetarians (28.4g compared with 19.9g, T value = -18.81,  $p < 0.00001$ ) and almost no animal protein (0.5g).

The fat intake of the cases was also not significantly different from that of the controls, even when uncorrected for total calorie intake (Z-score = -1.79, NS) although the Z-score decreased when total calorie intake is accounted for (Z-score = -1.30, NS)(see Table IV.4). The mean fat intake of cases was 76.1g compared with a value of 81.8g for controls (see Table IV.3). Non-vegetarians consumed significantly more total fat (80.2g) than their vegetarian counterparts (67.7g)(T value = 6.83,  $p < 0.00001$ ) and this significance was maintained when intake was corrected for calorie intake (T value = 6.90,  $p < 0.00001$ )(see Table IV.5).

The results for carbohydrate intake were similar to those for protein and fat intake. There was no significant difference between the total carbohydrate intake and percentage carbohydrate intake for cases and controls (Z-score for total carbohydrate intake = -1.28, NS, Z-score for percentage carbohydrate intake = 0.83, NS). The means and standard deviations reflect this (See Table IV.3). There is a significant difference between vegetarian carbohydrate intake and nonvegetarian intake. Vegetarian controls eat 47.0% of their calories in the form of carbohydrate while nonvegetarian controls eat only 41.4% of their calories as carbohydrates (T value = 16.0,  $p < 0.00001$ )(see Table IV.5).

The carbohydrate intake attributable to simple sugars was also determined and no significant difference between cases and controls was seen either for total sugar (Z-score = -0.79, NS) or percentage sugar intake (Z-score = 0.91, NS)(see Table IV.4). The

mean total sugar intake was 81.1g and 84.3g for cases and controls respectively with a mean percentage sugar intake of 18.4% and 17.6% respectively (see Table IV.3). Vegetarian controls ate proportionately more sugar than nonvegetarian controls with mean values of 21.8% and 17.6% respectively (T value = -10.3,  $p < 0.0001$ ).

The difference in fibre intake between cases and controls was not significant with a Z-score of -1.01 (see Table IV.4). The mean fibre intake of fibre was 17.4g for cases compared with 18.4g for controls (see Table IV.3). Fibre intake was significantly higher in vegetarian diets compared with nonvegetarian diets, 26.0g compared with 17.0g (T value = -19.7,  $p < 0.00001$ ) (see Table IV.5).

There was an enormous variation in the cholesterol intake of participants as demonstrated by a standard deviation of 152.2 for a mean of 294.9mg for the study population. PECAN analysis showed that cholesterol intake was not significantly different for cases and controls (Z-score = -1.44, NS) (see Table IV.4). The mean cholesterol intake of cases was 281mg compared with an intake of 308mg for the controls (see Table IV.3). Vegetarian controls ate significantly less cholesterol (170mg) than nonvegetarian controls (309mg) (T value = 13.7,  $p < 0.00001$ ) (see Table IV.5).

The above analyses were repeated after removal of all pairs where the case had previously undergone cholecystectomy as diets may have been altered by gallbladder removal and subsequent lack of coordination of bile acid release for digestion. Mean nutrient intakes were not significantly different after exclusion of these pairs (see Table IV.6). An earlier study had shown a difference in the effect of diet on gallstones according to age (Scragg et al, 1984a), so the analyses were also repeated for all case-control

Table IV.8 - Exogenous Oestrogen Use by Cases and Controls

Statistic	Oral Contraceptive		Hormone Replacement	
	Cases	Controls	Cases	Controls
Mean	0.65	0.67	0.32	0.54
Median	0.00	0.00	0.00	0.00
Std Dev.	2.17	2.37	1.16	3.21
Maximum	14.00	15.00	8.00	32.00
Minimum	0.00	0.00	0.00	0.00

Std Dev. = Standard Deviation  
 All figures are given in years.

Table IV.7 - Alcohol Intake for Cases & Controls,  
Nonvegetarian & Vegetarian Participants.

Group	Mean (g)	Standard Dev.(g)
Cases	8.9	15.4
Controls	7.8	10.3
Nonvegetarians	8.7	13.6
Vegetarians	5.3	6.0

Table IV.6 - Case and Control Nutrient Intake after Exclusion of Post-Cholecystectomy Cases and Matched Controls

Nutrient - Mean (S.D.) *	Cases	Controls
Total Calories (Kcal)	1694 (448)	1796 (413)
Total Protein (g)	61.9 (18.0)	67.1 (16.5)
Protein/Calories (%)	14.9 (3.58)	15.3 (3.65)
Animal Protein (g)	22.0 (14.8)	24.5 (14.5)
Anim. Product Prot. (g)	19.9 (9.22)	20.8 (9.58)
Non-animal Protein (g)	20.0 (7.63)	21.9 (8.07)
Total Fat (g)	75.1 (26.2)	80.9 (24.6)
Fat/Calories (%)	39.4 (6.68)	40.1 (5.63)
Total Carbohydrate (g)	188.6 (52.4)	200.4 (55.8)
Carbohydrate/Calories (%)	42.3 (8.41)	41.8 (6.64)
Simple Sugars (g)	79.4 (30.9)	84.7 (31.4)
S.Sugars/Calories (%)	18.1 (7.26)	17.7 (5.82)
Fibre (g)	17.3 (7.83)	18.8 (7.54)
Cholesterol (mg)	277 (149)	304 (152)

\*S.D. - Standard Deviation

pairs aged 50 years or less. 25 pairs remained after the exclusion of older women and no significant differences between the diets of cases and controls were shown in the younger women.

Alcohol intake was also determined using the FTDBIS programme and the wide range of habit for alcohol intake is reflected in the standard deviation of 13.1g for a mean intake of 8.4g for the study population. Cases, with a mean intake of 8.9g of alcohol did not drink significantly different amounts of alcohol compared with controls at 7.8g (Z-score = 0.72, NS)(see Table IV.7). Once again, however, the vegetarian intake was significantly different from that of the nonvegetarian women. Nonvegetarian women drank 8.7g of alcohol on average compared with a mean value of 5.3g for vegetarian women (T value = 3.66,  $p < 0.0005$ )(see Table IV.7).

Using multiple logistic regression analysis (PECAN) a combined score statistic was obtained for animal and vegetable protein, percentage fat, carbohydrate and sugar intake, fibre cholesterol and alcohol intake. With a Z-score of 7.97, the result was not significant. A separate score was calculated for simple sugar and fibre intake which was also not significant (Z-score = 1.415).

The highly statistically significant relationship between obesity and gallstone risk was shown previously (section IV.10.2.1). This relationship was not shown to be associated with any difference in total calorie intake using multiple logistic regression analysis (Z-score = -1.033).

Testing for significant differences between the mean nutrient intakes does not take into account individual nutrient distribution and histograms were constructed for this purpose.

Figure IV.2 - Individual Nutrient Intakes

Cases ———  
 Controls - - - -

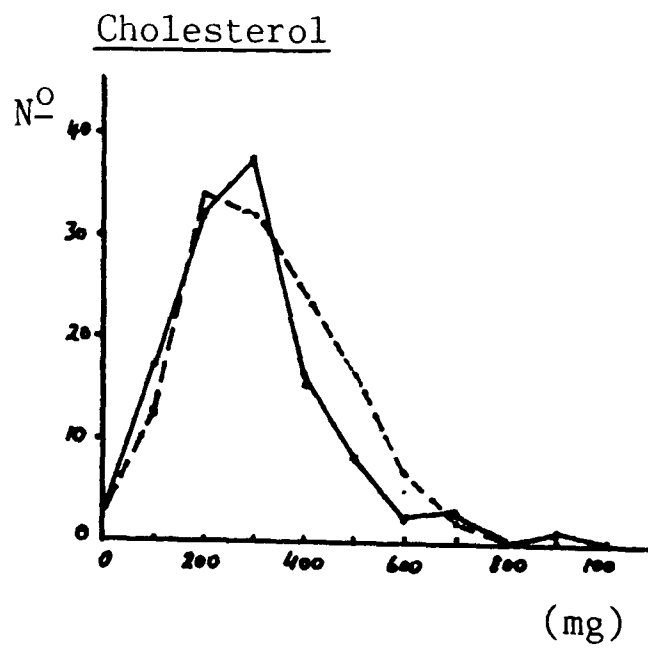
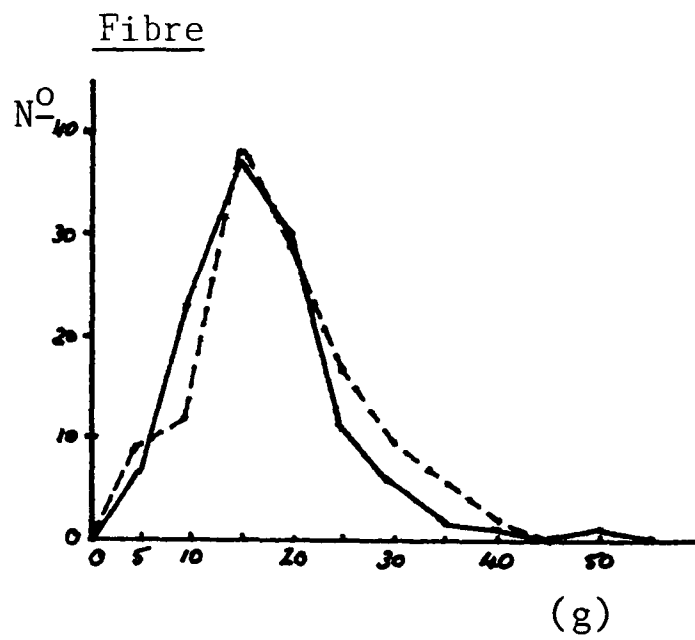
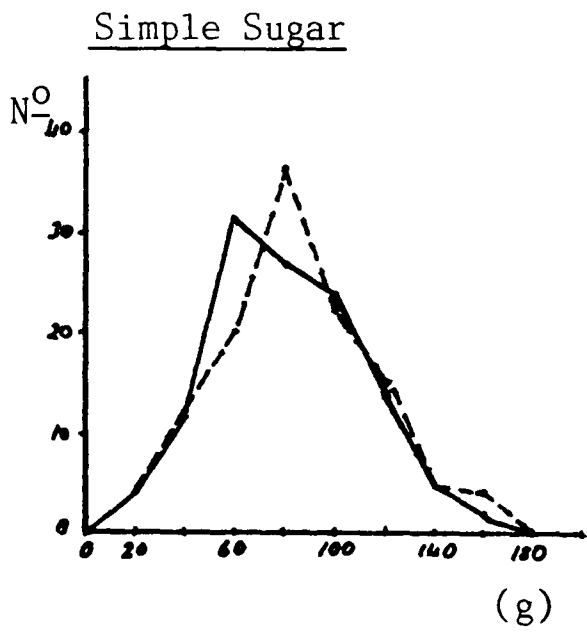
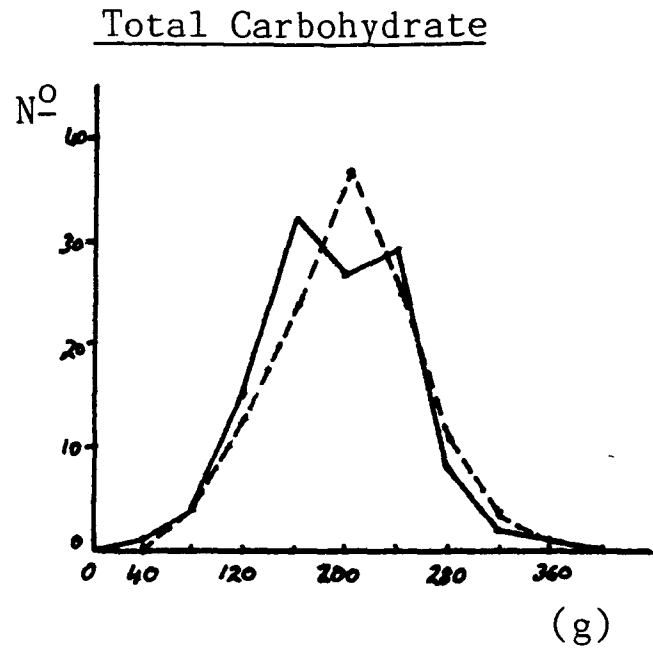
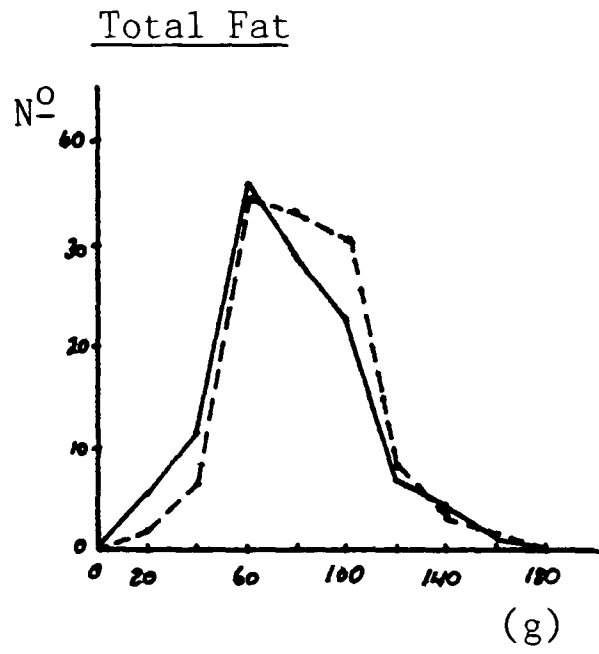
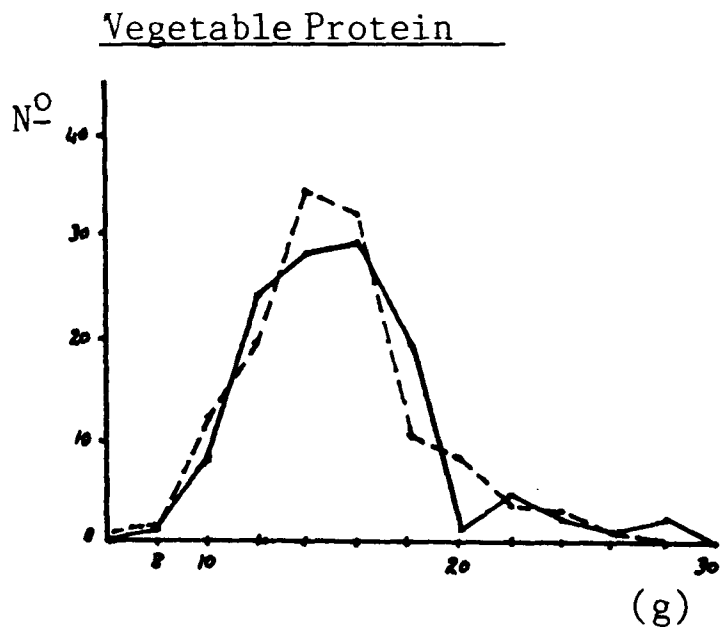
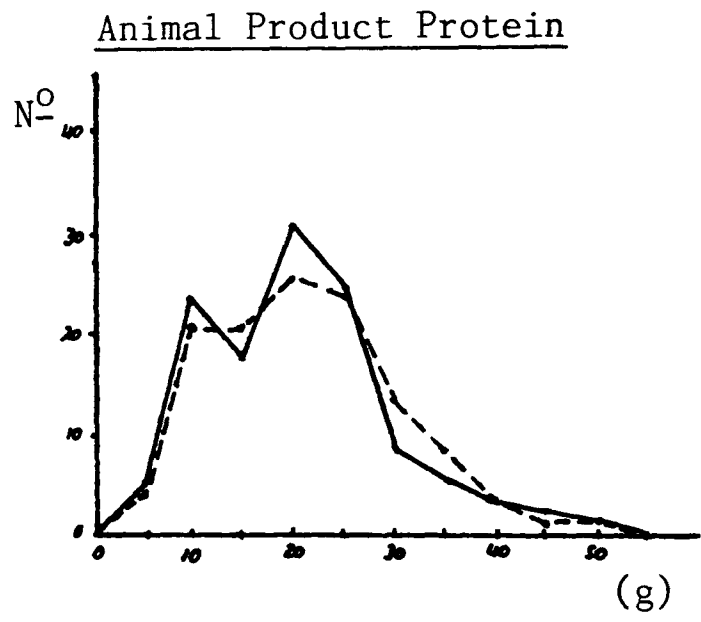
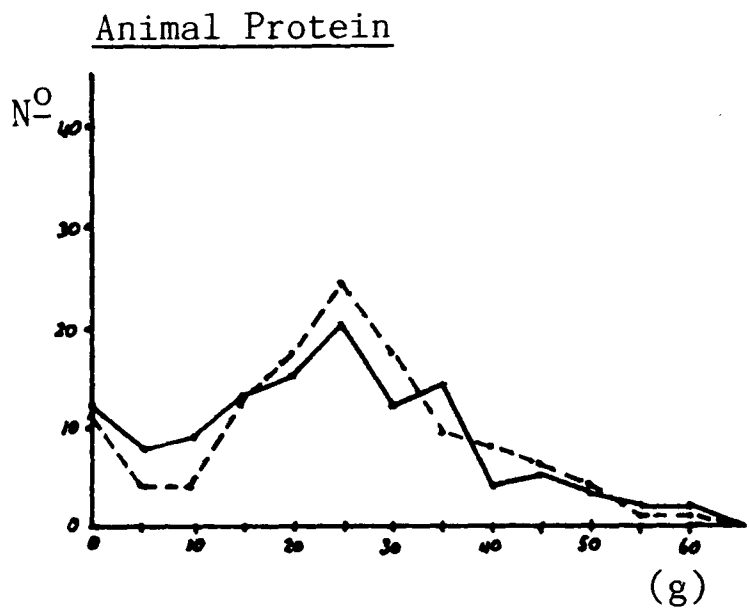
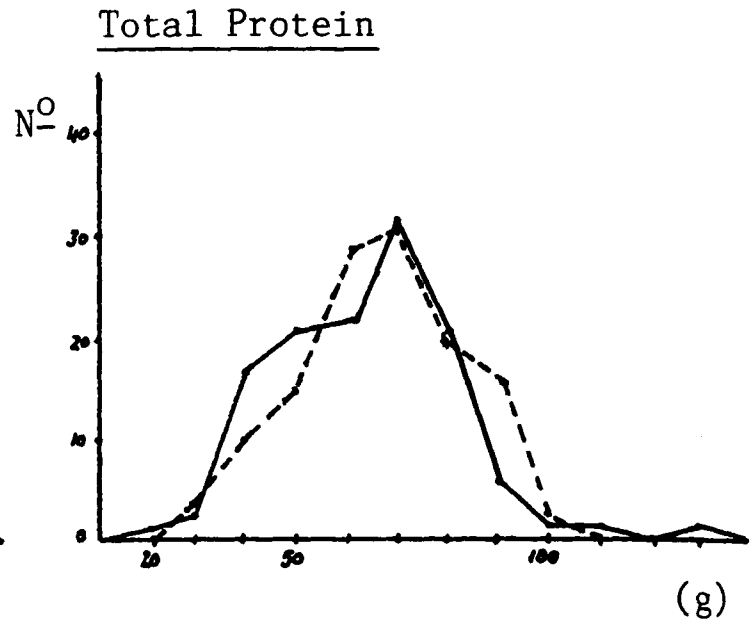
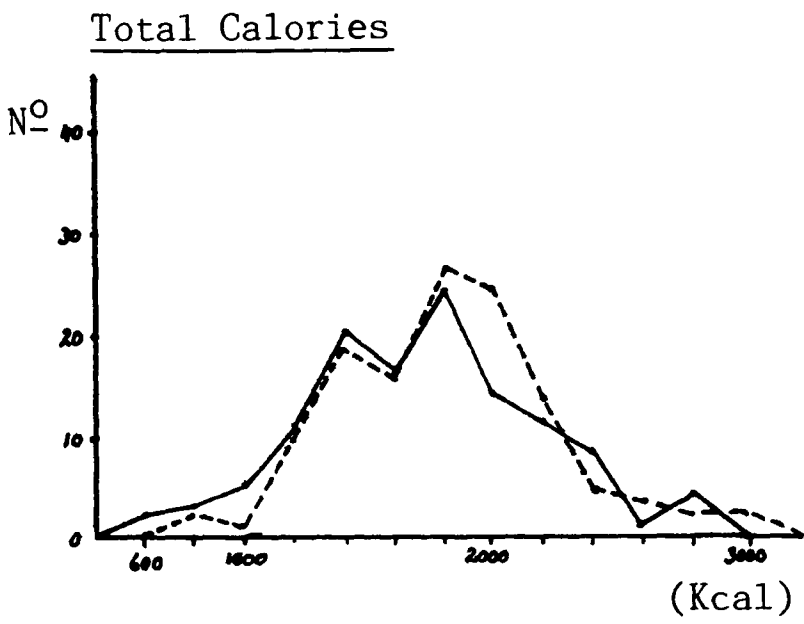


Figure IV.1 - Individual Nutrient Intakes

Cases ———  
 Controls - - - -



Figures IV.1 and IV.2 demonstrate the individual distribution histograms for all nutrients, comparing cases with controls. The figures clearly show that the distribution of individual intakes is virtually identical for all nutrients.

### 10.2.3 Exogenous Oestrogens

Each participant was questioned about intake of any form of oral exogenous oestrogens either in the form of an oral contraceptive or hormone replacement therapy. This information was recorded in terms of duration of use only. It was not possible to look at dosage effects as many participants could not remember brand names of their tablets, especially oral contraceptives. Cases were no more likely to have been prescribed the oral contraceptive pill than controls (Z-score = -0.097). Mean duration of

contraceptive usage was 7.8 months for cases and 8.0 months for controls but the standard deviation for each was in excess of 2 years showing the wide variation in pill usage. Most women, however, had never been prescribed an oral contraceptive and the median value for both cases and controls was zero. There was no significant difference between cases and controls for hormone replacement therapy (Z-score = -0.673, NS) and mean duration of usage was 3.8 and 6.5 months respectively. Again the median value for each group was zero. Table IV.8 summarises the simple statistics for exogenous oestrogen use.

#### 10.2.4 Smoking

Smoking habits of the participants were classified into five groups: "never-smokers", "ex-smokers", "light smokers" (<10/day), "moderate smokers" (10-20/day) and "heavy smokers" (>20/day). No relationship between smoking habits and the presence of gallstones was found (combined score statistic with 4 degrees of freedom = 1.375, NS).

Vegetarians again were significantly less likely to smoke and, in fact, none of the 24 vegetarian participants had ever smoked.

#### 10.2.5 Serum Lipids

Due to the study design it was not possible to obtain blood from every participant in the case-control study. Quite a few women objected to having blood taken and the unexpectedness of the request contributed to some of the refusals. The vegetarian participants were not asked for a blood sample because of the

Table IV.10 - Simple Statistics of Lipid Analyses for Case-Control Study

Lipid - Mean (S.D.)	Cases	Controls
Total Cholesterol (mmol/L)	5.28(0.92)	5.42(0.93)
HDL Cholesterol (mmol/L)	1.54(0.34)	1.55(0.30)
%HDL Cholesterol (%)	29.6(7.86)	29.6(7.72)
LDL Cholesterol (mmol/L)	3.20(0.78)	3.37(0.87)
%LDL Cholesterol (%)	60.3(8.13)	61.6(8.56)
VLDL Cholesterol (mmol/L)	0.56(0.46)	0.51(0.43)
%VLDL Cholesterol (%)	10.16(7.95)	8.86(6.72)

Table IV.11 - Simple Statistics for Cases by Symptoms

Lipid - Mean (S.D.)	Asymptomatic	Post-Cholecyst.
Total Cholesterol (mmol/L)	5.42(0.94)	4.89(0.74)
HDL Cholesterol (mmol/L)	1.53(0.32)	1.54(0.39)
%HDL Cholesterol (%)	28.6(6.29)	32.3(11.0)
LDL Cholesterol (mmol/L)	3.29(0.78)	2.95(0.75)
%LDL Cholesterol (%)	60.5(7.56)	59.7(9.80)
VLDL Cholesterol (mmol/L)	0.60(0.48)	0.40(0.39)
%VLDL Cholesterol (%)	10.86(8.12)	7.81(7.21)

Table IV.9 - Statistical Summary of Lipid Analyses for the Case-Control Study

Lipid	Z-Score	p Value
Total Cholesterol (mmol/L)	-1.142	0.253
HDL Cholesterol (mmol/L)	-0.559	0.576
%HDL Cholesterol (%)	0.326	0.745
LDL Cholesterol (mmol/L)	-1.341	0.180
%LDL Cholesterol (%)	-1.368	0.171
VLDL Cholesterol (mmol/L)	0.805	0.421
%VLDL Cholesterol (%)	1.470	0.142

necessity to conduct this part of the study by post. Therefore, out of the 228 nonvegetarian participants in this study, only 165 provided blood samples. Nine of these samples were haemolysed, leaving 74 case and 80 controls samples that could be analysed. Only 49 completed, matched pairs were obtained from these groups.

Using the regression analysis for matched pairs, there was no significant difference in serum cholesterol levels between cases and controls (Z-score = -1.142, NS)(see Table IV.9). The mean cholesterol level was 5.28 mmol/L for cases and 5.42 mmol/L for controls (see Table IV.10). Serum HDL and %HDL cholesterol values did not show any significant difference between the cases and controls (Z-scores = -0.559 & 0.326 respectively, NS)(see Table IV.9) and mean %HDL cholesterol was identical for cases and controls at 29.6% (see Table IV.10). Similar results were obtained for LDL and %LDL cholesterol with no significant difference between cases and controls (Z-scores = -1.341 & -1.368 respectively, NS)(see Table IV.9). The mean values and standard deviations of LDL and %LDL cholesterol are given in Table IV.10. Analysis of the final measurements, VLDL and %VLDL cholesterol, also failed to show any significant difference between the cases and controls (Z-scores = 0.805 & 1.470 respectively, NS)(see Table IV.9). The mean serum VLDL cholesterol value was 0.559 mmol/L for cases and 0.507 mmol/L for controls and %VLDL cholesterol was 10.2% and 8.9% respectively (see Table IV.10). If the cases were separated according to whether they had had a cholecystectomy or not it can be seen from Table IV.11 that asymptomatic cases had an identical serum lipid profile to the combined case group. Slight differences in the profile of post-cholecystectomy cases were

seen, particularly for total serum cholesterol (mean = 4.89 mmol/L), VLDL and %VLDL cholesterol (means = 0.40 & 7.81 respectively). However, these differences were not significant as evidenced by the standard deviations about each mean value.

### 10.3 Symptomatology

A checklist of possible symptoms attributable to upper gastrointestinal pathology was compiled and consisted of abdominal pain, abdominal discomfort, flatulence, nausea and vomiting, heartburn, acid regurgitation, waterbrash and fatty intolerance. A history of abdominal pain was considered as indicative of possible biliary origin if it was sited in the epigastrium and right upper quadrant. Abdominal discomfort was the term used to describe symptoms of bloating and abdominal distension without necessarily being associated with abdominal pain while flatulence described actual passing of wind. Heartburn encompassed the broader symptom complex of a burning discomfort or pain in the lower end of the oesophagus while acid regurgitation was used to describe the actual passage of acidic stomach contents into the oesophagus. If these contents flooded into the oropharynx the label "waterbrash" was used. Fatty intolerance is another broad term and was used to describe those symptoms of indigestion induced by the consumption of fatty foods. A colleague had expressed interest in the ability of cucumber to cause symptoms of indigestion so this was included at the bottom of the checklist.

If all the case-control pairs were included in the regression analysis, cases were significantly more likely to have suffered one or more episodes of epigastric abdominal pain than controls

Table IV.13 - Proportions of Cases and Controls Suffering from Specific Symptoms

Symptom	Cases			Controls All %
	Postchole* %	Asymptom* %	All %	
Abdominal Pain	90.3	20.9	39.3	16.9
Abdominal Discomfort	87.1	43.0	54.7	29.7
Flatulence	83.3	52.3	60.3	53.4
Nausea & Vomiting	75.0	20.7	33.9	17.8
Heartburn	55.5	43.0	45.2	39.0
Acid Regurgitation	41.1	21.8	25.0	24.6
Waterbrash	17.6	8.0	9.6	9.3
Fatty Intolerance	68.8	27.6	34.0	30.5
Cucumber Indigestion	62.5	28.9	31.4	32.2

\*Postchole - Postcholecystectomy

\*Asymptom - Asymptomatic

Table IV.12 - Statistical Summary of Symptomatology for the Case-Control Study

Symptom	All Pairs		Pairs excl. Chole.*	
	Z-Score	p Value	Z-Score	p Value
Abdominal Pain	3.535	0.001	1.063	NS
Abdominal Discomfort	3.507	0.001	1.362	NS
Flatulence	1.126	NS	-0.457	NS
Nausea & Vomiting	2.711	0.007	0.781	NS
Heartburn	1.539	NS	0.804	NS
Acid Regurgitation	0.210	NS	-0.522	NS
Waterbrash	0.180	NS	-0.277	NS
Fatty Intolerance	0.488	NS	-0.318	NS
Cucumber Indigestion	-0.207	NS	-0.522	NS

\* Cholecystectomised Case-Control Pairs.

(Z-score = 3.535,  $p < 0.001$ ) (see Table IV.12). 39.3% of cases had ever suffered from abdominal pain in the right upper quadrant compared with 16.9% of controls (see Table IV.13). They were also significantly more likely to have experienced abdominal discomfort (Z-score = 3.507,  $p < 0.001$ ) and nausea and vomiting (Z-score = 2.711,  $p < 0.01$ ). However, if matched pairs containing cases who had undergone cholecystectomy were removed from the analysis significance for all three symptoms was lost (Z-scores = 1.063, 1.362 & 0.781 respectively, NS). 20.9% of asymptomatic cases had ever suffered from abdominal pain compared with 16.9% of controls, 43.0% of cases had had abdominal discomfort and 20.7% had had nausea and vomiting compared with 29.7% and 17.8% respectively for the controls (see Table IV.13). Cases were no more likely to suffer from flatulence, heartburn, acid regurgitation, waterbrash, fatty intolerance or indigestion following ingestion of cucumber than controls whether or not pairs containing post-cholecystectomy were removed from the analysis. The statistics for these symptoms are summarised in Table IV.12 and the proportions are compared in Table IV.13.

#### **10.4 Discussion**

Once again obesity was shown to be an important risk factor in the formation of gallstones with cases much more likely to be obese than controls. This finding is in complete agreement with a large number of descriptive epidemiological studies detailed in the literature review (Section I.3.2.4) plus the findings of the prevalence survey described in section III of this thesis. A few studies, however, found the effect of obesity was limited to

younger age groups (Bernstein et al, 1973, Horn, 1956, Scragg et al, 1984a). The population of this study was restricted to women of 40 years of age and over and the strength of the relationship lends support to the belief that obesity plays a role in the aetiology of gallstones in women of all ages. Interestingly, the maximum weight statistic was a less sensitive index than current weight in terms of the effect of obesity. This may indicate a reduction in the tendency to form gallstones if weight loss occurs or, more importantly, a possible tendency to dissolve small gallstones with weight loss. That is, obese women who develop small gallstones and then go on to lose weight may dissolve these stones by producing bile with a lower lithogenic index. However, it may just reflect the inability of participants to remember their maximum weight accurately.

The dietary analysis was, perhaps, the most important part of the case-control study particularly considering the result of the prevalence survey which showed that vegetarian women were significantly less likely to develop gallstones. However, the dietary intake of the cases when analysed for specific nutrient intakes was not significantly different from that of the controls.

A number of studies have already considered the importance of total calories in the pathogenesis of gallstones. Sarles and his colleagues in several consecutive studies found that cases ate significantly more calories than the controls (Sarles et al, 1957, Hauton, 1966, Sarles et al, 1969) and their findings were supported by those of a later, Canadian study (Williams & Johnson, 1980). However, another Canadian study found that cases ate significantly fewer total calories than controls (Smith & Gee,

1979). One Australian study showed no difference in total intake between cases and controls (Wheeler et al, 1970) while another Australian study found a positive association between the prevalence of gallstones and high total calorie intake for subjects less than 50 years of age only (Scragg et al, 1984a). The results of this study support the findings of Wheeler et al (1970) but the population studied were all aged 40 or more. After exclusion of all case-control pairs aged over 50 years of age, there was still no significant difference in the diet of the cases compared with the controls but only 25 pairs were considered in this analysis so the age effect noted by Scragg and his colleagues cannot be discounted. Since obese women are more likely to have gallstones than women of normal weight, an increased total calorie intake for cases might have been predicted on this basis alone. However, obese people have actually been shown to eat less (Keen et al, 1979) illustrating how uncertain is the relationship between caloric intake and obesity.

Protein intake of cases and controls has not been previously described, perhaps because the total protein contribution to diet has not changed over the centuries. This is the first study to analyse protein intake according to its source of origin; a potentially important data source in view of the increased contribution of animal protein to the diet in developed countries. However, cases did not consume more animal or animal product protein than controls.

Clinical epidemiological and metabolic studies showed that total dietary fat was probably not an important epidemiological factor in the pathogenesis of gallstones despite evidence of its

potential gallstone-inducing effect from descriptive epidemiological studies such as those by Malhotra (1968), Wheeler et al (1970), Loftus Hills (1971) and Richardson et al (1973). The results from this study support the belief that total dietary fat intake is probably an unimportant factor as cases did not eat fat in significantly different quantities to controls. The consensus opinion on the importance of the polyunsaturated to saturated fat ratio (P/S ratio) in the aetiology of gallstones is far from certain. Unfortunately, coded information on the FTDBIS programme for different margarines had not been detailed enough to allow accurate analysis of fat intake in terms of P/S ratio. Cholesterol intake was determined, however, and there was no significant difference in intake between cases and controls. This is in keeping with the findings of other case-control studies. The Framingham study (Friedman et al, 1966) and Reid and colleagues (1971) found no difference also while Scragg and his group (1984a) found a positive correlation for female cases when compared with community controls only. As mentioned in the literature review, the few studies that have demonstrated a positive effect of either a high P/S ratio or high dietary cholesterol levels on biliary cholesterol saturation have only showed modest changes even with considerable dietary loading.

The outcome of the fibre results was one that was particularly eagerly awaited in view of the interest shown in the hypotheses put forward by Cleave, Burkitt, Trowell and Heaton blaming many western illnesses on the prevalent diet that is low in fibre and high in refined carbohydrate. Similarly, the simple

sugar intake of cases compared with controls was also anticipated particularly since Scragg and his group found a significant difference in the simple sugar intake of cases compared with controls (1984a). In their study a high intake of sugar in the form of drinks and sweets was associated with an increased risk of gallstones in both men and women. However, while cases ate proportionately more sugar and less fibre than controls, neither difference was statistically significant in this study. Simple sugar and fibre intake form an inverse relationship as was shown in sections I.3.2.5.3 and I.3.2.5.4 and it may be more accurate to view the two nutrients together. The fact that cases ate more sugar and less fibre than controls hints that the combined statistic may be important. However, using multiple logistic regression analysis on the PECAN programme, the combined score statistic obtained was not significant.

The final nutrient analysed was alcohol intake. Alcohol in moderate quantities has been implicated as a protective factor in the aetiology of gallstones in a number of epidemiological studies (Friedman et al, 1966, Wheeler et al, 1970, Sarles et al, 1969, Scragg et al, 1984a). Metabolic evidence points to the possibility of increased bile salt synthesis and secretion with alcohol (Marin et al, 1973, Nestel et al, 1976). The results in this case-control study, however, did not confirm the possible protective effect. Cases, in fact, consumed slightly more alcohol than their controls but the difference was not significant on logistic regression analysis.

The vegetarian diet provides a very interesting contrast to that of the nonvegetarians. Their intake differed significantly

for almost every nutrient analysed. They ate fewer calories and proportionately less protein than the nonvegetarian participants. Their animal protein intake was almost zero while they ate similar amounts of dairy and animal product protein and a great deal more vegetable protein. Their total and proportional fat and cholesterol intake were significantly less than the nonvegetarian women while their total and proportionate carbohydrate intake was significantly greater than the nonvegetarians. Similarly, their simple sugar intake was significantly greater as was their fibre intake so this group of people consumes larger quantities of both refined and unrefined carbohydrate. Therefore, we have studied a population whose dietary habits vary from the usual habits of women in the United Kingdom in almost every possible nutrient. They also drank significantly less alcohol. These results provide supportive evidence for the contributory role of diet in the formation of gallstones as vegetarian women were much less likely to have gallstones than nonvegetarian women, even when age and weight were taken into account. The fact that the case-control study was unable to elicit any dietary differences between the cases and the controls may have been due to the problem of a threshold effect where virtually all nonvegetarian, western women eat too much fat, animal protein and simple sugars and too little fibre and vegetable protein for any differences to be detected between women with gallstones and those without. Poor methodology could, perhaps, be blamed for the failure to detect any differences in the diet of the cases and controls but all cases were positively identified by ultrasound while controls were similarly identified as being free of gallstones and the dietary

diary was carefully piloted on a group of vegetarians and dietary intakes calculated from the diaries were shown to correlate well with intakes determined at interview. It is unlikely that the negative results can be due to methodological failure. The small numbers of the vegetarian participants in the case-control study precluded a detailed analysis of the vegetarian cases and controls alone. A much larger case-control study of vegetarians would be very informative as an indicator of the importance of the threshold effect as well as the importance of diet in the pathogenesis of gallstones. Similarly, a detailed dietary case-control study of women in a developing country might provide useful data.

The use of exogenous oestrogens either in the form of the oral contraceptive pill or as hormone replacement therapy was not associated with an increased risk of gallstone development in the first part of this study; the prevalence survey. It was not surprising, therefore, that the case-control study failed to show any relationship between gallstones and exogenous oestrogen intake either. However, it must be remembered that the women in this study were all aged 40 years or more and therefore relatively few had ever used the oral contraceptive pill or had had postmenopausal oestrogen therapy. The age distribution of this study may have been too old to detect any influence of exogenous oestrogens as recent studies point to the possibility that oestrogens promote the development of gallstones in susceptible women at an early age and who would otherwise have gone on to develop gallstones anyway (Layde et al, 1982, RCGP, 1982, Scragg et al, 1984a). This effect would also explain the positive results

reported by earlier studies who examined women who had only been taking oral contraceptives or hormone replacement therapy for a short time only (BCDSP, 1973, BCDSP, 1974). It would be interesting to know whether oestrogen-induced gallstones are more likely to cause symptoms because it has been shown, certainly in men, that a history of oestrogen intake is more likely to lead to cholecystectomy (Everson et al, 1982). Whether this is a result of increased investigation in men with upper gastro-intestinal tract symptomatology and taking oestrogens (stilboestrol), or an increased tendency to treat asymptomatic gallstones in these men or, perhaps, an increased tendency to cause symptoms is uncertain. Everson and his colleagues claimed that oestrogen therapy in men did not lead to an increased prevalence of gallstones despite the increased cholecystectomy rate. However, the symptomatology of oestrogen-induced gallstones is a side issue. Both studies within this thesis failed to show any long-term relationship between exogenous oestrogen use and the prevalence of gallstones; a finding that is increasingly well supported. The prevalence survey also failed to show that parity had any effect on the prevalence of gallstones.

In the Framingham study, smoking seemed to exert a protective effect on the development of gallstones (Friedman et al, 1966) while the opposite effect was reported in a more recent survey (Petitti et al, 1981). There was no sign of any effect of smoking upon the prevalence of gallstones in either the case-control study or the prevalence survey. Vegetarians, however, in keeping with their dietary and general health views, were much less likely to smoke than nonvegetarians.

Differences in serum lipid levels between gallstone cases and their controls have been shown in several studies. Triglyceride levels have been shown to be higher in cases than controls in two studies (Bell et al, 1973, Scragg et al, 1984c). Unfortunately, triglyceride levels could not be measured in this study because the participants were not fasted. It was felt that participation rates in the case-control study would drop if women knew beforehand that they were to be asked to give blood. High serum cholesterol levels have not been strongly implicated in the pathogenesis of gallstones. There was no relationship found between serum cholesterol levels and gallstone prevalence in three studies (Van der Linden, 1961, Friedman et al, 1966, Sampliner et al, 1970) and a negative relationship was shown in a recent study (Scragg et al, 1984c). The prevalence of gallstones is not increased in type IIa hyperlipidaemia which is associated with hypercholesterolaemia (Ahlberg et al, 1979). The results for serum cholesterol in this study showed a similar lack of association as did the results for LDL- and VLDL-cholesterol. HDL-cholesterol has been purported to play a protective role in the development of gallstones (Petitti et al, 1981, Thornton et al, 1981). Scragg and his colleagues (1984c) found that this protective effect disappeared when the confounding influences of triglyceride and insulin levels were accounted for. There was no significant difference in the HDL-cholesterol levels between cases and controls in this study. When post-cholecystectomy cases and their controls were excluded from the lipid analyses to rule out any effect of possible changes in serum lipids after cholecystectomy, the results were unchanged and all still insignificant. However,

the case-control analysis of serum lipids was hindered by the small numbers of blood samples available and potential differences may have been missed.

An important aspect of gallstone disease that was investigated in the case-control study was the relevance of upper gastro-intestinal symptomatology in the diagnosis of gallstones. Price, in his survey of 1963, showed that symptoms of abdominal pain, abdominal fullness and discomfort, nausea and vomiting, heartburn, and fatty intolerance were no more likely to occur in people with gallstones than those without. Similar findings were reported by Bainton and his colleagues (Bainton et al, 1976). However, classical teaching has always stated that gallstones are commonly associated with specific but low-grade "biliary symptoms" of nausea and vomiting, abdominal fullness and discomfort, and fatty intolerance eventually leading to the full-blown symptom complex of right-sided hypochondrial pain radiating around to the back, shoulder-tip pain and nausea and vomiting as in bile duct obstruction or acute cholecystitis. This study has been able to add important data to the discussion on the significance of "biliary symptoms". The study design was ideal in allowing comparison between women who were known to have gallstones and controls who were definitely free of gallstones. Moreover, the cases were selected from an "asymptomatic" general practice population so reflects the expected distribution of the disease in most British communities. The results highlighted very neatly the three symptoms commonly associated with an acute attack of biliary colic or acute cholecystitis - abdominal pain, abdominal discomfort and nausea and vomiting. However, once the

post-cholecystectomy cases were excluded along with their controls cases were no more likely to suffer from any of the above three symptoms than the controls. The less well-defined, low-grade symptoms of flatulence, heartburn, acid regurgitation and waterbrash, fatty intolerance and indigestion associated with ingestion of cucumber were no more likely to have occurred in the cases than the controls either before or after exclusion of the post-cholecystectomy cases. From these results, it can be inferred that the "inaugural symptoms" of gallstones as described by Moynihan in 1913 and lauded since are not related to the presence of underlying gallstones at all. This finding has extremely important implications for doctors when trying to assess whether or not gallstones are causing their owners enough discomfort to warrant cholecystectomy. Furthermore, there is increasing evidence that people who undergo cholecystectomy for low-grade "biliary symptoms" are not relieved of these symptoms by surgery.

### **10.5 Summary**

This thesis on the epidemiology of gallstones in women is comprised of two separate but related studies. The first study was specifically designed to determine the prevalence of gallstones in women in the community. Using an ideal screening tool; real-time ultrasonography, 652 nonvegetarian women and 130 vegetarian women were examined. The prevalence of gallstones in nonvegetarian women aged 40-70 years was shown to be 24.6% while, at 11.5%, gallstones were found to be significantly less common in vegetarian women ( $p < 0.05$ ). As well as diet, a number of other possible aetiological influences were investigated. Gallstones were shown to increase

with age and obesity ( $p < 0.01$  &  $p < 0.001$  respectively). Vegetarian women remained significantly less likely to have gallstones when age and body mass index were accounted for ( $p < 0.05$ ). Intake of specific nutrients could not be adequately analysed from the data collected in the postal questionnaire used in the prevalence survey except for fibre intake. There was no significant difference in fibre intake between women with gallstones and those without. However, vegetarian women were shown to consume more than one and a half times as much dietary fibre as their nonvegetarian counterparts. Another factor found to be associated with an increased risk of gallstones was a positive family history of the condition. Women with gallstones were significantly more likely to have a positive family history ( $p < 0.01$ ). Factors that have been purported previously to play a role in the aetiology of gallstones but were not shown to do so in the prevalence survey included parity, exogenous oestrogen intake and a history of smoking.

The case-control study was set up to obtain more detailed and accurate dietary information as well as further details of exogenous oestrogen intake. The opportunity was also taken to examine the influence of obesity more closely and to question participants about any history of upper gastro-intestinal tract symptoms. Only 119 age matched case-control pairs were included in this study.

Obesity was again shown to be a strong influence in the aetiology of gallstones and current weight was a more sensitive index of risk than maximum, non-pregnant weight. Despite the obvious differences in vegetarian and nonvegetarian diets and the

lower prevalence of gallstones in vegetarian women compared with nonvegetarian women, no differences between the diets of cases and controls could be shown. The failure to do so may reflect the presence of threshold levels of nutrients above which differences in gallstones risk cannot be shown. Exogenous oestrogen intake was also not shown to be significantly different between cases and controls.

The possibility that threshold levels for nutrient intakes exist plus the concept that exogenous oestrogens may cause the early development of gallstones in susceptible women who would have developed them at a later date anyway indicates the difficulties experienced by researchers in trying to determine the importance of aetiological factors in the pathogenesis of gallstones. Perhaps too much effort has been directed at populations in whom the people who are susceptible to the development of gallstones will almost certainly go on to develop them anyway as a result of the western diet and other influences. It might be more profitable to study less industrialized societies where susceptible people are not necessarily condemned to eventually developing gallstones. Case-control studies will then be better able to detect differences between cases and controls.

The prevalence survey also contributed very useful information about the likelihood that gallstones go on to cause symptoms. Although the only way to investigate natural history of gallstones properly is to carry out a prospective study of people with gallstones, the finding that more than 70% of all cases in this study were asymptomatic indicates that gallstones may not be the villains they have commonly been thought to be. Additionally,

the case-control study showed that a number of symptoms thought to be biliary in origin and therefore predictors of eventual complications such as cholecystitis or common bile duct obstruction were, in fact, no more likely to occur in cases than controls. The combined conclusion from these two studies is that asymptomatic gallstones should be left alone and that low-grade symptoms do not mean that gallstones are symptomatic. An understanding of this conclusion should lead to a decrease in the current cholecystectomy rates.

## **Publications & Abstracts**

Pixley FJ, Wilson DJ, McPherson K, Mann JI. Effect of vegetarianism on development of gall stones in women. British Medical Journal 1985; 291: 11-2.

Pixley FJ. The epidemiology of gallstones in women. Paper presented to the 28th Annual Meeting of the Society for Social Medicine, Oxford, September 19th, 1984.

Pixley FJ. The epidemiology and natural history of gallstones. Paper presented to the University of London Annual Course in Gastroenterology, Guy's Hospital, November 23rd, 1984.

Pixley FJ. The epidemiology of gallstones in women. Paper presented to the University of Oxford Advanced Gastroenterology Course, Oxford, January 6th, 1986. Paper incorporated as a chapter in "Advances in Gastroenterology" (in press).

Pixley FJ. The epidemiology and natural history of gallstones. In "Gallstone Disease and its Management", edited by Bateson MC (in press).

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## Appendices

Appendix A

OXFORD UNIVERSITY GALL BLADDER STUDY.

QUESTIONNAIRE

for office  
use only.

SECTION I - PERSONAL DETAILS:

1. Full Name Surname.....  
Forenames.....
2. Address .....
3. Date of Birth .....day.....month.....year
4. What is your marital status?
5. What is your occupation? Please could you describe this as accurately as possible. If you have retired, please write 'retired' along with your former occupation. ....
6. What is your husband's occupation? (if applicable)  
.....

		1			
					2
					4
					6
					10
					11
					12
					13

SECTION II - HEALTH:

7. What is your height? .....ft.....ins or .....cms
8. What is your weight? .....st.....lbs or .....Kg
9. How many pregnancies have you had?(including all that lasted over 20 weeks or more) .....
10. How old were you when the first child was born?
11. Have you ever had any of the following complaints?
  1. Diabetes YES/NO
  2. Crohn's Disease\* YES/NO
  3. Coeliac Disease\* YES/NO
  4. Other diseases of the stomach and/or bowel. YES/NO  
Please specify.....
12. Have you ever had any operations for any of the following conditions?

					16
					17
					20
					22
					23
					24
					25
					27

	Year				
Peptic Ulcer (Stomach Ulcer)					30
Gall Stones & other Gall Bladder Disease					33
Crohn's Disease					36
Appendicitis					39
Other Bowel Operations Please specify.....					43

\* If you haven't heard of these conditions then you don't have them!

**RECORD SHEET**

**A. GENERAL GUIDELINES**

**PLEASE READ THE INSTRUCTIONS CAREFULLY**

1. Please record ALL food and drink (except water) consumed during the day. Record the food at the time of eating it and NOT from memory at the end of the day. You should include all meals and snacks, plus extra sweets, drinks, etc. When recording food eaten at meals include any sauces, dressings or extras e.g. gravy, salad dressings, stuffings, as well as the main food.

2. If you do not eat a particular meal or snack, simply draw a line across the page at this point. This will show that you definitely have not eaten anything.

3. Please use the extra sheets provided if you do not have enough room to complete your days meals.

**B. DIETARY INFORMATION - GUIDELINES**

1. Please give details as to the **method of cooking** all foods e.g. grilled, boiled, roasted.

2. Give as many details as possible about the **type of food** that you eat:-

a). State brand of food where applicable.

b). Name type of biscuit, cake, cereal, - DIGESTIVE biscuit, FRUIT cake, Weetabix, etc..

c). Name type of cheese, fish, meat e.g. cheddar cheese, cod fillet, loin of pork

3. When indicating **quantity of food and drink** consumed the following suggestions may be helpful:-

a). For many foods such as **vegetables, cereals, and fruit**, a household measurement is adequate. State number of tsp., tbs., cups, etc. and whether heaped or level. Margarine and butter can be measured in tsp. or tbs., if you find this an easy method.

b). All **convenience foods** have their weight on the packaging and this can be quoted e.g. half a 15 oz. can baked beans.

c). **Bread, fruit loaves etc.** Indicate size of loaf - small, medium or large and whether thin, medium or thick slice.

d). **Cheese, fish and meat.** Weigh your portions sizes for these foods when practicable e.g. cheese in cheese roll or cold sliced meat in a salad. If this is not possible, e.g. if eating out or if you do not have the original weights of food please use the pictures on the attached sheet to indicate what sort of portion sizes you eat, e.g. you might have 1 slice of quiche size A, 1 portion of meat size B or 2 slices of cheese size C.

e). Use comparisons for describing portion sizes where this is easier e.g. potato - size of a hens egg, cheese - size of a matchbox.

If you follow these guidelines you will be able to show what you normally eat and drink. It is VERY IMPORTANT that you do not adjust what you eat and drink because you are keeping a record. This is very easy to do, but remember, we want the facts, not the perfect diet !

Record **ALL** food and drink consumed during the day including snacks, "nibbles", sauces and dressings.

Record 1) **method of cooking** food - e.g. **boiled** pasta

2) **type of food** - e.g. **boiled, wholegrain** pasta

3) **quantity of food** - e.g. **6Tbs.**boiled, wholegrain pasta

**DIETARY RECORD SHEET**

DAY 1    (WEEKDAY).DATE .....

LEAVE BLANK

MEAL/ SNACK	QUANTITY EATEN	DETAILS OF FOOD AND DRINK		
EARLY MORN				
B'FAST				
DURING MORN				
MID- DAY				

LEAVE BLANK

MEAL/ SNACK	QUANTITY EATEN	DETAILS OF FOOD AND DRINK		
DURING AFTER NOON				
EVENING MEAL				
DURING EVENING / BEDTIME SNACK				

Record **ALL** food and drink consumed during the day including snacks, "nibbles" sauces and dressings.

- Record 1) **method of cooking** food - e.g. **boiled** pasta  
 2) **type of food** - e.g. **boiled wholegrain** pasta  
 3) **quantity of food** - e.g. **6Tbs.** boiled wholegrain pasta

DAY 2      (WEEKDAY).      DATE .....

LEAVE BLANK

MEAL/ SNACK	QUANTITY EATEN	DETAILS OF FOOD AND DRINK		
EARLY MORN				
B'FAST				
DURING MORN				
MID- DAY				

LEAVE BLANK

MEAL/ SNACK	QUANTITY EATEN	DETAILS OF FOOD AND DRINK		
DURING AFTER NOON				
EVENING MEAL				
DURING EVENING / BEDTIME SNACK				

Record **ALL** food and drink consumed during the day including snacks, "nibbles", sauces and dressings.

- Record
- 1) **method of cooking** food - e.g. **boiled** pasta
  - 2) **type of food** - e.g. **boiled, wholegrain** pasta
  - 3) **quantity of food** - e.g. **6Tbs.** boiled, wholegrain pasta

DAY 3 ( SATURDAY ) DATE .....

LEAVE BLANK

MEAL/ SNACK	QUANTITY EATEN	DETAILS OF FOOD AND DRINK		
EARLY MORN				
B'FAST				
DURING MORN				
MID- DAY				

LEAVE BLANK

MEAL/ SNACK	QUANTITY EATEN	DETAILS OF FOOD AND DRINK		
DURING AFTER NOON				
EVENING MEAL				
DURING EVENING / BEDTIME SNACK				

Record **ALL** food and drink consumed during the day including snacks, "nibbles", sauces and dressings.

- Record
- 1) **method of cooking** food - e.g. **boiled** pasta
  - 2) **type of food** - e.g. **boiled, wholegrain** pasta
  - 3) **quantity of food** - e.g. **6Tbs.** boiled, wholegrain pasta

DAY 4 ( SUNDAY ) DATE .....

LEAVE BLANK

MEAL/ SNACK	QUANTITY EATEN	DETAILS OF FOOD AND DRINK		
EARLY MORN				
B'FAST				
DURING MORN				
MID- DAY				

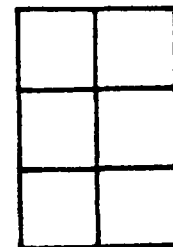
LEAVE BLANK

MEAL/ SNACK	QUANTITY EATEN	DETAILS OF FOOD AND DRINK		
DURING AFTER NOON				
EVENING MEAL				
DURING EVENING / BEDTIME SNACK				



SECTION I - GENERAL

1.NAME surname.....  
forenames.....  
2.ADDRESS.....  
.....  
3.DATE of BIRTH.....  
4.No. in HOUSE - adults.....  
children 1-4.....  
infants <1.....



SECTION II - HEALTH

1.HEIGHT.....  
2.WEIGHT - now.....usual?.....  
recent change?.....  
heaviest.....  
before cholecyst.....  
3.G.I.T. DISORDERS (if any).....  
.....  
age of onset.....  
treatment.....  
complications.....  
4.SURGERY.....  
year.....  
.....  
year.....  
5.OTHER ILLNESSES - hypertension...Rx.  
epilepsy.....Rx.  
diabetes.....Rx.  
other..... Rx.

6.GALLBLADDER - a.If post-cholecystectomy:

- Sx's prior to surgery:

.....

.....duration....

- precipitation of surgery:

.....

- persistent Sx's.....

b.If no Hx of surgery:

- Sx's.....

duration.....

frequency.....

SYMPTOMS ARE: abdominal pain  
abdominal fullness or discomfort  
flatulence  
nausea and vomiting  
heartburn  
acid regurgitation  
waterbrash  
fatty intolerance  
cucumber

7.DRUGS - Have you ever used oral  
contraceptives or any other sex  
hormone preparation at any time?

....Name.....	...duration...	...Reason...
.....	.....'til.....	.....
.....	.....'til.....	.....
.....	.....'til.....	.....

SECTION III - DIET

1. Do you eat or drink anything before  
breakfast?.....

2. What do you normally eat for breakfast?

Fruit/Fruit Juice.....sweetnd...

Cereals - brand.....

freq.....

Cooked - type.....

freq.....

Bread(inc rolls) - type.....

freq.....

no.....

thick.....

Beverages.....no.....milk/sugar

Other - .....

supplements.....

3. Do you eat or drink anything during the  
morning? Drink.....

Food.....

4. What do you normally eat for lunch &/or  
dinner Soup/Starter.....

Meat - freq.....

lean/fatty.....

cook/meth.....

Fish - freq.....

cook/meth.....

Cheese - freq.....  
     cream cheese.....  
     hard cheese.....  
     soft cheese.....  
     Edam/Gouda.....  
     low fat cheese.....  
     processed.....  
 Sauces/Gravy.....amt.....  
 Eggs - freq.....cook/meth.....  
 Bread/Subs - type.....  
     freq.....  
     slices.....  
 Veg/Salad - peas.....  
     greens.....  
     b/beans,s/corn.....  
     carrots/root.....  
     tomatoes.....  
 Dressing - type.....  
     amt.....  
 Potatoes - cook/meth.....  
     freq.....  
 Pulses - freq.....  
 Rice/Pasta - type.....  
     freq.....  
 Fruit - fresh/tinned/dried  
     freq.....

Puddings - freq.....  
ice/cream.....  
yoghurt.....

Beverages - type.....  
freq.....

5. Do you normally eat or drink anything during  
the afternoon? Drink.....  
Food.....

6. Do you normally eat or drink anything before  
going to bed? Drink.....  
Food.....

7. How much milk would you normally drink each  
day?.....Full/Fat.....  
Semi-Skimmed.....  
Skimmed.....  
Evaporated.....

8. How much sugar would you consume every day?  
.....

9. What fats do you normally use for cooking?

butter  
lard/dripping  
margarine - brand.....  
vegetable oil  
sunflower oil  
corn oil  
soya oil  
olive oil

What brands do you usually use?

How often do you have fried food including  
breakfast per week?.....

How much butter/marg do you use daily as a spread?.....

10.What type of flour do you usually use?

- white
- wholemeal
- mixture - %.....
- other

11.How many cups of tea/coffee per day?.....

12.Do you eat nuts?.....freq.....

13.Do you eat crisps?.....freq.....

14.Do you eat biscuits?.....freq.....

15.Do you eat cakes/pastries?.....freq.....

16.Do you eat sweets/chocolates?...freq.....

17.Do you smoke?.....how often?.....

type.....

duratn.....

18.Do you drink alcohol?.....type.....

amt.....

freq.....

DRAFT (1)

OXFORDSHIRE HEALTH AUTHORITY

Appendix D

Telephone Oxford (0865) 64711

John Radcliffe Hospital

Headington

Oxford

OX3 9DU

ur ref  
ur ref: DJW/SWD

20 October 1982

Dear Sir/Madam

We would be grateful if you would consider taking part in a Research Project organised by the Department of Radiology and the Department of Community Medicine and General Practice.

Your doctor has requested that you attend for an x-ray examination of the gallbladder known as an oral cholecystogram. We would like to perform a second examination of the gallbladder at the same visit using an ultrasound machine. This is a safe, painless procedure that does not involve the use of x-rays or radiation. It is routinely used as an alternative to the oral cholecystogram and uses the same method as that used to look at developing babies in their mother's womb. The procedure only takes a few minutes and does not involve the use of drugs or injections.

The results of your oral cholecystogram will be reported to your doctor in the normal way as will any additional information obtained by the ultrasound examination. The strictest confidence will be observed and individual identities will not be included in the research.

Please ask if you have any questions. Should you not feel able to participate in the study your oral cholecystogram will, of course, be completed in the normal way.

Yours sincerely

Signature has been removed

Appendix D

## DEPARTMENT OF COMMUNITY MEDICINE &amp; GENERAL PRACTICE

Professor of Social & Community Medicine  
M.P. Vessey, MD., FRCP. (Ed.), FFCM.

Clinical Reader in General Practice  
G.H. Fowler, BM., FRCGP., DCH., DRCOG.

Dear.....,

We are writing to ask you whether you would consider taking part in a research project on gall stones. This general practice and the Department of Community Medicine and General Practice <sup>are collaborating</sup> in the study. Gall stones is a very common condition in women but very little is known about the causes. Our study has two aims. We are hoping to find out more about what causes gall stones and in particular whether the condition is related to food. The other aim is to find out exactly how common the condition really is. Because many people with gall stones do not develop symptoms for many years a special screening test is used to detect these stones. This is a very simple test known as ultrasound. It is very quick, lasting only about 3 minutes, does not involve X-Rays or injections and is entirely painless and safe. It is, in fact, the same test that is now used so often to look at developing babies in their mother's womb.

Taking part in the project involves completing the very simple, attached questionnaire and having the 3 minute ultrasound at the John Radcliffe Hospital. We would be extremely grateful for your help in this project. If you are prepared to help would you please complete the enclosed questionnaire and let us know if the given time for your ultrasound appointment does or doesn't suit you.

If you have any questions then please don't hesitate to contact Dr Fiona Pixley at the Gibson Laboratory Buildings, The Radcliffe Infirmary, Oxford. (phone number 511562). We would be happy to provide reimbursement for the travel expenses to and from the John Radcliffe and assure you, of course, that all information is treated with the strictest confidence.

Yours sincerely,

Signatures have been removed

UNIVERSITY OF OXFORD

DEPARTMENT OF COMMUNITY MEDICINE & GENERAL PRACTICE

Professor of Social & Community Medicine  
M. P. Vessey, M.D., FRCP. (Ed.), FFCM

Clinical Reader in General Practice  
G. H. Fowler, BM., FRCGP., DCH., DRCOG.

Dear .....,

We are writing to ask you whether you would consider taking part in a research project on gallstones. This general practice and the Department of Community Medicine and General Practice are collaborating in the study. Gallstones is a very common condition in women but very little is known about the causes. Our study has two aims. We are hoping to find out more about what causes gallstones and in particular, whether the condition is related to food. The other aim is to find out exactly how common the condition really is. Because many people with gallstones do not develop symptoms for many years a special screening test is used to detect these stones. This is a very simple test known as ultrasound. It is very quick, lasting only about 5 minutes, does not involve X-Rays or injections and is entirely painless and safe. It is, in fact, the same test that is now used so often to look at developing babies in their mother's womb.

Taking part in the project involves completing the simple, attached questionnaire and having the ultrasound done at the Radcliffe Infirmary. We have carried out the same study on another general practice in Oxford already and the results look most promising. We would be extremely grateful for your help in this project. If you are prepared to help would you please complete the enclosed questionnaire and let us know if the given time for your ultrasound appointment does or doesn't suit you.

If you have any questions then please don't hesitate to contact Dr Fiona Pixley at the Gibson Laboratories Building, The Radcliffe Infirmary, Oxford. (Phone number 511562). We would be happy to provide reimbursement for the travel expenses to and from the Radcliffe and assure you, of course, that all information is treated with the strictest confidence. Hoping to hear from you soon,

Yours sincerely,

John Chadwick & Fiona Pixley's signatures have been removed

We have made a provisional booking for your ultrasound as shown below on the detachable form, along with directions to find the Radiology Department. This, of course, can be altered if it doesn't suit you as we fully understand that a fair amount of travel may be involved. If the date doesn't suit you please let us know in the space provided and fill in an alternative time. It would be helpful if you could try and come during the particular time and days specified as these are the times that the Radiology Department has very kindly allowed us to have use of the ultrasound machine. We will confirm alternative appointments with you.

The time given IS / IS NOT suitable

An alternative time is, and give a few if possible:

- MONDAY ..... at 5.15, 5.30, 5.45, 5.00.
- THURSDAY..... at 5.15, 5.30, 5.45, 5.00.
- TUESDAY ..... at 5.15, 5.30, 5.45, 5.00.
- WEDNESDAY..... at 5.15, 5.30, 5.45, 5.00.

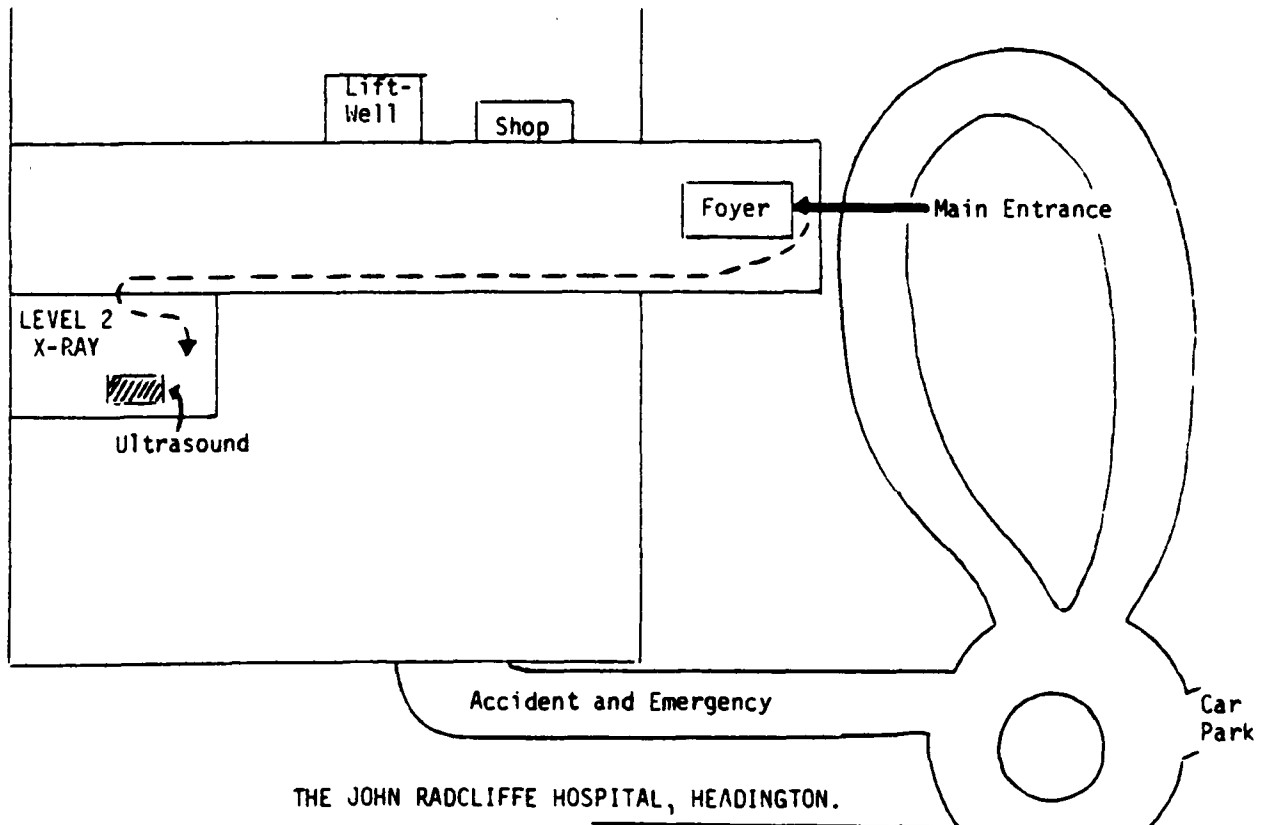
Thank you very much for your time and effort. They have been and will be greatly appreciated.

Yours sincerely,

*Simon Pickley*

please detach here to retain your appointment time and directions on how to get there.

Would you please be at LEVEL 2 X-RAY DEPARTMENT on .....198.....



UNIVERSITY OF OXFORD  
DEPARTMENT OF COMMUNITY MEDICINE & GENERAL PRACTICE

Professor of Social & Community Medicine  
M. P. Vessey, MD., FRCP(Ed)., FFCM., FRCGP.

Clinical Reader in General Practice  
G. H. Fowler, BM., FRCGP., DCH., DRCOG.

Dear .....

We are writing to ask you whether you would consider taking part in a research project on gallstones. As you may remember, a previous study carried out in this department by Dr John Gear was able to show that vegetarians had a lower rate of diverticular disease of the large bowel compared with meat-eaters. This was shown to be due to the higher intake of fibre in the diet of vegetarians. Gallbladder disease is a very common condition in women and although very little is known about its causes, it seems to be a fibre-related disease also. Our study has two aims. We hope to be able to find out more about what causes gallstones and, in particular, whether the condition is related to diet. The other aim is to find out exactly how common the disease really is. Because many people with gallstones do not have symptoms for many years, this will be done using a special but very simple test known as ultrasound. The test lasts about 3 minutes, does not involve X-Rays or injections and is entirely painless and safe. It is, in fact, the same test that is now used so often to look at developing babies in their mother's womb. We are, therefore, taking a random selection of people from the Vegetarian Study who live reasonably close to Oxford to investigate gallstones as related to dietary aspects.

Taking part in this study involves completing the very simple, attached questionnaire and having the ultrasound done at the John Radcliffe Hospital. We would be extremely grateful for your help in this project. If you are prepared to help would you please complete the enclosed questionnaire and let us know if the possible times for the ultrasound examination do or do not suit you?

If you have any questions then please contact Dr Fiona Pixley at the Gibson Laboratory Buildings, The Radcliffe Infirmary, Oxford (phone number below). We would be happy to provide reimbursement for the travel expenses to and from the John Radcliffe and assure you, of course, that all information is treated in the strictest confidence.

Yours sincerely,

Signature removed

UNIVERSITY OF OXFORD

DEPARTMENT OF COMMUNITY MEDICINE & GENERAL PRACTICE

Professor of Social & Community Medicine  
M. P. Vessey, M.D., FRCP. (Ed.), FFCM.

Clinical Reader in General Practice  
G. H. Fowler, BM., FRCGP., DCH., DRCOG.

Dear .....,

We are writing to ask you whether you would consider taking part in a research project on gall stones. As you may remember, a previous study carried out in this department by Dr John Gear was able to show that vegetarians had a lower rate of diverticular disease of the large bowel compared with meat-eaters. This was shown to be due to the higher intake of fibre in the diet of vegetarians.

Gall bladder disease is a very common condition in women and although very little is known about its causes it seems to be a fibre related disease also. Our study has two aims. We hope to be able to find out more about what causes gall stones and in particular whether the condition is related to diet. The other aim is to find out exactly how common the disease really is. Because many people with gall stones do not have symptoms for many years, this will be done using a special but very simple test known as ultrasound. The test lasts 3 minutes, does not involve X-Rays or injections and is entirely painless and safe. It is, in fact, the same test that is now used so often to look at the developing babies in their mother's womb.

We have presented the details of this study to the Doctors Latto who are very interested in the potential results. We have therefore contacted you via either the Vegetarian Society or the Vegetarian Study being carried out by this department and hope that you feel able to participate in it. Taking part in the study involves completing the simple, attached questionnaire and having an ultrasound examination done. We are planning to take our ultrasound machine to the Royal Berkshire Hospital in early December for several days and would like to see as many ladies as possible in that time. If you are prepared to help would you please complete the questionnaire and let us know what day(s) in the first week in December would suit you. I will contact you with further details as soon as possible.

If you have any questions then please contact Dr Fiona Pixley at this department, (phone number 511562). We would be happy to provide reimbursement for any travel expenses to Reading or arrange for an ultrasound examination at the John Radcliffe Hospital in Oxford if that suits you better. We assure you, of course, that all information is treated in the strictest confidence.

Yours sincerely,  
*Signature removed*

UNIVERSITY OF OXFORD  
DEPARTMENT OF COMMUNITY MEDICINE & GENERAL PRACTICE

Professor of Social & Community Medicine  
M.P. Vessey, MD., FRCP. (Ed.), FFCM.

Clinical Reader in General Practice  
G.H. Fowler, BM., FRCGP., DCH., DRCOG.

Dear.....,

Recently I sent you a letter regarding gall bladder disease and dietary aspects. The letter included a dietary and health questionnaire and an appointment date for an ultrasound examination. As yet, I haven't received your questionnaire so I don't know whether the appointment time and date suited you. It is possible that the letter was lost in the post, therefore I am sending another questionnaire and appointment with a reply-paid envelope.

I fully understand the time and effort involved in completing such a questionnaire and undergoing an ultrasound examination but in a survey of this type it is most important that as many replies as possible are received. I would be most grateful if you could find the time to answer the questions and return the form to me as your cooperation will greatly assist my research. Please, don't hesitate to contact me if you have any questions or doubts.

Thank you very much for your help,

Yours sincerely,

Signature removed

UNIVERSITY OF OXFORD  
DEPARTMENT OF COMMUNITY MEDICINE & GENERAL PRACTICE

Professor of Social & Community Medicine  
M.P. Vessey, MD., FRCP. (Ed.), FFCM.

Clinical Reader in General Practice  
G.H. Fowler, BM., FRCGP., DCH., DRCOG.

Dear .....,

I have been sending out information recently regarding a gall bladder study that I am carrying out. It is an unusual study in that I am looking at women who are generally perfectly healthy so I fully understand that it may seem a rather troublesome event for you to participate in. Although I would like as many people as possible to join the study, it would be overly optimistic to expect everyone to be able to come. If you feel it isn't possible for you to participate I would appreciate it though, if you would let me know this as we don't know whether to continue sending correspondence to you.

Yours sincerely,

Signature removed

UNIVERSITY OF OXFORD  
DEPARTMENT OF COMMUNITY MEDICINE & GENERAL PRACTICE

Professor of Social & Community Medicine  
M. P. Vessey, M.D., FRCP. (Ed.), FFCM

Clinical Reader in General Practice  
G. H. Fowler, BM., FRCGP., DCH., DRCOG.

Dear .....

We have virtually finished the first part of our study on gallbladder disease in the community that you so kindly participated in last year. So far the results have been extremely interesting with gallstones occurring far more commonly than previously believed yet usually without causing any problems at all. In fact, we believe that most gallstones will remain silent for one's lifetime.

You probably remember filling in a dietary questionnaire that gave us some very helpful information on various dietary aspects. Unfortunately, though, dietary assessment is difficult and we have found that we need more information to be able to analyse the effect of diet on gallstone formation accurately. We hate to bother you again after you were so helpful last time but we would greatly appreciate it if you would help us in this next stage.

We have designed a dietary diary which we would like you to complete. It has full instructions on the first two pages and requires completion over two week days and a Saturday and Sunday. If you would start the diary on the day after you receive this letter then we will visit you some time later to collect it and ask a few general dietary questions. There is a detachable section on this letter. would you complete it to let us know the best way to contact you and return it to us? We can then arrange a convenient time to visit you to collect the diary.

We do appreciate that this will involve an effort on your part but if you wouldn't mind completing the diary we would be very grateful. If you have any queries at all, please don't hesitate to contact us on 511562. We look forward to hearing from you,

Yours sincerely,

Signature removed

Dr Fiona Pixley

Kath Bunch

Signature removed

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Name .....

I would / would not like to help in the dietary assessment study.

Contact number / address is (if you would like to help) .....

.....

UNIVERSITY OF OXFORD  
DEPARTMENT OF COMMUNITY MEDICINE & GENERAL PRACTICE

Professor of Social & Community Medicine  
M. P. Vessey, MD., FRCP(Ed)., FFCM.

Clinical Reader in General Practice  
G. H. Fowler, BM., FRCGP., DCH., DRCOG.

Dear.....,

We have virtually finished the first part of our study on gallbladder disease and diet that you so kindly participated in either last year or the year before. So far the results have been extremely interesting with gallstones occurring far more commonly than previously believed yet usually without causing any problems at all. Even more interestingly, we have found that gallstones are about half as common in vegetarian women.

You probably remember filling in a dietary questionnaire that gave us some very helpful information on various dietary aspects. Unfortunately, though, dietary assessment is difficult and we have found that we need more information to be able to compare the vegetarian diet with the omnivorous diet more accurately. We hate to bother you again after you were so helpful last time but we would greatly appreciate it if you would help us in collecting some further dietary data.

We have designed a dietary diary that is very similar to one developed for the Vegetarian Study and would like you to complete it. It has full instructions on the first two pages and requires completion over two week days and a Saturday and Sunday. If you would start the diary on the Thursday after you open this letter and also complete the other more general questions included with it then return the completed diary to me, I can then contact you again to clarify any foodstuffs which I may not be sure about. All the omnivorous women who have participated in this part of the study lived in Oxford so I was actually able to visit them to make sure that all the necessary details were included. Unfortunately, as you ladies are from all over England, it is not possible to visit you and I will be relying on your own accuracy (which is usually far better than omnivorous women!). The most important items to remember are accurate quantities, brand names and if your meals include casseroles etc., could you write down briefly what the ingredients are?

We do appreciate that this diet will involve quite an effort on your part but if you wouldn't mind completing the diary we would be very grateful. If you have any queries at all, please don't hesitate to contact us on (0865)511562. We look forward to hearing from you,

Yours sincerely,

Dr Fiona Pixley,

Dr Jim Mann.

UNIVERSITY OF OXFORD

DEPARTMENT OF COMMUNITY MEDICINE & GENERAL PRACTICE

Professor of Social & Community Medicine  
M. P. Vessey, MD., FRCP(Ed)., FFCM.

Clinical Reader in General Practice  
G. H. Fowler, BM., FRCGP., DCH., DRCOG.

Dear .....

I do apologise for having to write you again regarding the dietary study I am carrying out as part of my research into gallbladder disease. I have almost finished this dietary survey now which means that the whole study is all but completed so I would really appreciate it if you would let me know whether or not you are able to help me in this second part.

The information that we have been able to collect from this dietary survey has been invaluable in determining more accurately the fat, fibre and total caloric intake of participants to allow us to compare ladies with gallstones with those who had none. As the vegetarian ladies have about half the incidence of gallstones, it is very important that we can get more accurate information on how your diet differs from omnivorous diets.

I do appreciate that a diary like this can be time-consuming and therefore a bother so if you feel that it isn't possible for you to complete the diary, I will quite understand - would you let me know though? If you would like to participate, would you send the diary back making sure that the important details like brand-names, quantities and recipes or ingredients are included in the diary?

Thank you very much for your interest and I do hope that you will find it possible to participate in this dietary survey.

Yours sincerely,

Dr Fiona Pixley.

UNIVERSITY OF OXFORD  
DEPARTMENT OF COMMUNITY MEDICINE & GENERAL PRACTICE

Professor of Social & Community Medicine  
M. P. Vessey, MD., FRCP(Ed)., FFCM.

Clinical Reader in General Practice  
G. H. Fowler, BM., FRCGP., DCH., DRCOG.

Friday, June 29th.

Dear

I do apologise yet again for having to bother you again regarding my study of gallbladder disease and diet. It is just that I have virtually finished seeing all the ladies in Dr Blasewicz's practice now and would like to start analyzing results. I would really appreciate it if you decided that you would like to participate in this second and final section of my study as the results look very interesting but will be even more valuable if more people were able to join.

If you feel that completing a four-day dietary diary really isn't possible then that is perfectly understandable but I would really appreciate it if you would let me know of your decision by returning the slip below.

Thanks very much indeed for your help so far and I'm sorry to be sending another letter,

Yours sincerely,

Dr Fiona Pixley.

Please detach here and return the form below

I am / am not interested in participating in the dietary survey section of the gallbladder study.

Name .....  
Contact Telephone No .....  
(if participating)