THE ROLE OF NATURAL SALICYLATES IN FOOD INTOLERANCE

by

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in the University of Sydney.

PREFACE

The work described in this thesis was initiated in order to investigate adverse reactions to food in sensitive individuals. All experiments were personally carried out by the author between April 1977 and September 1986.

None of the material has previously been presented for the purpose of obtaining any other degree.

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ABSTRACT

During the 1960's and 1970's several authors reported that manipulation of diet to exclude certain additives and natural chemical substances led to apparent clinical benefit in a number of conditions, however, available diets were largely empirical. In April 1977, the present study commenced with the development of an elimination diet, in order to investigate the role of food in recurrent idiopathic urticaria/angioedema. Stable asymptomatic remission was first established on this baseline diet, prior to challenge with test substances encapsulated in clear gelatine. Following completion of the challenge protocol, long-term dietary modification based on individual oral provocation results was advised.

The initial clinical experience in 76 patients indicated that natural salicylates (aspirin) were one of the main groups of compounds which could precipitate recurrent idiopathic urticaria/ angioedema, however, the literature revealed that published information about their presence in food was limited. Consequently, a systematic analysis of the total salicylate content of commonly eaten foods was undertaken using thin layer chromatography and high performance liquid chromatography, after which the elimination diet was modified and charts were constructed which could be used by salicylate-sensitive individuals to control the total daily dose of salicylate consumed.

It then became apparent that adverse reactions to food may cause not only symptoms involving the skin (recurrent idiopathic urticaria/ angioedema), but also of the gastrointestinal tract, respiratory tract and/or central nervous system. Clinical observation suggested that in these patients a variety of other food additives and natural compounds might be implicated in provoking some of their symptoms. The challenge battery was therefore expanded and made double-blind to allow for the extensive and objective investigation of all patients presenting with more subjective symptoms (such as migraine, irritable bowel and neuropsychiatric symptoms). Over 3000 patients with varying manifestations of food intolerance have now been investigated. Susceptibility appears to be familial, more widespread than generally appreciated and there is strong circumstantial evidence to suggest that reactions are pharmacological in nature (rather than immunological). Reactions appear to be dose-related, and sensitive patients may exhibit withdrawal and supersensitivity, as well as tachyphylaxis and tolerance after reintroduction. Overall, natural salicylates are the single commonest substance to produce reactions when tested by double-blind oral challenge. Most patients are sensitive to multiple substances (between 2 and 10 commonly), and the effects can be additive.

ABBREVIATIONS

ARS	Anne Ruth Swain
ASA	Aspirin
BDH	British Drug House
BFSTA	Bis (Trimethylsilyl) Trifluoroacetamide
$CaCl_2$	Calcium Chloride
CNS	Central Nervous System
GABA	Gamma-Aminobutyric Acid
GLC-MS	Gas Chromatography-Mass Spectrometry system
HCl	Hydrochloric Acid
HPLC	High Performance Liquid Chromatography
H_3PO_4	Phosphoric Acid
IBS	Irritable Bowel Syndrome
$KHSO_4$	Potassium Bisulphate
$MgSO_4$	Magnesium Sulphate
MSG	Mono Sodium Glutamate
$NaHCO_3$	Sodium Bicarbonate
NaOH	Sodium Hydroxide
NH&MRC	National Health and Medical Research Council
NSW	New South Wales
RIU/AO	Recurrent Idiopathic Urticaria/Angioedema
RPAH	Royal Prince Alfred Hospital
SD	Standard Deviation
SE	Standard Error
SPSS-X	Statistical Packages for Social Sciences, Number 10
TBAP	Tetrabutylammonium Phosphate
TLC	Thin Layer Chromatography
USA	United States of America

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CONTENTS

CHAPTER 1

INTRODUCTION

This study commenced at Royal Prince Alfred Hospital (RPAH) in April 1977, after Dr. R.L. Clancy (Staff Immunologist) became interested in the possible role of dietary substances in the pathogenesis of recurrent idiopathic urticaria and angioedema (RIU/AO). During the 1960's and 1970's several authors had reported that a number of chemicals present in food could precipitate acute exacerbations of RIU/AO and that diets designed to exclude these substances often induced prolonged remission (Warin, 1960; Moore-Robinson & Warin, 1967; Champion et al., 1969; James & Warin, 1970; Lockey, 1971; Juhlin et al., 1972; Michaelsson & Juhlin, 1973; Warin & Champion, 1974; Doeglas, 1975; Thune & Granholt, 1975; Warin, 1976; Warin & Smith, 1976; Ros et al., 1976; Settipane et al., 1976; Doeglas, 1977). The chemicals that were implicated included the naturally occurring salicylates in food as well as artificially added preservatives and colourings.

RIU/AO is a common condition generally treated symptomatically with a variety of antihistamines, the side effects of which are often limiting factors. As a result of the favourable findings reported by Warin and Smith (1976) Dr. Clancy decided to investigate dietary management as an alternative, and approached Ms M. Hosking (Head Therapeutic Dietitian) in order to develop a suitable elimination diet. At this time the author was consulted while working as an intern dietitian in the Dermatology Ward at RPAH. Under the guidance of Ms M. Hosking and Ms J. Rogers (Head Food Services Dietitian), and after reviewing the available literature on diet and RIU/AO, an elimination diet was formulated and the study commenced (Gibson & Clancy, 1978).

Review of Literature Leading up to This Study

It had been known since the turn of the century that aspirin ingestion could precipitate acute urticaria (Hirschberg, 1902) and more recently Calnan (1957, 1964) noted that aspirin could also trigger recurrences in many patients with RIU/AO. Warin (1960) had reported that 22 of 70 patients with RIU/AO developed exacerbations after administration of aspirin. Subsequently, Moore-Robinson and Warin (1967) reported an incidence of 22% in 228 patients and Champion et al. (1969) found that 21% of 268 patients with RIU/AO reacted to aspirin. James and Warin (1970) gave test doses of aspirin to 96 patients with RIU/AO in a "single blind" manner, and 37 of these developed an urticarial reaction to the challenge.

In 1959, 1969 and 1971, Lockey reported the role of azo dyes derived from coal tar, particularly the yellow dye tartrazine, in RIU/AO. Juhlin et al. (1972) showed that in seven out of eight aspirin-sensitive patients similar exacerbations occurred with tartrazine, as well as with certain benzoates used as preservatives. In 1973 Michaelsson and Juhlin reported 52 patients who were challenged with aspirin, sodium benzoate, 4-OH benzoic acid, and three azo dyes tartrazine, sunset yellow and new coccine. Of these, thirty five reacted to aspirin, twenty seven to benzoic acid compounds and twenty seven to azo dyes. Doeglas (1975) had performed similar provocation tests, and in 23 patients with RIU/AO known to react to aspirin, 30% reacted to tartrazine, 17% to sodium benzoate and 15% to 4OH benzoic acid. Using a battery of challenge tests Thune and Granholt in 1975 and Warin and Smith, and Ros et al. in 1976 also identified a group of RIU/AO patients who reacted to salicylate, tartrazine, sodium

benzoate and 4-OH benzoic acid. Settipane et al. (1976) challenged 38 patients with tartrazine in whom there was an eight percent positive response.

Several of the above authors had reported that diets designed to exclude foods containing salicylates and/or additives induced prolonged remission of urticaria in those patients who had shown a positive response to oral challenge with these compounds (Michaelsson and Juhlin, 1973; Doeglas, 1975; Warin, 1976; Warin & Smith, 1976; Ros et al., 1976; Doeglas, 1977). Seventy-five percent of the patients studied by Warin and Smith (1976) became either asymptomatic or improved after being on an appropriate diet for a two month period. This improvement was similar to the results obtained by Michaelsson and Juhlin (1973) and Doeglas (1975). In 1976, Ros et al. found that in 59 patients with RIU/AO who reacted to challenge with salicylates, preservatives and azo dyes, a diet designed to reduce consumption of these items resulted in complete remission in 24% and improvement in 57%.

Design of the Elimination Diet at RPAH

Our approach to the identification of dietary chemicals which contribute to the pathogenesis of RIU/AO was similar to that of Warin and Smith (1976). An important difference, however, was our attempt to establish a stable asymptomatic remission prior to challenge, using a baseline diet constructed to exclude all the test substances (Gibson & Clancy, 1978). This approach had the dual advantage that patients

would be able to discontinue antihistamines, which might otherwise mask challenge reactions, as well as reducing background symptoms from substances included in a normal diet which might otherwise produce false positive results. The low response rate with the lactose placebo in this study emphasized the importance of removing this background "noise" and contrasts with the experience of other workers (Michaelsson & Juhlin, 1973; Doeglas, 1977).

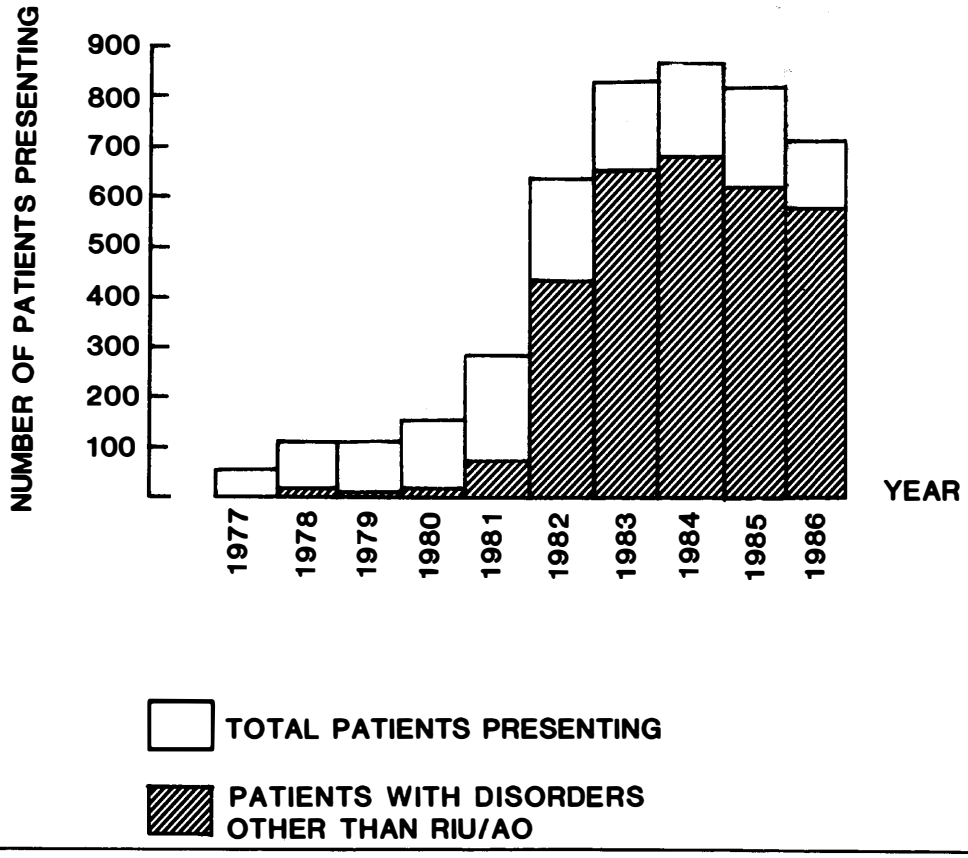
We began with a diet based mainly on the elimination diets of Rowe (1944, 1972), Shelley (1964), Feingold (1968) and Warin (1976) designed to exclude the common food "allergens" as well as salicylates, preservatives and azo-dyes, but clinical experience with our first two patients suggested that this information was inadequate. Since pineapple and pears were the only fruits allowed, both patients consumed large amounts of pineapple juice and fresh pineapple, and found that this appeared to exacerbate their symptoms. A more detailed review of the literature revealed that, where available, analytical data on the content of salicylates and benzoates occurring naturally in foods was incomplete, scattered, and sometimes contradictory (Table 2.6), and much of the published information was not relevant to the Australian diet. It was therefore decided to undertake a systematic analysis of the total salicylate content of commonly eaten foods under the supervision of Professor A.S. Truswell in the Human Nutrition Unit, University of Sydney. This work, described in Chapter 2, was completed in 1983 and led to a number of modifications of our original elimination diet (Gibson & Clancy, 1978; Allen et al., 1984).

The Clinical Spectrum of Food Intolerance

Dr. Clancy left RPAH in 1978, and clinical evaluation of patients undergoing dietary elimination and challenge testing continued with Professor A. Basten, Drs. P. Gatenby, S. Krilis and S. Van Nunen. The clinical features and results of immunological and other investigations in the first 76 patients with RIU/AO were published in 1980 (Gibson & Clancy).

FIGURE 1.1

TOTAL PATIENTS SEEN AT THE ALLERGY CLINIC 1977-1986



In 1980 Dr. R.H. Loblay joined the RPAH Allergy Clinic as Clinical

Immunologist. By the following year it was becoming increasingly evident to us, as well as others (Juhlin, 1981), that patients with RIU/AO sometimes also experienced abdominal pain, diarrhoea, headache, respiratory and/or constitutional symptoms when undergoing blind challenge with various food substances. It was found that elimination of the relevant foods sometimes resulted in dramatic improvement in chronic symptoms of this kind, even when RIU/AO was only a minor component of the clinical presentation.

Since 1981 there has been a gradual change in the spectrum of patients attending the RPAH Allergy Clinic (Figure 1.1). This was partly due to the dissemination of the protocol to other hospitals and practitioners who were then able to manage patients with uncomplicated RIU/AO in the same way, so that those referred to RPAH tended to have more complex or unusual clinical presentations. Another factor was the increasing public interest in "food allergy" which in the late 1970's and early 1980's was widely promoted by fringe and alternative practitioners as being responsible for a vast array of symptoms and diseases (Feingold, 1975; Airola, 1977; Mackarness, 1980; Crook, 1984). By 1982 the Allergy Clinic at RPAH had become well known for its interest in food intolerance, and many patients were therefore referred with a variety of clinical problems, which they suspected might be attributable to dietary factors. These included migraine, irritable bowel syndrome (IBS), asthma and eczema, as well as patients who experienced symptoms referable to multiple organ systems, often together with vague constitutional symptoms suggestive of psychoneurosis. This latter group of patients was

designated as having "systemic" symptoms. A Paediatrician, Dr. V. Soutter, also began attending the Clinic in 1981, and a significant number of "hyperactive" children have undergone dietary evaluation since then.

Clinical observations suggested that in patients with syndromes other than RIU/AO milk, wheat, natural amines (Hanington, 1967), monosodium glutamate (MSG) (Kwok, 1968; Schaumberg et al., 1969), and a variety of other food additives might also be implicated in provoking some of these symptoms, and a more stringent elimination diet was designed for the investigation of such patients. At the same time the range of challenge substances was extended, and starch and sucrose were introduced as placebos in place of lactose, which was sometimes found to provoke abdominal symptoms. Since many of these additional symptoms were of a subjective nature the challenge protocol was made "double-blind", with numbered challenge capsules administered in an arbitary order. The clinical characteristics, dietary investigation and management of patients with these syndromes other than RIU/AO are described in Chapter 6. In the final Chapter the results of the previous chapters are discussed. Observations about the range of food chemicals implicated, the symptoms provoked on challenge and the clinical behaviour of reactions provided valuable clues as to the underlying mechanisms of adverse food reactions.

CHAPTER 2

SALICYLATE ANALYSIS

INTRODUCTION

Analyses described in this Chapter were initiated because of lack of available information on natural salicylates in food, as outlined in Chapter 1. Initially after contacting Juhlin (1978) it was decided to follow his previously established methods using Thin Layer Chromatography (TLC), but this proved unsatisfactory. After a careful review of the literature a number of different extraction methods were tried, and with suitable modifications good chromatographs were eventually obtained. However, it soon became obvious that this method, even at best, could not provide reliable quantitative estimates of salicylate concentration, and it was therefore decided to change to High Performance Liquid Chromatography (HPLC). At that time HPLC had only been used for assaying aspirin in tissues and body fluids, and benzoates added to foods as preservatives. To measure natural salicylates present in foods at one or two orders of magnitude lower concentrations required a considerable amount of preliminary work to modify existing methods.

MATERIALS AND METHODS

Development of Extraction Method

Preliminary work extracting acetylsalicylic acid, sodium salicylate, sodium benzoate and 4-OH benzoic acid for TLC based on Horwitz (1975) gave limited recoveries which were only qualitative and so further development was undertaken to ensure maximal extraction of naturally occurring salicylates in foods and beverages. These methods involved various techniques summarized in the following table (Table 2.1).

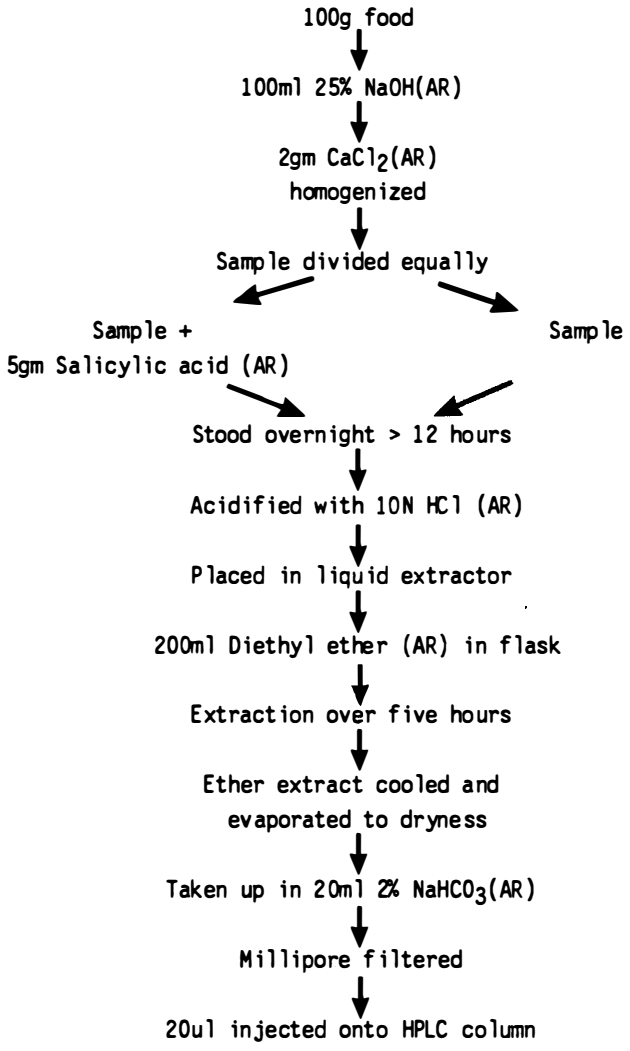
TABLE 2.1

METHODS OF EXTRACTION INVESTIGATED

Methods of extraction investigated	1	2	3	4	5
References used in development of extraction method	salicylic acid (Horwitz, 1975)	benzoic acid (Horwitz, 1975)	salicylic acid (Guppy et al., 1977-1978)	benzoic acid (Murphy & Stutte, 1978)	Sep Pak C18 cartridge by Waters
Steps in method:					
1. Amount of food	50g	150g	50g	5g	5g
2. Reagents added to food		methylene dichloride (AR) + 2N HCl (AR)			
3. Homogenize food	yes	yes	yes	yes	yes
4. Reagents added to food	5g $CaCl_2$(AR) 10% NaOH(AR) stand 2 hours	15g NaCl(AR) 10% NaOH(AR) stand 2 hours		3ml 2% acetic acid (AR) heated 100C for 10 min. 8ml 2N HCl (AR) heated 100C 1 hr	
5. Centifuge	yes	yes	no	no	yes
6. Filter (paper)	no	no	yes	yes	no
7. Reagents added	25% HCl(AR)	25% HCl(AR)			
8. Extraction	Diethyl ether	Diethyl ether	Distilled H_2O	Diethyl ether	
9. Identification	TLC	TLC			
	HPLC	HPLC	HPLC	HPLC	

FIGURE 2.1

EXTRACTION METHOD FOR HPLC



The methods by Guppy et al. (1977-1978) and Horwitz (1975) gave reasonable extraction, however all these methods developed emulsions which were difficult to clear, so a modified Horwitz (1975) method was developed with extraction via a liquid extractor [Figures 2.1, 2.2(b), 2.2(c)]. This modified method used stronger sodium hydroxide (NaOH) i.e. 25% NaOH (AR) overnight instead of 10% NaOH (AR) for two

hours with the aim of completing the hydrolysis of all salicylate esters to free salicylate. Then the acidification step was improved using concentrated hydrochloric acid (HCl)(AR) to bring the pH to 2.0. Finally, the extraction procedure was changed from ether extraction by a separating funnel giving a 50% recovery of added standards to ether extraction via glass extractors over five hours resulting in good recoveries of added standard salicylic acid due to the lack of emulsion formation [Figures 2.2(b), 2.2(c)].

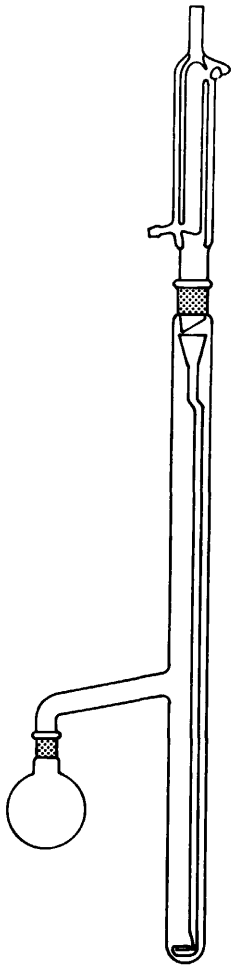
FIGURE 2.2(a)

EXTRACTION EQUIPMENT

Homogenization of each food sample by commercial blender prior to extraction.

FIGURE 2.2(b)

EXTRACTION EQUIPMENT



Liquid extractor with condenser and round bottomed flask

Food Extract Preparation for Analysis

Foods and beverages purchased from local stores or donated by the RPAH kitchen for analysis were analysed for salicylate by the method outlined in Figure 2.1. Samples weighing a hundred grams were homogenised in a commercial blender, along with 100mls 25% NaOH (AR) and

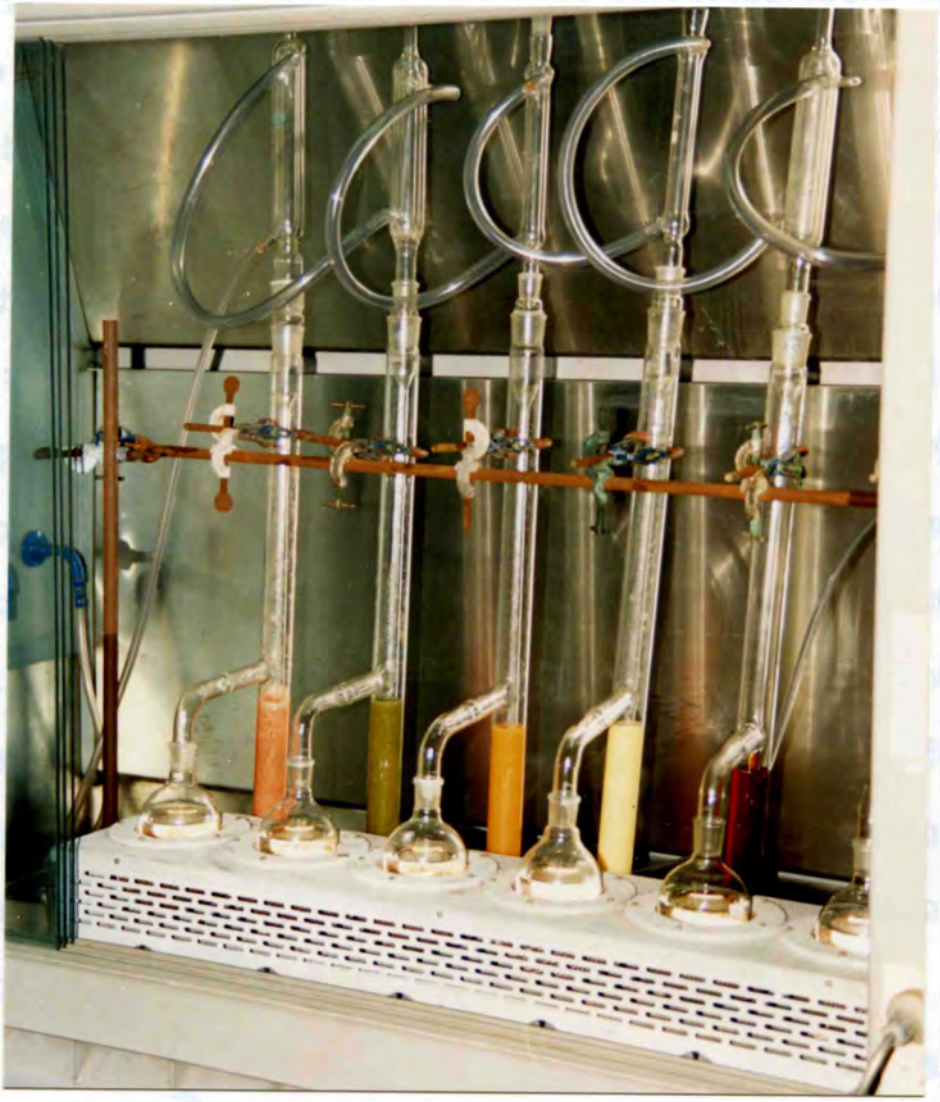
two grams of calcium chloride ($CaCl_2$)(AR) [Figure 2.2(a)]. Duplicate samples corresponding to 50gm food or beverage were weighed out. To one sample of each pair, five milligrams of salicylic acid (AR) standard was added. Both samples were allowed to stand overnight. The two homogenates were then acidified with 10N HCl (AR) to pH<2 and placed in separate liquid extractors. Two hundred millilitres of diethyl ether (AR) were placed in a round bottomed 500ml flask, along with several glass boiling beads to avoid bumping. The flask was then placed in a heating mantle and connected to the liquid extractor and condenser. Extraction was carried out for five hours [Figure 2.2(c)]. The ether extract was then allowed to cool and evaporate to dryness in the fume cupboard. The sample was then taken up in 20ml 2% sodium bicarbonate ($NaHCO_3$)(AR) and filtered through 0.45 micron millipore filters prior to analysis by HPLC.

Development of TLC Procedure for Salicylate Detection

Silica gel TLC separation after hydrolysis and extraction have been reported to give excellent separation of acetylsalicylic acid, sodium salicylate, sodium benzoate and 4-OH benzoic acid (Juhlin, 1978). Several solvent systems were tried (Baldwin et al., 1960; Ganshirt & Morianz, 1960; Baldwin et al., 1961; Ganshirt, 1963; Copius-Peereboom & Beekes, 1964; Gossele & Srebrnik-Friszman, 1966; Pinella et al., 1966; Khemani & French, 1969; Chiang, 1969; Gossele, 1971; Tjan & Konter, 1972; Zweig & Sherma, 1972; Juhlin, 1978) as well as visualization by a range of stains (Copius-Peereboom & Beekes, 1964; Gossele & Srebrnik-Friszman, 1966; Chiang, 1969; Gossele, 1971; Juhlin, 1978). The final method used is outlined in Figure 2.3 below.

FIGURE 2.2(c)

EXTRACTION EQUIPMENT



Total salicylate obtained from each food sample by liquid extraction

TLC of Food and Beverage Extracts

Forty grams of kieselgel type 60 (AR) by Merck was shaken for two to three minutes with 80mls of distilled water to give a homogeneous

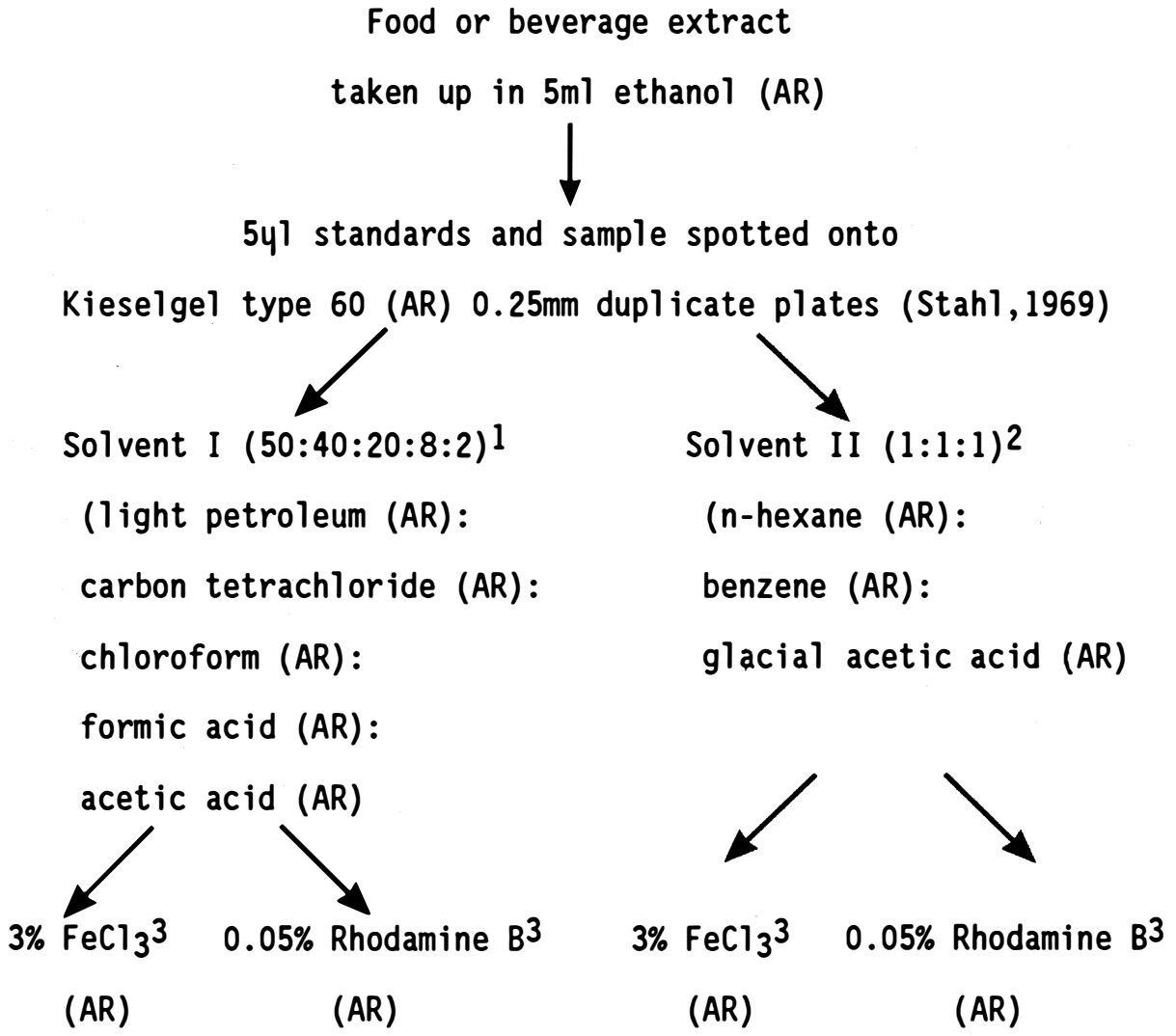
suspension, which was spread evenly with a Desaga applicator on five glass plates (20cms by 20cms) to a thickness of 0.25mm. The plates were dried in an oven at 100°C for half an hour and allowed to cool in a dessicator. Five microlitres of 0.01g/l ethanolic solutions (AR) of acetylsalicylic acid (AR), sodium salicylate (AR), sodium benzoate (AR) and 4-OH benzoic acid (AR) were applied to each TLC plate by a SGE microlitre syringe. Sample application to duplicate plates was made on a line three centimetres from the lower edge, and the sample was developed by the ascending method. In order to ensure the equilibration of the vapour in the chamber, the inside was lined with filter paper soaked in the solvent. The development was continued until the solvent reached five centimetres from the upper edge. Duplicate plates were run in the two mobile phases separately under normal conditions (room temperature and a relative humidity between 35% and 70%). The duplicate plates were sprayed with one of two sprays for identification of acetylsalicylic acid, sodium salicylate, sodium benzoate and 4-OH benzoic acid (Figure 2.3).

Validation Of TLC Method

Multiple extractions of two foods, roast beef and milk, shown to contain no acetylsalicylic acid, sodium salicylate, 4-OH benzoic acid or sodium benzoate were carried out to validate the extraction method.

FIGURE 2.3

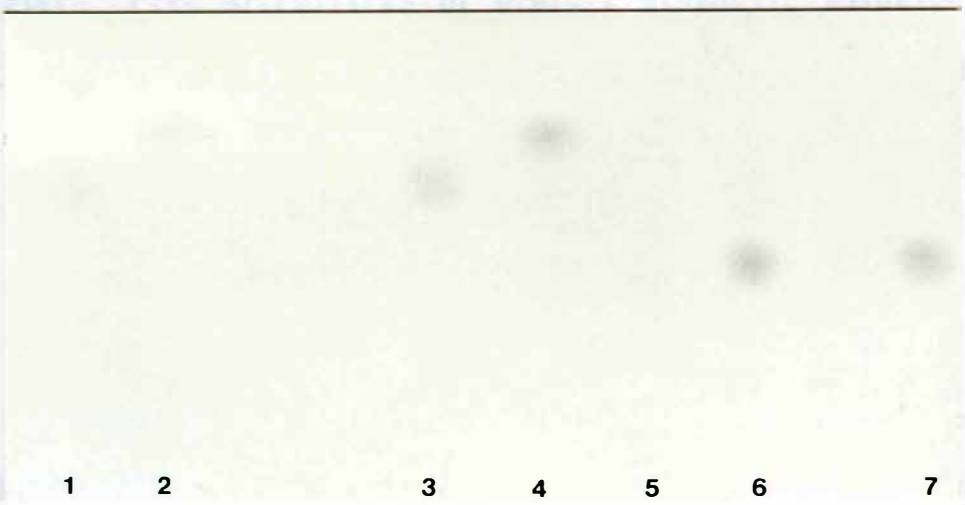
TLC OF FOOD AND BEVERAGE EXTRACTS



1. Gossele, 1971
2. Chiang, 1969
3. Copius-Peereboom & Beekes, 1964

FIGURE 2.4(a)

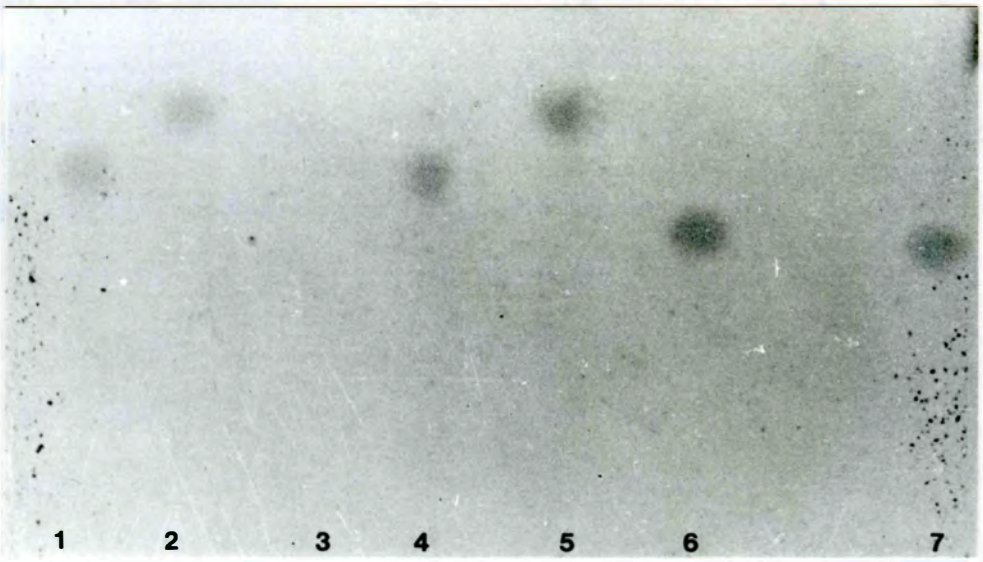
TLC PLATES OF STANDARD COMPOUNDS ELUTED WITH ROAST BEEF EXTRACTS



1. Aspirin
2. Sodium salicylate
3. Roast beef + aspirin
4. Roast beef + sodium salicylate
5. Roast beef
6. Roast beef + 4-OH benzoic acid
7. 4-OH benzoic acid

FIGURE 2.4(b)

TLC PLATES OF STANDARD COMPOUNDS ELUTED WITH MILK EXTRACTS



1. Aspirin
2. Sodium salicylate
3. Milk
4. Milk + aspirin
5. Milk + sodium salicylate
6. Milk + 4-OH benzoic acid
7. 4-OH benzoic acid

Development of HPLC Procedure for Salicylate Detection

The HPLC method used to analyse the salicylate content of foods and beverages was based on those reported by other workers for the quantitative analysis of benzoic and salicylic acid as naturally occurring compounds (Nelson, 1973; Smyly et al., 1976; Das Gupta, 1977; Murphy & Stutte, 1978; Leuenberger et al., 1979; Jandera & Engelhardt, 1980; Wehr, 1980) across a pH range from 4.5 to 7.4.

HPLC Separation

Duplicate food extracts one of which was spiked, the other unspiked with standard salicylic acid (AR) were injected into a HPLC for analysis. This HPLC system consisted of a Varian 5060 pumping system with Rheodyne 7125 injection valve. The column was a Waters uBondpak C18 reverse phase column, 300mm by 4.6mm, fitted with a precolumn packed with Vydac RP P389 packing material. The eluent was monitored with a Varian Vari-Chrom variable wavelength detector with wavelength at 235nm connected to a Varian CDS 111 computing integrator and Houston Omniscribe recorder.

Optimum separation occurred with a solvent of 300ml methanol (unichrom), 700ml glass distilled water, 10ml tetra butylammonium phosphate (TBAP)(unichrom) and 10g phosphoric acid (H_3PO_4)(AR) made to pH 7.0 with 25% NaOH (AR). The separation was programmed isocratically at ambient temperature with a flow programme which resulted in a back pressure of 90-150 atmospheres. The flow rate was 1.0ml/min for seven minutes, increasing to 2.0ml/min over the next three minutes. A wavelength of 235nm was chosen because it gave the best resolution and specificity (Leuenberger et al., 1979).

FIGURE 2.5

HPLC EQUIPMENT

Specificity of Salicylate Identified by HPLC

Specificity of the salicylate peak was checked by two methods. First, 20 phenolic compounds chemically similar to salicylic acid and which might occur in foods were chromatographed by HPLC. Second, the salicylate peaks from 11 foods were collected and analysed by a Gas Chromatography-Mass Spectrometry system (GLC-MS)(Eglington et al., 1968) by Mr M. Smythe in the Chemistry Department, Sydney University. The foods were two vegetables (maize and radish), two fruits (red currants and dates), three herbs or spices (curry, cumin and rosemary), two sugary foods (honey and licorice), one sample of wine and one sample of tea.

Samples of sodium salicylate (AR) were run on the HPLC to determine retention times, to optimize conditions for collecting the salicylate peak. Peaks from foods with retention times identical to the salicylate standard were collected, acidified with 1ml 3M HCl (AR) extracted with 10ml diethyl ether (AR), three times and washed with 10ml distilled water, three times. The ether solution was drawn off, dried over anhydrous magnesium sulphate ($MgSO_4$) and filtered through cotton wool into a 1ml "Reactivial" (Regis). The volume was evaporated to 10µl with dry nitrogen under gentle heat. One hundred microlitres bis (trimethylsilyl) trifluoroacetamide (BFSTA) (Regis) was added in a dry environment and refluxed gently for one hour. The solution was reduced to 10µl under dry nitrogen and injected into the Gas Chromatograph (GLC). The column of the GLC, a Pye 104, was glass 2m by 6mm, packed with OV 17 (3%) on Chromosorb W (100/120 mesh). Helium flow through the column was 30 ml/min. The GLC was interfaced (via an AEI glass jet separator) to an AEI MS-30 mass spectrometer operating at 4KV with an ionization voltage of 70ev. The chromatographic trace was produced by the total ion current monitor of the mass spectrometer run at 20ev except when scanning (10 second/decade) when the ionizing voltage returns to 70ev.

Calculations of Salicylate Content of Foods Analysed by HPLC

Peak retention times and peak area (McCoy et al., 1984) were monitored and computed automatically by the integrator; initial peak identification was based on retention times and comparison with the standards as well as co-chromatography with the standards. Since

retention times alone are not sufficient for positive identification, salicylate peaks were collected and spectra of the chromatographic peaks were also obtained. Concentrations of salicylate were determined from the slope of the calibration plots in which peak area was plotted against amount injected. The detector responses were found to be linear over the entire working range.

Results from triplicate or multiple extractions were used to compute the total salicylate per 100gm food sample. Extraction efficiencies were calculated. They varied with the composition of the food. Fruits, vegetables, condiments and beverages gave extraction rates of greater than 85%, compared with cereals and protein foods for which the extraction rate was approximately 60%.

Validation was carried out by extracting several foods more than 10 times along with samples spiked with salicylic acid (AR). Several foods were selected which varied in physical attributes and which by previous analysis had been found to contain salicylate in amounts that were relatively low (carrots and pumpkin), medium (orange and pineapple) or high (thyme). The foods had varied physical attributes.

The salicylate contents of the multiple samples of these foods were calculated and then subjected to statistical analysis. The mean, standard deviation (SD) and standard error (SE) for the salicylate content of each food was determined from which the 95% confidence interval was calculated (Hays, 1973; Petrie, 1978; Sachs, 1984).

The 95% confidence interval for the mean salicylate content of each food, determined the normal range of salicylate present in each food i.e. the chance of the mean not being contained within this range would be at most five percent (Figure 2.6). A 95% confidence interval for a mean value is the interval from (mean - 1.96SE) to (mean + 1.96SE) where 1.96, corresponding to a 95% confidence level, is called the critical value and was obtained from tables for the standard normal distribution.

FIGURE 2.6

FORMULA FOR CALCULATION OF 95% CONFIDENCE INTERVAL

$$\text{Mean} = \bar{x} = \frac{\text{salicylate contents of food samples } (x)}{\text{number of extractions } (n)}$$

$$\text{Standard deviation of the sample} = SD = \sqrt{\frac{\sum (x - \bar{x})^2}{n - 1}}$$

$$\text{Standard error (estimated)} = SE = \frac{SD}{\sqrt{n}}$$

95% Confidence interval for the mean is (L,H) where:

$$L = \bar{x} - 1.96SE$$

$$H = \bar{x} + 1.96SE$$

RESULTS

Salicylate Identified in Foods by TLC

Some 56 foods were extracted with ether (Table 2.1) and analysed by TLC (Figure 2.3). Acetylsalicylic acid alone was found in 14 of the food extracts, while both acetylsalicylic acid and sodium salicylate were detected in 23 of those foods examined. A further 19 foods contained no appreciable amounts of acetylsalicylic acid or sodium salicylate (Table 2.2). However a subsequent sensitive specific method of HPLC combined with an efficient liquid extraction procedure has shown that eight of these foods contained appreciable salicylate.

The TLC method gave limited results as it was only able to identify but not quantify the presence of acetylsalicylic acid, sodium salicylate, 4-OH benzoic acid and sodium benzoate in a food sample.

In the selection of foods analysed by TLC, the results showed a small number which contained only acetylsalicylic acid, while most foods contained both acetylsalicylic acid and sodium salicylate in varying amounts. The pure acetylsalicylic acid standard and the acetylsalicylic acid present in food extracts showed degradation to salicylate on the plate if a delay occurred between plate application and elution. Acetylsalicylic acid is very unstable in aqueous solutions breaking down to salicylate. It was therefore difficult to quantify accurately the specific amounts of acetylsalicylic acid or sodium salicylate present in each food extract so a HPLC method was then developed to investigate this more thoroughly.

TABLE 2.2

SALICYLATE IDENTIFIED BY TLC

Food	Acetylsalicylic Acid	Sodium Salicylate	Food	Acetylsalicylic Acid	Sodium Salicylate
Almond	+	-	Nutmeg	+	+
Apple	+	+	Onions *	-	-
Banana	-	-	Orange juice	+	-
Beans	+	-	Parsley	+	+
Beer	+	-	Pawpaw	-	-
Black pepper	+	+	Peaches	+	+
Bread	-	-	Pears	+	+
Capsicum	+	+	Pears	-	-
Carrot	+	+	Peas *	+	+
Chocolate	-	-	Peppermint	+	-
Cider *	-	-	Pineapple juice	+	-
Cinnamon	+	+	Potato	-	-
Cloves	+	+	Pumpkin *	-	-
Cocoa	-	-	Pumpkin seeds *	+	+
Coconut	+	+	Red wine	-	-
Coffee	+	-	Roast beef	-	-
Coke	+	-	Salmon	+	-
Cucumber	+	+	Sherry	+	+
Dates	+	-	Sultanas	+	+
Gin	-	-	Tea	+	+
Honey *	-	-	Tomato paste	+	+
Lettuce	-	-	Tomato sauce	+	+
Licorice	+	+	Vinegar	+	-
Malt vinegar *	-	-	Whiskey	-	-
Mango	+	-	White grapes *	+	+
Milk	-	-	White wine	+	+
Mint	+	+	Zucchini	+	-
Mustard	+	-			

* Salicylate identified in foods by HPLC (in small amounts except honey which was a different brand)

FIGURE 2.7

GRADED DOSES OF STANDARD COMPOUNDS*

Figure 2.7(a) Graded Doses of Aspirin*

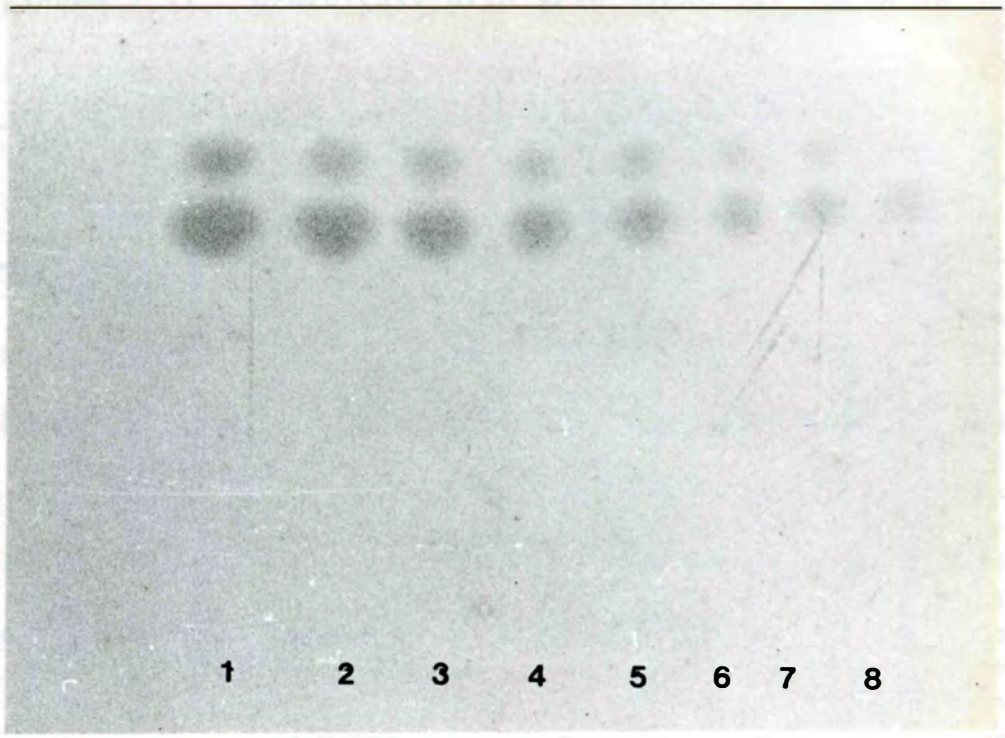


Figure 2.7(b) Graded Doses of Sodium Salicylate*

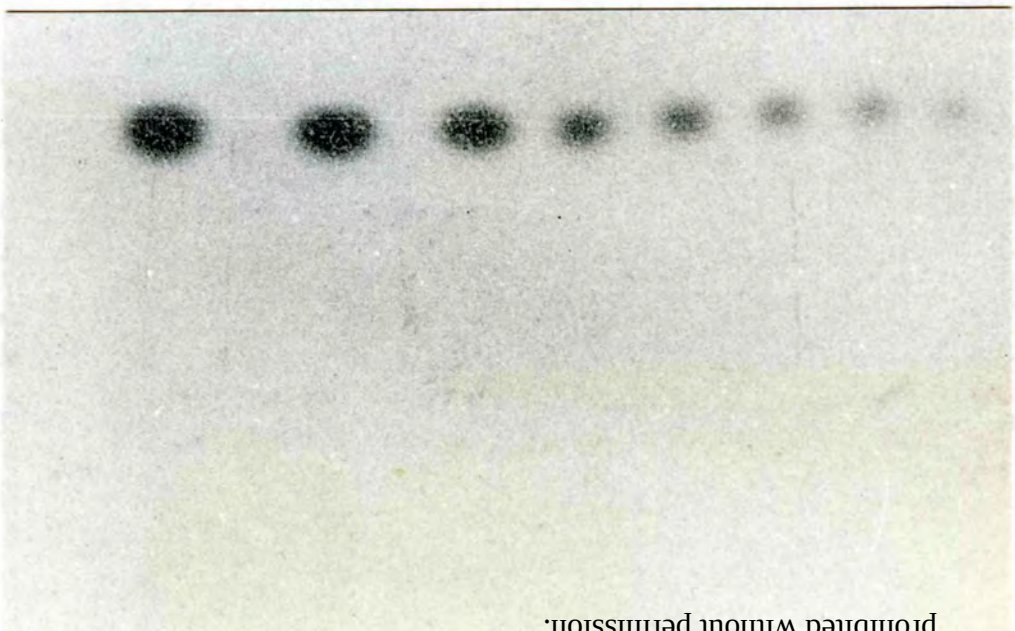
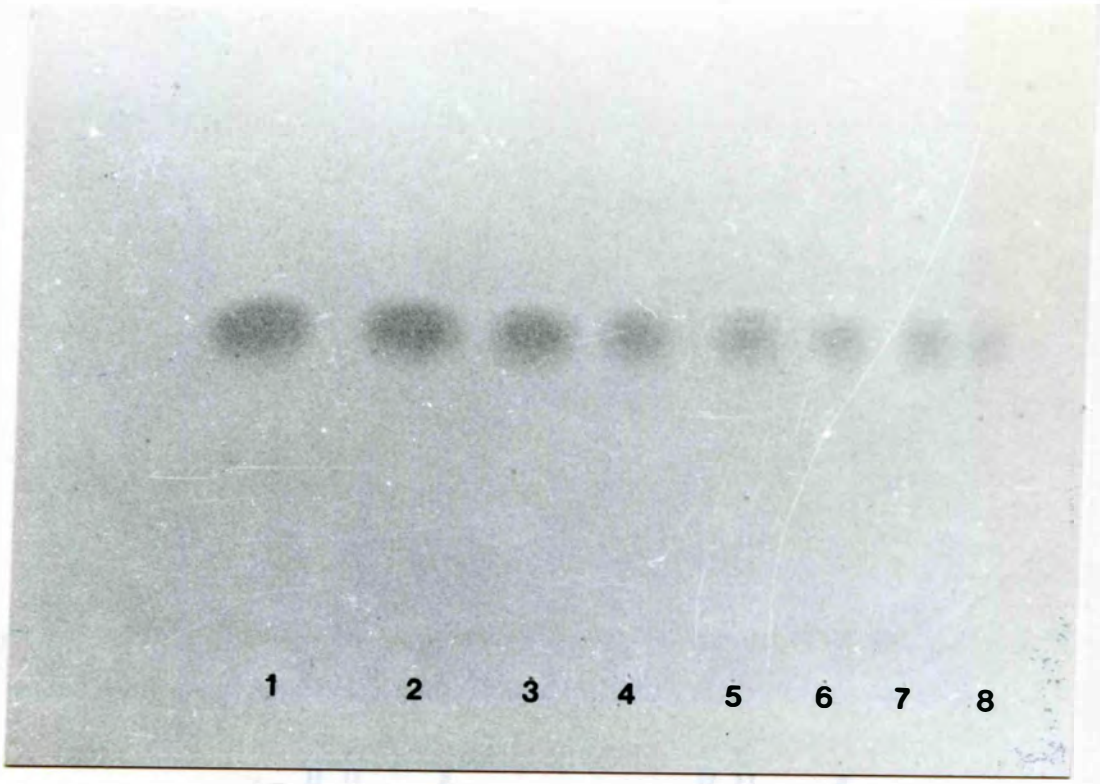


FIGURE 2.7

GRADED DOSES OF STANDARD COMPOUNDS*

Figure 2.7(c) Graded Doses of 4-OH Benzoic Acid*



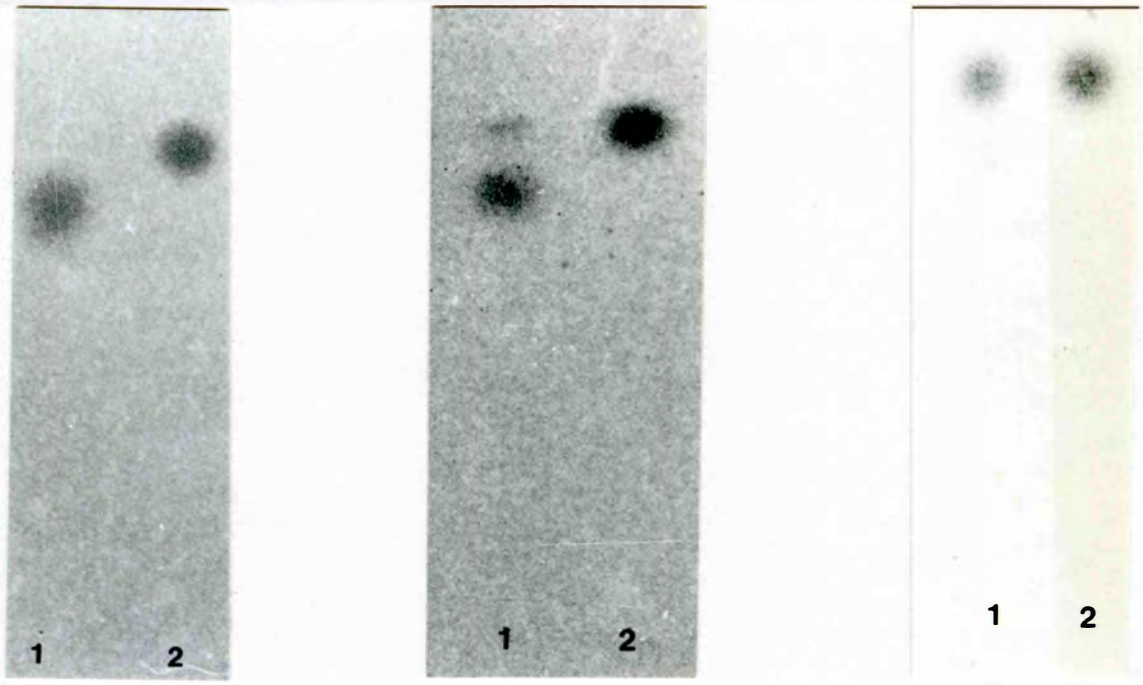
* Graded doses of standard compounds are 10µg, 20µg, 30µg, 40µg, 50µg, 100µg, 150µg, 200µg respectively.

Validation of Salicylate Identified by HPLC

Specificity of the salicylate was checked by comparision with several phenolic compounds chemically similar to salicylic acid and which might occur in foods. All 20 compounds were taken up in 2% $NaHCO_3$ (AR) and had retention times different from the retention time of salicylic acid.

FIGURE 2.8

HYDROLYSIS OF ASPIRIN TO SODIUM SALICYLATE ON THE TLC PLATE PRIOR TO ELUTION



(A) (B) (C)

Aspirin (lane 1) and sodium salicylate (lane 2) were plated at various times after extraction.

(A) Aspirin peak undegraded

(B) Partial degradation of aspirin peak

(C) Complete degradation of aspirin to salicylic acid

TABLE 2.3

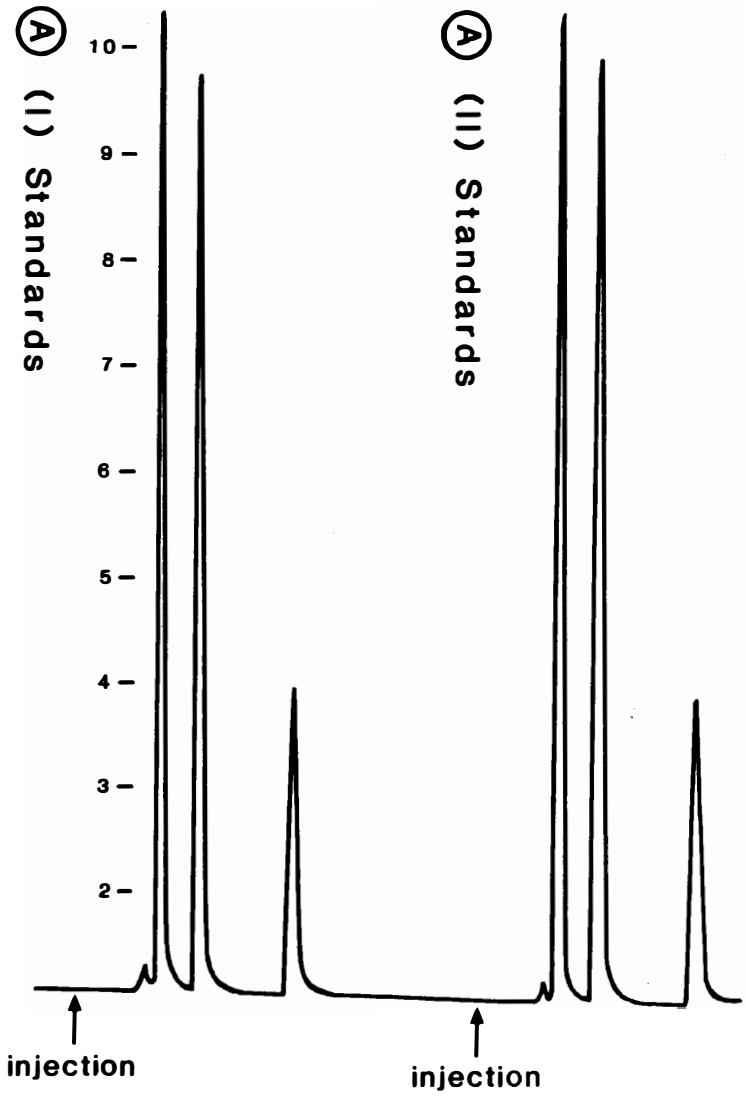
RETENTION TIMES OF SEVERAL PHENOLIC COMPOUNDS

Compound	Retention Time (minutes)
P-Amino benzene	2.46
Para amino benzoic acid	3.20
Phthalic acid	3.31
Salicylamide	3.32
Salicin	3.43
4OH benzoic acid	3.46
Vanillic acid	3.51
Sulphosalicylic acid	4.68
Anthranilic acid	6.01
Gentisic acid	6.75
Acetylsalicylic acid	7.10
Benzoic acid	8.20
Catechol	8.23
Theophylline	8.34
Quercetin	11.30
Vanillin	11.72
Acetanilide	14.41
Phenol	14.81
Salicylic acid	16.81
Coumarin	18.88
Methyl salicylate	58.43

Solvent: 20% methanol, 80% glass distilled water, 0.01 mmol phosphoric acid, 0.0025 mmol TBAP pH 7.0

FIGURE 2.9

STANDARD SALICYLATE AND BENZOATE PEAKS BY HPLC



The salicylate peaks from 11 foods were collected and Mr M. Smythe analysed them by GLC-MS in the Mass Spectrometry Unit of the School of Chemistry at Sydney University. All the peaks from the HPLC were found to contain a significant amount of salicylate.

TABLE 2.4

FOOD SALICYLATE ANALYSIS BY HPLC AND THEN GLC-MS

Foods Analysed by GLC-MS	Salicylate Present
Sweetcorn	yes
Radish	yes
Red currants	yes
Dates	yes
Curry	yes
Cumin	yes
Rosemary	yes
Capillano honey	yes
Barratts licorice	yes
Red wine	yes
Darjeeling tea	yes

Extraction Reproducibility

Multiple extractions of several foods with low, medium and high levels of salicylate were carried out to validate the results obtained. The mean salicylate content of each food was calculated along with the standard deviation and the standard error.

TABLE 2.5

MULTIPLE EXTRACTIONS OF FOODS

Food	No of Extractions	Mean	S.D.	S.E	95% Confidence Interval
Potato	20	0	0	0	(0, 0)
Pumpkin	36	0.12	0.016	0.113	(0.115, 0.125)
Carrots	40	0.23	0.045	0.007	(0.216, 0.244)
Pineapple	28	2.10	0.246	0.047	(2.009, 2.191)
Orange	35	2.39	0.502	0.085	(2.224, 2.556)
Thyme	22	183	45.205	9.638	(164.11, 201.89)

Total Salicylates in Food

It was found that most fruits contained considerable amounts of salicylate. Most berry fruits are significant sources of salicylate, with a range from 0.76mg/100g for mulberries to 4.4mg/100g for raspberries. Dried fruits have relatively high salicylate contents compared with their fresh counterparts because of the water removal during the drying process. Apples showed considerable variation of salicylate content between varieties. The salicylate content of citrus fruits ranged widely from 0.18mg/100g to 2.39mg/100g. Those fruits low in salicylate often have a less piquant flavour. Some

herbs and spices were found to contain very high amounts per 100g, e.g. curry powder, paprika, thyme, garam masala and rosemary. Within the beverage group, salicylate content varied widely among the teas and coffees. Teas are an important source of salicylate in the usual diet and so 18 different brands and varieties were analysed. All contained more than 1.9mg/100ml except decaffeinated tea, which contained only 0.37mg. Salicylate is soluble in methylene chloride, a solvent commonly used for extraction of caffeine. Table 2.6 includes data for nine coffees. All contain less than 0.96 mg salicylate per 100ml. Licorice and peppermint candies and some honeys contain salicylates. Cereals, meat, fish and dairy products contain none or negligible amounts.

TABLE 2.6

TOTAL SALICYLATES IN FOODS

Food	Type	State*	Salicylate mg/100g (this study)	References
Fruit				
Apple	Golden delicious	f	0.08	
	Red delicious	f	0.19	65(.004)
	Granny smith	f	0.59	
	Jonathan	f	0.38	
	Ardmona	c	0.55#	65(.004)
	Mountain Maid	j	0.19#	94(<.01),10
Apricot		f	2.58	65(.003) 94(<.01)
	Ardmona	c	1.42#	
	Letona	n	0.14#	94(<.01)
Avocado		f	0.60#	
Banana		f	0#	65(.005),64
Blackberry	John West	c	1.86	54,57

Continued

Food	Type	State*	Salicylate mg/100g (this study)	References
Blueberry	Socomin	c	2.76	42,85
Boysenberry	John West	c	2.04	
Cantelope	Rockmelon	f	1.50#	
Cherry	sweet	f	0.85	94(<.01),34 81,93
	John West	c	2.78	
	Morello Sour	c	0.30	93
Cranberry	S & W	c	1.64	4,5
		s	1.44	
Currants	blackcurrant	fr	3.06	65(.0005),1 2,3,57,93
	red currant	fr	5.06	93
Custard apple		f	0.21	
Dates		f	3.73	
	Cal-Date	d	4.50#	
Figs		f	0.18	94(.019)
	S & W Kadota	c	0.25	
	Calamata string	d	0.64	
Guava	Gold Reef	c	2.02	
Grapes	Red Malaita	f	0.94	10,59,73
	Sultana	f	1.88	57
	S & W light seedless	c	0.16	
	Berri Dark	j	0.88	66(.004)
	Sanitarium Light	j	0.18	
	currants I.P.C	d	5.80	10,18,57,59
	raisins A.D.F.A	d	6.62#	94(.046)
	sultana	d	7.80	
Grapefruit		f	0.68#	65(.01) 83,8(-)
	Berri	j	0.42	
Kiwi fruit		f	0.32	65(.002),92
Lemon		f	0.18#	19,80,83, 8(-)
Loganberry	John West	c	4.40	
Loquat		f	0.26	
Lychee		c	0.30	
Mandarin		f	0.56#	94(<.01)
Mango		f	0.11	
Mulberry		f	0.76	
Nectarine		f	0.49	65(.004)
Orange		f	2.39#	65(.007),36 83,8(-)
	Berri	j	0.18#	36(.003),19 94(<.01),36

Continued

Food	Type	State*	Salicylate mg/100g (this study)	References
Passionfruit		f	0.14#	56,99
Pawpaw		f	0.08#	32
Peach		f	0.58#	65(.003),10 12,26,49
	Letona	c	0.68#	65(.005) 94(.08)
	Letona	n	0.10#	94(.046)
Pear	Packham (with skin)	f	0.27#	
	Packham (no skin)	f	0#	
	William (with skin)	f	0.31#	
	Letona Bartlett	c	0#	65(.001) 94(<.01)
Persimmon		f	0.18#	
Pineapple		f	2.10#	94(<.01),36
	Golden Circle	c	1.36	65(.006)
	Golden Circle	j	0.16#	36(.008) 94(<.01)
Plum	Blood (red)	f	0.21	
	Kelsey (green)	f	0.095	65(.003),81
	Wilson (red)	f	0.11	
	S.P.C.dark red	c	1.16	
	Letona prunes	c	6.87	65(.034) 94(.061)
Pomegranate		f	0.07	
Raspberries		f	5.14	65(.003),10 54,57,59,67
		fr	3.88	
Rhubarb		f	0.13	
Strawberry		f	1.36#	65(.004),10 18,54,55,57, 59,74,84
Tamarillo		f	0.10	65(.004)
Tangelo		f	0.72	8(-)
Watermelon		f	0.48#	65(.007)
Youngberry		c	3.06	
Vegetables				
Alfalfa		f	0.70	16,17
Asparagus		f	0.14#	94(<.01)

* key to abbreviations page

Continued

Food	Type	State*	Salicylate mg/100g (this study)	References
Asparagus	Triangle Spears	c	0.32#	
Bamboo shoots	Sunshine	c	0	
Beans	blackeye	d	0	
	Borlotti	d	0.08	
	broad,vicia faba	f	0.73	62
	brown	d	0.002	
	green French	f	0.11#	94(.015) 65(.008)
	lima	d	0	94(<.01)
	mung	d	0	
	soya	d	0	6,51,52,75
	soya grits	d	0	
Beansprouts		f	0.06	
Beetroot		f	0.18#	94(<.01),82
	Golden Circle	c	0.32#	65(.007)
Broccoli		f	0.65#	
Brussel sprouts		f	0.07	
Cabbage	green	f	0#	66(.001) 94(<.01),70
	red	f	0.08#	
Carrots		f	0.23#	94(.035),82
Cauliflower		f	0.16#	65(.007)
Celery		f	0#	82
Chicory		f	1.02	72(-)
Chives		f	0.03	72(-)
Choko	(Chayote)	f	0.01#	
Cucumber	(no peel)	f	0.78#	71
	Aristocrat gherkin	c	6.14#	71
Eggplant	(with peel)	f	0.88#	71
	(no peel)	f	0.30#	
Endive		f	1.90	72(-)
Horseradish	Eskal	c	0.18	70
Leek		f	0.08	
Lentil	brown	d	0	
	red	d	0	
Lettuce		f	0#	72(-)
Marrow	(Cucurbita pepo)	f	0.17	
Mushroom		f	0.24	
	Champignon	c	1.26	
Okra	Zanae	c	0.59	
Olive	black Kraft	c	0.34#	29
	green Kraft	c	1.29#	29
Onion		f	0.16#	72(-)
Parsnip		f	0.45	

Continued

Food	Type	State*	Salicylate mg/100g (this study)	References
Peas	chickpea	d	0	
	green	f	0.04#	65(.002),71 94(<.01)
	green split pea	d	0	
	yellow split pea	d	0.02	
Peppers	green chilli	f	0.64#	
	red chilli	f	1.20#	
	yellow-green chilli	f	0.62#	
	sweet, green (capiscum)	f	1.20#	65(.004),13 71
Pimiento	Arson sweet red	c	0.15	
Potato	white (with peel)	f	0.12#	65(.006),14 23
	white (no peel)	f	0#	
Pumpkin		f	0.12#	94(<.01),71
Radish	red,small	f	1.24#	82
Shallots		f	0.03	
Spinach		f	0.58	94(.01),72
		fr	0.16#	
Squash	baby	f	0.63	
Swede		f	0	
Sweet corn		f	0.13#	65(.01)
	Mountain Maid niblets	c	0.26#	65(.073)
	Mountain Maid creamed	c	0.39#	65(.082) 94(.03)
Sweet potato	white	f	0.50#	65(.004)
	yellow	f	0.48#	65(.004)
Tomato		f	0.13#	65(.005),15 94(.01),21, 38,44,63,71
	Letona	c	0.53#	94(.014)
	Goulburn Valley	j	0.10#	36(.005-.02)
	Heinz	j	0.12#	94(.016),21
	Letona	j	0.18#	
	Campbell	p	0.57#	65(.007),21
	Leggo	p	1.44#	
	Tom Piper	p	0.43#	
	Heinz	sp	0.54#	65(.008)
	Kiora	sp	0.54#	36(.003-.05)
	P.M.U.	sp	0.32#	
	Fountain	sc	0.94#	36(.19-.24)
	Heinz	sc	2.48#	65(.005)
	I.X.L.	sc	1.06#	94(<.01-.03)
	P.M.U.	sc	0.98#	24,36
	Rosella	sc	2.15#	

Continued

Food	Type	State*	Salicylate mg/100g (this study)	References
Turnip		f	0.16#	
Watercress		f	0.84#	
Zucchini		f	1.04#	71(-)

* key to abbreviations page

Condiments

Allspice	powder	d	5.20	
Aniseed	powder	d	22.80	11,60,61
Bay leaf	leaves	d	2.52	
Basil	powder	d	3.40	
'Bonox'		l	0.28	
Canella	powder	d	42.60	
Cardmon	powder	d	7.70	
Caraway	powder	d	2.82	28
Cayenne	powder	d	17.60	
Celery	powder	d	10.10	
Chili	flakes	d	1.38	
	powder	d	1.30	
Cinnamon	powder	d	15.20#	10,27,41,59
Cloves	whole	d	5.74	25,28
Coriander	leaves	f	0.20	
Cumin	powder	d	45.00	
Curry	powder	d	218.00	
Dill		f	6.90	
	powder	d	94.40	
Fennel	powder	d	0.80	
Fenugreek	powder	d	12.20	
Five spice	powder	d	30.80	
Garam masala	powder	d	66.80	
Garlic	bulbs	f	0.10#	65(.008),72
Ginger	root	f	4.50	
Mace	powder	d	32.20	
'Marmite'	Sanitarium	p	0.71#	
Mint	common garden	f	9.40#	
Mixed herbs	leaves	d	55.60	
Mustard	powder	d	26.00	
Nutmeg	powder	d	2.40#	60
Oregano	powder	d	66.00	
Paprika	hot powder	d	203.00	
	sweet powder	d	5.70	
Parsley	leaves	f	0.08#	72,82
Pepper	black powder	d	6.20#	
	white powder	d	1.10#	

Continued

Food	Type	State*	Salicylate mg/100g (this study)	References
Pimiento	powder	d	4.90	
Rosemary	powder	d	68.00	
Saffron	powder	d	0	
Sage	leaves	d	21.70	
Soy sauce		l	0	
Tabasco Pepper	McIlhenny	sc	0.45	39
Tandori	powder	d	0	
Tarragon	powder	d	34.80	
Turmeric	powder	d	76.40	
Thyme	leaves	d	183.00	
Vanilla	essence	l	1.44	
Vinegar	malt	l	0	
	white	l	1.33	
Worcestershire	sauce	l	64.30	
'Vegemite'	Kraft	p	0.81#	
* key to abbreviations page				
Drinks				
'Aktavite'		pw	0	
Cereal coffee	Bambu	pw	0.15	
	Dandelion	pw	0.08	
	'Ecco'	pw	0	
	'Natures Cuppa'	pw	2.26	
	'Reform'	pw	0.38	
Coca-Cola		l	0.25	
Coffee	Andronicus Instant	pw	0	10,59,86
	Bushells Instant	pw	0.21	76(-)
	Bushells Turkish	pw	0.19	
	Gibsons Instant	pw	0.12	
	Harris Mocha Kenya	b	0.45	
	Harris Instant I	pw	0	
	Harris Instant II	pw	0.10	
	International Roast	pw	0.96	
	Maxwell House Inst	pw	0.84	
	Moccona Instant	pw	0.64	
	Moccona Decaf	pw	0	
	Nescafe Instant	pw	0.59	
	Nescafe Decaffeinated		0	
	Pablo instant	pw	0	
	Robert Timms Inst	pw	0.16	

Continued

Food	Type	State*	Salicylate mg/100g (this study)	References
Herbal tea	camomille	b	0.06	
	fruit	b	0.36	
	peppermint	b	1.10	
	rose hip	b	0.40	
'Milo'		pw	0.01	
'Ovaltine'		pw	0	
Rose hip syrup	Delrosa	l	1.17	
Tea	Asco	b	6.40	22,30,35,37
	Billy	le	2.48	40,47,53,58
	Burmese Green	le	2.97	68,69,79,87
	Bushells	b	4.78	89,90,91,95
	Golden Days Decaf	b	0.37	102,103,104
	Harris	b	4.00	
	Indian Green	le	2.97	
	Peony Jasmine	le	1.90	
	Old Chinese	le	1.90	
	Tetley	b	5.57	
	Twinings:			
	Earl Grey	b	3.00	
	English Breakfast	b	3.00	
	Darjeeling	l	4.24	
	Irish Breakfast	b	3.89	
	Lapsang Souchong	b	2.40	
	Lemon Scented	b	7.34	
	Orange Pekoe	l	2.75	
	Prince of Wales	b	2.97	

* key to abbreviations page

<u>Cereals</u>

Arrowroot	powder	d	0	
Barley	unpearled	d	0	78
Buckwheat	grains	d	0	100
Maize	meal	d	0.43	
Millet	grains	d	0	
	hulled grains	d	0	
Oats	meal	d	0	
Rice	brown grains	d	0#	46
	white grains	d	0#	
Rye	rolled	d	0	
Wheat	grains	d	0	52,67,76(-)

* key to abbreviations page

Continued

Food	Type	State*	Salicylate mg/100g (this study)	References
Nuts and Seeds				
Almonds		f	3.00	8
Brazil nuts		f	0.46	
Cashew nuts		f	0.07	
Coconut	dessicated	d	0.26	
Hazelnuts		f	0.14	
Macadamia nuts		f	0.52	
Peanuts	unshelled	f	1.12	51,52
	Sanitarium butter	p	0.23	
Pecan nuts		f	0.12	
Pine nuts		f	0.51	
Pistachio nuts		f	0.55	
Poppyseed		d	0	
Sesame seed		d	0.23	
Sunflower seed		d	0.12	
Walnuts		f	0.30	
Water chestnut	Socomin	c	2.92	
Sugars				
Carob	powder	d	0#	
Cocoa	powder	d	0#	
Honey	Allowrie	l	2.50	
	Aristocrat	l	3.70	
	Capillano	l	10.14	
	Mudgee	l	3.90	
	'No Frills'	l	11.24	
Golden syrup	C.S.R.	l	0.10#	
Maple syrup	Camp	l	0	
Sugar	white granulated	d	0	
Molasses	C.S.R.	l	0.22	58
Confectionery				
Caramel	Pascall Cream	d	0.12	
Licorice	Barratts	d	9.78	
	Giant	d	7.96	
Peppermints	Allens Strong Mint	d	0.77	60
	Allens 'Koolmint'	d	7.58	
	Lifesavers	d	0.86	
	'Minties'	d	1.78	
	Allens 'Steamrollers	d	2.92	

* key to abbreviations page

* key to abbreviations page

Continued

Food	Type	State*	Salicylate mg/100g (this study)	References
Dairy				
Cheese	blue vein	f	0.05	
	Camembert	f	0.01	
	Cheddar	f	0	
	cottage	f	0	
	Mozarella	f	0.02	
	tasty cheddar	f	0	
Milk	fresh fullcream	l	0#	31
Yogurt	fullcream	f	0	
Meat, Fish and Eggs				
Beef		f	0#	96
Chicken		f	0#	
Egg	white	f	0	
	yolk	f	0	
Kidney		f	0	96
Lamb		f	0#	
Liver		f	0.05	
Oyster		f	0	
Pork		f	0	
Prawn		f	0.04	
Salmon	Lunchtime Pink	c	0	
Scallop		f	0.02	
Tripe		f	0	
Tuna	Seakist	c	0	
Alcoholic Drinks				
Beer	Reschs Dinner Ale		0.35	45
	Tooheys Draught		0.23	
	Tooths Sheaf Stout		0.32	
Cider	Bulmer's Dry		0.17	
	Bulmer's Sweet		0.19	
	Lilydale Dry		0.17	
	Mercury Dry		0.16	

* key to abbreviations page

* key to abbreviations page

Continued

Food	Type	State*	Salicylate mg/100g (this study)	References
Liquers	Benedictine		9.04	
	Cointreau		0.66	
	Drambuie		1.68	
	Tia Maria		0.83	
Port	McWilliams Royal Reserve		1.40	
	Stonyfell Mellow		4.20	
Sherry	Lindemans Royal Reserve sweet		0.56	
	Mildara Supreme Dry		0.46	
	Penfolds Royal Reserve sweet		0.49	
Spirits	brandy - Hennessy		0.40	
	gin - Gilbey's		0	
	rum - Bundaberg		0.76	49
	rum - Captain Morgan		1.28	
	vodka - Smirnoff		0	
	whiskey - Johnnie Walker		0	45
Wines	Buton Dry Vermouth		0.46	7,20,33,45
	Kaiser Stuhl Rose		0.37	97,98
	Lindemans Riesling		0.81	
	McWilliams Dry White Wine		0.10	
	McWilliams Cabernet Sauvignon		0.86	
	McWilliams Private Bin Claret		0.90	
	McWilliams Reserve Claret		0.35	
	Penfolds Traminer Riesling Bin 202		0.81	
	Seaview Rhine Riesling		0.89	
	Stonyfell Ma Chere		0.69	
	Yalumba Champagne		1.02	

* key to abbreviations page

Legend for Table 6.2 Total Salicylates in Food

* b = bag, c = canned, d = dried, f = fresh, fr = frozen, j = juice, l = liquid, le = leaves, n = nectar, p = paste, pw = powder, sc = sauce, sp = soup.

Most trade names are those of products of various Australian companies.Some varieties of foods also are Australian.

For coffee, milligrams salicylate per 100 ml made from 2 gm powder in 100 ml water.For tea, milligrams salicylate per 100 ml infusion made from two standard tea bags (4 gm dry leaves).

* Edible portions
# Multiple extractions

Published studies on the salicylate content of food are listed numerically. Values obtained when given are recorded in brackets in mg/100g and when no salicylate was (-).

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Foods not analysed in this study but analysed in the literature

Chestnut	101	Malt	76(-)
Chokeberry	43	Muskmelon	48
Cloudberry	9	Sapodilla fruit	50
Cottonseed oil	51	Spearmint	60
Gooseberry	85		

Peel of Fruits and Vegetables

The values of salicylate in foods varied if the peel was included in the analyses. The peel was found to be a concentrated source of salicylate in foods that were extracted both with and without their peel (Table 2.7). In addition gherkin was shown to have a high salicylate content compared with peeled cucumber. This was due in part to the salicylate content of the skin as well as the salicylate present in the vinegar and spices used in pickling. This finding proved important in the clinical context where patients with RIU/AO were eating pears as the only fruit allowed on the elimination diet (Chapter 3). Such patients often consumed pears in considerable quantities, and those who were salicylate sensitive often did not remit completely until they were instructed to eat only peeled pears.

TABLE 2.7

EFFECT OF PEEL ON THE SALICYLATE CONTENT OF FOODS[1]

Foods	Food with Peel	Food with no Peel
Granny smith apple	0.59	0.55
Cucumber	-	0.78
Potato	0.12	0
Pear	0.27	0
Peanuts	1.12	0.23

1. Salicylate concentrations are expressed as mg/100g.

Comparison of Fresh, Canned and Juiced Foods

Fruit juices contain only the liquid fraction of fruit, discarding the skin and flesh, resulting in low levels for the juice compared with the fruit flesh (Table 2.8). However, this table was compiled from fruits and juices which were derived from many sources which means that the results expressed are only an indication of the variation which can occur depending on the source and variety of fruit examined. From the table, fruit juice has a lower salicylate content than its fresh fruit counterpart. However, this is misleading in a clinical context because fruit juice is usually consumed more often and in larger amounts than fresh fruit. Consequently, salicylate sensitive patients have found that consumption of both fruit and fruit juice must be controlled in order to remain asymptomatic.

TABLE 2.8

COMPARISON OF FRESH, CANNED AND JUICED FOODS[1]

Food	Juice[2]	Fresh	Canned[2]
Apple	0.19	0.59	0.55
Orange	0.18	2.39	-
White grape	0.18	1.88	0.16
Red grape	0.88	0.94	-
Pineapple	0.16	2.10	1.36
Apricot	0.14	2.58	1.42
Tomato	0.18	0.13	0.53

1. Salicylate concentrations are expressed as mg/100g.
2. For source of canned and juice see Table 2.6 for brands.

Concentration of Salicylate in Tomato Products

Concentration of the food with the loss of water led to an increase in salicylate content. Several tomato products from different sources were analysed giving a range of values (Table 2.9). However, the results showed that concentration of a food with the loss of water and the further concentration of flavours with the addition of herbs and spices led to a marked increase in salicylate content. In sens- itive patients this concentration of foods can mean that very small amounts of a food item can lead to a marked reaction.

TABLE 2.9

CONCENTRATION OF SALICYLATE IN TOMATO PRODUCTS[1]

Tomato Product[2]	Salicylate mg/100g
Tomato sauce	0.94 - 2.48
Tomato paste	0.43 - 1.44
Tomato soup	0.32 - 0.54
Tinned tomato	0.53
Tomato juice	0.10 - 0.18
Fresh tomato	0.13

1. Salicylate concentrations are expressed as mg/100g.
2. For source of tomato products see Table 2.6 for brands.

DISCUSSION

Extraction and Analysis

Salicylates were first extracted from the bark of the Willow tree (Salix) in the 1820's, although the medicinal properties of this and other plants were known in Hippocratic times (Reviewed in Gross & Greenberg, 1948). Advances in organic chemistry during the 19th century enabled the French pharmacist Leroux in 1829 to isolate in the pure form salicin, the active ingredient in Willow bark (Leroux, 1830). In 1831, salicylaldehyde was distilled from the flowers of Spirea Ulmaria by the Swiss pharmacist, Pagenstecher. He subsequently transmitted this information to the German chemist, Lowig, who in 1835 produced salicylic acid by oxidation of salicylaldehyde (Tschirch, 1917). In 1838, Piria also obtained salicylic acid by hydrolysis and oxidation of salicin. In the 1840's and 1850's, following the isolation of methylsalicylate from oil of wintergreen, salicylic acid was prepared by the action of phosphorous perchloride on methylsalicylate by the French chemist Cahours and the Scottish chemist Cooper.

In the late 19th and early 20th centuries salicin was indentified in Poplar and Willow trees and in the Black Law (Squibb, 1875; Beck, 1891; Jowett & Potter, 1902; Sajous & Sajous, 1916-1932; Ghosh, 1925; Lortat-Jacob, 1925; Evans et al., 1945; Iwamoto et al., 1945). Methylsalicylate glycosides were found in Birch and Beech trees as well as in Gaultheria procumbers, partridge berry, chequerberry, wintergreen, Gaultheria hispidula, wild pansy, milk wort, bay tree, Indian licorice, soap berry, olive, madder, jasmine,

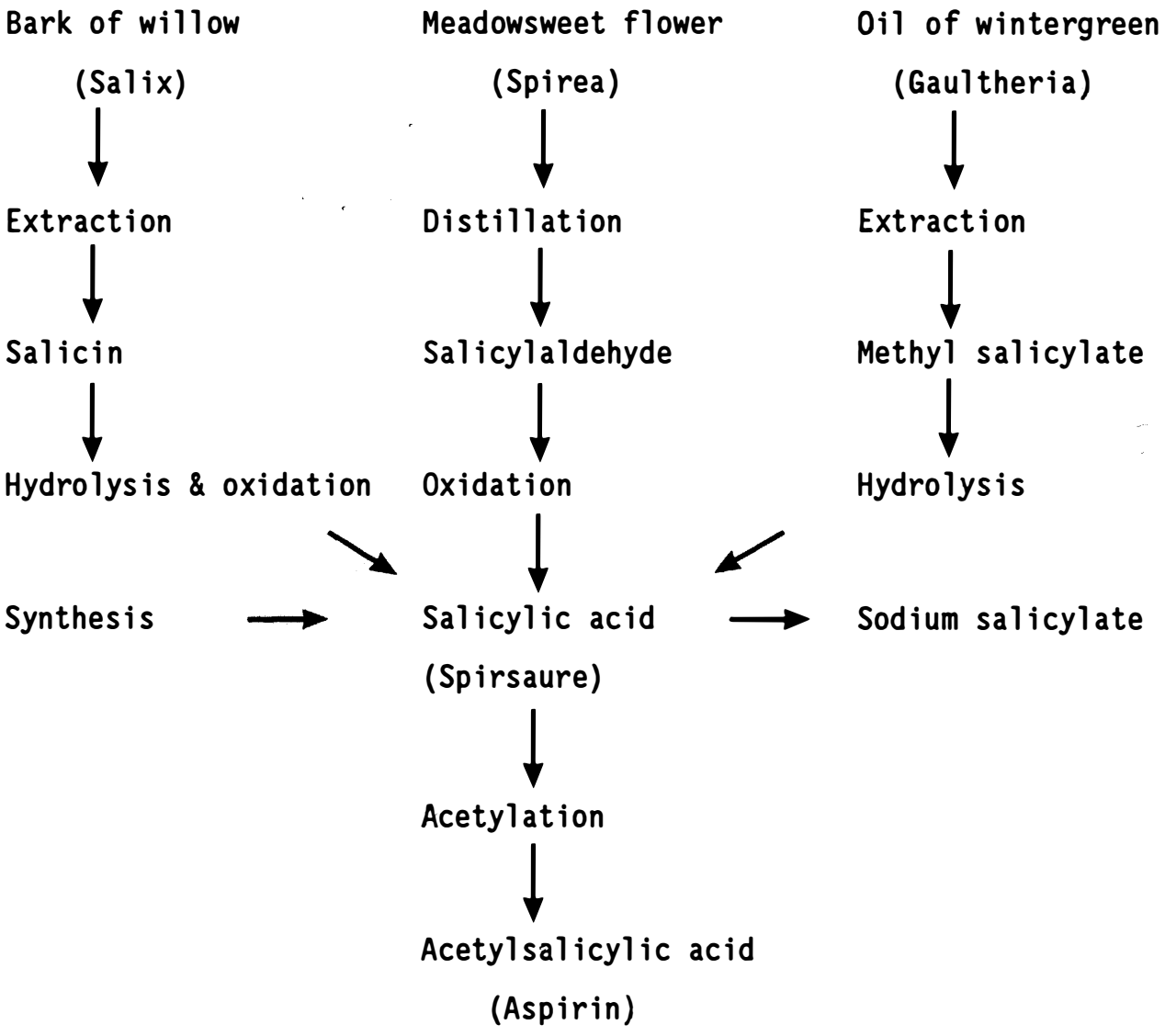
myrtle, linden, buckthorn, various grasses, coffee, and in the following botanical orders: Leguminosae, Euphorbiaceae, Bixineae, Cupuliferae and Erythrixyleae (Longmore, 1798; Bigelow, 1818; Proctor, 1842-1843, 1843, 1844; Cahours, 1843; Anon., 1882; Anon., 1888; Stokvis, 1895, Prebble, 1894; Editorial, 1898; Walters, 1899; Lortat Jacob, 1925; Tailleur, 1902; Cone, 1903; Winters, 1904, 1907; Pancoust & Pearson, 1909; Hilpert, 1913; Self, 1915; Sajous & Sajous, 1916-1932; La Wall, 1920; Ghosh, 1925; Autenreith; 1928; Perutz, 1930; Arny & Fischelis; 1937; Morel, 1951; Onishi & Yamamoto, 1955, 1956; Ibrahim & Towers, 1960; Tomaszewski, 1960; Ibrahim, 1964; Pridham & Young, 1964; Pearl & Darling, 1964, 1965; Julkunen-Tiitto, 1985; Gaydou et al., 1986). In addition, they have been found in the woodruff, marigold, hyacinth, tulip, yucca, clover, yellow bird's nest, meadowsweet, ipecacuanha, mignonette, ammoniac plant, Americeae family and pansy (Bigelow, 1818; Buchner, 1854; Wicke, 1854; Squibb, 1875; Anon, 1888; Griffiths, 1889; Binz, 1897; Desmouliere, 1904; Lortat-Jacob, 1925; Perutz, 1930; Schlimme, 1943; Wood & Osol, 1943).

Up to the early 1950's the standard method for identification of salicylates were colorimetric (Gross & Greenberg, 1948; Rainsford, 1984). With the development of TLC (Izmailov & Schraiber, 1938; Stahl et al., 1956; Stahl, 1958, 1969) and GLC (James & Martin, 1952) it became possible to develop more sensitive methods of detection, and this was the initial approach adopted in the present study. Quantification was attempted by densitometry, but this proved unreliable since the readings often did not reflect the density of

spots as estimated by visual inspection. HPLC (Johnson & Stevenson, 1977), which was first introduced for analysis of salicylates in tissues and body fluids in the late 1960's (Peng et al., 1978) proved to be a much more reliable and quantitative method for analysis of food extracts in the present study.

FIGURE 2.10

HISTORICAL EXTRACTION OF SALICYLATE FROM PLANTS (COLLIER, 1963)



Extraction methods have changed little since the earliest attempts by European chemists in the late 19th and early 20th centuries (Gross & Greenberg, 1948; Rainsford, 1984). When the present study commenced the standard method, as outlined in the Association of Analytical Chemists Handbook (Horwitz, 1975), was to shake the homogenized material in a separating funnel so as to transfer salicylates from the aqueous to the organic solvent phase. It was soon found that this method was rather inefficient, requiring large quantities of food and solvent, and forming emulsions which were difficult to separate. A more efficient method was therefore developed, where the homogenized food was first hydrolysed in 25% NaOH overnight, followed by acidification to pH<2.0. This resulted in extensive plant tissue breakdown, with all bound salicylate esters being freed from the cell structure (Harbourne, 1980; Newby et al., 1980). The food sample was then extracted with liquid extractors over 5 hours which avoided the formation of emulsions and allowed for more complete mixing of food and solvent than in previous methods published leading to a more efficient extraction. The results obtained by this method yielded considerably higher salicylate concentrations than those reported in the literature, i.e about 10 times higher for pineapple juice but more than 100 times higher for pineapples (Table 2.6). This can be explained partly by the fact that earlier workers did not employ such stringent techniques as the efficient liquid extraction procedure, but more likely the higher levels were due to the conversion of conjugated salicylates to free salicylic acid so that the total salicylate in the foods was measured (Harbourne, 1980). Total benzoates were successfully extracted along with total salicylates (Table 2.6). but as they were eluted close to the solvent front they

could not be accurately quantified. (Estimates in some foods gave values for total benzoic acid of 100 times the amount of total salicylates).

Distibution

Salicylates in food were first reported in Lancet (Anon., 1903). They were identified in small amounts in the common fruit strawberry. Subsequently, Desmouliere (1904), Paul (1917), Dodge (1918) and Perutz (1930) also found salicylates in apple, anise, cherry, grape, cinnamon, nutmeg, orange, peppermint, plum, raspberry, spearmint, and since then there have been at least 100 reports of their presence in a variety of fruits and vegetables, aniseed and almonds (Table 2.6). No previous study has, however, systematically analysed the salicylate content of all commonly eaten foods, and since this information was found to be essential for the dietary management of patients with RIU/AO such an analysis was undertaken by the author. In all, 333 foods were extracted and analysed, and significant salicylate concentrations were found in a wide variety of fruits and vegetables, herbs and spices, nuts, beverages, and miscellaneous plant-derived foods.

(a) Fruits and Vegetables

A range of salicylate values in fruits and vegetables was found to be dependent on the species of the plant, ageing/ripening of the fruits or vegetables and extensive processing (cooking, canning, freezing). For example, several varieties of apples exhibit a range of salicylate contents with golden delicious containing 0.08mg/100g compared with red delicious with 0.19mg/100g and granny smiths having a value of 0.59mg/100g. Those varieties which gave a less piquant flavour were found to be often lower in salicylate content.

Similarly, the salicylate content of fruits and vegetables was found to vary with ripening as this process leads to a change in the chemical composition and therefore the flavour of fruit and vegetables.

Heating by conventional cooking methods does not have an appreciable effect on the content of available salicylate in food. However, commercial processing that results in the extensive breakdown of plant tissue can result in an increase of available salicylate, for example fresh corn compared with processed corn products (Table 2.10). This is why the severe treatment with sodium hydroxide and hydrochloric acid used in the extraction method affected the fibre structure of the fruits and vegetables leading to a release of bound salicylate.

Another physical characteristic which had an influence on the salicylate content in the sample we examined was the peel where the concentration of salicylate is higher than in the fruit or vegetable flesh e.g. potato, pear, eggplant, peanut, apple (Table 2.7). Since the peel is the physical barrier between the outside environment and the flesh and seed of all fruits and vegetables it may be that the higher salicylate content is acting in a protective capacity. At the beginning of this century it was known that salicylic acid was a very effective antimicrobial agent and, indeed, it was used as a preservative in Europe until reports of adverse reactions led to its prohibition in the 1950's (Lueck, 1980).

When fruits or vegetables are crushed for juice the peel and fibre content are discarded which results in a lowering of the salicylate content supporting the notion that salicylate is bound in the fruit or vegetable fibre which is discarded during processing (Table 2.8). An average of four pieces of fruit is needed to make one glass of fruit juice showing that considerable amounts of salicylate are discarded in the pulp after fruit juice manufacture. However, the ease of ingestion of vast quantities of fruit juice compared with pieces of fresh fruit often means individuals still consume a large quantity of salicylate in the form of juice.

Concentration of salicylate in foods can be seen with the range of tomato products analysed. Tomato sauce, soup, paste exhibit high levels of salicylate compared with the fresh tomato (Table 2.9). This is due to their concentration during processing and the addition of spices which even though their use is in small amounts they can still make a significant contribution to dietary salicylate.

(b) Beverages

Beverages like tea, coffee and their herbal or cereal counterparts are all high sources of salicylate, being derived from plant matter. Cereal coffees are based on chicory and beetroot, while herbal teas rely for flavour on various herbs and natural flavours like peppermint for their considerable salicylate content.

Methylene dichloride is a solvent that was commonly used in the decaffeination of coffees and teas. Both caffeine and salicylate are readily soluble in it. Consequently decaffeinated tea contains markedly reduced salicylate compared with tea and decaffeinated coffee is completely devoid of salicylate.

Alcoholic beverages exhibit a range of salicylate content depending on the raw products used in their manufacture and their processing. Fermentation does not significantly increase the salicylate content of beverages with grape juice being comparable to wine and apple juice comparable to cider in salicylate content. Spirits like vodka, whiskey and gin are very poor sources of salicylate because their raw ingredients are low and distillation removes any salicylate present. Alternatively, liquers are very high sources of salicylate due to their raw ingredients e.g. Tia Maria (chocolate), Drambuie (herbs), Contreau (orange peel) and Benedictine (herbs).

(c) Protein Rich Foods (Meat, Poultry, Fish, Eggs, Dairy, Legumes)

Protein foods from both animal or vegetable origins were generally low sources of salicylate. All dried legumes analysed had less than 0.08mg/100g in the dry state. Beef, lamb, pork, chicken, oysters, fish and dairy products all had negligible salicylate content. Liver was found to contain 0.05mg/100g perhaps indicative of the animals' food intake from plant sources.

(d) Cereals

Salicylate levels in nine whole grain cereals were negligible, with the exception of a yellow maize meal which contributed 0.43mg/100g.

(e) Sweets

Little research had been done previously on the amount of salicylate in popular sweets and confectionery. However, Porsch et al. (1965) and others have identified salicylate in anise and mint. Therefore a representative number of licorices and peppermints were analysed along with some other popular sweets.

Values for caramels, cocoa and carob were negligible. The peppermints contained variable amounts. It would appear that high salicylate contents in mint candies come mostly from additional flavorings like methylsalicylate. Mint sweets may also be used in the manufacture of licorices, to which anise is added for flavour, a high source of salicylate.

TABLE 2.10

SALICYLATE CONTENT OF CORN PRODUCTS

Corn Product	Salicylate Content
Maize meal	0.43mg/100g
Fresh corn	0.13mg/100g
Canned niblets	0.26mg/100g
Canned creamed corn	0.39mg/100g

Conclusions

It was surprising to find, as a result of the analyses performed, that salicylates (including aspirin) are much more widely distributed than hitherto suspected. Significant levels were present in most fruits and vegetables, herbs and spices, and plant-derived foods and beverages, as well as in unexpected foods such as honey, Vegemite, confectionery and a variety of nuts (Table 2.6). In fruits and vegetables the concentration depended on the species (and in some cases

variety), ageing, ripening, processing and preparation. As shown by the TLC data (Figure 2.8) aspirin itself comprises a significant, if not major, proportion of the total salicylates present, although this is difficult to quantify precisely because of rapid break-down during extraction and analysis. Salicylates were absent from animal-derived foods such as fish, meats and dairy products, and from most grains. As will be discussed in Chapter 10, salicylates are products of secondary plant metabolism, and along with other benzoic acid derivatives are probably synthesized to a greater or lesser extent by most plants, including those commonly eaten as foods.

The salicylate levels measured in the present study are higher than those reported elsewhere, probably due to the more efficient extraction procedure and the use of HPLC. It was previously thought that the normal diet contained such small amounts of salicylate as to be clinically irrelevant (South, 1976, 1976, 1977, 1979, 1980; Samter, 1977), but calculations based on the levels measured here indicate that an average Australian diet contains of the order of 10 to 50 mg per day, and some individuals may consume up to 100 mg per day. Furthermore, salicylate-containing foods also appear to be rich sources of other benzoic acid derivatives, many of which cross-react with aspirin in sensitive individuals (Chapter 3 & Chapter 6).

CHAPTER 3

DIETARY INVESTIGATION OF RECURRENT IDIOPATHIC URTICARIA/ANGIOEDEMA

INTRODUCTION

Urticaria caused by nettles and insect bites was recognized in Hippocratic times, although its modern name was not coined until the late 18th century. It is not clear exactly when foods were first associated with attacks of RIU/AO, but angioedema caused by eggs was described by Donati in the 16th century (Schadeweldt, 1981), and Wilson, in the 18th century, was said to have sometimes traced its cause by "omitting first one and then another article of food" (Bateman, 1813). In the late 19th and early 20th century it was widely recognized that foods, as well as drugs, fevers, physical and emotional factors could all contribute to the aetiology of urticaria, and by the 1950's almost every author writing on the subject included a long list of foods known to precipitate symptoms (Warin & Champion, 1974). However, it was not until Lockey implicated the azo-dye tartrazine that more interest was taken in identifying the relevant food constituents (Lockey, 1959, 1969, 1971, 1977).

Aspirin as a drug was known to be capable of provoking urticaria and angioedema as early as 1902 (Hirschberg, 1902), and although salicylates were known to be present in various plants and fruits (Chapter 2) it took over 50 years before therapeutic diets were introduced with the idea of avoiding exposure to natural salicylates (Shelley, 1964; Feingold, 1968; Lockey; 1971; Noid et al., 1974; Olivier, 1974; Warin, 1976). As outlined in Chapter 1, these provided a starting point for the present study, but it was soon evident that knowledge of the distribution of natural salicylates was inadequate.

The results of the salicylate analyses described in Chapter 2 made it possible to identify many previously unrecognized sources of dietary salicylates, so that a more effective elimination diet could be developed.

This chapter describes the results of dietary investigation in 1,349 patients with RIU/AO, representing the largest series reported to date, using a standard elimination diet and blind challenge protocol which was developed for use on an outpatient basis. The stringency of salicylate exclusion, along with close contact between patient and dietitian, resulted in excellent compliance and a higher success rate than generally reported in the literature.

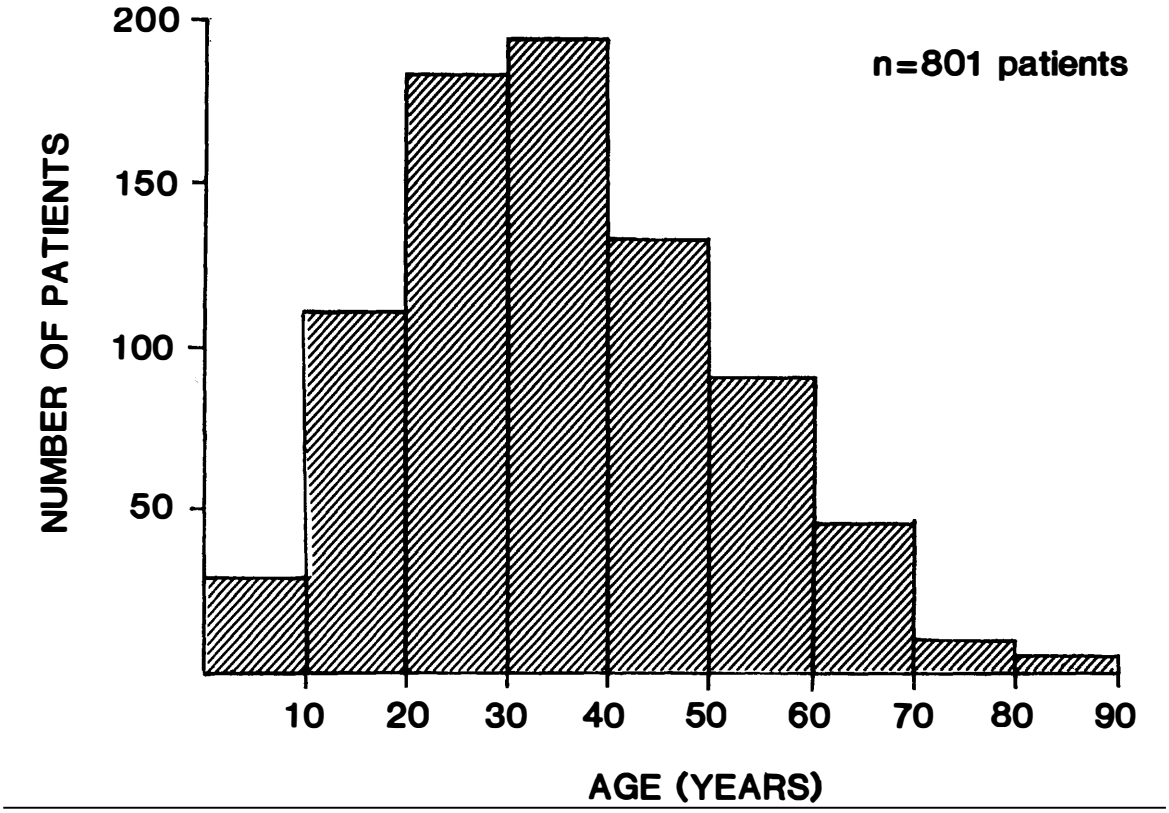
MATERIALS AND METHODS

Patients

Between April 1977 and September 1986, 1349 patients with RIU/AO presented to the Allergy Clinic at RPAH, referred generally by their family doctor or a dermatologist. Patients were aged between one and 86 years (Figure 3.1) with RIU/AO of six weeks to 60 years duration (Figure 3.2). Sixty two percent were female and 38% male; 43.7% presented with urticaria only, 17.6% with angioedema only, and 38.7% with both urticaria and angioedema. The patients with "physical" precipitants such as cold, sun exposure or pressure generally also experienced "idiopathic" attacks, and were investigated and managed along the same lines as other patients with RIU/AO.

FIGURE 3.1

HISTOGRAM OF THE AGES OF RIU/AO PATIENTS



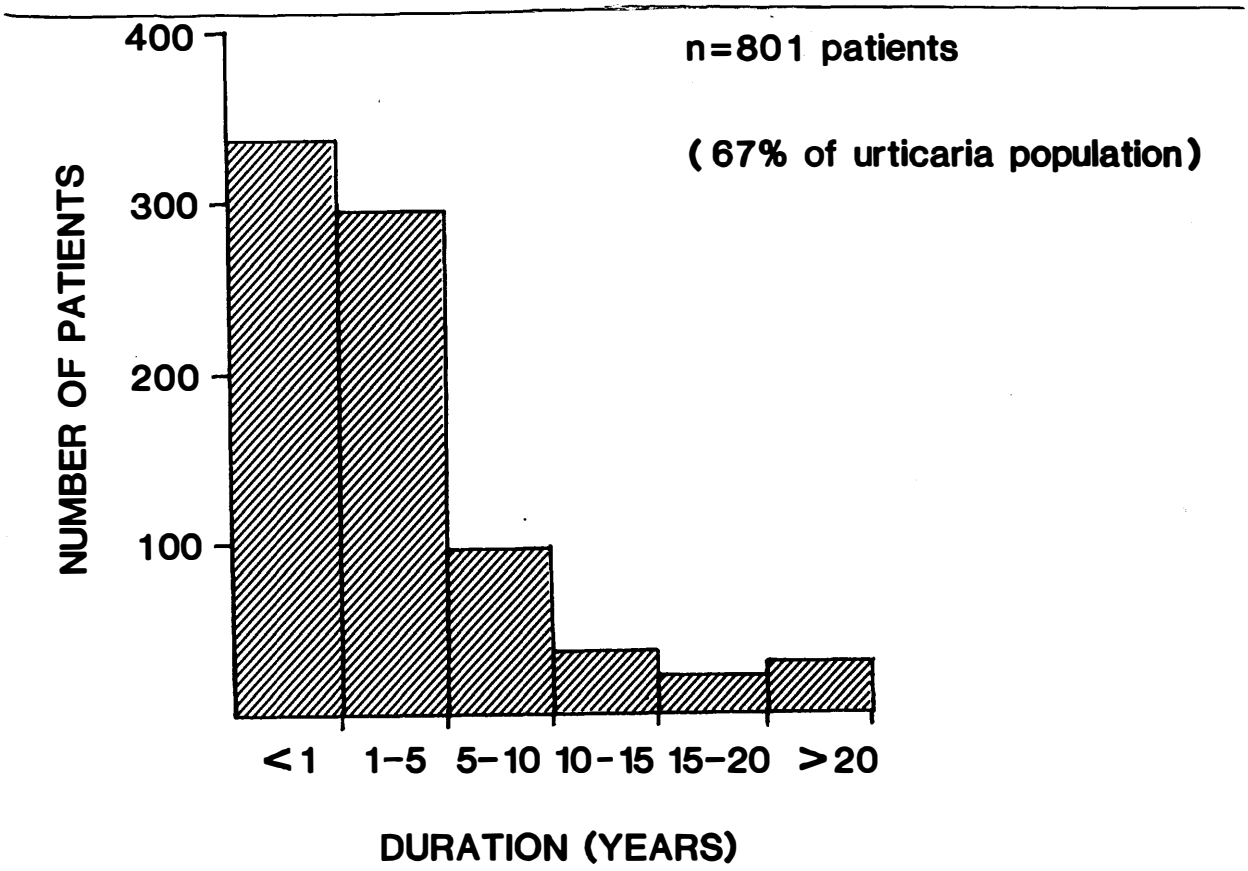
Clinical Evaluation

Each patient was evaluated by a physician to exclude underlying systemic disease before undergoing dietary investigation (Gibson & Clancy, 1980). Rare patients found to have urticarial vasculitis or C1-esterase inhibitor deficiency were managed with appropriate

medical therapy, and those with atypical symptoms were referred to the dermatologist attending the clinic for further evaluation. Individuals with a history of asthma underwent respiratory assessment before taking any challenges, which were administered under appropriate medical supervision (depending on their degree of bronchial hyper-reactivity). Patients with a history of laryngeal oedema and/or systemic anaphylaxis were routinely admitted to hospital for challenge.

FIGURE 3.2

HISTOGRAM OF THE DURATION OF RIU/AO



Derivation of the Elimination Diet

As outlined in Chapter 1, the elimination diet was developed to exclude food additives and natural salicylates in patients with RIU/AO. The Pure Food Act (1908, 1937) and ingredient labelling (National Health and Medical Research Council, [NH&MRC], 1986) were used to determine those foods containing artificial colourings and preservatives (Briggs & Wahlquist, 1985; Hanssen & Marsden, 1986). In the cases where labelling was limited, industry practice was ascertained. For example, pasta is generally coloured with carotene, a natural vegetable extract but some brands may be coloured with one or more permitted artificial colours. It soon became clear from clinical observations that industrial practice did not always correspond to the legal requirements, and when suspicion fell upon a particular product information was obtained from the Food Technologist concerned, or from Mr. Bill Porter at the NSW Health Commission.

In the early stages dairy products were excluded because of their possible contamination with penicillin, and bread was excluded because yeasts had been implicated as a cause of RIU/AO by Holti (1966 & 1967) and James and Warin (1971). By late 1980 clinical observations indicated the need for a revision of the elimination diet. Dairy products, eggs, and unpreserved bread rarely if ever proved to be a significant problem for patients once the correct precipitants were identified, and were therefore re-introduced into the baseline diet, making it much more palatable. At the beginning of 1982, and again in early 1983, laboratory analyses of food salicylate content (Chapter 2) resulted in further modifications of the diet as shown in Table 3.1.

TABLE 3.1

MODIFICATIONS OF THE ELIMINATION DIET

Foods	Time Periods *			
	I	II	III	IV
Pear skin	+	+	+	-
Pepper	+	+	+	-
Carrots	+	+	-	-
Honey	-	+	+	-
Coffee (certain brands)	+	+	-	-

+ = included; - = excluded

* Time periods:

I. April 1977 to January 1979.
II. January 1979 to January 1982.
III. January 1982 to January 1983.
IV. January 1983 to January 1986.

The elimination diet which finally developed was a useful tool in the management of patients with RIU/AO and contained adequate protein, fat and carbohydrate provided that the dietary recommendations were followed (NH&MRC, 1986). However, the level of ascorbic acid was particularly low because of the restricted range of fruits and vegetables so the use of an uncoloured, unflavoured multivitamin was advised (Myadec by Parke Davis, Multivit Six by Glaxo, Elevit RDI by Roche).

All acetylsalicylic acid-based medications (Dispirin, Alka-seltzer, Aspirin and compound analgesics) were prohibited. Most flavourings used in toothpastes, cough lozenges, flavoured medications and syrups contain salicylate and so suitable alternatives were prescribed. Products containing oil of wintergreen (Decorub, Deep Heat), a concentrated source of methyl salicylate, were forbidden as were some perfumes used to scent many toiletries because they contain amyl and benzyl salicylate (Morel, 1951; Bedoukian, 1981; Toda et al., 1983).

Medications which were allowed included uncoloured antihistamines and other uncoloured medications which did not contain acetylsalicylic acid, methylsalicylate or their analogues. With other drugs, if suitable white medications were not available and therapy could not be interrupted, patients were instructed to wash the artificial colouring off the surface by rubbing gently under running water. Capsules could be opened if necessary, and the powdered contents taken, discarding the coloured gelatine capsule.

Patient Instruction about the Elimination Diet

Following confirmation of a diagnosis of RIU/AO each patient was interviewed by the dietitian and a detailed food history taken. At this interview the elimination diet programme was discussed with both the patient and the person responsible for preparing meals. The author was responsible for the project and trained Gabrielle Boyd and Jenny McQueen to assist in the dietetic management of the RIU/AO patients. Gabrielle Boyd assisted from April 1983 to February 1984 and Jenny McQueen from April 1984 to September 1986.

Patients were given information about the food sources of natural salicylate (Swain et al., 1985), benzoate, brewers yeast and the artificial colours and preservatives (NH&MRC, 1986). This gave each patient a perspective as to the possible dietary precipitants of their symptoms of RIU/AO. The importance of compliance was stressed, emphasizing that only those foods listed were to be eaten, as dietary infractions could lead to recurrence of symptoms and false positive or confusing challenge results. Detailed instructions and explanations were given about which foods to eat and which foods to avoid, along with the reasons for each inclusion and exclusion of food on the diet. Patients were warned that their attention to detail was critical to the success of the programme.

Practical advice on shopping, food preparation and how to vary the diet was given, as well as a list of recipes for preparation of palatable meals. The management of restrictions to lifestyle were also dealt with. Detailed advice was given about lunches and snacks, takeaway food, eating out at restaurants, dinner parties and other social occasions (weddings, parties, sporting events) all of which provided practical information which was critical for patient compliance and confidence. At the same time patients were advised about vitamin supplements, medications, cosmetics and toiletries.

Frequent review of the patients' progress over the telephone was often necessary for encouragement during the early stages of the programme, clarification of dietary instructions, practical advice about day to day problems, and what to do about exacerbations of their symptoms. On average each patient would make contact with the author at least once per week over the time that they were on the elimination diet.

Patients who experienced marked reduction or complete relief of symptoms for five consecutive days after a minimium of two weeks on the elimination diet telephoned the dietitian for their challenge capsules (see below) which were sent by mail. Those with a history of laryngeal oedema, anaphylaxis or asthma were given their challenges in graded doses under appropriate medical supervision (Appendix 5).

If there was no significant improvement after two weeks, the diet was discussed over the telephone to ensure that the instructions had been understood, and to check compliance. Those patients who showed no improvement after six weeks on the elimination diet were asked to return for review by the physician. In some cases further restriction of wheat and milk products was successful, but in the majority a normal diet was resumed along with antihistamine therapy as required. Patients who could not comply with the elimination diet for social, family or other reasons did not undergo challenge, and were treated symptomatically.

Challenges

The battery of test substances comprised those compounds reported as major precipitants of RIU/AO by Warin and Smith (1976) and sodium metabisulphite which is widely used in drinks, liquid and moist consistency foods and some dried fruits (NH&MRC, 1986). Challenge sets were initially prepared by the Pharmacy Department at RPAH and later by the author Anne Ruth Swain (ARS). The test substances (Appendix 3) were supplied by Searle (Ajax chemicals) and BDH

(British Drug House chemicals) and encapsulated in clear gelatine capsules supplied by Parke Davis. Filled capsules were packed separately into numbered plastic vials (Melewish). Between April 1977 and January 1983 the challenge compounds were numbered in a standard sequence, and thereafter they were packaged in an arbitary order which varied from one set to the next. The challenge compounds used have an indefinite shelf-life when stored in a cool dry place and it was therefore possible to prepare them in batches of 200 sets at a time.

The challenge set was updated at intervals as shown in Table 3.2. The initial challenge set of compounds based on Warin and Smith (1976) was modified to exclude penicillin after it was found that this was not detectable in Australian dairy products and was therefore unlikely to be of clinical significance. The challenge battery was also expanded to include sodium salicylate and sodium metabisulphite. Sodium salicylate was added for comparison with aspirin, while sodium metabisulphite was added as it is widely used as a preservative in similar foods to benzoates. Initially the only placebo was a single uncoloured challenge, lactose, but in January 1979 a coloured placebo, B carotene, was added to the challenges as a control for the yellow tartrazine capsule. In January 1982 the dose of tartrazine was increased from 10mg to 30mg, which is equilavent to the amount of azo dye in one food item, for example one Icey Pole (NH&MRC, 1986). Twelve months later the protocol was made double blind by numbering the challenges in an arbitary sequence, and at the same time starch was substituted for the lactose placebo. In the late 1983 it was noted that a new batch of B carotene obtained from a different

supplier had a very strong smell of carrots; this appeared to coincide with a sudden increase in the reaction rate to this second "placebo" (Table 3.8) and it was therefore omitted from the challenge battery in May 1984.

TABLE 3.2

DEVELOPMENT OF THE CHALLENGE PROTOCOL

Dates	Modifications to Challenge Protocol *
January 1979	Addition of sodium salicylate (A&B doses), sodium metabisulphite, carotene
January 1981	Omission of penicillin
January 1982	Increase tartrazine dose to 30mg
January 1983	Randomized sequence, double blind protocol Substitution of starch for lactose as a placebo Single dose (300mg) for both aspirin and sodium salicylate
May 1984	Omission of carotene

* From April 1977 to January 1979 the challenge protocol consisted of lactose, tartrazine, sodium benzoate, 4-OH benzoic acid, brewers yeast, penicillin, aspirin.

The challenge doses for the four challenge periods are shown below (Table 3.3).

TABLE 3.3

CHALLENGE DOSE (MG)

Challenge Compounds	Time Periods[1]			
	I	II	III	IV[9]
Lactose[2]	700	700	700	-
B Carotene & lactose[3]	700	700	700	700
Tartrazine[4]	10	10	30	30
Sodium benzoate	500	500	500	500
4-OH benzoic acid	200	200	200	200
Brewers yeast[5]	600	600	600	600
Penicillin[6]	250	-	-	-
Acetylsalicylic acid[7]	450	450	300	300
Sodium salicylate[7]	-	450	300	300
Sodium metabisulphite[8]	-	500	500	500
Starch & B carotene	-	-	-	700

1. Time periods:
   - I. April 1977 to January 1979.
   - II. January 1979 to January 1982.
   - III. January 1982 to January 1983.
   - IV. January 1983 to January 1986.
2. Lactose placebo replaced by starch placebo.
3. B carotene placebo filler changed from lactose to starch. Challenge finally omitted because of contaminated source.
4. Tartrazine dose increased from 10mg to 30mg in Jan'82, equivalent to one coloured food item.
5. Brewer's yeast was made by Cenovis Health Company Pty. Ltd.
6. Penicillin deleted as not a significant amount in milk.
7. Comparison between sodium salicylate and acetylsalicylic acid, given in two doses 150mg first, then 300mg two hours later if no reaction. Changed to single dose of 300mg in double blind set.
8. Addition of sodium metabisulphite, a widely used preservative.
9. Single blind challenge order changed to double blind Jan'83 onwards.

Challenge Instructions

Challenges were commenced after at least two weeks on the elimination diet, once there had been five consecutive days free of symptoms. Numbered capsules were taken in the morning half an hour before or two hours after breakfast (for children the capsule contents were mixed with golden syrup if they were unable to swallow the capsules). Challenges were spaced by at least 48 hours to allow for delayed reactions, and any response to challenge was followed by a pause of a further three symptom-free days before proceeding to the next challenge, since patients often experience a temporary refractory period during which they are unresponsive to further challenge. Each patient was provided with diary sheets and recorded in detail the time each challenge was taken, and if a reaction occurred the time of onset, type and severity of symptoms and their duration.

Challenges were taken at home except in patients with laryngeal oedema and asthma for whom challenges of acetylsalicylic acid, sodium metabisulphite and tartrazine were supervised. Patients with very mild asthma were supervised by their local general practioner. Those with mild-moderate asthma were challenged under supervision at the Allergy Clinic, patients with severe asthma and laryngeal oedema were admitted into hospital for challenge with graded doses of acetylsalicylic acid, sodium metabisulphite and tartrazine (Appendix 5).

Symptom Diary

Patients were given a diary and instructed to keep a detailed record of (a) all food and beverages consumed, (b) the type and duration of all symptoms experienced, (c) details of all food and capsule challenges taken, and (d) the dose and type of medications taken when necessary.

FIGURE 3.3

SAMPLE PATIENT SYMPTOM DIARY

	BREAKFAST	MID MORNING	LUNCH	MID AFTERNOON	TEA	EVENING
DIETARY INTAKE FOR: DAY: DATE:	2 Weetbix milk coffee	2 coffee biscuits coffee	1 boiled egg 2 bread coffee	coffee	roast lamb potato, lettuce custard, pear	2 coffee biscuits coffee
SYMPTOMS: (Type & Severity) MEDICATIONS AND/OR CHALLENGES TAKEN: (Time taken, reaction time and symptoms)	hives on legs and buttocks, swelling around eyes none				hives on legs and buttocks swelling around eyes	
DIETARY INTAKE FOR: DAY: DATE:	2 toast golden syrup coffee	pear	1 chicken sandwich pear	2 coffee biscuits coffee	2 chops potatoes pear	2 coffee biscuits coffee
SYMPTOMS: (Type & Severity) MEDICATIONS AND/OR CHALLENGES TAKEN: (Time taken, reaction time and symptoms)	hives on legs and buttocks easing, swelling subsiding around eyes				hives on legs easing, slight swelling around eyes	

Interpretation of Challenge Reactions

Challenges were initially given single-blind as a numbered sequence, but by the end of 1982 both the physicians and the dietitians had become so familiar with the standard sequence that there was a danger of biased interpretation with subtle reactions. Accordingly, in January 1983 the protocol was made double-blind, with the challenge substances being numbered in an arbitary order for each patient.

After completion of challenges patients were seen by the dietitian who reviewed their diary and recorded the results. The two other dietitians, Gabrielle Boyd and Jenny McQueen, were trained for nine to twelve months by the author in order to ensure uniformity of interpretation. A challenge was considered positive if there was a recurrence of urticaria and/or angioedema within 48 hours, and reactions were almost always clear-cut. Three patterns were commonly observed: acute, intermediate and delayed as described below in the results section.

If the response to any challenge was uncertain it was repeated as a capsule or three-day open food challenge (Appendix 6). This method also provided a check when challenges were taken too quickly in succession due to the eagerness of the patient to complete the tests. Under these circumstances overlap of challenges could lead to confusion about which one was responsible for a particular reaction, or conversely a false negative could be recorded if the subsquent challenge was taken during the refractory period.

Following completion of the protocol a modified therapeutic diet was prescribed based on the final assessment, and a follow-up appointment made to review progress six weeks later. In this way care was taken that patients were not over or under-restricted because of misleading results.

Long Term Dietary Management

After six weeks, gradual liberalization of the therapeutic diet by food chemical groups was encouraged in an attempt to induce tolerance by raising the threshold for triggering symptoms (Appendix 10). Patients sensitive to natural salicylates were then encouraged to liberalize their diet by taking very small amounts of the foods containing moderate amounts of salicylate every third day for two weeks and then if there was no adverse reaction increasing the frequency to every second day for two weeks and then to every day. If there was still no adverse reaction the patient was encouraged to increase the amount as tolerated provided there were no adverse reactions. In patients with a mild degree of sensitivity it was sometimes possible to return eventually to a virtually normal diet without relapse.

Re-Challenge of RIU/AO Patients

In order to test the reproducibility of the challenge protocol, 142 patients with RIU/AO who had completed the elimination diet and challenge protocol at least 12 months before, were contacted by telephone and asked if they would be prepared to repeat testing with the challenges. Those who agreed, provided they had been free of symptoms, were mailed a second set of challenges which were sent out

in batches every three to six months between October 1978 and May 1981. The re-challenge compounds and their dosages are as outlined in Table 3.4. Patients were asked to maintain a strict exclusion diet during the re-challenge period.

TABLE 3.4

CHALLENGE BATTERY FOR RE-CHALLENGE OF RIU/AO PATIENTS

Challenge	Dose
Aspirin & sodium salicylate	300mg & 300mg
Sodium benzoate & 4-OH benzoic acid	500mg & 200mg
Sodium metabisulphite	500mg
Tartrazine	10mg
Brewers yeast	600mg
Lactose	700mg

Records and Statistical Analysis

For each patient undergoing dietary investigation a file was created comprising clinical details, dietary history, progress notes and challenge reactions. For the 698 patients who underwent blind challenges, results were tabulated in summary form on a spreadsheet

which was updated at regular intervals, and used as the basis for subsquent statistical analysis. Details of date of presentation, age, sex, duration and nature of symptoms (urticaria, angioedema or both), and challenge results were entered into a VAX 8600 computer, using files created so as to be compatible with the Statistical Package for Social Sciences, Version ten (SPSS-X). Unless otherwise stated, this program package was used for all statistical analyses.

(a) 95% Confidence Intervals

The proportion (p) of patients reacting to each of the challenge compounds was tabulated, and 95% confidence intervals calculated as follows:

$$1.96\sqrt{\frac{p(1-p)}{n}}$$

By the Central Limit Theorem (Sachs, 1984) the number of patients tested (n=614) was sufficiently large to justify assuming that the proportion in each case was normally distributed.

(b) McNemar's Test

Since each patient was tested with multiple challenge substances, reactions to which may not be completely independent, the overall results for each compound compared with placebo were analysed by McNemar's test (1947). This test compares the frequency of positive and negative reactions to each challenge, with those to the placebo,

the null hypothesis being that the frequencies differ no more than expected by chance:

		Placebo	
		+	-
Active	+	a	b
Challenge	-	c	d

The frequencies b and c are thus expected to have the same value as 0.5(b+c); the more b and c deviate from this expected value, the less confidence can be placed in the stated null hypothesis. The significance of a difference between these values was determined by calculating Chi-square by McNemar's formula and consulting a Chi-square table while assuming one degree of freedom:

$$\text{Chi-square} = \frac{(b-c)^2}{b+c+1}$$

Since the number of patients tested was in all instances more than 30, the correction of Bennett and Underwood (1970) was not required.

(c) Bon Ferroni Correction

For a single comparison between active and placebo challenges a P value of <0.05 would be considered significant. However, with multiple challenges per patient the probability of achieving this value by chance increases with each comparison, and the significance level must be adjusted accordingly. The Bon Ferroni method sets a

significance level of P <0.05/N, where N refers to the number of comparisons. In the present case, N=7 (since there are 7 active challenges) and the significance level required is therefore P <0.007.

(d) Chi-Square Test (Contingency Table)

Since the elimination diet and challenge protocol were modified during the course of the present study, the results were analysed separately for each of four time periods (Table 3.8). Changes in reaction frequency for each challenge substance were analysed by constructing contingency tables to compare observed and expected values (Sachs, 1984):

Observed values

	time periods				
	I	II	III	IV	total
+ ve	a	d	g	j	x
- ve	b	e	h	k	y
total	c	f	i	l	z

Expected values

	time periods			
	I	II	III	IV
+ ve	$\frac{cx}{z}$	$\frac{fx}{z}$	$\frac{ix}{z}$	$\frac{lx}{z}$
- ve	$\frac{cy}{z}$	$\frac{fy}{z}$	$\frac{iy}{z}$	$\frac{ly}{z}$

The Chi-square value was then calculated as follows:

$$\text{Chi-square} = \Sigma \frac{(\text{observed value} - \text{expected value})^2}{\text{expected value}}$$

(e) Chi-Square Test (Goodness-Of-Fit)

In those patients who were re-challenged the results were tabulated according to whether each challenge produced the same response or a different response on re-testing. To determine whether the sample of 77 patients who were re-challenged was a representative one, a 2*2 contingency table was constructed comparing the initial results for each challenge in the sample ("observed") with those of the whole RIU/AO group tested during the same time period ("expected"):

	+ ve	- ve
observed	a	b
expected	c	d

The Chi-square goodness-of-fit statistic was then determined as follows:

$$\text{Chi-square} = \frac{\Sigma(\text{observed values} - \text{expected values})^2}{\text{expected values}} = \frac{(a-c)^2}{c} + \frac{(b-d)^2}{d}$$

P values were derived from tables of the distribution of the Chi-Square statistic, with one degree of freedom.

For each patient in whom the challenges were repeated, the results were tabulated according to whether the response to each challenge substance was positive or negative on both occasions, with four possible categories: +/+, +/-, -/+, and -/-. The null hypothesis was that the challenge findings in each case were due to chance

alone. Expected proportions were calculated from the known figures in RIU/AO patients as a whole, assuming the same frequency of positive and negative reactions to occur at random in both the first and second challenge series. The observed results were compared with those "expected" by constructing a 2*4 contingency table:

	+/+	+/-	-/+	+/+
observed	a	b	c	d
expected	e	f	g	h

and in each case, Chi-square was calculated as follows:

$$\text{Chi-square} = \Sigma \frac{(\text{observed} - \text{expected})^2}{\text{expected}}$$

P values were derived from Chi-square tables, with three degrees of freedom.

(e) Newman-Keuls Test

To determine whether there was a tendency for reactions to cluster with particular challenge compounds, the reaction frequencies were ranked and comparisons made between all pairs using the criterion of the least significant difference. With multiple comparisons, the probability of finding "significant" differences by chance increases, and the Newman-Keuls method was used to avoid drawing erroneous conclusions (Snedecor & Cochrane, 1967, pages 233-237). The analysis was performed by Associate Professor R. Berry (Commonwealth Institute of Health, University of Sydney) using his own Fortran program written for the purpose.

RESULTS

Of the 1349 patients, 1193 were prescribed the elimination diet. Remission of symptoms was experienced by 698 patients (58.5%) who were subsequently challenged. No improvement was reported in 80 patients (6.7%) and 415 (34.8%) patients did not persist with the elimination diet (Table 3.5).

The remaining 156 patients were given a "low chemical diet" (low salicylate, low preservative, low artificial colour, low brewer's yeast, (Appendix 8) as it was felt that they would be unable to cope with the very rigid dietary restrictions and challenge procedure. The low chemical diet restricted only the commonest food chemical precipitants of RIU/AO, resulting in a less rigid diet.

Patients who responded favourably to the elimination diet generally became asymptomatic within one to two weeks. The majority of those on regular antihistamines were able to withdraw medication by the end of the first week without experiencing an exacerbation, but sometimes residual symptoms would require a few more days to subside. The challenges were only undertaken when the patient had been free of all symptoms, off all antihistamines, for at least five consecutive days, and after a minimum of two weeks on the elimination diet.

TABLE 3.5

DIETARY RESULTS OF RIU/AO PATIENTS ON THE ELIMINATION DIET

Dietary Results	Number of Patients
Improvement	698
No improvement	80
Failed to complete	415
"Low chemical" diet[1]	156
Total	1349

1. "Low chemical" diet = low salicylate, preservative, artificial colour, brewers yeast diet.

Challenge Results

Challenge reactions generally followed three patterns: acute (onset half to two hours, duration one to two hours); intermediate (onset five to eight hours, duration up to 24 hours); delayed (onset 24-48 hours, duration two or three days up to a week or more). The challenge results are presented in Table 3.6. The percentage of patients reacting to each of the active challenge compounds compared with placebo was analysed using McNemar's test, since each individual was subjected to multiple challenges. Even allowing for multiple testing, using the Bon Ferroni technique, the response to the entire set of challenges compared with placebo was highly significant

(P <0.0001).If a P-value of <0.05 is taken as being the significance level for the number of patients reacting to a single challenge substance compared with placebo, the significance level for seven such comparisons is P <0.007 (Bon Ferroni technique, Materials and Methods).

TABLE 3.6

TOTAL CHALLENGE RESPONSE OF PATIENTS WITH RIU/AO

(TIME PERIODS I,II,III,IV COMBINED[1])

Challenge Compounds	% Positive Response	95% Confidence Limits (±)	P Value compared with Lactose
Salicylates (total)	60.9	3.9	< .0001
Acetylsalicylic acid	52.6	4.0	< .0001
Sodium salicylate	41.4	3.9	< .0001
Benzoates (total)	47.0	4.0	< .0001
Sodium benzoate	34.1	3.8	< .0001
4OH benzoic acid	32.9	3.7	< .0001
Sodium metabisulphite	39.2	3.9	< .0001
Tartrazine	33.9	3.7	< .0001
Brewers yeast	30.3	3.6	< .0001
Lactose	6.8	2.0	

1. Time periods I,II,III,IV combined = April 1977 to January 1986

In order to determine whether there was a different pattern of reactivity in patients with urticaria alone versus those with angioedema the RIU/AO population was divided into three sub-groups and the results tabulated for each separately (Table 3.7).

TABLE 3.7

CHALLENGE RESPONSES (%) IN PATIENTS PRESENTING WITH URTICARIA, ANGIOEDEMA OR BOTH

Challenge Compounds	Urticaria Alone	Angioedema Alone	Urticaria & Angioedema
Acetylsalicylic acid	54.9	48.0	51.9
Sodium salicylate	45.1	35.6	38.0
Sodium benzoate	36.9	32.3	31.7
4-OH benzoic acid	36.0	27.1	31.9
Sodium metabisulphite	45.0	31.3	35.5
Tartrazine	37.8	30.5	31.0
Brewers yeast	32.7	29.3	28.0
Lactose	6.6	9.1	6.4

Since there were several significant changes in both the baseline elimination diet itself (Table 3.1) and the challenge protocol (Table 3.2), the results were also tabulated and analysed separately for each of the time intervals, as outlined in Table 3.8.

TABLE 3.8

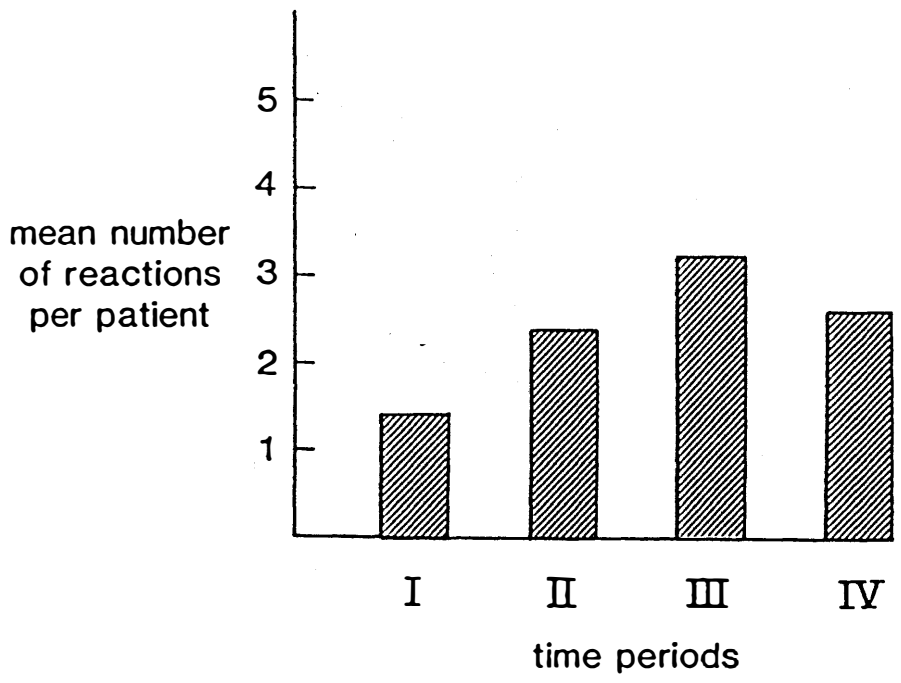
PATIENTS REACTING TO CHALLENGE IN DIFFERENT TIME PERIODS (%)

Challenge Compounds	Time Periods[1] I	II	III	IV
All salicylates	51.6	59.9	71.6	61.3
Acetylsalicylic acid	51.6	51.9	54.7	52.8
Sodium salicylate	-	39.3	56.5	34.8
All benzoates	31.9	46.6	54.6	51.4
Sodium benzoate	20.9	33.2	38.1	39.8
4OH benzoic acid	23.1	31.9	43.3	33.7
Sodium metabisulphite	-	36.0	45.3	40.0
Tartrazine	27.5	29.1	46.9	36.7
Brewers yeast	16.5	28.6	41.7	33.5
Lactose	3.3	8.0	7.3	-
Starch	-	-	-	6.0
B Carotene / starch	-	-	-	12.0
Total patients challenged	93	241	97	183
Total reactions to challenges	137	577	311	475
Mean reactions per patient	1.47	2.39	3.21	2.58
P value		<.0005	<.005	<.1

1. Time periods:
   - I. April 1977 to January 1979.
   - II. January 1979 to January 1982.
   - III. January 1982 to January 1983.
   - IV. January 1983 to January 1986.

To determine if there was a significant difference between the reaction rates in each successive time period the means of the number of reactions per patient were compared using the Chi-square test. There was a highly significant increase in the mean reaction rates following the changes made in January 1979 (Period II) and those made in January 1982 (Period III). In each case there was an increase in the stringency of salicylate exclusion on the baseline diet, and the findings therefore suggest that this may have led to an increased sensitivity to the challenges generally. Comparisons between individual challenge compounds (Chi-square test) showed that the most significant changes ($P < 0.05$) occurred with tartrazine (when the dose was increased) and to the salicylates, benzoates and brewers yeast (corresponding to theincreased salicylate restriction). In January 1983 the challenges were altered to a double blind protocol, and this was accompanied by a fall in the mean reaction rate which did not, however, reach statistical significance ($0.1 > P > 0.05$).

FIGURE 3.4 MEAN NUMBER OF REACTIONS PER PATIENT



Cross-Reactivity between Challenge Substances

There appears to be confusion in the literature about the degree to which aspirin and tartrazine may cross-react in patients with RIU/AO (Stevenson et al., 1986). In order to define this more clearly, data from the present study was analysed by constructing a series of two by two contingency tables of reactivity to various pairs of challenge compounds. Cross-reactivity was determined by calculating the probability of reacting to one challenge, given either a positive or negative reaction to the other, and the differences were analysed by a Chi-square test (Table 3.9). Results are shown for reactivity to each compound according to whether patients were challenge-positive or negative to aspirin alone, sodium salicylate alone, or either/both salicylate/s (Top panel, Table 3.9) and also according to whether patients were positive or negative to sodium benzoate alone, 4-OH benzoic acid alone or either/both benzoate/s (Bottom panel, Table 3.9). There were highly significant correlations between aspirin, sodium salicylate and both benzoates. Another striking correlation was between reactivity to sodium metabisulphite and both the salicylate and benzoate challenges, a finding which has not previously been reported. It is interesting to note, however, that cross-reactivity between aspirin and tartrazine did not reach statistical significance, although tartrazine reactivity did correlate significantly with responses to sodium salicylate and both benzoates.

Table 3.9 Footnote:

* P values shown as <0.0000 are below the limits given in available statistical tables.

TABLE 3.9

CROSS-REACTIVITY BETWEEN CHALLENGE SUBSTANCES (%)

CHALLENGE COMPOUNDS	ASPIRIN				SODIUM SALICYLATE				ANY SALICYLATE			
	+ve	-ve	X^2	P (<)	+ve	-ve	X^2	P (<)	+ve	-ve	X^2	P (<)
Aspirin					73.6	34.8	68.4	.0000				
Sodium salicylate	59.7	22.1	68.4	.0000								
Any salicylate												
Sodium benzoate	39.8	27.5	10.1	.0015	45.8	31.0	10.6	.0011	39.4	25.6	12.0	.0005
4-OH benzoic acid	39.9	24.4	16.1	.0001	47.1	26.1	22.0	.0000	39.6	21.8	20.4	.0000
Any benzoate									54.2	34.6	21.8	.0000
Sodium metabisulphite	50.2	27.3	27.2	.0000	57.1	26.8	43.0	.0000	50.0	21.4	40.0	.0000
Tartrazine	37.0	30.5	2.8	.0965	44.3	29.3	11.0	.0009	36.7	30.0	3.1	.0798
Brewers yeast	33.4	26.0	3.9	.0486	39.1	28.6	5.6	.0181	32.3	46.6	2.3	.1277
Lactose	8.7	5.2	2.0	.1528	10.4	6.0	2.2	.1398	9.0	4.0	4.0	.0457

CHALLENGE COMPOUNDS	SODIUM BENZOATE				4-OH BENZOIC ACID				ANY BENZOATE			
	+ve	-ve	X^2	P (<)	+ve	-ve	X^2	P (<)	+ve	-ve	X^2	P (<)
Aspirin	61.2	47.5	10.1	.0015	63.9	46.2	16.1	.0001	60.7	44.7	15.3	.0001
Sodium salicylate	50.6	35.3	10.6	.0011	55.6	33.1	22.0	.0000	50.0	31.8	16.1	.0001
Any salicylate									70.7	51.9	21.8	.0000
Sodium benzoate					60.5	20.6	93.7	.0000				
4-OH benzoic acid	59.0	19.5	93.7	.0000								
Any benzoate												
Sodium metabisulphite	60.6	26.2	56.8	.0000	61.7	26.6	57.1	.0000	57.0	20.9	67.6	.0000
Tartrazine	46.8	26.6	24.4	.0000	50.5	25.1	38.0	.0000	45.0	23.6	30.6	.0000
Brewers yeast	38.8	25.5	11.2	.0008	37.2	26.3	7.2	.0074	35.3	25.4	6.8	.0093
Lactose	8.3	6.0	0.9	.3543	8.3	6.1	0.8	.3847	8.4	5.4	1.6	.2083

The question then arose as to whether there might be subgroups within the RIU/AO patients in whom particular challenge substances have a tendency to cluster. This might have important implications for understanding the mechanisms by which certain chemical compounds, but not others, cause urticarial reactions in predisposed individuals (Chapter 10). By ranking the reaction frequencies with each of the challenges, it was possible to apply the Newman-Keuls test to study this issue. Overall, no significant clustering of reactions was found, although salicylates did stand out from the other substances as provoking symptoms significantly more frequently than any other single substance or group of substances (data not shown).

Re-Challenge of RIU/AO Patients

In order to determine the reliability of the challenge protocol, 142 patients previously tested were sent a second challenge battery 12 months later. Of these 77 agreed to undergo re-challenge (Table 3.10).

Fifty patients either refused because they were frightened of experiencing a severe reaction (six individuals), or initially agreed to participate but then failed to take the second set of challenges. Since the 77 patients who agreed could have been self-selected, their initial challenge results were compared to those of the RIU/AO group as a whole to determine whether the re-challenge patients were a representative sample. Using the Chi-square goodness-of-fit test, the only significantly different challenge results were with salicylates (42% on those agreeing to re-challenge versus 52% in the whole larger group reacting, respectively; $0.01 < P < 0.05$).

TABLE 3.10

RE-CHALLENGE OF 77 PATIENTS WITH RIU/AO

Challenge Sets	Number of Patients
Total challenge sets sent	142
Not taken	50
Changed address	14
Died	1
Challenges taken	77

The responses to re-challenge of the 77 patients studied were tabulated together with their reactions to the initial battery and classified as "same" (+/+ or -/-) or "different" (+/- or -/+) responses (Table 3.11).

Overall, of the 462 re-challenge tests, 406 reactions were the same (87.9%) and 56 were different (12.1%) from the responses recorded after the first set of challenges. The statistical analysis of these figures is outlined in detail in the Materials and Methods section. The results were highly significant for all challenges ($P < 0.0005$) except with metabisulphite, where the numbers tested were small (29 patients). Furthermore, when the discordant results are examined closely there is a striking tendency for initially positive reactions to be negative on re-challenge, but not the reverse ($P < 0.0005$ by

McNemar's test). This implies that the discordant re-challenge results are more likely to be a true indication of a loss of sensitivity rather than to a lack of reliability of the challenge procedure itself.

TABLE 3.11

RESPONSES TO RE-CHALLENGE

Challenges	Same Response +/+ or -/-	Different Response +/-	-/+
Salicylates (total)	58 (75%)	19 (25%)	0
Benzoates (total)	68 (88%)	9 (12%)	0
Sodium metabisulphite	73 (95%)	4 (5%)	0
Tartrazine	67 (87%)	10 (13%)	0
Brewers yeast	72 (94%)	5 (6%)	0
Lactose	77 (100%)	0 (0%)	0

Physical Urticarias

Although physical factors such as pressure were commonly found to exacerbate symptoms in patients with RIU/AO, a few patients (1%) presented with urticaria precipated only by physical stimuli. Because of the clinical overlap these patients also underwent dietary investigation.

Ten patients with cold urticaria were placed on the elimination diet and were asked to perform daily ice-cube tests (at rotating sites) to assess their cold-sensitivity. Of these, four became asymptomatic and underwent blind challenge, four showed no change, and two were unsure of the outcome. Three of the four diet-sensitive patients reacted to salicylate challenge (by developing urticaria in response to a cold stimulus) and one reacted to tartrazine.

Three patients with isolated dermographism were also placed on the elimination diet, and two became asymptomatic. One of these was challenged and reacted to erythrosine alone and this patient has remained free of symptoms provided she avoids coloured foods. The other diet-responsive patient was a six-year-old girl who also suffered from asthma and lived in the far west of NSW. She was therefore considered unsuitable for challenge, but remains well on a diet low in salicylates and additives.

DISCUSSION

Despite the publication of numerous studies over the past 20 years (Table 3.12) there are still wide divergences of opinion as to the role of food in precipitating RIU/AO. Although much of the early literature came from the USA (Lockey, 1959, 1969, 1971; Samter & Beers, 1967, 1968; Settipane & Pudupuakkan, 1975; Settipane et al., 1976; Settipane, 1977, 1983) remarkably little of this information appears to have had any impact on clinical practice in North America (Mathews, 1980; Small et al., 1982; Saryan, 1983; Margolis & Nisi,

1985). In Britain and Europe, largely as a result of the work of Warin and Juhlin (Table 3.12), there has been increasing awareness of the role of salicylates and food additives in RIU/AO (BMJ Editorial, 1976, 1981; Ormerod, 1984), although the practical application of these findings is still viewed as questionable (Lancet Editorial, 1981).

In Australia, partly as a result of the present study, there is a more general appreciation of the role of diet in RIU/AO and, in particular, of the importance of naturally occuring compounds as well as additives (Roberts-Thompson et al., 1984; Truswell, 1985; Walls, 1986). In the ten years since the elimination diet and challenge programme was first introduced at RPAH the protocol has been requested by 575 general practitioners, specialists and dietitians, and is in use in over 20 teaching hospitals throughout Australia (Table 3.13).

These figures are probably a reflection of the fact that the protocol used at RPAH can be readily applied in routine clinical practice, given an understanding of the basic principles and the chemical composition of the common foods. In most published studies formal dietary investigation is generally regarded as only being feasible in a research institution, leaving individual practitioners to manage their patients on an empirical basis. The protocol developed during the present study involves a single outpatient visit for consultation

with the physician and explanation of the elimination diet and challenges by the dietitian. In most cases the patient is then able to carry out the entire testing procedure at home, keeping in regular telephone contact with the dietitian (and physician when necessary) in order to discuss any uncertainities or confusion about the instructions. After completion of the challenges (usually 4-6 weeks later) patients are asked to return for follow-up with their diary sheets, at which time a suitably modified long-term diet can be prescribed.

Compliance with this protocol has been very good, with 65% of the patients returning for follow-up. Many factors contributed to this, including the provision of clear-cut instructions about permissible foods as well as those to avoid, the effectiveness of the elimination diet itself in relieving symptoms of RIU/AO and the ability to carry out challenge testing on an out-patient basis. Ready telephone access to the dietitian was also important since patients often needed to check whether some unlisted food was permissible, or to ask advice about social occasions, recipes, medications and other practical details.

Modifications to the baseline elimination diet in 1982, with the addition of bread, milk and eggs (which were only rarely implicated), improved its palatibilty considerably and made adherence easier for patients, although there was no noticeable change in the compliance rate.

TABLE 3.12

PUBLISHED DIETARY STUDIES IN RIU/AO

Author	Year	Number of Patients	Control	Challenge Results: Tartrazine	4-OH Benzoic Acid	Sodium Benzoate	Aspirin	History of Aspirin	Dietary Response: Number of Patients	Total	Partial
Lockey	1948	4		100					4		+
Warin	1960	70					30	29			
Samter & Beers	1967	40		8				100			
Moore-Robinson & Warin	1967	228	0				22	49			
Lockey	1969	6		100					6		+
James & Warin	1970	96					41	10			
Lockey	1971	4		100				50	4		+
Juhlin et al.	1972	8	0	71	86	29		100	8	14	86
Michealsson & Juhlin	1973	52	0	37	40	42	67	33	16	81	6
Thune & Granholt	1975	100		21	6	10	34		100	12	50
Doeglas	1975	23		30	17	23	26	32	27		63
Settipane et al.	1976	38	0	8			0	26	38		34
Warin & Smith	1976	108		13	5	11	41		38		75
Ros et al.	1976	75		67	59	59	79	36	75	24	57
Doeglas	1977								18		67
Kaaber	1978	65		5	3	3			23	44	30
Fujita et al.	1978	57			23	23					
Neuman et al.	1978	30		23							
Mikkelsen et al.	1978	61		11							
August	1979	86		23	22	22			22	45	23
Meynadier et al.	1979	24		46	25	25			98	80	12
Lindemayer & Schmidt	1979	90		19	29	29			90	20	55
Wutrich & HackiHerrman	1980	81		21	18	18					
Gibson & Clancy	1980	65	0	26	34	34	54		65	75	15
Valverde et al.	1980								258	61	22
Wutrich & Fabro	1981	306		6	6	6			51	31	57
Juhlin	1981	330	5	18	11	11	22	10			
Kirkhof et al.	1982	100		15	8	8	23		41	44	29
Merk & Goerz	1983	25		24							
Hannuksela	1983	137		1	4		18				
Doeglas	1983	271		10	11						
Verschave et al.	1983								67		73
Giam & Rajan	1983	36		14	6	6	36				
Botey et al.	1984	9					100	33			
Allen et al.	1984			30	35	17	61				
Loblay & Swain	1986	826	7	35	55	55	62		826	70	
Supramaniam & Warner	1986	43	2	26	9	9	2		43		93

TABLE 3.13

ELIMINATION DIET PROTOCOL REQUESTS

Location	Medical Practitioners	Teaching Hospitals	Dietitians
NSW	243	11	90
Interstate	113	10	86
Overseas	32	7	11

Findings of the Present Study

In the present study 58.5% of patients presenting with RIU/AO experienced remission of symptoms within one to two weeks on the elimination diet. Of the remainder, 34.8% did not return for follow-up, but when subsquently contacted by questionnaire a significant proportion had in fact continued to modify their diet in order to control symptoms (Chapter 4). Only 6.7% of patients returned for follow-up had not experienced any improvement in symptoms on the elimination diet. It is clear, therefore, that diet is a relevant precipitating factor in the great majority of patients with RIU/AO, and that it is the single most important factor in nearly 60%.

In most studies reported to date dietary response rates have only been documented in relatively small numbers of patients, with variable results (Table 3.12). Although most diets used sought to

exclude artificial preservatives and colourings, only Juhlin (1977, 1980, 1981, 1985), Thune and Granholt (1975), Giam and Rajan (1983) and Botey et al. (1984) made any attempt to restrict salicylate intake, and even here this was done in a rather incomplete and haphazard fashion.

Challenge protocols also varied greatly (Table 3.12). Some were carried out on a normal diet (Warin 1960,1976; Hannuksela, 1983), and most others required restriction of additives only for periods of 3 days to one week before the challenges were commenced (with the exception of Superamaniam and Warner, 1986). In the present study it was found that the stringency of salicylate exclusion in the baseline diet had a significant impact on the reaction rate to the various challenge compounds, and this may in part account for the higher response rates documented. Increased sensitivity appears to occur with all challenge compounds (Table 3.8) and is reflected by an increase in the mean number of reactions per patient (Table 3.8). Possible reasons for this will be discussed in Chapter 10.

Two further differences in the challenge protocol used here are of relevance. Firstly, clinical experience indicates that patients become more sensitive to challenge over a two-week period of dietary restriction, and a lowered reaction-threshold may therefore have contributed to the high frequency of positive challenges. Secondly, patients are often found to be in a refractory state for up to 48 hours after a positive reaction to a previous challenge. Almost all other studies administered challenges at daily intervals without allowing for a refractory period, so that reaction rates are likely to have been significantly under-estimated.

The response to challenge is known to be dose-dependent (Warin, 1960; Moore-Robinson & Warin, 1967; James & Warin, 1970) and this effect can be seen when the dose of tartrazine was changed from 10mg to 30mg (periods II and III, Table 3.8). A cumulative effect can also be seen when the challenge sequence was changed from a fixed order, with aspirin and sodium salicylate following sodium benzoate and 4-OH benzoic acid, to a random order (periods III and IV, Table 3.8). Salicylates (2-OH benzoic acid derivatives) and benzoates are closely related compounds which appear to frequently cross-react (Table 3.9), and it is therefore not suprising that consecutive challenges with these compounds leads to an increased reaction frequency.

Reactions to the chemical compounds were also seen to be interrelated by cluster analysis with a high degree of cross reactivity between 4-OH benzoic acid, sodium benzoate, tartrazine, aspirin and brewers yeast. These compounds and their metabolites all have a basic structure which may account for their common action.

A high degree of reproducibilty of the challenge results was evident from the observation that 88% of challenges produced the same result when re-tested in a subgroup of 77 of the RIU/AO patients (Table 3.11). Interestingly, in those instances where re-testing produced discordant results the pattern was invariably +/- rather than -/+ (Table 3.11), suggesting a true loss of reactivity rather than random variability. This raises the important question of whether such patients may have experienced either "spontaneous" or diet-induced remission of sensitivity. From the available data it is not possible to determine if this was the case, although at follow-up there was no significant difference in the degree of dietary restriction maintained by these patients (Chapter 4).

Physical Urticarias

Urticaria precipitated by physical factors such as cold, pressure, exercise, sunlight, etc. is generally regarded as a nosologically separate group of disorders, implying differences in aetiology (Warin & Champion, 1974; Champion et al., 1986; Czarnetzki, 1986). Among these only dermographism occurs commonly in patients with RIU/AO and in the present study this symptom was found to disappear and reappear in relation to dietary change in exactly the same way as in the patients with the "idiopathic" lesions. This observation prompted dietary investigation of all patients seen at the allergy clinic with physical urticarias, and although the numbers were relatively small it is clear that dietary factors modify the reaction to physical stimuli in a significant proportion. Possible mechanisms of this interaction will be discussed in Chapter 10.

New Findings of This Study

This study has contributed to the further documention that food chemicals are very important precipitants of RIU/AO and has further found that the incidence of food induced RIU/AO previously reported has probably been under-estimated. This appears to have occurred because most of the studies on the precipitants of RIU/AO have not considered food as a precipitant and those who have, have not been carried out with such stringent dietary restrictions prior to challenge.

In this study, the effect on RIU/AO of sodium metabisulphite was also investigated as it is one of the most widely used food preservatives today and previously its role in RIU/AO had not been studied.

Amongst the physical urticarias, cold urticaria was found to be the one that was most significantly affected by diet. This effect was studied in a small number of patients, in whom the naturally occurring salicylates in food taken on a chronic basis resulted in exacerbating the effect of the patients cold urticaria.

The results of this study indicate that food may be a more important precipitant of RIU/AO than prevously thought. Furthermore, the results would seem to indicate that food-related RIU/AO may more commonly be due to the small molecular weight substances naturally and artificially found in food than to food proteins as previously thought. This has important implications as to the mechanisms behind the development of RIU/AO. For this reason the study is now investigating in detail the effect of amines (tyramine and B phenylethylamine), monosodium glutamate and sodium nitrate as precipitants of RIU/AO. From January 1986 the challenge battery was modified so that the relevance of these compounds in the RIU/AO patients could be investigated thoroughly. Up to September 1986, 21 patients have completed this challenge set and 44% have reacted to amines, 37% to monosodium glutamate and 63% to sodium nitrate.

CHAPTER 4

DIETARY MANAGEMENT AND FOLLOW-UP

INTRODUCTION

Following the identification and elimination of dietary precipating factors in patients with RIU/AO, it was often observed that certain incriminated foods could be safely re-introduced whereas others would lead to a recurrence of symptoms. Clinical observations suggested that the foods most likely to precipitate recurrences of urticaria were those containing the highest concentrations of salicylates, and in order to confirm this impression it was decided to survey patients who had completed the elimination and challenge programme. By July 1983, 591 patients had completed the protocol from a total of 843 presenting for investigation. Initially an attempt was made to contact these patients by telephone, but this proved difficult in practice, so it was decided to send a standard questionnaire to each by mail. This was followed up three to four months later by a second questionnaire to those patients who had not replied.

From the replies it did indeed appear that high-salicylate foods were commonly implicated in causing recurrent symptoms. Furthermore, it had become clinically apparent that recurrences showed a cumulative dose-dependence, with symptoms often appearing gradually after several days of regular consumption of sub-threshold amounts of aspirin from a variety of food sources. In order to provide practical guidelines for dietary management in such patients, a chart was prepared listing foods according to salicylate-content, taking into account the quantities likely to be consumed as an average serving-size. This subsquently proved extremely useful in helping patients

to liberalize their diet maximally without developing cumulative effects, as well as enabling them to identify the most likely causes of acute recurrences.

When the replies from the initial questionnaire were collated and tabulated it became apparent that at least three quarters of the patients surveyed were still maintaining a restricted diet six months to six years after initial presentation. This was surprising since the remission rate in uncomplicated urticaria is said to be of the order of 50% within the first six months, although a significant minority may still be symptomatic after ten years (Champion et al., 1969). Consequently, the question of outcome in patients who failed to complete the elimination and challenge programme (approximately 30% of the total) also became of interest. It had previously been assumed that the majority of those who did not return for follow-up had not experienced any improvement in symptoms after an initial period of dietary restriction, placing them in a "diet-resistent" category. In these cases it seemed likely that other, as yet unidentified aetiological factors were responsible for the presence of continuing symptoms. Other possibilities considered were that some patients had found the dietary protocol too difficult or demanding and preferred to continue symptomatic drug treatment, or that they had experienced spontaneous remission and no longer needed to pursue dietary investigation. Thus, in November 1985, 304 such patients (23.5% of those seen up to that time) were surveyed by questionnaire to determine what proportion were still symptomatic and how many were still on a restricted diet.

MATERIALS AND METHODS

Duration of Symptoms

From the replies to the first questionnaire the total duration of symptoms was estimated in all patients who were able to return to an unrestricted diet. This was calculated by adding together the duration of symptoms at presentation (recorded in the case history notes) to the time taken to be able to return to a normal diet without recurrence of symptoms. The results were plotted by a life-table method, making allowance for those in whom the disease was still active (Champion et al., 1969). Confidence limits (95%) were calculated as described in Chapter 2.

Questionnaires

The first questionnaire was designed to ascertain the long-term effectiveness of dietary management in patients who had completed the elimination and challenge programme. The forms used are shown in Appendix 13. Patients were asked whether or not symptoms had recurred since dietary elimination and challenge testing. If symptoms had not recurred they were questioned about their current dietary restrictions; if symptoms had recurred they were asked to give details of the severity, duration, relationship to food, dietary compliance, dietary indiscretions, current dietary practices along with use of relief medications.

The second questionnaire was mailed to those patients who presented to the clinic with RIU/AO but did not complete the elimination and challenge testing programme (Appendix 14). Patients were asked

whether or not they had started the elimination diet and if it had been effective. They were also asked whether they had returned to a "normal" diet or whether they had continued dietary restriction, and if so which foods they avoided.

Statistical Analysis

Regression analysis was performed on a VAX 8600 computer using SPSS-X on the results of those patients who reported that they were free of symptoms and following an unrestricted diet. The duration of symptoms was compared with the patients' sex, age, reaction to the salicylate challenge and presenting symptoms of urticaria alone, angioedema alone or both together.

The degree of ongoing dietary restriction reported in the questionnaire replies was examined in relation to (a) presence or absence of symptoms, and (b) the relationship or otherwise of recurrences to foods. The Chi-square contingency test was used since these factors are independent variables.

Construction of Salicylate Charts

The levels of salicylate found in food presented in Chapter 2 were used to construct charts which could be used by patients to control the total daily dose of salicylate consumed. Each food was ranked by taking into account both its salicylate concentration and the amount that would be commonly eaten in an average serving. Foods were thus grouped according to whether they would provide a "negligible", "low", "moderate", "high" or "very high" salicylate dose per serve.

negligible : no detectable salicylate

low : < 0.1 mg salicylate per serve

moderate : 0.1 - 0.5 mg salicylate per serve

high : 0.5 - 1.0 mg salicylate per serve

very high : > 1.0 mg salicylate per serve

* ONE SERVE: Fruits: One item (apple, orange, etc.)
One slice (watermelon, rockmelon, pineapple, etc.)
One cupful (150g)(sultanas, berries, grapes, etc.)

Vegetables: Equivalent of one cupful (150g)

Nuts: One half cupful (80g)

Sweets: One tablespoon

Herbs/spices: One teaspoon

Drinks: One glass or cup (150ml)

TABLE 4.1

SALICYLATE CHART

VEGETABLES

Negligible	Low	Moderate	High	Very High
bamboo shoot	brussel sprout	asparagus	alfalfa sprout	capsicum
cabbage	chive	beetroot	broadbean	champignon
celery	choko	broccoli	cucumber	chicory
lettuce	green beans	carrot	eggplant	endive
potato(peeled)	green peas	cauliflower	watercress	gherkin
swede	leek	marrow		hot pepper
	mungbean spout	mushroom		olive
dried beans	red cabbage	onion		radish
dried peas	shallot	parsnip		tomato products
brown lentils		pumpkin		
red lentils		spinach		zucchini
		sweetcorn		
		sweet potato		
		turnip		

Continued

FRUIT

Negligible	Low	Moderate	High	Very High
banana	golden delicious apple (peeled)	custard apple	avocado	apricot
pear (peeled)	pawpaw	fig	grapefruit	blackberry
	pomegranate	lemon	granny smith apple	blackcurrant
		loquat	jonathan apple	blueberry
		mango	kiwi fruit	boysenberry
		pear (with peel)	lychee	cherry
		persimmon	mandarin	cranberry
		red delicious apple	mulberry	currant
		rhurbarb	nectarine	date
		tamarillo	passionfruit	grape
			peach	guava
			tangelo	loganberry
			watermelon	orange
				pineapple
				plum
				prune
				raisin
				raspberry
				redcurrant
				rockmelon
				strawberry
				sultana
				youngberry

HERBS and SPICES

Negligible	Low	Moderate	High	Very High
	garlic		allspice	aniseed
	malt vinegar		bay leaf	canella
	parsley		cardamon	cayenne
	saffron		carraway	cumin
	soy sauce		cinnamon	curry
	tandori		cloves	dill
	vanilla		ginger	five spice
			nutmeg	garam masala
			pepper (black)	mace
			pepper (white)	Marmite
			pimiento	mint
			white vinegar	mixed herbs
				mustard
				oregano
				paprika
				rosemary
				sage
				tarragon
				turmeric
				Vegemite
				worster sauce

Continued

NUTS

Negligible	Low	Moderate	High	Very High
poppyseed	cashews	brazil		almond
		coconut		waterchestnut
		hazelnuts		
		macadamia		
		peanuts		
		pecans		
		pinenuts		
		pistachio		
		sesame seeds		
		sunflower seeds		
		walnuts		

SWEETS

Negligible	Low	Moderate	High	Very High
carob	caramels	molasses		honey
cocoa	golden syrup			licorice
maple syrup				peppermints
white sugar				

BEVERAGES

Negligible	Low	Moderate	High	Very High
COFFEE	COFFEE	COFFEE		TEA
Andronicus	Harris instant	Harris Mocha		all brands
Pablo instant	Bushells instant	International		peppermint
decaffeinated	Bushells Turkish	Roast instant		
	Robert Timms	Moccona instant		CEREAL COFFEE
OTHER	instant	Nescafe instant		Nature's cuppa
Aktavite				
Milo	TEA	TEA		ALCOHOL
Ovaltine	camomille	decaffeinated		liqueur
	rosehip	fruit		port
ALCOHOL				rum
gin	CEREAL COFFEE	CEREAL COFFEE		wine
vodka	dandelion	Reform		
whisky	Ecco			
	Bambu	OTHER		
		coke		
		fruit juice		
		rosehip syrup		
		ALCOHOL		
		beer		
		brandy		
		cider		
		sherry		

Assessment of Nutritional Adequacy

In patients on a highly restricted diet where there was concern about nutritional adequacy a three to five day diet history was recorded and analysed by computer, using the "Soda" programme (Version 1.2, R.J. Hartley, 1982; Computer Models, P.O. Box 280, Bentley, WA). This provides an estimate of protein, fat, carbohydrate, kilojoules, vitamin A, thiamin, riboflavin, niacin, vitamin C, iron and calcium intake.

RESULTS

Natural History of RIU/AO

The total duration of disease was calculated for those patients with RIU/AO who were asymptomatic and following a normal diet on follow-up and plotted as a life table (Figure 4.1(a)), and for comparison, the data of Champion et al. (1969) is reproduced in the Figure 4.1(b). Although the proportion of patients entering remission during the first five years was much lower in the present study, the figures are very comparable at ten and 20 years after onset. Regression analysis was performed in order to look for a correlation between duration of disease and age, sex, response to salicylate challenge or disease pattern (urticaria alone, angioedema alone or both together). No significant relationship was found with any of these parameters.

FIGURE 4.1(a)

LIFE TABLE PLOT OF RIU/AO PATIENTS IN PRESENT STUDY

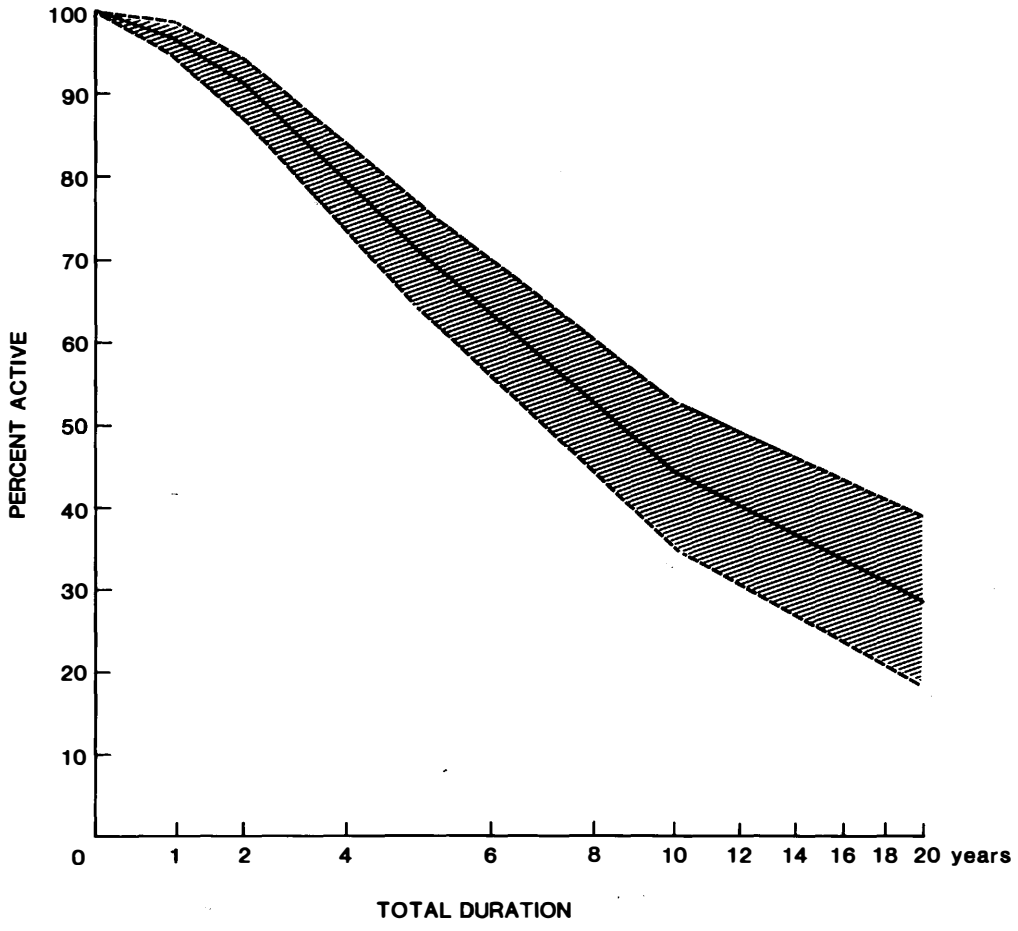


FIGURE 4.1(b)

LIFE TABLE PLOT OF RIU/AO PATIENTS (CHAMPION ET AL., 1969)

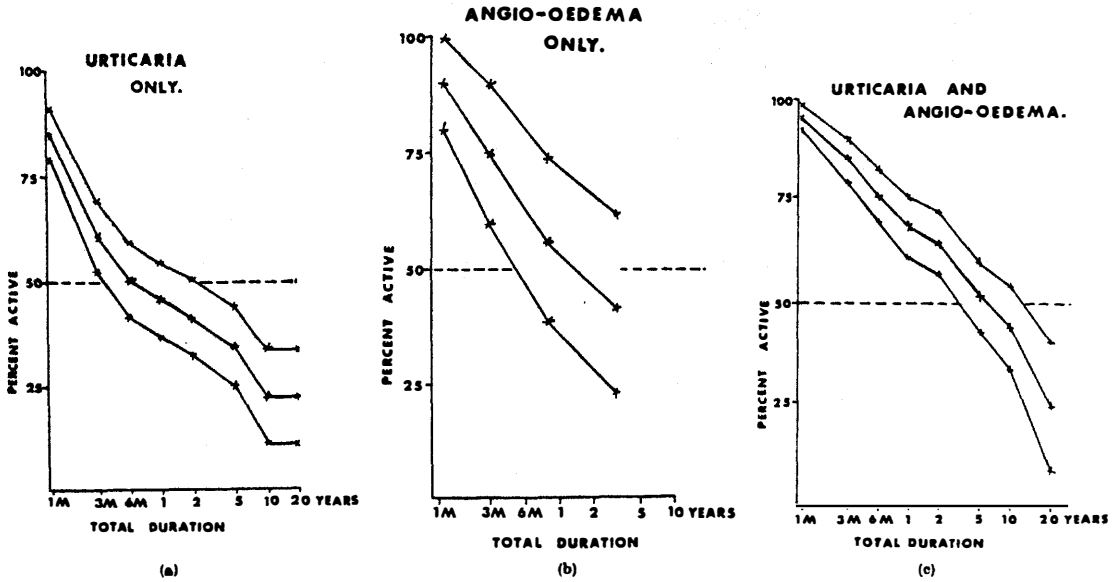


FIG. 4.—The expected percentage of patients active, with 95% confidence limits, by the total duration of disease (log scale).

Patients who Completed the Elimination Diet and Challenge Programme

Up to July 1983, 843 patients with RIU/AO had presented to the RPAH Allergy Clinic. Of these, 581 had completed the elimination diet and challenge programme, and during 1984 their progress was evaluated by a telephone and mailed questionnaire, or at clinic attendances. Altogether, 383 patients (66%) were successfully contacted, the remainder being lost to follow-up due to change of address, death or failure to reply (Table 4.2).

From the questionnaire replies it was evident that over half the patients surveyed were still symptomatic, and of these the majority experienced recurrences precipitated by foods (Table 4.3). There was a significant difference in dietary restriction pattern between patients in remission and those with recurrences ($P < 0.0001$, Chi-square test of independence), and in the latter group there was a strong correlation between the degree of dietary restriction and the occurrence of food-related symptoms ($P < 0.0001$).

On the questionnaire patients were asked to list foods they had identified as causing recurrences. The foods reported are shown in Table 4.4 (most commonly implicated) and Table 4.5 (implicated by only one or two patients). In almost every instance each food could be identified as a source of salicylates, colours or preservatives, often in combination, and in some cases together with amines and/or MSG (Chapter 6).

TABLE 4.2

RESPONSE TO FOLLOW UP QUESTIONNAIRE OF RESPONDERS

Patients Surveyed		Number of Patients
Number of patients surveyed (total)		581
Telephone		141
Questionnaire		222
Clinic review		20
Lost to follow-up (Total)		198
Change of address	154	
No reply	42	
Died	2	

TABLE 4.3

MAINTENANCE DIETARY PRACTICES OF DIETARY RESPONDERS (%)

Outcome at Follow-Up	Number of Patients	Maintenance Diet		
		Unrestricted	Partial Restriction	Highly Restricted
Remission	138	41%	32%	27%
Recurrences:				
Related to food	200	3%	24%	73%
Unrelated to food	45	78%	17%	4%

TABLE 4.4

COMMON PROVOKING FOODS

Foods	Chemical Content *	No. of Patients	Foods	Chemical Content *	No. of Patients
Wine	s,a,m,(p)	43	Sweets	(s,a,p,c)	6
Tomato	s,a,m,	30	Salads	s,(a)	6
Colours	c,(s,a,m,p)	23	Stone fruit	s	6
Fruits &			Tomato sauce	s,a,m	6
Vegetables	s,(a,m)	22	Nuts	s,a,(p)	6
Preservative	p,(s,a,m,c)	20	Vegemite	s,a,m	6
Lemonades	p,(c,s)	19	Seafood	(s,a,m,p,c)	5
Tea	s	18	Zucchini	s	4
Italian dishes	s,a,m	16	Dried fruit	s,(p)	4
Chocolate	a,(s,c)	15	Honey	s	4
Citrus	s	14	Strawberry	s	4
Beer	s,a	12	Mushroom	s,a,m	4
Spices	s	12	Meat Pie	s,a,m,p,c	4
Fruit juice	s,(p)	11	Icecream	(s,a,p,c)	4
Salicylates	s	10	Fish & chips	a,p,(c,s,m)	4
Chinese dishes	m,(s,a,p)	8	Bread	(s,p,c)	4
Cucumber	s	8	Xmas pudding	s,a,(p,c)	4
Pineapple	s	7	Xmas cake	s,a,(p,c)	4
Cheese	s,(p)	7	Rockmelon	s	3
Preserved meat	p,a,(s,m,c)	7	Lemon	s,a	3
Restaurant -			Tomato paste	s,a,m	3
meals	s,a,(m,p,c)	7	Coconut	s,a	3
Pineapple	s,a	7	Twisties	s,a,m,c	3
Cheese	a,(m,p,s,c)	7	Pickles	s,a,(m,p,c)	3
Apple	s	7	Flavoured-		
Capsicum	s	6	yogurt	s,a,p,c	3
Curry	s,(a,m,p,c)	6	Spicey chicken	s,a,m	3

* s = salicylate, a = amine, m = glutamate, p = preservative, c = colour

TABLE 4.5

LESS COMMON PROVOKING FOODS (LESS THAN 3 PATIENTS)

Foods	Chemical Content *	Foods	Chemical Content *
Apple pie	s,(p,c)	Lebanese dip	s,m
Avocado	s,a	Malt	
Blackberries	s,a	Mango	s
Beetroot	s	Mint sauce	s,a,(p,c)
Broccoli	s,m	McDonalds meals	s,a,(m,c,p)
Cheesecake	s,(a,c,p)	Onion	s
Chilli	s	Packet gravy	s,a,m
Coffee	(s)	Peas	s
Cornflakes	s	Pork	a
Carrot	s	Raspberries	s
Chicken soup	s,a,m,c	'Ribena'	s,p
Dried beans	(p)	Rhubarb	s
Egg		Sardines	a
Eggplant	s,a	Seasonings	a,s,m,(c)
Fish paste	s,a,(m,c,p)	Smoked salmon	a,(p)
Frozen foods	(s,a,m,c,p)	Stock cubes	s,a,m,c
Fruit buns	s,a,p,(c)	Taco shells	s,(a,m,p,c)
Grapes	s,a,m	Tripe	a
Herbs	s	Vinegar	s,a
Jelly	s,c,(a)	W'cester sauce	s,a,m

* s = salicylate, a = amine, m = glutamate, p = preservative, c = colour

From a total of 200 patients who reported that recurrences were related to food, 187 were able to recall and list one or more foods that they had identified as precipitants of RIU/AO. A total of 489 foods were reported of which 465 (95%) correlated directly with the patients' challenge responses (Table 4.6).

TABLE 4.6

FOOD RELATED RECURRENCES COMPARED WITH CHALLENGE RESULTS

Number of patients reporting food reactions		187
Number of food reactions reported		489
Total food reactions corresponding to challenge reactions		465
Salicylate	366	
Preservative	110	
Tartrazine	56	
Brewers yeast	39	

Of these, 79% were attributable to salicylate and 24% to the preservatives (benzoic acid and sodium metabisulphite). Some reports implicated cheese and chocolate in individuals sensitive to salicylates, preservatives and and/or tartrazine, but on closer examination these referred to composite products which also contained salicylates and additives (e.g. processed cheese, soft-centered chocolates, "Cherry Ripe" and "After Dinner" mints). However, in 24 instances the foods implicated did not correspond to challenge reactions (Table 4.7). In the light of subsequent experience (Chapter 6), it is likely that these patients were also sensitive to amines, MSG, nitrates and

propionate and possibly other substances, which were not routinely included in the challenge set for patients with RIU/AO.

TABLE 4.7

FOOD REACTIONS TO COMPOUNDS NOT TESTED

Foods Reported Causing Reactions	Compound Responsible	Foods Reported Causing Reactions	Compound Responsible
Bacon	a,n	Chocolate	a,(s,p,c)
Bread	(s,a,p)	Corned beef	a,p
Cheese	a,(m,p,c)	Ham	a,p
Chicken	(m,s,a)	Mushrooms	a,s,m
Chicken soup	(m,s,a,c,p)	Pork	a
Chinese meals	(m,s,a)	Sardines	a

* s = salicylate, a = amine, m = glutamate, p = preservative, c = colour

Patients who Failed to Complete Dietary Investigation Programme

From a total of 1294 patients presenting with RIU/AO to the RPAH Allergy Clinic up to November 1985, 304 failed to return for follow-up. In order to ascertain the outcome in this group, each patient was sent a questionnaire by mail, and 119 replies were received. Of the remainder, 73 had changed address and 112 failed to reply.

Of those 119 who replied to the questionnaire 101 patients had commenced the elimination diet, 41 of whom improved symptomatically even though they did not continue through the challenge programme. Sixty-one percent of the patients reporting improvement on the elimination diet said they were still restricting their diet to some extent at the time of the survey, compared with only 28% of the 60 patients who failed to show improvement ($P < 0.001$ by the Chi-square test). By contrast, of the remaining 18 patients who did not start the elimination diet, only two patients indicated that they were restricting their diet when surveyed.

Overall, amongst patients who failed to complete the elimination and challenge programme the questionnaire response rate was 51% (Table 4.7). From a comparison of the replies with those of patients who had completed the programme (Table 4.2) it was evident that a similar spectrum of foods was incriminated by both groups in triggering recurrent attacks of urticaria (Table 4.8).

Patients who implicated "sugar" were commonly referring to sweet foods when questioned specifically, most of which contain salicylate (in the flavour), as well as preservatives and colourings in most cases. These patients were also asked whether they had experienced recurrent symptoms up to the time of survey. Sixty-four (53.8%) of those answering the questionnaire (with 30 patients avoiding certain foods) were still symptomatic, whilst 55 had suffered no recurrence (although 14 of these also continued to restrict their diet).

TABLE 4.8

COMMON PROVOKING FOODS OF PATIENTS WHO DID NOT COMPLETE ELIMINATION AND CHALLENGE PROGRAMME

Food	Chemical Content*	No of Patients	Food	Chemical Content*	No of Patients
"Preservatives"	p	16	Dairy products	a,(p,c)	5
"Colours"	c	13	Soft drinks	s,a,p,c	4
"Salicylates"	s	9	Orange	s,a	4
Fruit	s,a,p	9	Red meat	a	4
Alcohol	s,a,m,p	8	Not stated		4
Spices	s	6	Garlic	(s,a,m)	3
"Sugar"	(s,a,p,c)	6	Bread	p,(s,c)	2
Artificial flavour	(s,a,m)	6	Aspirin	s	2
Antioxidant	p	5	Tomato	s,a,m	2
Vegetables	s,a,m	5	Onion	s	2
Coffee	s	5	Strawberry	s	2

* s = salicylate, a = amine, m = glutamate, p = preservative, c = colour

DISCUSSION

There is little information in the literature documenting the natural history of RIU/AO. The largest, and most widely cited series is that of Champion et al. (1969) in which 554 patients were identified retrospectively through hospital records, and surveyed by questionnaire. Figures from this study showed that between 25% and 50% of patients had remitted within 6 months of onset (depending on whether they suffered from urticaria alone, angioedema alone, or both), although a significant minority had remained symptomatic for over ten years. (For comparison, the findings of Champion et al., 1969 are reproduced in Figure 4.1(b)). Earlier studies of Urbach and Gottlieb (1946) and Warin (1954) in smaller numbers of patients quoted even higher figures for the remission rate in the first six months, probably reflecting the inclusion of many cases of acute urticaria where symptoms subside within two to three weeks in about 90% of patients (Czarnetzki, 1986, page 31). More recently Levine (1975) made brief reference to his own earlier series of 88 patients with chronic urticaria, in which two-thirds of those contacted were in remission three years or more after presentation. The results of the present study indicate a rather lower remission rate than that described by Champion et al. (1969), with 50% of our patients still experiencing symptoms more than eight years after the onset, and 30% with urticaria still active after 20 years. This difference could be a result of patient selection bias towards a more chronic population at the RPAH Allergy Clinic, or due to the more thorough exclusion of patients with acute urticaria in a prospective study such as this.

An important issue not addressed adequately in previous studies is the role of avoidance of dietary precipitants in the control of RIU/AO. In the present study patients are defined as being in "remission" if they are asymptomatic, and remain so on an unrestricted diet. Many of those surveyed here would have learned to avoid specific foods known to trigger recurrences and would not have been classified as "active" by Champion et al. (1969), thus yielding more favorable figures for the remission rate. Regression analysis showed no significant correlation between duration of symptoms and age, sex, aspirin (ASA) reactivity or pattern of symptoms (urticaria alone, angioedema alone or both together). It is not possible to determine whether dietary restriction accelerates "remission" (defined as a loss of sensitivity to food and other triggering factors) without having studied a control group on a normal diet. However, using the data of Champion et al. (1969) as historical controls it does seem that the slope of the life-table analysis is similar to that observed in the present study (Figures 4.1 and 4.2), implying that dietary restriction is likely to have prevented symptom recurrences, but not necessarily to have accelerated the loss of underlying sensitivity.

The observation that amongst the patients surveyed here, recurrences were food-related in the great majority, most of whom continued to restrict their diet, supports the clinical value of the dietary elimination and challenge protocol in patients with RIU/AO, as outlined in Chapter 3. Moreover, the foods implicated generally corresponded to the challenge responses in individual patients, although in a few instances foods containing amines, MSG, nitrates or propionates were implicated. As outlined in Chapter 6, some patients with RIU/AO are sensitive to these substances when challenged, and they are now included as part of the routine challenge battery.

The foods most commonly found to trigger recurrences were those which contained salicylates (Table 4.6). At first sight it may seem that the amount of salicylate present naturally in foods (Chapter 2) would be too small to produce a significant effect, even in sensitive individuals, and that the dose used in challenge testing (300-600 mg of ASA) does not accurately reflect a normal dietary stimulus. However, the results of the present study strongly suggest that salicylate sensitivity as defined by reaction to a single high-dose challenge does indeed correlate closely with clinical symptoms triggered by salicylate containing foods. There are two possible explanations for this. Firstly, foods that contain salicylate are generally rich in other cross-reacting compounds, particularly benzoic acid and its derivatives (indeed salicylates are themselves 2-OH-benzoate derivatives), and these are often present at concentrations one or two orders of magnitude greater than ASA alone (Chapter 10). Thus, ASA challenge reactivity may be regarded as a marker of sensitivity to a range of closely related chemical compounds which may be present in milligram (or greater) amounts in certain foods. Secondly, even small amounts of salicylate such as found in an average Australian diet (estimated at being of the order of ten milligram per day; Chapter 2) may have cumulative effects over a period of time. Indeed, it has been found that ten to 25 mg of ASA daily for one to two weeks can reproduce the acute inhibitory effects of standard doses on platelet function (Weksler et al., 1985; Patrono et al., 1985). Sinzinger et al. (1984) has shown that as little as one milligram of ASA daily for seven weeks can have significant in vitro effects.

At a clinical level salicylate-containing foods were quite often seen to have cumulative effects in patients with RIU/AO. This became particularly obvious once the charts were drawn up listing foods according to salicylate content in an average serving. After the completion of challenge testing each patient who reacted to ASA was provided with these charts and given instructions to begin gradual liberalization after a six-week period of stabilization on their maintenance diet (Appendix 10). This involved the re-introduction of foods containing low levels of salicylate, initially in small quantities and at infrequent intervals; if no symptoms appeared, patients were instructed to increase the amounts eaten and frequency, and then to move on to foods in the "moderate" column (Table 4.1). In this way a patient was able, by trial-and-error to establish a threshold daily dose, beyond which symptoms would reappear. In many cases recurrence was insidious rather than acute, and it was therefore often necessary to return patients to the baseline "salicylate-free" diet for a two or three week period, allow symptoms to subside, and then begin liberalizing the diet more gradually. The importance of the dose of salicylate ingested was further illustrated by the reactions of patients to different forms of the same food. For example, some individuals could safely tolerate a slice of fresh tomato, but would react to the more concentrated substances in tomato sauce or tomato paste (in which salicylate content is often further increased by the addition of herbs and spices).

Using this approach, most patients were able to achieve a reasonable degree of variety in their diet without experiencing a recurrence of chronic symptoms. The extent of ultimate liberalization of the diet

was generally determined by each individuals' dose-threshold which often rose gradually with continued exposure, so that some patients could ultimately tolerate a virtually normal diet without a major recurrence of symptoms. In most cases, however, patients still found it necessary to limit their intake of foods with the highest concentrations of salicylate (e.g. wine, spices, Vegemite, strawberries, oranges, stone fruits and pineapple).

Re-introduction of additive-containing foods was often more difficult, presumably because of the higher concentrations of these substances present in processed foods, compared with the sub-threshold levels of ASA occurring naturally in many fruits and vegetables. Patients were therefore instructed to read labels carefully when shopping, and to be particularly cautious with soft drinks, fruit juices, dried fruits and other fruit-based, liquid and moist consistency foods (which are usually preserved with metabisulphite or benzoate).

Practical dietetic advice was an important aspect of successful long-term management in food-sensitive patients. For example, when eating out at restaurants or dinner-parties adverse reactions could often be prevented by avoidance of gravies, sauces and spices, as well as salicylate-rich vegetables which often accompany meat dishes, and by the judicious choice of wines taken in small amounts. If, for social reasons, avoidance was not possible on certain occasions, an anti-histamine taken after the meal was recommended to prevent a severe reaction. Similarly, travelling patients were advised to contact

airlines in advance for provision of suitable meals, and to discuss their requirements with hotel and restaurant staff (where it is almost always possible to obtain simple, plain roasts and grills).

Patients were also advised to avoid ASA-containing medications and to be cautious with other non-steroidal anti-inflammatory drugs which may provoke idiosyncratic reactions. Those sensitive to tartrazine were advised to avoid coloured capsules and tablets where possible; if necessary capsules could be opened and only their contents taken, and the coloured coating of tablets could be removed by rubbing the surface gently under running water.

Nutritional status could sometimes be compromised in patients on a highly restricted diet, but supplements were rarely required in patients with RIU/AO. If any doubt existed, a three to five day diet history was recorded and analysed using the "Soda" computer programme (Version 1.2, R.J. Hartley, 1982; Computer Models, P.O. Box 280, Bentley, Western Australia).

CHAPTER 5

SALICYLATE PHARMACOKINETICS

INTRODUCTION

As outlined in Chapter 4, follow-up of patients with RIU/AO showed that foods were frequently incriminated as a cause of recurrent symptoms. Furthermore, there was a very good correlation between the salicylate content of the foods reported and the results of blind challenge with ASA in individual patients, suggesting that the natural salicylates present in a wide variety of plant foods are indeed capable of producing clinical effects. From the laboratory analysis described in Chapter 2 it was calculated that an average daily diet contains between ten milligram and 100 mg of natural salicylate, but how much of this is actually absorbed is unknown. Indeed, the harsh treatment of foods required for efficient extraction (Chapter 2) suggests that much of the salicylate present in food may be chemically bound (e.g. as esters) or physically sequestered (Harbourne, 1980; Newby et al., 1980), and the extent to which this may be released in vivo by the normal digestive processes is as yet uncertain. It was therefore decided to conduct a feeding study, using normal subjects, in which urinary excretion of salicylates and their major metabolites would be measured as an index of the bioavailability of dietary salicylate.

Having established that dietary salicylates are indeed absorbed physiologically, the question arose as to why certain individuals may react idiosyncratically to these substances. It is generally agreed that the mechanism is unlikely to be attributable to immunological hypersensitivity (Chapter 10). Two other broad possibilities were therefore considered: (i) the patients with RIU/AO may have an

abnormality of salicylate absorption, metabolism or excretion, or (ii) they may have abnormal target-organ responsiveness. In order to test the first of these hypotheses a pharmacokinetic study was devised to compare serum levels and urinary excretion of salicylates and their metabolites in ASA-sensitive RIU/AO patients and normal controls. The results clearly indicated that salicylate disposition is normal in such individuals, at least with respect to their handling of a single high dose, making it likely that their abnormality lies in the end-organ response.

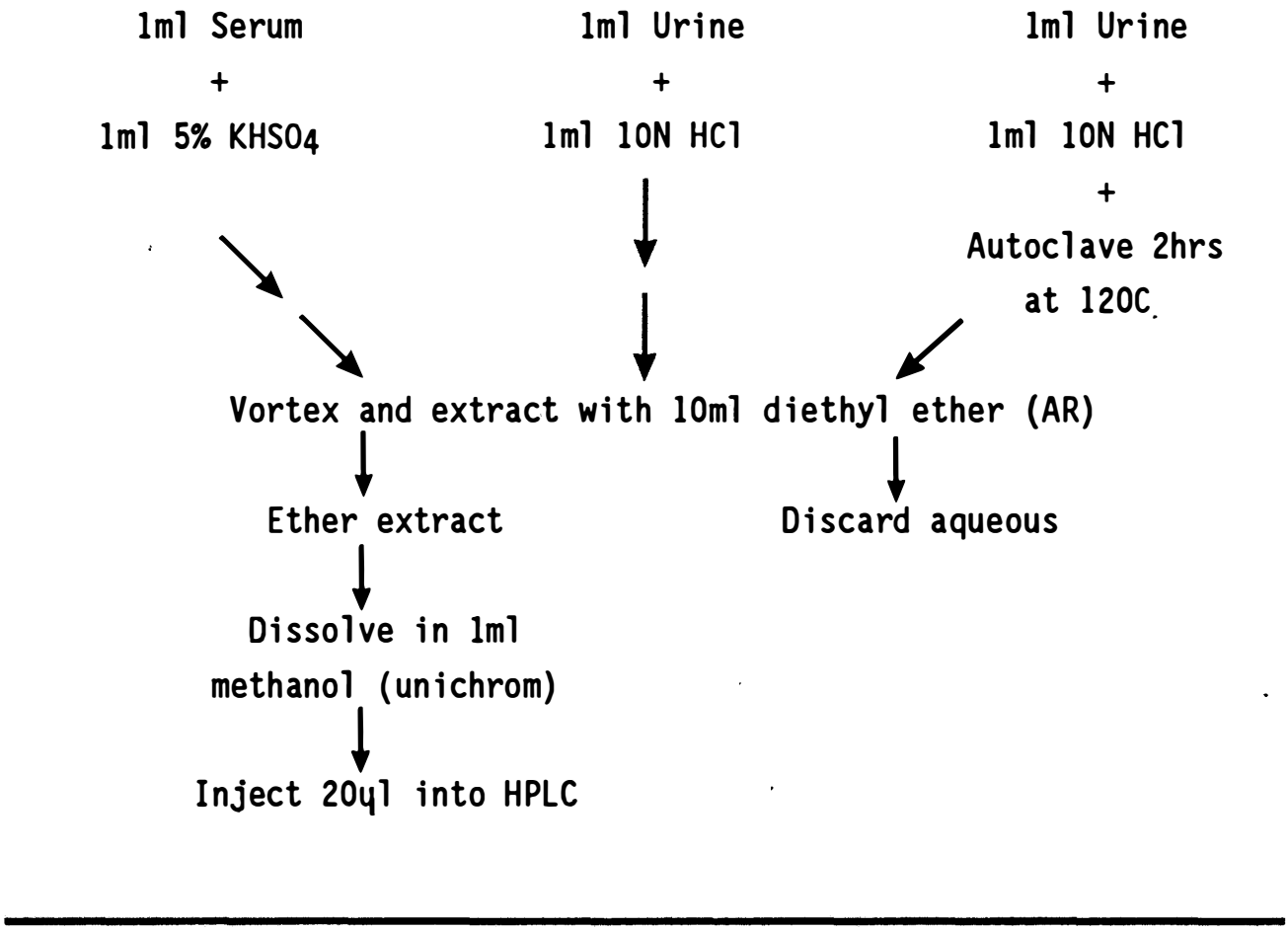
MATERIALS AND METHODS

Extraction of Salicylate in Urine

The concentration of total salicylic acid in urine was assayed by a modification of the method by Day et al. (1981). Samples were acidified and heated in order to break down all the major metabolites of aspirin (gentisic acid, salicyluric acid, salicylic acid, salicyl phenolic glucuronide and salicylacyl glucuronide) to salicylate, for which standards were available. Duplicate one millilitre aliquots of urine were acidified with one millilitre of 10N HCl (AR), sealed in a glass ampoule and heated to 120°C for two hours. Thereafter the acidified urines were extracted with five millilitres of diethyl ether (AR) by mixing on a vortex for 30 seconds and the ethereal extract pipetted into a storage vial and evaporated to dryness at room temperature in the fume cupboard. The residue was taken up in one millilitre methanol (unichrom) prior to injection of 20µl onto the HPLC.

FIGURE 5.1

SALICYLATE EXTRACTION IN URINE AND SERUM



Method of Salicylate Extraction from Serum

Salicylic acid in plasma was extracted by modifications of the manual method of Rowland and Riegelman (1967) as this gave the least emulsion formation of the extraction methods published that were tried (Brodie et al., 1944; Lange & Bell, 1966; Larsson & Fuchs, 1974; Bekersky et al., 1977; Caterson et al., 1978; Chrastil & Wilson, 1978; Peng et al., 1978; Cham et al., 1979; De Boer et al., 1979; Cham et al., 1980; Sioufi & Pommier, 1980; Ou & Frawley, 1982). One millilitre of serum was pipetted into a 15ml

conical centrifuge tube containing one millilitre five percent potassium bisulphate ($KHSO_4$)(AR) solution, (which acted as an acidifying medium and adjusted the pH to two) extracted with five millilitres diethyl ether (AR) by vortexing for 30 seconds and then centrifuged at 2500 rpm in an IEC centra refrigerated centrifuge at minus five degrees centigrade. The ethereal extract was pipetted into a storage vial and evaporated to dryness at room temperature in the fume cupboard. The residue was taken up in one millilitre of methanol (unichrom) prior to injection of 20ml into the HPLC.

HPLC Development

In order to conveniently investigate the disposition of salicylic acid in plasma and urine a direct, specific and sensitive assay was sought. The variety of published assay methods (Brodie et al., 1944; Schacter & Manus, 1958; Chirigos & Udenfriend, 1959; Umberger & Fiorese, 1963; Rowland & Riegelman, 1967; Thomas et al., 1973; Rance et al., 1975) presented problems as to their ease of operation, directness, specificity, and sensitivity required for the analysis of a large number of samples. HPLC provided a method that would simultaneously permit the separation and quantification of acetylsalicylic acid metabolites from serum and urine (Bekersky et al., 1977; Blair et al., 1978; Caterson et al., 1978; Peng et al., 1978; Cham et al., 1979; Cham et al., 1980; Day et al., 1981; Ou & Frawley, 1982). The use of a fluorescent detector rather than ultra violet detector maximized sensitivity whilst minimizing the background due to biological impurities (Bekersky et al., 1977; Blair et al., 1978; Day et al., 1986, 1987). Extensive sample clean-up was eliminated by this method and enabled rapid analysis of a large number of samples.

HPLC Separation

HPLC was carried out using a Varian 5060 pumping system with Rheodyne 7125 injection valve. A stainless steel column 125mm by 4.6mm, was packed with Spherisorb 5um ODS packing fitted with a precolumn packed with Vydac RPP389 packing material. The eluent was monitored with a Schoeffel FS 970LC fluorometer and a GM 970 monochromater operated at 315nm for excitation and emission greater than 389nm (Day et al., 1986). The isocratic mobile phase used was a mixture of methanol : water : glacial acetic acid (20 : 80 : 1.6) (Bekersky et al., 1977; Caterson et al., 1978; Cham et al., 1979; Cham et al., 1980; Day et al., 1981; Day et al., 1986) at a constant flow of two millilitres per minute. The fluorescence detector sensitivity was connected to a Varian CDS 111 computing integrator and Houston Omniscribe recorder.

Twenty microlitres of extracted urine and serum samples were injected onto the HPLC along with standards. Retention times and areas under the peak were monitored and computed automatically by the integrator. Identification was based on retention time and fluorescence wavelength compared with the standards, as well as by co-chromatography with the standards.

Dietary Study

(a) Subjects

Twenty nine healthy volunteers from the Dietetics Department and Immunology Department, RPAH, and the Human Nutrition Unit, University of Sydney, gave informed consent to participation in the study. The subjects were adults, aged 19 to 55 years, and consisted of 11 males

and 18 females. None had been taking any medication for the previous two weeks.

(b) Study design

Subjects were allowed to eat normally until the evening before the study began, but were asked not to take any aspirin-containing medications. After an overnight fast each was supplied with a low-salicylate diet for two days and then allowed to return to a normal diet for two weeks. At the end of this time, a high-salicylate diet was provided for a further two days, followed by a return to normal diet once again. At the beginning of each test diet, subjects were asked to empty their bladder before breakfast, and to collect all urine passed over the next three days in 24-hour aliquots. Collection bottles were preserved with thymol (BP) which does not interfere with salicylate estimations. At the end of each 24-hour collection, urine volume was measured and an aliquot placed in a specimen jar for storage at -20C.

(c) Food Preparation

All meals were prepared by the author (ARS) and supplied to the subjects (usually) in the Metabolic House. Savoury foods, desserts and cakes were prepared, packaged and frozen in advance; meat, eggs, salads, fruit, vegetables, sandwiches and tarts were freshly prepared the day before they were required. The high-salicylate diet was designed with an emphasis on fruits, vegetables and condiments known to be rich in salicylates (Chapter 2), providing an estimated 88 mg per day, whereas the "low" salicylate diet consisted of foods with no detectable salicylate content (Table 5.1).

TABLE 5.1

DIETARY INTAKE ON LOW AND HIGH SALICYLATE DIETS

Low Salicylate Intake	mg/serve	High Salicylate Intake	mg/serve
Weetbix + milk + sugar	0	Orange juice	1.00
Poached egg	0	Dried fruit + honey	8.55
Toast + butter + G.syrup	0	Toast + butter + honey	2.74
Andronicus coffee + milk	0	Tetley tea (1 bag)	2.79
Plain oatmeal biscuit	0	Almond + date slice	5.37
Andronicus coffee + milk	0	Tetley tea (1 bag)	2.79
Egg and lettuce sandwich	0	Savoury meat loaf	7.08
Peeled pear	0	Carrot + raisin salad	2.69
Banana	0	Cherry almond tart	6.06
		Tetley tea (1 bag)	2.79
Plain oatmeal biscuit	0	100g sultanas + raisins	7.21
Andronicus coffee + milk	0	Tetley tea (1 bag)	2.79
Lamb chops	0	200ml orange juice	1.00
Potato chips	0	Curry + boiled rice	16.31
Lettuce	0	Almond apricot pie	4.46
Tinned pears	0	Dried fruit + honey	5.02
Baked rice custard	0	Icecream	
Plain oatmeal biscuit	0	Almond + date slice	5.37
Andronicus coffee + milk	0	Tetley tea (1 bag)	2.79
Total salicylate (mg)/day	0		88.27

(d) Statistical Analysis

The 24-hour urinary salicylate levels in the periods on the high and low salicylate diet were compared using the Paired Student's T-Test. The data were paired for each of the three days and the differences were assumed to be normally distributed.

Aspirin Challenge Study

(a) Subjects

Two groups were studied : salicylate-sensitive patients with RIU/AO and normal control subjects. Patients with RIU/AO were chosen from among those investigated prior to August 1984 in the Allergy Clinic at RPAH; all had responded to the elimination diet and were shown to be salicylate sensitive on blind challenge. Patients known to be compliant and reliable were contacted, and after explanation of the protocol 23 individuals consented to participate in the study. When tested previously, five patients had reacted to aspirin alone and the remaining 18 had reacted to aspirin and between one and seven other challenges. Nine males (39%) and 14 females participated in the study, their ages ranging from 15 to 70 years (median age 38). The duration of RIU/AO prior to presentation ranged between four weeks and 36 years (median three years).

Control subjects were ten healthy female volunteers with no history of RIU/AO, from the Department of Dietetics and Metabolic Ward at RPAH. Each was asked to abstain from aspirin-containing medications and to follow a low salicylate diet for two weeks before the study in order to ensure an equivalent salicylate intake to that of the subjects with RIU/AO.

(b) Study Design

The low salicylate diet followed by all subjects was the same as that used for the management of patients with RIU/AO (Chapter 3, Appendix 7). After an overnight fast the subjects presented to the metabolic ward at RPAH. On arrival, each was asked to empty their bladder for a baseline urine measurement, and a 21-gauge indwelling "butterfly" needle was placed in a suitable forearm vein. The needle was flushed with heparinized saline to maintain patency, and before collection of each sample approximately two millilitre of blood was aspirated and discarded to clear the needle. Each subject was then given one 300mg soluble ASA tablet dissolved in approximately 200ml of water, and blood and urine samples taken at the following intervals:

Blood: 0, 3, 6, 10, 15, 20, 25, 30, 40, 50, 60, 75, 90, 105 minutes, 2hr, 2.5hr, 3hr, and then hourly up to 10 hr. (Rowland et al., 1972).

Urine: hourly for 10 hours (Graham et al., 1977). At the end of the day patients and controls were given a bottle to take home and asked to collect urine overnight, ending the collection at 24 hours.

Blood samples of 10ml were allowed to clot in plain plastic tubes at room temperature. The blood was then centrifuged at four degrees centigrade, serum pipetted off and stored at -20°C until assayed. The urine was collected in 500ml glass beakers, measured, and stored in sterile plastic specimen jars at -20°C until assayed. A water intake

of 200ml/hr was maintained for ten hours and a light breakfast, lunch and dinner were allowed at two hours, five hours and ten hours after administration of the ASA.

RESULTS

Dietary Study

The total salicylate excretion in each 24-hour period on the low and high salicylate diet was calculated and tabulated for individual subjects (Table 5.2). Two of these (W.B and A.T.) took part in the low salicylate diet period but were unable to complete the study as they were overseas at the time. In a further three patients one or two urine samples were discarded as the frozen aliquots thawed when the freezer was accidently left open.

For each day of the low and high salicylate diet period, the mean, standard deviation (SD) and standard error (SE) of the total urinary salicylate, was calculated for the subjects as a group (Table 5.2 and Figure 5.2). The mean excretion of salicylate on the "low" diet decreased significantly from a baseline of 81.66 mg to 37.47 mg over the three days studied ($P < 0.002$). Conversely, on the "high" diet, mean salicylate excretion increased from 96.32 mg to 114.34 mg ($P < 0.002$). Overall, the difference between day one on the "low" and "high" diet period was not significant ($P < 0.2$), but on days two and three the differences increased to 53.7 mg and 76.87 mg respectively ($P < 0.001$).

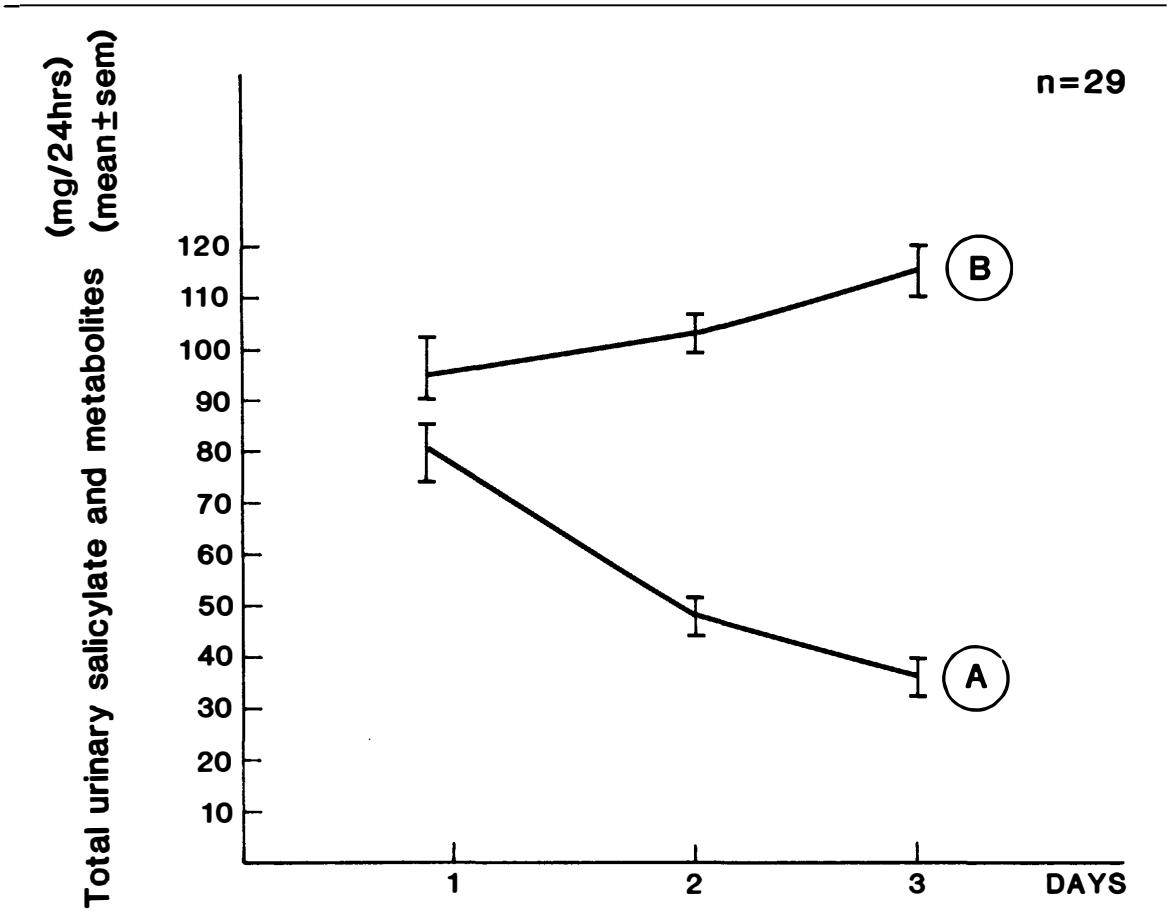
TABLE 5.2

TOTAL SALICYLATE EXCRETED (MG/24 HOUR URINE)

Subjects	Low Salicylate Day 1	Low Salicylate Day 2	Low Salicylate Day 3	High Salicylate Day 1	High Salicylate Day 2	High Salicylate Day 3
B.R	28.80	18.83	2.41	37.23	106.80	176.80
S.H	137.18	42.87	22.58	107.67	119.64	113.86
J.H	42.62	85.73	69.56	122.67	112.70	120.80
H.B	71.60	67.17	71.47	173.98	133.01	111.94
T.G	119.56	45.40	49.48	94.20	115.30	99.10
Y.F	76.09	72.75	72.28	96.80	99.48	89.78
M.P	147.60	41.73	48.33	98.68	82.65	117.36
A.S	35.50	25.52	-	131.70	150.12	91.56
S.V	130.13	53.27	28.48	54.36	105.28	126.54
M.A	107.60	68.37	55.91	115.50	121.73	-
M.O	89.33	4.48	2.66	-	75.80	-
N.S	60.90	54.00	55.67	44.39	125.96	132.12
D.M	69.54	33.41	5.41	145.82	87.84	59.49
K.B	43.51	37.54	45.15	87.29	78.68	79.20
J.K	43.37	23.13	13.92	101.35	103.66	144.20
G.B	93.32	76.84	53.48	67.99	96.25	85.05
A.S	83.50	51.71	18.02	83.90	102.61	135.12
W.B	81.90	53.07	37.96	-	-	-
I.D	94.18	46.12	11.88	95.47	109.40	184.00
P.W	70.54	27.83	33.44	81.27	92.04	118.99
M.D	127.36	67.50	79.12	114.96	117.41	60.20
G.T	32.94	22.09	1.96	49.21	64.50	96.60
P.D	78.48	59.15	60.59	112.30	88.46	80.60
S.S	101.49	92.13	24.39	122.40	100.44	148.40
S.D	80.94	43.01	61.90	133.89	65.95	122.90
V.C	121.09	63.84	53.00	38.76	136.99	148.95
D.T	84.86	80.85	29.12	149.04	98.78	124.53
R.L	27.15	28.14	21.40	43.38	74.96	90.30
A.T	86.95	27.48	18.90	-	-	-
Mean	81.66	48.76	37.47	96.32	102.46	114.34
S.E	6.26	4.03	4.49	7.23	4.13	6.39

FIGURE 5.2

TOTAL SALICYLATE EXCRETION



A. Urinary salicylate of patients on low salicylate diet

B. Urinary salicylate of patients on high salicylate diet

Aspirin Challenge Study

In order to determine whether patients with RIU/AO might have an abnormality of ASA pharmokinetics, 23 salicylate-sensitive patient and ten controls were given a simple oral dose of 300 mg ASA, whilst

on a low-salicylate diet, and their blood and urine collected over a 24 hour period. The results of serum salicylate levels are shown in Figure 5.3, and urinary excretion in Figure 5.4, in each case with the control values superimposed over those of the test subjects. No significant differences were seen in either serum or urinary salicylate levels at each time point, suggesting that there is unlikely to be any abnormality in the absorption, metabolism or excretion of a single dose of ASA in patients with RIU/AO.

FIGURE 5.3

SERUM SALICYLATE LEVELS

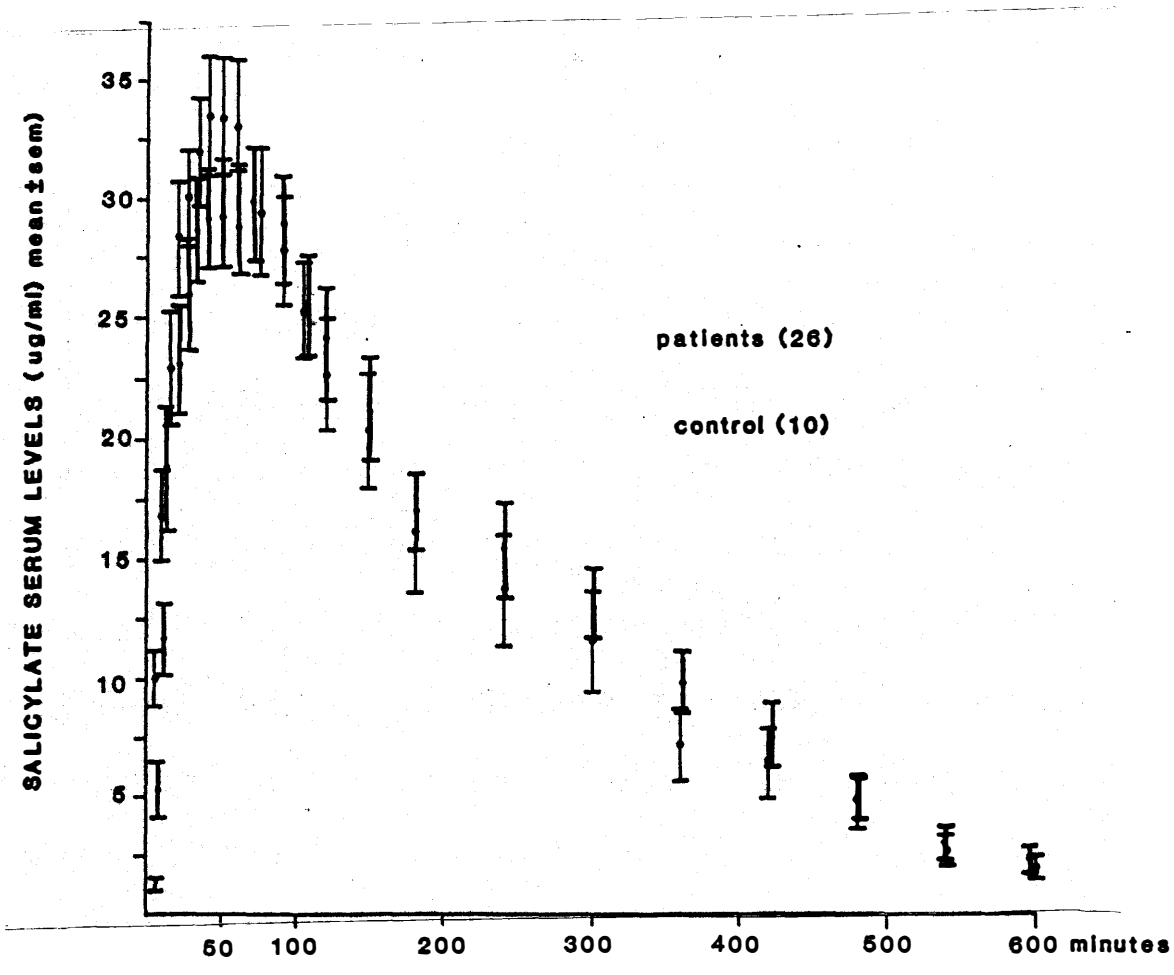
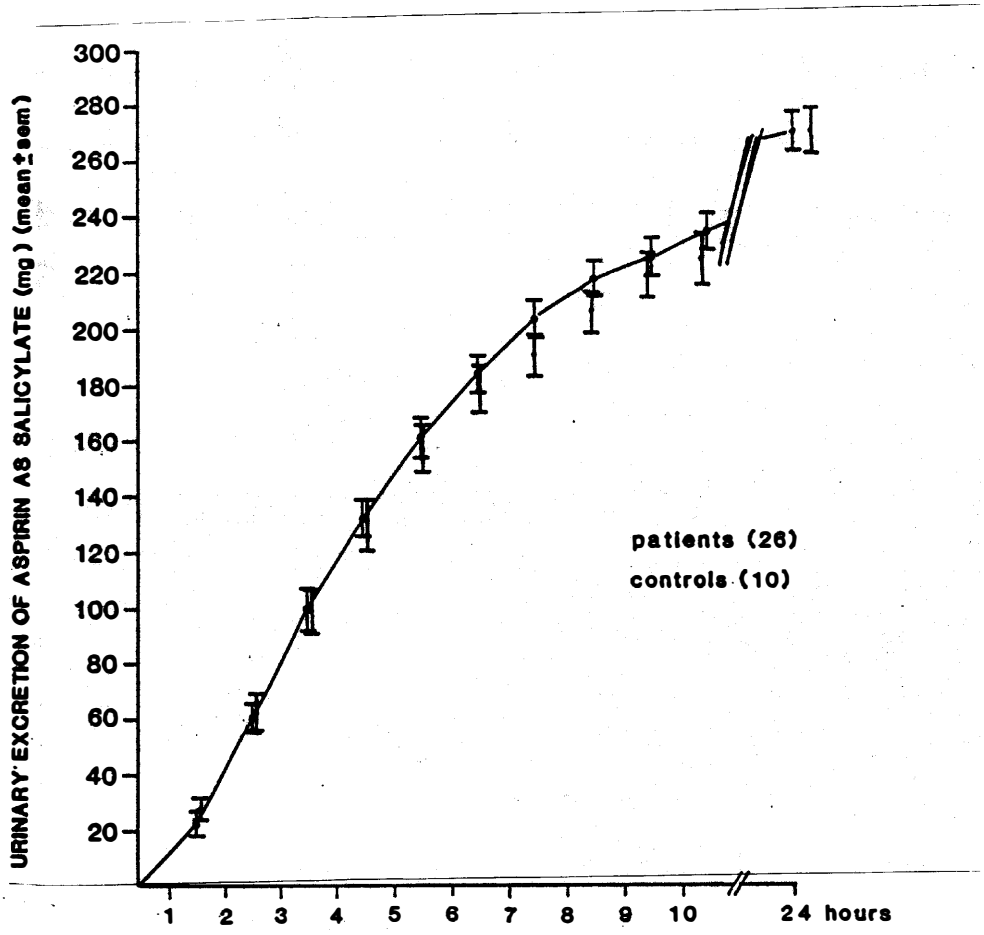


FIGURE 5.4

URINARY SALICYLATE EXCRETION



DISCUSSION

The absorption, distribution and metabolism of pharmologically administered salicylates is exceedingly complex (reviewed by Rainsford, 1984, pp. 32-66). Aspirin and salicylic acid have been the most extensively studied: they are weak acids, and are rapidly absorbed as the non-ionized forms at low pH in the stomach. Salicylic acid appears in the serum more rapidly, partly because of its higher partition coefficient and stronger binding to plasma proteins, and

partly because of the delay in transport of ASA due to its interaction with mucosal esterases. Plasma levels increase with first-order kinetics, reaching a peak between 20 minutes and 2 hours after a single oral dose, although there is a high degree of inter-subject variability (Rowland et al., 1972). More than 90% of the salicylate and 85% of ASA in plasma is bound to albumin, but this can be modified by age, dietary protein intake, pH, plasma lipids, drug therapy and disease states (including hypersensitivity; Storm van Leeuwen, 1924). There are thought to be two binding sites: a primary one resembling that for tryptophan and its derivatives, and a secondary site rather like that occupied by long-chain fatty acids and anionic azo-dyes. Salicylates may competitively displace these, as well as other bound drugs, hormones and metabolites (Davison, 1971). Free and bound forms are in dynamic equilibrium and the free concentration determines both therapeutic and adverse effects (Rainsford, 1984).

Aspirin is rapidly hydrolysed to salicylate by esterases in the gastrointestinal tract, blood, liver, kidney and other organs. More than 50% of an orally administered dose may be extracted and/or metabolized on first pass through the liver. Metabolism occurs through hydroxylation or conjugation, summarized in Figure 5.5. The capacity for formation of glycine- and glucuronide conjugates is limited, and can be competitively inhibited by other phenols, which may therefore be an important factor in determining toxicological effects. Excretion of all metabolites is almost entirely via the urine; salicylate itself undergoes glomerular filtration, passive proximal reabsorption and active tubular secretion, and the latter

can be inhibited by competing acids (including benzoate metabolites). Reabsorption is strongly pH-dependent with clearance increasing dramatically above urine pH levels of 6.5 (Davison, 1971).

The main parameters governing salicylate pharmacokinetics are shown in Figure 5.6. Rate constants can be calculated from mathematical models comprising two or more compartments (Rowland et al., 1972), although the situation becomes very complex with chronic administration. Thus, the steady-state plateau levels achieved by regular ingestion increase more than proportionately with increasing dose, as does the time required to reach the plateau (Levy, 1968; Levy & Tsuchiya, 1972), probably due to saturation of the pathways responsible for biotransformation to salicyluric acid and salicylphenolic glucuronide (Levy, 1965, 1966, 1978).

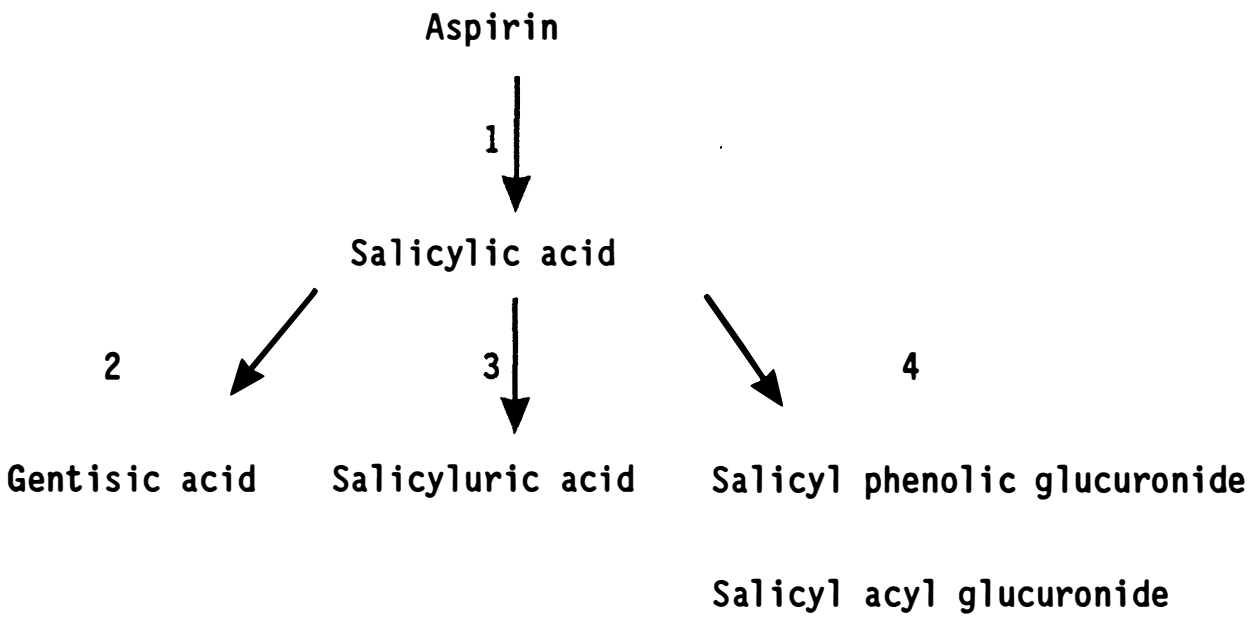
The disproportionality is more marked with respect to free than bound salicylate due to decreased binding by albumin with increasing concentrations (Levy, 1980). In addition to this there are marked inter-individual variations in salicylate pharmacokinetics, with a four-fold range of areas under the concentration-time curve.

The degree to which salicylates in foods are present as free ASA and salicylic acid, versus chemically bound esters or the physically sequestered compounds is unknown. The feeding study described in this Chapter was designed to examine the bioavailability of dietary salicylates by measuring their urinary excretion products on a high and low-salicylate diet (Swain et al., 1985). No previous study has attempted to examine the gastrointestinal absorption of dietary salicylates, although many studies suggest that they can produce

significant clinical effects. Interestingly, many pharmacologists have noted small but detectable levels in the serum of individuals not exposed to any salicylate-containing medications, and this has usually been attributed to "background noise" in the assay system rather than to genuine absorption from dietary sources (R.O. Day, personal communication).

FIGURE 5.5

SALICYLATE METABOLISM

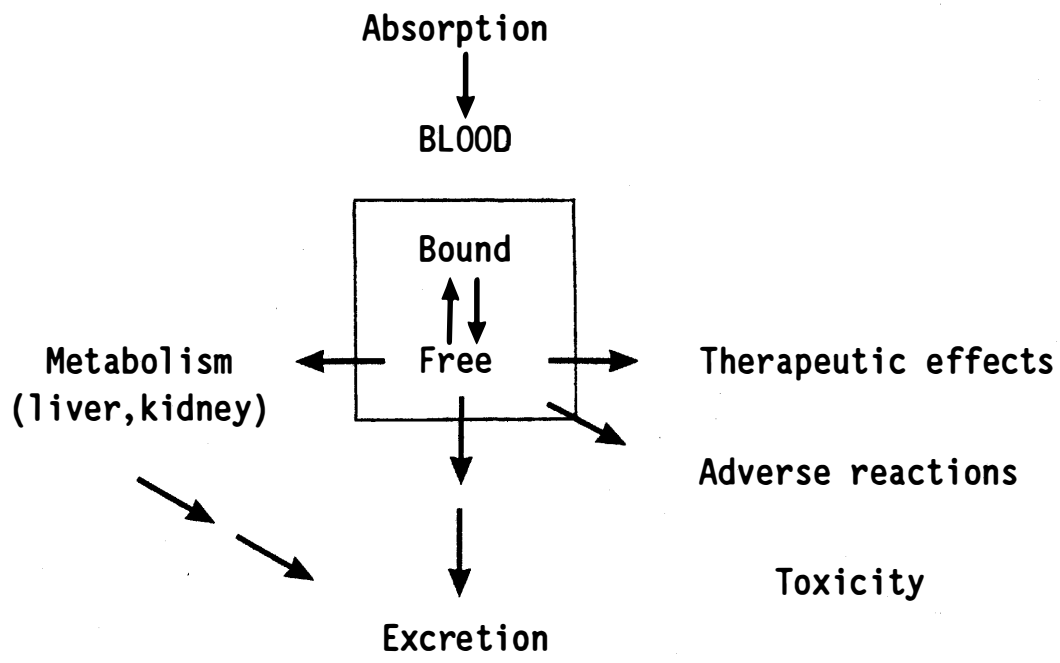


1. Spontaneous, aspirin esterases or protein acetylation
2. Hydroxylation (hepatic microsomal cytochrome P450)
3. Glycine conjugation (liver, kidneys)*
4. Glucuronic acid conjugation (gastrointestinal tract, liver)*

(* capacity limited processes)

FIGURE 5.6

SALICYLATE BIODISTRIBUTION



Baseline levels of total urinary salicylates in the subjects studied here were approximately 80 - 100 mg per day (range 29 to 149; Table 5.2), and assuming these to be steady-state values they can be taken to reflect the amount of salicylate absorbed per day from the gastro-intestinal tract. Although the bowel microflora are capable of synthesizing certain phenolic substances there is no evidence that they are capable of producing salicylates. It is therefore reasonable to conclude that the baseline excretion is equivalent to the amount absorbed from the normal daily diet. In retrospect it would have been interesting to have taken a diet history from the each of the subjects before they entered the study in order to estimate their daily intake. Nevertheless, the excretion of the group as a whole

corresponds to the calculated salicylate content (10-100 mg) of an average Australian diet (Chapter 2), suggesting that the great majority is indeed released by the normal digestive process and is available for absorption.

During the two days on a low salicylate diet mean urinary excretion fell progressively to approximately half the baseline; similarly, on the high salicylate diet excretion increased by almost 20 mg/24 hour and had clearly not yet reached a plateau on day three. Given the complexities of salicylate pharmacokinetics it may well have taken several more days or even longer, to reach a steady-state in each case. It therefore remains for future studies to determine the extent to which salicylates can be completely eliminated from the body on a low salicylate diet, and the extent to which they may accumulate on a salicylate-rich diet.

Inter-individual variability in salicylate pharmacokinetics is well documented, and can be quite marked, as outlined above (Levy, 1980). It was therefore of interest in the present study to ask whether pharmacogenetic differences might be a significant factor in determining the susceptibility of certain individuals to aspirin-induced RIU/AO.

This question was approached by comparing the pharmacokinetics of ASA in salicylate-sensitive RIU/AO patients with that of a normal control group. All subjects followed a low-salicylate diet for a 2 week period, at the end of which they give a single dose of 300 mg of ASA orally, and serum and urinary salicylate levels were measured at

appropriate intervals over the next 24 hours. As in other studies serum levels peaked at around 60 minutes and declined exponentially over the next 10 hours; urinary excretion increased progressively over this time, and remained near plateau levels overnight (Graham et al., 1977; Levy, 1978). No significant differences were seen in the RIU/AO patients compared with the control group (Figure 5.3 and Figure 5.4). This finding makes it unlikely that abnormal ASA metabolism is the reason for salicylate intolerance in RIU/AO, although in view of the complexities of salicylate accumulation kinetics outlined above, patients given regular ASA doses may have shown significant differences after several days. However, the fact that 20 out of the 26 RIU/AO subjects developed urticaria within 36 hours of the single ASA test dose, whilst their serum and urinary salicylate levels were no different from controls, suggests that the abnormality is more likely to lie at the level of end-organ responsiveness (Chapter 10).

INTRODUCTION

Although this study was begun with the intention of defining the role of diet in RIU/AO, clinical experience in the first three years indicated that food reactions could also provoke a number of other symptoms in susceptible individuals. Initially we, like Juhlin (1981), observed that some patients presenting with RIU/AO also suffered other concomitant symptoms, such as headache, abdominal pain, rhinitis or asthma, and that these symptoms could be provoked by specific challenges. Later, as the spectrum of the Allergy Clinic population changed (Chapter 1) it became clear that these non-cutaneous manifestations of food intolerance could occur as isolated syndromes, or in various combinations.

During this time it also became evident that a number of patients experienced vague constitutional and/or neuropsychiatric symptoms in conjunction with their somatic complaints, and that these too could subside with dietary restriction and reappear with subsequent challenge. The increasing numbers of patients presenting with subjective symptoms made it imperative to use a double-blind challenge protocol which included suitable placebos. As a result the challenge battery developed for investigation of RIU/AO was modified in 1981. A number of other substances not previously tested in urticaria, but implicated in the literature as causes of various non-cutaneous adverse reactions, were included (Table 6.1), along with a change to

sucrose and starch as placebos. In addition, the challenges were numbered in an arbitrary order which varied from one patient to the next, thus minimizing observer bias when assessing the challenge reactions.

Since that time approximately 3,000 patients have attended the Allergy Clinic for dietary investigation of non-urticarial conditions including migraine, irritable bowel syndrome, asthma and eczema, as well as certain controversial syndromes such as "hyperactive" behaviour disturbances in children and vague symptoms suggestive of psychoneurosis in adults. This chapter outlines the results of dietary investigation in such patients, and provides a broad view of the clinical spectrum of food intolerance within which RIU/AO may be viewed as one amongst several inter-related syndromes.

MATERIALS AND METHODS

Modification of Elimination Diet and Challenge Programme

The elimination diet used for RIU/AO patients (Chapter 3) was further modified and restricted with the exclusion of wheat and milk products for patients presenting with eczema, asthma, irritable bowel syndrome, aphthous ulcers, migraine, hyperactivity and systemic symptoms (Appendix 2). At the same time the range of challenge compounds was extended, and starch and sucrose were introduced as placebos (Tables 6.1 and 6.2).

TABLE 6.1

ADDITIONAL SUBSTANCES IMPLICATED IN ADVERSE REACTIONS TO FOOD

Food or Substance	Literature
Milk	Harrison et al., 1976; Atherton et al., 1978; Blumenthal et al., 1981; Francis, 1982;
Wheat	Ferguson, 1976; Dodge, 1980; Francis, 1982;
Eggs	Meara, 1965; Atherton et al., 1978; Francis, 1982; Ford & Taylor, 1982
MSG	Kwok, 1968; Schaumberg et al., 1969;
Nitrate	Henderson & Raskin, 1972; Moneret-Vautrin et al., 1980;
Antioxidants	Juhlin et al., 1972; Juhlin, 1981;
Amines	Bethune et al., 1963; Hanington, 1967; Hanington, 1980; Moneret-Vautrin et al., 1979; Kalish, 1981;
Sorbic acid	NH&MRC, 1986
Sodium propionate	NH&MRC, 1986
Erythrosine	Augustine & Levitan, 1980;

TABLE 6.2

MODIFICATIONS TO CHALLENGE SET

Dates	Modifications to Challenges
November 1981	Double blind protocol
	Placebos:
November 1981	starch
January 1982	sucrose
November 1981- May 1984	B carotene (160a)*
	Multiple acetylsalicylic acid doses:
May 1984- January 1986	two doses of acetylsalicylic acid
	Preservatives:
November 1981- January 1982	propyl gallate (310)*
May 1984	addition of: sodium propionate (281)*
November 1981- May 1984	reduction of: sodium nitrate (251)*, sodium nitrite (250)*
	Artificial colours:
May 1984	addition of: erythrosine (127)*
	Miscellaneous:
November 1981- May 1984	bakers yeast

* Code number for additives in food (NH&MRC, 1986)

The challenge set was made double-blind with two placebos in order that the results obtained would be as objective as possible. The dose effect was studied with the acetylsalicylic acid challenge where several doses were randomly given to patients. Similarly three colour challenges and a composite colour and preservative challenge were given to a random selection of patients so that correlations could be made. Propionic acid was added to the set after a pilot study was performed as it is a widely used preservative in yeast-leavened bakery items. Several challenges were omitted: bakers yeast because it was found to be denatured by baking, B carotene as it was found to be contaminated and propyl gallate as it was an oil and difficult to encapsulate. The dose of the challenge of sodium nitrate and sodium nitrite was reduced as some patients experienced severe reactions.

Patients were instructed to commence the challenge procedure of food and capsule challenges after at least two weeks on the elimination diet with five asymptomatic days. Open challenges with milk and wheat (as well as eggs in those with eczema) were taken first, and these foods were added to the baseline elimination diet if there was no obvious reaction. Double blind chemical challenges including placebos were then administered (Appendix 4). Challenge capsules were taken in the morning half an hour before or two hours after a breakfast (for children the capsule contents were mixed with golden syrup or mashed potato if they were unable to swallow the capsules). The capsules were spaced by at least 48 hours to allow for delayed reactions and any response to challenge was followed by a pause of a

further three symptom free days before proceeding to the next challenge, since patients were often found to experience a temporary refractory period during which they are unresponsive. Some challenges were given divided into "A" dose and a "B" dose, representing a small dose ("A") and a larger dose ("B") of the same or related compounds. These were taken on the same day, the small dose ("A") first and if there was no reaction within two hours the second dose ("B") was taken. However, if a noticeable reaction occurred to dose "A" the subsequent dose "B" was not taken.

In some patients, double blind capsule challenge was not practical or feasible and in these cases open food challenge were carried out. This mainly occurred in (a) very young children who were unable to swallow the capsules or the powder, (b) patients who were terrified by the thought of taking capsules or their effect or (c) patients who lived out of town without a close general practitioner who could supervise their challenges. Open challenges consisted of specially selected foods according to chemical composition (Appendix 6). Patients were asked to record the time, type and quantity of food taken, and if a reaction occurred the type and severity of symptoms and their time of onset and duration.. With a positive food challenge, re-challenge was performed three times to confirm the reaction. When a food challenge provoked a reaction the patient was instructed to wait until symptoms had cleared and then allow a further three days before taking the next food challenge. If no symptoms occurred the next food challenge was taken after 48 hours.

Clinical and Dietary Evaluation

Each patient was assessed by a physician at the Allergy Clinic before embarking on dietary investigation and was assigned to a clinical category as shown in Table 6.3

TABLE 6.3

SPECTRUM OF PATIENTS SEEN AT THE ALLERGY CLINIC FROM APRIL 1977 TO SEPTEMBER 1986

Primary Diagnosis	Number of Patients
RIU/AO (Chapter 3)	1349
Eczema	233
Asthma	251
Irritable bowel syndrome	358
Aphthous ulcers	46
Migraine	237
Hyperactivity	301
Systemic	809
Unclassified	817

Eczema:

All patients had been previously seen by a general practitioner, paediatrician and/or dermatologist, and most had been prescribed topical steroids and moisturizers at some stage. A diagnosis of atopic eczema was made on clinical grounds, based on the history,

typical appearance and distribution of skin lesions, a family history of atopy, and in some cases measurement of total and specific IgE. If there was any doubt about the diagnosis patients were seen by the Allergy Clinic dermatologist before dietary intervention. In addition to the standard elimination diet, the common food allergens (eggs, milk and wheat) were also routinely excluded from the diet in the patients with eczema initially. Because of severe inflammation and excoriation patients generally took 6 to 8 weeks to settle, and in the meantime were encouraged to continue using topical therapy. Unless there was a clear-cut history of specific food allergy each patient routinely underwent open egg, milk and wheat challenge; foods tolerated without any recurrence of symptoms were added to the baseline diet before the administration of double-blind chemical challenges. Any reactions were treated topically if necessary, and allowed to settle completely before any further challenges were administered.

Asthma:

Regardless of whether previous investigation had been carried out all patients with a history of asthma underwent formal spirometry and testing of bronchial reactivity to histamine. Based on the dose of histamine required to provoke a fall in $FEV_{1.0}$ of >20%, patients were classified as having mild, moderate or severe bronchial hyperactivity (Yan et al., 1983). If dietary triggering factors were suspected by the patient and/or physician, patients were placed on the elimination diet and asked to monitor daily peak-flow rates and/or symptoms. Improvement was assessed according to both subjective and objective parameters, including chest tightness, exercise tolerance, nocturnal wheezing, medication requirements, peak-flow rates and/or spirometry.

Challenges were supervised according to the degree of bronchial hyperreactivity as measured at presentation: those with mild reactivity to histamine were challenged either at the clinic or under the supervision of their general practitioner, those with moderate hyperreactivity were challenged in the Allergy Clinic or under the supervision of a respiratory physician, and those with severe hyperreactivity were admitted to hospital for challenge. The challenge protocol for asthmatics was modified to allow for graded dosages as outlined in Appendix 5.

Irritable Bowel Syndrome (IBS):

Aproximately two thirds of such patients were referred to the Allergy Clinic by a gastroenterologist, and most of the remaining patients had previously been investigated with a barium meal and/or endoscopy. The approach to clinical evaluation at the Allergy Clinic corresponded to that described by Kruis et al. (1984), although a numerical score was not assigned. If there was any uncertainty about the diagnosis at presentation, patients were referred to a gastroenterologist before undergoing dietary investigation.

Mouth Ulceration:

Approximately two-thirds of the patients presenting with "idiopathic" aphthous ulceration were referred by Professor M. Jolly, Department of Oral Surgery, School of Dentistry, University of Sydney. Each patient was assessed by a physician at the Allergy Clinic to exclude underlying organic disease before undergoing dietary investigation. On the elimination diet symptoms often improved markedly within two to six weeks, but in severe cases complete resolution could take up to 12 weeks.

Migraine:

Patients with migraine were mostly referred by their general practitioner although approximately one-third had previously been investigated and treated by a neurologist. Diagnostic criteria and grading of severity were as described by Lance (1986). Most patients suffered from "common" migraine, with or without episodic "classical" migraine attacks, and in general only those with moderate or severe headaches occurring more than twice a month were sufficiently motivated to warrant dietary investigation. On the elimination diet "significant" improvement was considered to have occurred if there was (a) complete resolution of headaches, (b) cessation of a need for medication, or (c) a reduction in severity and/or frequency of headaches by 50% or more.

"Hyperactive" Children:

Three hundred and one children with behaviour disturbances presented to the Allergy Clinic, often having been diagnosed as "hyperactive" and generally seeking dietary investigation because of suspected food reaction. The problems associated with this as a diagnostic entity are considered in detail below (see Discussion). Each child was screened by a paediatrician, Dr. V. Soutter, at the Allergy Clinic before undergoing dietary assessment. The most common presentation was of an over excitable, irritable child who cried easily and exhibited unpredictable mood swings and/or sleep disturbances. The parents often described the child as being unresponsive to the usual disciplinary measures when "out of control", and as being generally "difficult to live with". As in other series, three-quarters of the children were boys, the average age being seven years. Although

"soft" neurological signs were sometimes present, none of the children had major abnormalities such as epilepsy or mental retardation. Almost all families had previously sought help from one or more other sources without success (for example child psychologist, paediatrician, psychiatrist, community health centre or baby health clinic). About half had previously tried the Feingold diet, often with partial benefit, or sometimes with initial improvement followed by relapse.

In general, a non-judgemental attitude was adopted towards parents' beliefs concerning their child's behaviour. Unless contraindicated (see below), the elimination diet was used as a screening procedure to select children who were (a) more likely to be diet-sensitive, and (b) sufficiently compliant to undergo double-blind challenge testing. For several reasons, psychometric tests and behaviour ratings were not carried out. Behavioural changes were often complex, variable and intermittent, and it was felt that those in most intimate contact with the children, namely parents, were in the best position to make a judgement. Futhermore, as pointed out by Weiss (1985, 1986) children with situational hyperactivity are often able to exert a degree of voluntary control over their behaviour during the first two or three encounters with a physician, or at school, accounting for the fact that in previous studies parent ratings have not always agreed with doctor and/or teacher ratings. Finally, parents were often very stressed by having to cope with one or more difficult children, and good compliance would have been more difficult to achieve with a complex protocol. To ensure scientific validity of

the outcome, reliance was therefore placed on the double-blind placebo-controlled challenge protocol (which corresponds to the guide lines suggested by the Nutrition Foundation Advisory Committee Report, 1980).

In families where there was a poor mother-child relationship formal elimination and challenge testing was not recommended. Children who exhibited sociopathic behaviour or hostility towards the mother, making compliance unlikely, were given general advice about avoidance of additives and problem foods on an empirical basis. A similar approach was adopted in cases where it was suspected that a strict diet might be used by the mother as an instrument of child abuse (Meadow, 1977).

Children with behaviour disturbances who underwent formal dietary testing were prescribed the same elimination diet as that used for RIU/AO (Appendix 1) unless they suffered gastrointestinal symptoms, in which case wheat and milk were also restricted initially (Appendix 2). For children, a special approach was required both at the initial interview and throughout the diet, with much time spent discussing in detail how to cope with school lunches, tuckshop, birthday parties, weekends at friends, treats, takeaway food and cooking for the rest of the family. Many parents chose to challenge their children in the school holidays so that it would cause the least disruption to their progress at school and so they could best monitor their response.

Adults with "systemic" symptoms:

Patients were classified as having "systemic" symptoms if there were two or more organ systems involved, and if there were constitutional symptoms present such as headache, malaise, lethargy and/or myalgia. Since symptoms of this kind are quite non-specific patients were carefully screened to exclude organic pathology and overt psychiatric illness. Where indicated on clinical grounds, appropriate laboratory tests were carried out or psychiatric assessment was arranged. The clinical characteristics of this heterogeneous group of patients are described further in the results and discussion sections below.

Patient Management

Following confirmation of a diagnosis of eczema, asthma, irritable bowel syndrome, aphthous ulcers, migraine, hyperactivity or systemic symptoms by the physician each patient was interviewed by a dietitian who outlined the elimination diet in detail (Chapter 3). Patients were advised to take an uncoloured multivitamin and a calcium supplement and were given detailed instructions about permissable "over the counter" medications.

In these patients frequent contact and with the dietitian over the telephone was necessary for encouragement, reiteration of dietary instructions and practical advice about day to day issues which resulted in excellent compliance (Table 6.4).

Patients who experienced marked reduction or complete relief of symptoms for five consecutive days after a minimium of two weeks on

the elimination diet telephoned the dietitian for their challenge capsules which were sent by mail.

For those patients who presented with asthma, migraine, irritable bowel syndrome, hyperactivity and systemic symptoms in whom there was no improvement after six weeks of strict dieting on the elimination diet, continued restriction was not found to be helpful and these patients were instructed by the dietitian as to how to gradually resume a normal diet. They added foods back by chemical food groups every three days until a full diet was reached. A few patients experienced an exacerbation of their symptoms with the addition of some of the food groups. Patients with aphthous ulcers and eczema often took longer to resolve, showing minimal improvement in the initial six weeks with continuing improvement over a further six weeks so that marked improvement had occurred after 12 weeks, at which time they were ready to be challenged.

Following completion of the challenge protocol, reactions were noted by the dietitian and the double blind code was broken. Long term dietary modification based on individual oral provocation results was advised, avoiding only those foods which contained incriminated chemicals. After six weeks, gradual liberalization of foods by chemical grouping was encouraged in an attempt to induce tolerance and raise the threshold for triggering symptoms. Patients were encouraged to liberalize the natural compounds to which they had shown a sensitivity i.e. natural salicylates, amines and monosodium glutamate by taking very small amounts on an intermittent basis and then increasing the frequency and amounts as tolerated (Appendices 10, 11, 12).

Statistical Analysis

McNemar's test for correlated proportions as outlined in detail in Chapter 3 was used to compare each chemical challenge response with all the other compounds given so that any significant correlations between the chemical substances and placebos could be made for each syndrome.

The McNemar's test was also used to determine if the dose effect of a small and large dose of aspirin given to 239 patients was of any significance.

RESULTS

Of the 2235 patients, 1930 were prescribed the elimination diet. Nine hundred and seventy two patients (50.4%) improved on this diet and were challenged with the chemical challenges, 84 (4.3%) patients did not improve. Eight hundred and seventy four patients (45.3%) did not persist with the elimination diet (Table 6.4).

A further 494 patients commenced a less rigid "low chemical diet" (low salicylate, low amine, low glutamate, low preservative, low artificial colour, low brewers yeast) after medical and dietetic assessment suggested that they would be unlikely to cope with the very rigid dietary restrictions and challenge procedure. The low chemical diet restricted only the commonest food chemicals that may precipitate symptoms.

TABLE 6.4

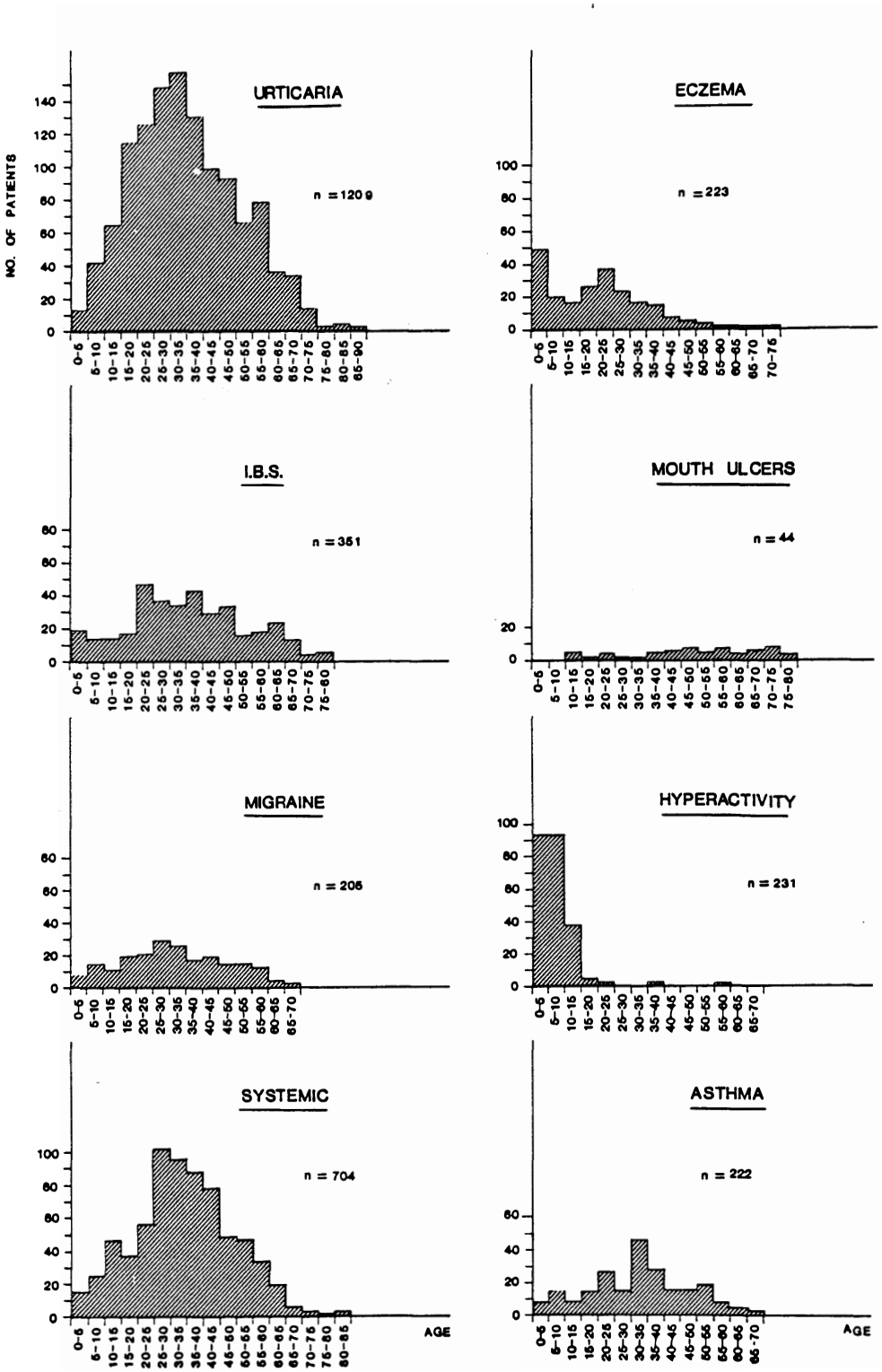
TOTAL PATIENTS

Syndrome	Number of Patients	Number of Patients Responding	Modified Diet No Challenges	Failures	Dropouts (No Follow-Up)	Percentage Response
Urticaria	1349	698	156	80	415	58.5%
Eczema	234	80	37	9	107	40.5%
Asthma	251	65	104	4	78	44.2%
IBS	358	157	29	21	148	48.2%
Mouth ulcers	46	23	15	2	6	74.2%
Migraine	237	109	9	11	108	47.8%
Hyperactivity	301	136	45	2	118	53.1%
Systemic	809	403	62	35	309	53.9%
Total patients	3584	1670	650	164	1289	53.6%

The presenting age of patients ranged from a few weeks to 86 years of age. The spectrum of ages for each syndrome are shown in the series of histograms in Figure 6.1 below.

FIGURE 6.1

AGE DISTRIBUTION OF PATIENTS



The sex ratio amongst the adults showed a preponderance of females which was most striking in those with "systemic" symptoms (Table 6.5 and Table 6.6). Whereas in those patients presenting with "hyperactivity" only 27% were female.

TABLE 6.5

SEX RATIOS OF TOTAL PATIENTS PRESENTED

Presenting Syndrome	Male (%)	Female (%)
Urticaria	37	63
Eczema	39	61
Asthma	37	63
Irritable bowel syndrome	31	69
Mouth ulcers	19	81
Migraine	25	75
Hyperactivity	74	26
"Systemic"	23	77

TABLE 6.6

SEX RATIOS OF PATIENTS WHO RESPONDED TO DIETARY ELIMINATION

Presenting Syndrome	Male (%)	Female (%)
Urticaria	38	62
Eczema	31	69
Asthma	35	65
Irritable bowel syndrome	31	69
Mouth ulcers	18	82
Migraine	30	70
Hyperactivity	73	27
"Systemic"	26	74

Challenge Results

Patients who became asymptomatic or who achieved markedly reduced symptoms on the elimination diet were challenged (Table 6.7). The challenge battery was updated throughout so that important comparisons could be made between syndromes with respect to colour and aspirin dose. For this reason the total number of patients challenged with each compound are presented by syndrome.

The frequency of positive challenge reactions in the various patients groups is shown in Table 6.8. Of the compounds tested the one most frequently incriminated was salicylate but cross reactions between many substances was common and idiosyncratic. The responses to each chemical challenge were compared with the placebos starch and sucrose using McNemar's test for dependent variables (Chapter 3). And for all those challenges which were taken by the majority of patients (salicylate, amines, MSG, preservatives, nitrates, antioxidants, tartrazine and brewers yeast), the reaction rates were found to be significantly different from the placebos starch and sucrose with $.002 < P < .00001$. The response rate to a challenge was then calculated listing only those patients who had experienced a recurrence of their presenting symptoms (Table 6.8).

Almost all patients react to more than one substance, averaging from 2.1 in those presenting with aphthous ulcers to 6.4 in those with "systemic" symptoms. The latter behave clinically as the most sensitive group of patients in whom even very minor deviations from their prescribed diet can cause reactions.

TABLE 6.7

TOTAL NUMBER OF PATIENTS CHALLENGE TESTED

	Urticaria	Eczema	Asthma	IBS	Mouth Ulcers	Migraine	Hyperactive Children	Systemic
Total Cases	62	80	58	159	23	109	136	403
Active								
Salicylate	57	58	61	152	4	104	92	394
Amines	55	56	23	156	3	102	98	380
MSG	57	45	41	147	4	95	82	370
Preservative	61	71	45	148	22	107	131	378
Antioxidant	56	42	22	141	4	98	82	362
Propionate	27	27	13	88	2	34	42	129
Nitrate	55	43	22	146	3	98	79	364
Tartrazine	60	70	61	150	22	106	125	379
Erythrosine	23	24	7	86	2	35	44	121
Brewers yeast	57	67	41	137	21	103	117	359
Gluten	55	40	29	140	21	93	80	356
Lactose	56	55	42	125	22	102	105	353
Wheat	60	80	50	155	21	108	136	397
Milk	60	79	52	151	22	108	135	394
Placebo								
Starch	56	50	27	144	21	104	84	369
Sucrose	54	41	28	124	3	98	81	364

TABLE 6.8

CHALLENGE RESPONSES (PRESENTING SYMPTOMS ONLY) (%)

	Urticaria	Eczema	Asthma	IBS	Mouth Ulcers	Migraine	Hyperactive Children	Systemic
Active								
Salicylate	48	51	42	62	74	51	68	63
Amines	29	50	30	39	0	52	36	55
MSG	33	31	32	48	0	54	39	57
Preservative	25	44	40	39	18	51	51	57
Antioxidant	29	19	27	38	0	33	33	46
Propionate	19	22	8	37	0	32	24	46
Nitrate	38	40	41	47	0	58	44	55
Tartrazine	23	33	30	36	18	43	50	48
Erythrosine	35	38	57	40	50	31	43	49
Brewers yeast	16	30	20	32	10	40	31	42
Gluten	2	15	3	16	0	7	1	14
Lactose	9	15	7	18	0	11	8	16
Wheat	3	16	0	17	0	5	2	15
Milk	0	22	2	20	0	5	5	16
Placebo								
Starch	5	6	7	8	0	8	4	8
Sucrose	2	0	0	5	0	7	1	7

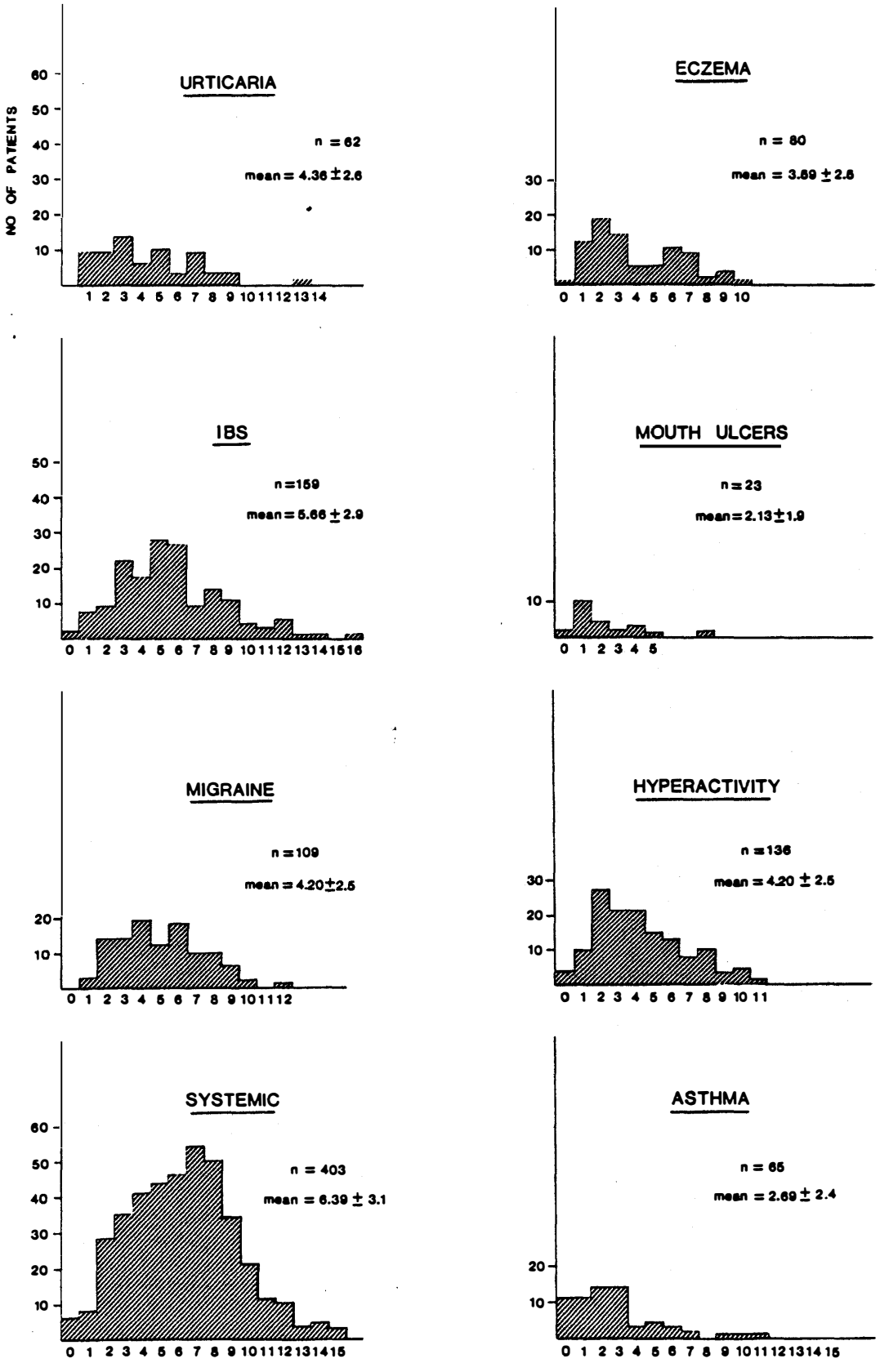
TABLE 6.9

CHALLENGE RESPONSES (ANY SYMPTOMS)(%)

	Urticaria	Eczema	Asthma	IBS	Mouth Ulcers	Migraine	Hyperactive Children	Systemic
Active								
Salicylate	66	63	55	71	91	61	80	76
Amines	44	54	52	53	33	59	52	65
MSG	53	47	42	61	75	61	48	67
Preservative	48	59	51	51	73	59	66	70
Antioxidant	41	24	46	46	75	37	44	55
Propionate	30	33	23	44	0	41	38	55
Nitrate	58	51	64	52	33	66	65	67
Tartrazine	38	44	46	46	46	49	59	59
Erythrosine	35	58	71	47	50	37	57	60
Brewers yeast	25	42	29	45	29	42	41	49
Gluten	6	15	7	17	0	14	1	20
Lactose	16	18	10	22	5	14	10	21
Wheat	5	19	0	19	0	5	5	19
Milk	5	28	6	21	0	7	10	24
Placebo								
Starch	7	8	7	1	10	11	6	10
Sucrose	7	0	4	5	0	7	4	7

FIGURE 6.2

NUMBER OF POSITIVE REACTIONS



The clinical spectrum of challenge reactions is illustrated for acetylsalicylic acid, amines, MSG, preservatives (sodium benzoate, 4OH benzoic acid, sorbic acid, sodium metabisulphite), antioxidants (BHT, BHA) and nitrates (sodium nitrate, sodium nitrite). In each group of patients the symptoms provoked are mainly confined to the organ system involved at presentation, with an identical pattern being seen when reactions to any of the compounds are tabulated in the same way. When each clinical group is compared with the overall population it becomes evident that each of the relevant food chemicals can provoke a variety of symptoms, depending on the particular pattern of target organ susceptibility in each individual.

TABLE 6.10

SYMPTOMS PROVOKED PER CHALLENGE

(A) SALICYLATE CHALLENGE

RESPONSE (%)	SYNDROME						
	Urticaria	Eczema	Asthma	IBS	Migraine	Hyperactivity	Systemic
Urticaria	53	0	5	8	3	10	10
Eczema	0	41	0	1	0	2	1
Asthma	2	2	43	1	0	0	5
IBS	12	5	13	60	15	28	37
Migraine	16	12	8	13	49	9	33
CNS*	2	9	10	9	6	63	27
Lethargy	11	0	5	11	5	5	34
Myalgia	4	2	0	6	2	0	18

(B) AMINE CHALLENGE

RESPONSE (%)	SYNDROME						
	Urticaria	Eczema	Asthma	IBS	Migraine	Hyperactivity	Systemic
Urticaria	29	2	4	4	2	2	8
Eczema	0	50	0	1	0	2	1
Asthma	0	2	30	1	1	1	3
IBS	7	4	4	38	13	17	27
Migraine	9	11	9	12	52	10	33
CNS*	9	2	9	9	7	36	17
Lethargy	4	4	9	6	8	7	23
Myalgia	4	2	9	3	2	0	14

(C) MSG CHALLENGE

RESPONSE (%)	SYNDROME						
	Urticaria	Eczema	Asthma	IBS	Migraine	Hyperactivity	Systemic
Urticaria	33	2	2	7	5	2	11
Eczema	0	31	0	1	0	1	1
Asthma	0	4	32	3	0	4	5
IBS	11	7	12	47	12	18	31
Migraine	12	11	12	14	54	7	36
CNS*	9	11	12	7	6	39	22
Lethargy	4	0	2	12	6	7	25
Myalgia	2	2	0	3	2	1	14

(D) PRESERVATIVES (BENZOATES, SORBATE & METABISULPHITE) CHALLENGE

RESPONSE (%)	SYNDROME						
	Urticaria	Eczema	Asthma	IBS	Migraine	Hyperactivity	Systemic
Urticaria	25	7	2	6	6	8	10
Eczema	0	44	0	0	0	2	1
Asthma	3	0	40	1	2	1	7
IBS	10	3	11	38	19	18	31
Migraine	18	7	9	18	50	5	34
CNS*	7	7	4	6	8	51	21
Lethargy	5	0	2	9	6	8	24
Myalgia	2	0	0	5	2	1	14

(E) ANTIOXIDANT (BHA & BHT) CHALLENGE

RESPONSE (%)	SYNDROME						
	Urticaria	Eczema	Asthma	IBS	Migraine	Hyperactivity	Systemic
Urticaria	29	2	0	2	5	1	6
Eczema	2	19	0	0	0	0	1
Asthma	0	0	3	1	0	0	3
IBS	7	5	14	38	10	13	23
Migraine	13	5	9	8	33	5	25
CNS*	4	2	9	7	5	33	18
Lethargy	4	5	5	6	5	5	20
Myalgia	4	2	0	3	2	1	10

(F) SODIUM NITRATE/ NITRITE CHALLENGE

RESPONSE (%)	SYNDROME						
	Urticaria	Eczema	Asthma	IBS	Migraine	Hyperactivity	Systemic
Urticaria	38	2	0	3	5	1	7
Eczema	0	40	0	1	0	1	1
Asthma	2	2	41	0	2	1	3
IBS	11	7	23	47	28	28	34
Migraine	16	14	14	14	58	15	37
CNS*	9	9	23	8	4	44	16
Lethargy	6	5	5	8	10	15	21
Myalgia	0	0	9	5	2	0	14

(G) TARTRAZINE CHALLENGE

RESPONSE (%)	SYNDROME						
	Urticaria	Eczema	Asthma	IBS	Migraine	Hyperactivity	Systemic
Urticaria	23	6	5	7	2	5	7
Eczema	0	33	2	0	0	2	1
Asthma	2	6	30	1	2	1	2
IBS	8	1	8	36	11	18	23
Migraine	10	6	10	10	43	5	29
CNS*	8	10	2	6	7	50	18
Lethargy	5	1	5	5	7	8	21
Myalgia	2	1	2	3	2	0	13

* CNS = Central nervous system

The dose effect of aspirin was studied in a total of 230 patients who were randomly given a challenge battery which contained two separate aspirin challenges: a high dose (1200mg) and a low dose of (either 150, 200, 300 or 500mg). The results are shown in Table 6.11.

TABLE 6.11

DOSE EFFECT OF ASPIRIN

Low dose Aspirin	1200 mg Aspirin	
	Positive	Negative
Positive	24.3%	33.5%
Negative	10.8%	31.4%

As the number of patients given each dose was small the groups were combined into small versus large dose for statistical analysis by McNemar's test for dependent groups. The test showed that their was a dose related effect with a significant difference between the response rate to the low and high doses $P < .0005$. Patients results were then compared on the basis of their positive or negative response to salicylate (Figures 6.3 and 6.4).

FIGURE 6.3

RESPONSE TO SALICYLATE AND OTHER COMPOUNDS

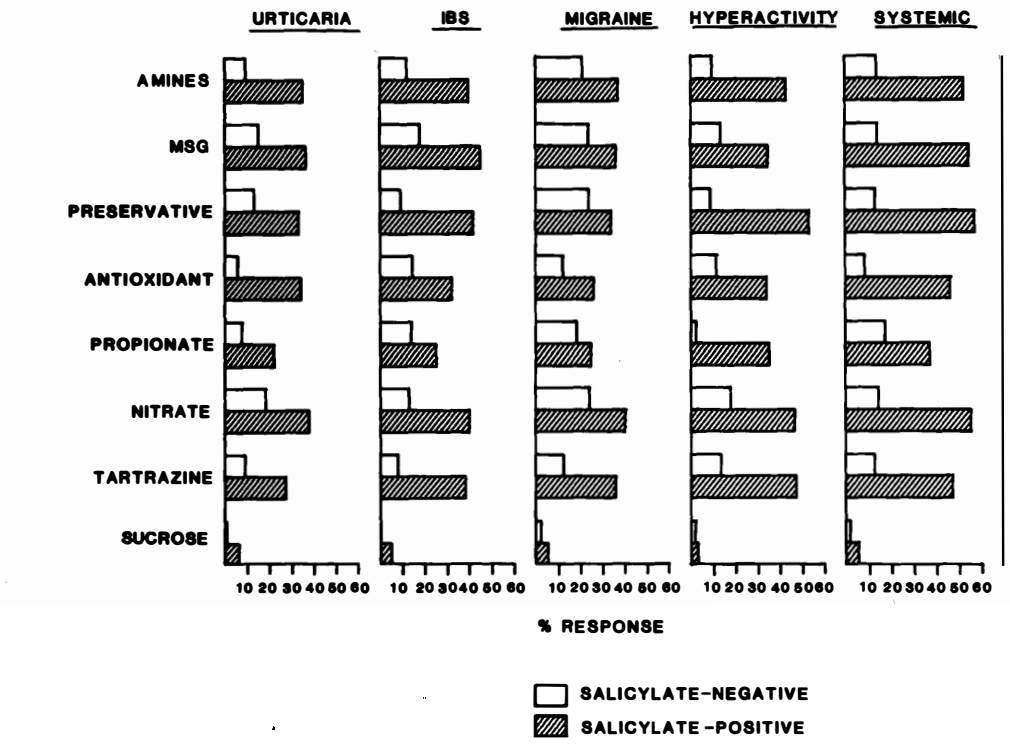
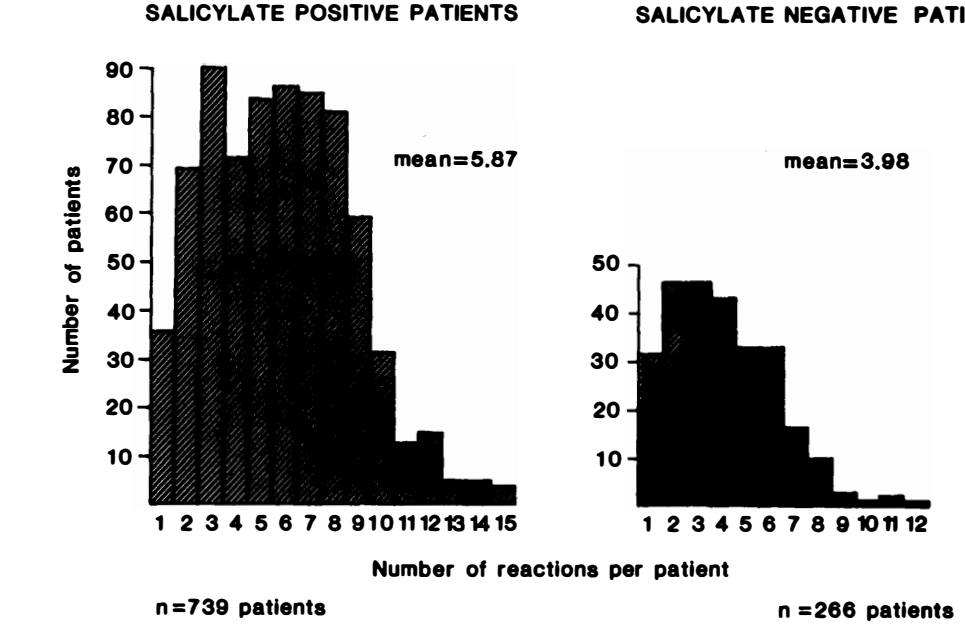


FIGURE 6.4

TOTAL NUMBER OF REACTIONS TO CHALLENGE PER PATIENT

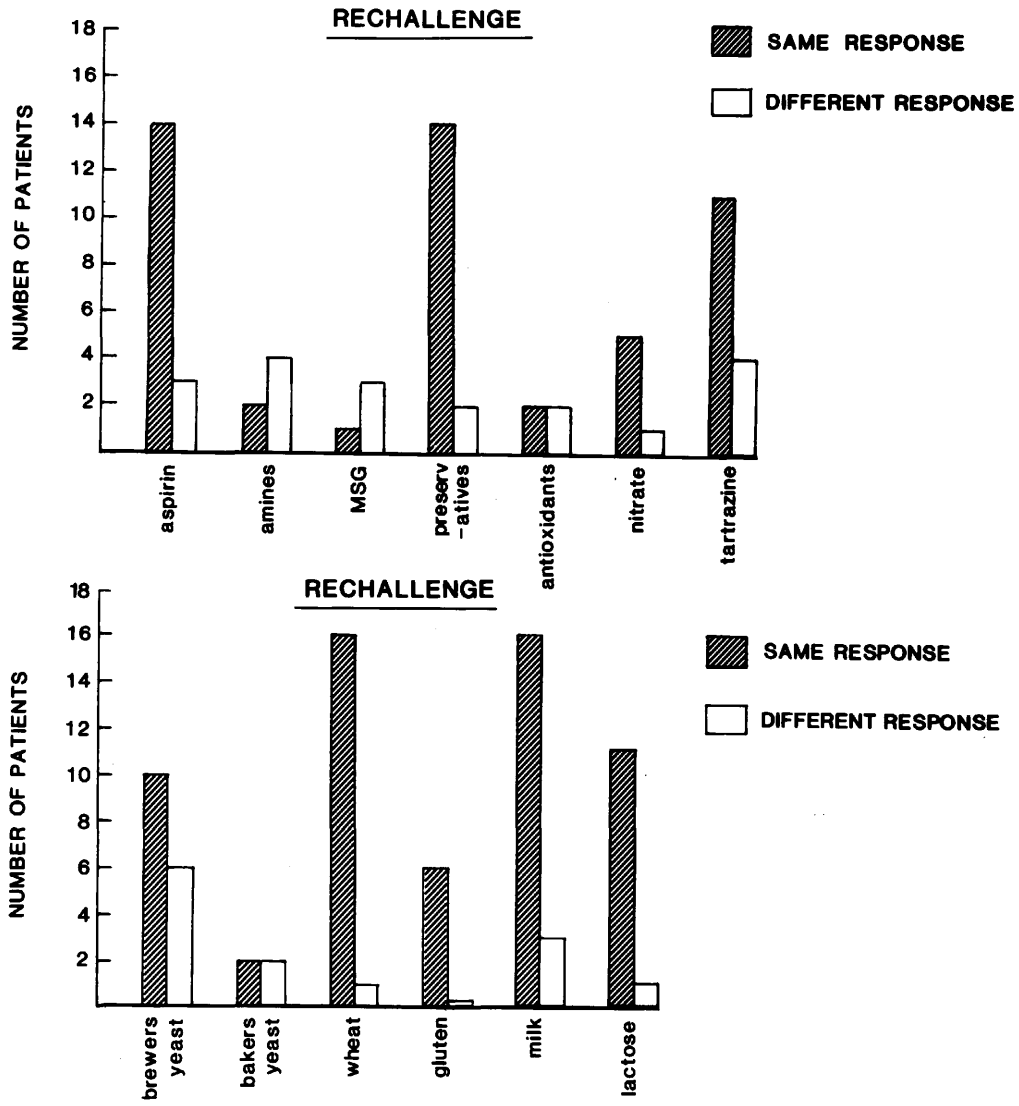


Re-challenge Response to Challenge

Nineteen patients elected to repeat the elimination diet and challenge procedure. Overall the reproducibility was found to be 77% with a range of 25% to 100%.

FIGURE 6.5

RE-CHALLENGE RESPONSE TO CHALLENGES (%)



DISCUSSION

A review of the recent literature reveals a good deal of confusion and controversy surrounding the subject of adverse food reactions, the historical background of which is discussed in Chapter 9. Terminology is itself an important source of confusion since the word "allergy" is often used inappropiately to describe any adverse reaction, regardless of mechanism (Anderson, 1986). Some authors, such as May (1986), dismiss as psychological or erroneous the association of foods with any symptoms other than those of a classical allergic reaction (anaphylaxis, urticaria, angioedema, asthma, eczema and gastrointestinal disturbances), on the basis that immunological mechanisms have not been demonstrated. Others, such as Speer (Speer, 1978, 1983; Speer et al., 1981), have recognized an association between reactions to acetyl salicylic acid, additives and various food "allergens", but because most of the patients studied were atopic, and symptoms such as asthma and urticaria were common, Speer (1975) regards such reactions as allergic manifestations, despite the consistent failure of most investigators to demonstrate aspirin-specific IgE by skin prick or RAST testing (Krilis et al, 1981).

Amongst "orthodox" authors, Moneret-Vautrin draws the clearest distinction between true, immunologically mediated food allergy, and pharmacological food intolerance or idiosyncracy, mediated by "... known, suspected or unknown pharmacological mechanisms" (Moneret-Vautrin, 1983). He refers to pharmacological reactions as "false food allergies" or "pseudo-food allergies" since they sometimes

provoke urticaria or asthma, and can therefore "masquerade" as allergic reactions. Although his views are in substantial agreement with those presented here, his choice of terminology is unfortunate since it is likely to leave a casual reader with the impression that such reactions are imaginary.

In the discussion which follows, each of the clinical syndromes in which food intolerance was implicated in the present study will be considered separately. Space does not permit an exhaustive review of the literature in each area, and therefore only the most relevant references will be cited, along with selected review articles. A historical overview will be given in Chapter 9.

Asthma

Aspirin intolerance has been recognized as a triggering factor in urticaria and asthma since the turn of the century (Hirschberg, 1902) and with the exception of Speer (1981) most authors now view this as a pharmacological idiosyncracy (Settipane, 1983; Asad et al., 1984; Van Arsdel, 1984; Slepian et al., 1985). Tartrazine has also been incriminated as a cause of urticaria and asthma, following the original anecdotal reports of Speer (1958) and Lockey (1959) nearly 30 years ago. Since then several studies have suggested an association between aspirin and tartrazine reactivity in asthmatics (Samter & Beers, 1967, 1968; Settipane & Padupakkam, 1975; Stenius & Lemola, 1976; Spector et al., 1979; Weber et al., 1979), although this relationship has been questioned by others (Stevenson et al., 1986).

More recently it has become apparent that all asthmatics are sensitive to the effects of inhaled SO_2, a common atmospheric pollutant, and a proportion are sensitive to ingested SO_2 in the form of metabisulphite used as a preservative (Koenig et al., 1980; Sheppard et al., 1980; Freedman, 1980; Baker et al., 1981; reviewed by Boushey, 1982; Stevenson and Simon, 1984; and Bush et al., 1986). Ingested metabisulphite can release SO_2 from the fluid phase in the oropharynx, and may also circulate to the lungs as the sulphite radical (HSO_3^-). In those asthmatics sensitive to ingested metabisulphite, reduced tissue levels of sulphite oxidase may be partly responsible for increased vulnerability to SO_2, although heightened sensitivity of the cholinergic bronchoconstrictor mechanism has also been postulated (Stevenson & Simon, 1984).

Finally, MSG has also been reported as a cause of "Chinese restaurant asthma" in a small number of patients, although their reactivity to other substances has not as yet been documented, and the mechanism is unknown (Allen & Baker, 1981).

In the present study 61 asthmatic patients underwent single blind challenges with aspirin and tartrazine, and a proportion were also challenged with other compounds in the standard challenge battery (Table 6.3). At the time of writing the challenges routinely administered to asthmatics were aspirin, tartrazine, metabisulphite (in solution), MSG and placebo (Appendix 5). As a rule, moderate to severe asthmatics (as defined clinically and by bronchial responsiveness to histamine) were challenged under observation at RPAH, and those with mild asthma were supervised by their family doctor.

Overall, 42% of patients reacted to aspirin, 30% to tartrazine, 40% to metabisulphite and 32% to MSG (Table 6.8). In twenty-four of these individuals (who were challenged under supervision at RPAH) and a further eight patients challenged in hospital since September 1986, strict criteria were applied in recording a positive reaction, i.e. >20% fall in FEV_1. In these 32 patients the prevalence of reactions were: aspirin - 41%; metabisulphite - 47%; MSG - 26%; tartrazine - 13%. Seven patients did not react to any of these four challenge compounds, and only three reacted to both aspirin and tartrazine.

Aspirin sensitivity has been reported in between four and 28% of asthmatic patients generally, and in up to 78% of a highly selected population with nasal polyps (reviewed by Slepian et al., 1985), but it is difficult to make direct comparisons with the present study because of variations in referral patterns and selection criteria. Nevertheless, it is possible to conclude that the frequency of aspirin intolerance is similar to that described in other series, and moreover, that although it is quite common for individuals to react to both aspirin and tartrazine, the association is no greater than that expected by chance. Thus, of the 25 patients who reacted to aspirin, 10 reacted to tartrazine and 15 were negative, and of the 36 who did not react to aspirin, eight reacted to tartrazine and 28 were negative. Chi-squared analysis of these figures shows no statistically significant association between aspirin and tartrazine reactions ($P > 0.2$). Similarly, there was no significant association between aspirin and metabisulphite challenges ($P > 0.3$), although nearly half the patients reacting to sulphite challenge also reacted to aspirin, contrary to the claims of Stevenson and Simon (1984).

Eczema

Schloss (1915) was one of the first investigators to draw attention to the relationship between food allergy and atopic eczema, but the issue later became confused by the finding that skin tests with foods correlated poorly with clinical sensitivity. Nevertheless, the clinical importance of diet, at least in a proportion of cases, has been increasingly recognized in recent years (Atherton et al., 1978; Juto et al., 1978; Hill & Lynch, 1982; Sampson, 1983; Sampson & Jolie, 1984). Estimates of prevalence vary between 0% and 25% (Heiner, 1981), and in highly selected series may be up to 59% (Sampson & McCaskill, 1985).

It is generally agreed by all reviewers that the most commonly incriminated foods are eggs, milk, wheat, peanuts and fish, and that the mechanism is IgE-mediated (Atherton, 1981; Rasmussen, 1984; Sampson, 1986). The diagnostic usefulness of skin prick tests or RAST, however, is controversial. Our approach to skin testing is similar to that of Sampson (1986): a negative result effectively rules out immediate food hypersensitivity, and is therefore useful in cases where the history is equivocal, but because of the high frequency of positive skin tests in patients without clinical food sensitivity they have poor predictive value.

Of 234 patients with eczema seen at the RPAH Allergy Clinic, 80 under went food and chemical challenge. Those with a clear history of egg hypersensitivity (20%) were not challenged with eggs, and of the remainder nine percent reacted to open egg challenge. All patients

took open challenges with milk and wheat, 22% and 16% respectively reporting a flare of their eczema. If eggs, milk and/or wheat were tolerated these foods were re-introduced into the elimination diet before commencing double-blind chemical challenges. The prevalence of reactions to salicylates, amines, preservatives, and other substances in the challenge battery, was very comparable to that observed in patients with RIU/AO (Table 6.8), an observation which has not previously been reported. Indeed, of the 80 patients who underwent chemical challenge all reacted to at least one active substance, the average being between three and four (Figure 6.2). One ten year old boy reacted only to lactose amongst the double-blind challenges, and he also reacted to milk on open challenge. Interestingly, amongst patients reacting to milk, nearly three-quarters reacted to the double-blind lactose challenge, raising the possibility that some of these may not be IgE-mediated. Skin testing with lactose should help resolve this question in the future.

The findings presented here suggest that in patients with atopic eczema, a combination of IgE-mediated food allergy and pharmacological idiosyncracy may be involved in pathogenesis in a proportion of cases. Indeed, the possibility exists that chemical intolerance may be a prerequisite for disease expression, and that a high IgE level, with increased mast cell releasability, is the predisposing factor which renders an individual prone to developing eczema rather than urticaria (Chapter 10).

Migraine

Reports of a link between certain foods and headache date back to Hippocratic times. With increasing knowledge about mechanisms of hypersensitivity during the first half of the 20th century, and following the introduction of skin testing, many authors arrived at the conclusion that migraine was an allergic disorder. In these studies skin testing, elimination diets and/or challenges were used for diagnosis, and the foods most commonly implicated were milk, wheat and chocolate (reviewed by Monro, 1982; 1987).

In 1967 Hanington first published evidence that pharmacologically active amines in certain foods could cause vascular headaches, and since that time there has been continuing debate about the relative importance of allergy versus chemical idiosyncracy in the aetiology of migraine (reviewed by Hanington, 1983). The role of tyramine has been disputed in some studies (Moffett et al., 1972; Zeigler & Stewart, 1977; Shaw et al., 1978; Congden & Forsyth, 1979; Kohlenberg, 1982) and supported by others (Bonnet & Lepreux, 1971; Ghose et al., 1977; Glover et al., 1983). Reviewing the subject, Raskin (1981) finds the evidence supporting tyramine sensitivity in migraine to be "persuasive". Nevertheless, some authors continue to regard food induced migraine as an allergic disorder (Monro et al., 1980, 1984; Grant, 1979; Wilson et al., 1980; Egger et al., 1983; Mansfield et al., 1985), although Merrett et al. (1983) could find no correlation between total and specific IgE or IgG4 in patients with "dietary" versus "non-dietary" migraine.

In the present study 237 patients with migraine were evaluated and placed on the elimination diet, and of these 109 became either headache-free, or reported sufficient subjective improvement to warrant undergoing systematic challenge testing (Table 6.4). The pattern of responsiveness was strikingly similar to that found in patients with the other clinical syndromes studied (Table 6.8). Over half (52%) of the patients tested experienced headache when challenged with tyramine and phenylethylamine, which along with the high prevalence of sensitivity to MSG (54%) and nitrate (58%) supports the findings of Hanington and others, as discussed above. Interestingly, 51% of our patients also reacted to salicylates, a finding which has not previously been documented. As with RIU/AO, each patient exhibited an individual pattern of reactions, to between one and 12 of the substances tested (mean=4.2, SD=2.5, Figure 6.2).

With hindsight, the somewhat confusing findings of previous studies can now be reconciled. The wide range of substances to which the patients with migraine react when challenged accounts for the fact that such a diversity of foods, including many not normally considered as highly allergenic, have been implicated. For example, Monro et al. (1980) and Egger et al. (1983) identified cheese, chocolate, pork, fish, shellfish, orange, tomato, cola, peanuts, banana, mushrooms, corn, apple, rhubarb, tea, coffee and wine. These all contain amines and/or salicylates (Appendices 10, 11). The low incidence of reactions to wheat and milk in our patients contrasts with the findings in several of the studies reviewed by Monro (1987). This could be a result of different referral patterns and the patient selection methods, or due to other methodological differences (see discussion of "hyperactivity" below, and Chapter 10).

Irritable Bowel Syndrome (IBS)

Although gastrointestinal symptoms such as vomiting, abdominal pain and diarrhoea, have been recognized symptoms of acute allergic reactions to food since the turn of the century (Portier & Richet, 1902), the role of diet in chronic or recurrent "functional" bowel disorders is much more controversial. There is an astonishingly wide range of views on the pathogenesis of IBS (Lancet Editorial, 1975, 1984). At one end of the spectrum, patients are said to be anxious, dependent, hysterical or otherwise deranged, their symptoms being largely imaginary or grossly exaggerated (Almy, 1977; Fielding, 1977). It has been suggested that such individuals may experience minor symptoms, which are common amongst people who regard themselves as healthy (Thompson & Heaton, 1980; Drossman et al., 1982), but as a result of "learned illness behaviour" in childhood are more likely to seek medical attention (Mechanic et al., 1982; Whitehead et al., 1982; Ford, 1983; Sandler et al, 1984). Others consider these symptoms to be genuine, but produced by the response of the gut to stressful life events, conscious or unconscious psychological conflict, anxiety or aggression (reviewed by Ford, 1986). At the other end of the spectrum is the view that IBS is an organic disorder of gut function resulting either from an intrinsic abnormality of the smooth muscle or its neurohumoral control mechanisms (Snape et al., 1976).

Claims that IBS can be caused by food "allergy" date back to the 1920's, at which time the disorder was known as "mucous colitis" (Duke, 1921; Vaughan, 1922; Rowe, 1944). The issue has long been a controversial one, prompting Ingelfinger et al. (1949) to lay down

specific criteria for the diagnosis of gastrointestinal allergy: (i) demonstration that symptoms are caused by contact with a specific substance that is innocuous to the bulk of the population, (ii) evidence of an immune mechanism in pathogenesis, and (iii) demonstration of lesions or functional changes in the gut. More recently it has been suggested that IBS may occur, at least in a proportion of cases, on the basis of non-immunological food intolerance (Buisseret et al., 1978; Cooper et al., 1980; Ferguson, 1982; Alun Jones, 1982). Despite this a number of authors have failed to appreciate the distinction between immunological hypersensitivity and non-immune mechanisms, concluding that failure to confirm an allergic aetiology excludes food as a cause of symptoms in IBS (Bentley et al., 1983; Farah et al., 1985). The shortcomings of such studies have been pointed out by Alun Jones et al. (1983), and are discussed in detail below.

In the present study 358 patients with IBS were evaluated, 329 of whom were placed on the standard elimination diet. Of these 159 (48%) reported complete loss of symptoms or significant improvement, and underwent double-blind challenge (Table 6.1). The results followed the same pattern as in other patient groups, with salicylates most commonly reproducing symptoms (62%), followed by MSG, nitrates, amines and food additives (Table 6.8). The symptoms most frequently provoked by challenges were nausea, malaise, abdominal pain, bloating and diarrhoea. As with RIU/AO patients, those with IBS who proved to be diet-sensitive could generally be managed successfully by long-term restriction of their dietary intake of foods containing the relevant substances (Chapter 8).

Mouth Ulcers

Recurrent aphthous ulceration is a common condition which may affect up to 20% of the population, and is characterized by small painful lesions occurring singularly or in crops. Although mouth ulceration can occur in connective tissue diseases, bullous skin diseases, Behcet's syndrome, inflammatory bowel disease, deficiency states and cyclic neutropenia, the majority of cases are "idiopathic". A variety of factors have been implicated, including a genetic predisposition, endocrine factors, viral infections, autoimmunity, psychological factors and socio-economic status (reviewed by Challacombe, 1987). In recent years, attention has shifted to specific food allergies or intolerances as a cause of recurrent aphthous ulceration, including gluten (Walker et al., 1980; Wray, 1981; Wright et al., 1986), dairy products (Thomas et al., 1973; Wright et al., 1986), and a number of miscellaneous foods (Hay & Reade, 1984). Although many of these authors have speculated about possible immunological mechanisms, none have presented any clear evidence of a true allergic basis of this condition. Wray et al. (1982) found that some patients release histamine from basophils in response to specific foods, but this did not correlate well with clinical findings. Furthermore, even though challenges provoked mouth ulceration in selected patients, removal of the relevant foods from the diet did not prevent recurrences in the long-term. On the other hand, Wright et al. (1986) found six patients in whom prolonged and relentless mouth ulceration responded dramatically to avoidance of gluten, milk or azo-dyes, and who were well over a follow-up period of between one and four years. Two other groups have also incriminated gluten but none of the patients studied were found to

have any clinical or histological evidence of coeliac disease (Walker et al., 1980; Wray, 1981). Hay and Reade (1984) found four patients whose mouth ulceration resolved or improved markedly with a diet based of the Rowe elimination diets, and recurrences were variously provoked by figs, cheese, tomato, vinegar, lemon, pineapple, mustard, milk and wheat flour. Because of the observation of a latent period of between eight and 72 hours, these authors speculate that the mechanism may involve a delayed (Type IV) hypersensitivity response, but no evidence was presented to support this.

In the present study, 46 patients were referred to the RPAH Allergy Clinic for assessment of possible food intolerance as a contributory factor. Of these, 31 were placed on the standard elimination diet, and 15 were given an empirically modified diet (Table 6.3). Twenty-three patients (74%) became symptom-free on the elimination diet and were systematically challenged. None of the patients tested reacted to open food challenge with wheat or milk, or to double-blind challenge with gluten, in contrast to previously published studies (Walker et al., 1980; Wray, 1981; Wright et al., 1986). However, all 23 patients reacted to one or more of the chemical challenges, by far the commonest being salicylates (Table 6.8). Even those who failed to react to a single dose of ASA often developed recurrences of mouth ulceration (sometimes together with urticaria) when salicylate-containing foods were taken over a longer period of time, suggesting a cumulative dose-dependent effect. Challenges other than colourings and preservatives rarely provoked reactions, but the numbers tested were too small to draw any valid conclusions (Table 6.8).

It is interesting to note that patients with the most intractable and long-standing mouth-ulceration (referred from the Dental Hospital) generally took longest to settle on the elimination diet, sometimes requiring as long as three months. This was unlikely to be coincidental, since challenges regularly provoked acute recurrences with ulceration often lasting for two weeks or more in such cases.

As an incidental issue, 11 patients referred from the Dental Hospital because of burning sensations in the mouth (Lancet Editorial, 1978) underwent dietary investigation. Only one appeared to improve, but she reacted to all the challenges given including the placebos (data not shown).

Neuropsychiatric Syndromes

By far the most heated of the many controversies surrounding the whole subject of food intolerance revolves around the question of whether food reactions can provoke neuropsychiatric symptoms. The historical roots of this issue (see Chapter 9) date back more than 70 years when Hoobler (1916), reported that unusual restlessness was common in allergic children. Later, Duke (1921) and Shannon (1922), claimed that food allergy could be responsible for various "neuropathic" manifestations such as nervousness, irritability, weakness, hypotension, sleep disturbance, hyperactivity, behaviour disorders, moodiness, poor concentration and learning disabilities. In the 1950's Speer (1954) coined the term "allergic tension-fatigue syndrome" to describe what he and others believed to be symptoms characteristic of an allergic reaction involving the brain (Wooton,

1934; Turnbull, 1943; Jones, 1949; Davison, 1952; Crook, 1963; Leonard, 1966; Wolf, 1971; Weinberg & Tuchinda, 1973; reviewed by Speer, 1983). Meanwhile, another school of thought (now referred to as "Clinical Ecology") arose following the earlier work of Rowe (1931), claiming that "non-reaginic food allergy" (Coca, 1942) or "masked food allergy" (Rinkel, Randolph & Zeller, 1951) was responsible for a wide array of vague, subjective symptoms.

Many orthodox allergists reject these claims, pointing out that they are based on anecdotal clinical impressions and unproven diagnostic methods (May, 1986). Nevertheless, they attracted widespread interest from the media, the public, and certain segments of the medical profession during the 1970's, due in part to the publication of two popular books: "Why your child is hyperactive" (Feingold, 1975) and "Not all in the mind" (Mackerness, 1976). The results of the present study do indeed lend a degree of support to these claims, but as will be outlined below (and in Chapter 9) the conclusions drawn from the data are not necessarily the same. Since the clinical problems encountered in children are somewhat different from those in adults, the two areas will be discussed separately.

(a) Hyperactive Children

By 1960, an average of ten articles were appearing each year on the "hyperactive child syndrome," increasing to over 100 per year by 1975, and more than 2000 per year by 1980 (Ross & Ross, 1982). It was first described by Hoffman (1845) as a syndrome consisting of

"hyperactivity, impulsivity, distractability and excitability", and has been given many different labels since that time, including hyperkinesis, minimal brain dysfunction and Strauss syndrome, and is currently referred to in the Diagnostic and Statistical Manual of Mental Disorders (DSM-III, 1980) as "Attention deficit disorder, with hyperactivity". The clinical picture of the "hyperactive" child is widely recognized, and generally not disputed by investigators and clinicians. However there has been a great deal of debate about its aetiology, and about the validity and clinical usefulness of according these symptoms the status of a diagnostic entity (Shaffer & Greenhill, 1979; Rutter, 1982). The most extreme viewpoint is that there has been an unhealthy collusion between physicians, teachers and drug companies in "medicating" difficult children who are normal, but who may be reacting to under-staffed schools or poor homes by being disruptive (Schrag & Divoky, 1975). A more reasoned argument has been put forward by Schechter (1982), who believes that, even though there may be a small number of genuinely disturbed children requiring medical attention, either on the basis of a neurological deficit, a developmental delay or a temperamental attribute, the apparent epidemic of so-called hyperactivity has been exaggerated by a number of sociological factors. He considers the most significant of these to be the breakdown of the traditional extended family in the post-war era and the "medicalization" of child-rearing which took place after the publication of Benjamin Spock's book (Commonsense book of baby and child care by Spock, 1945).

Until recently the issue of diagnostic and predictive validity was confused by the apparently widely differing prevalence of hyperactivity in Britain (1/1000) compared with the United States (where between five percent and 20% of school-age children are said to be affected (reviewed by Lipton et al., 1979). It is now recognized that the prevalence is in fact similar in both countries, but there is a tendency for British workers to exclude hyperactive-aggressive children, and label them as having "conduct disorder" (Taylor, 1986). This problem has been further compounded by confusing pervasively and situationally hyperactive children, with the result that physician's, teacher's and parent's assessments may be quite divergent (discussed in detail by Weiss, 1985). Thus, despite continuing debate, recent studies suggest that the hyperactive child syndrome has both concurrent and predictive validity and may indeed be considered a valid diagnostic entity (Weiss, 1985, 1986; Lancet Editorial, 1986).

In 1973, Feingold presented a paper at a meeting of the American Medical Association claiming that up to 50% of hyperactive children became well or improved markedly on a diet free of additives and natural salicylates (Feingold, 1973). This idea captured the public imagination, and following the appearance of his book (Feingold, 1975) more than 100 parent self-help organizations sprang up in the USA, UK and Australia, advocating the "Feingold Diet". During the next decade Feingold's claims generated enormous controversy. The issue became strongly politicized, mainly because of the emphasis placed on food additives, with protagonists calling for special

labelling laws and a ban on the use of foods containing additives in school canteens, while official statements denied any safety difference between natural and artificial food substances (reviewed by Lipton et al., 1979).

The literature on diet and hyperactivity is large, and has been extensively reviewed by several authors (Sieben, 1977; Taylor, 1979; Lipton et al., 1979; Conners, 1980; Dickerson & Pepler, 1980; Ribon & Joshi, 1982; Egger, 1987). The numerous clinical trials designed to test Feingold's hypothesis fall into three groups: (i) uncontrolled, open clinical trials of the additive-free diet; (ii) blind clinical trials of the diet; (iii) double-blind challenge experiments with or without cross-over of test and placebo diets. In general, open trials have supported Feingold's claims, whereas controlled clinical trials have yielded much less impressive results (Lipton et al., 1979). Up to 1980 there were four open trials reporting beneficial effects of the Feingold diet, both in Australia (Cook & Woodhill, 1976) and the USA (Salzman, 1976; Brenner, 1977; Harper et al., 1978). Several controlled studies involving blind challenges with food colours showed encouraging results in pilot studies, but subsequently failed to confirm their preliminary findings (Harley & Matthews,1977, Harley et al., 1978; Levy & Hobbes, 1978; Levy et al., 1978; Conners et al., 1976, 1978; Goyette et al., 1978; Mattes & Gittelman-Klein, 1978, 1980). In some series, despite the negative findings overall, a small number of children were identified in whom challenges appeared to provoke a consistent adverse reaction compared to placebo (Harley et al., 1978; Williams et al., 1978; Rowe et al., 1979; Weiss et al., 1980; Swanson & Kinsbourne, 1980).

As a result of widespread concern about Feingold's claims, the Nutrition Foundation in the USA established a National Advisory Committee on Hyperkinesis and Food Additives charged with the task of encouraging and evaluating controlled studies testing his hypothesis. In its final report (1980) the Committee concluded that "...studies already completed provide sufficient evidence to refute the claim that artificial food colourings, artificial flavourings and salicylates produce hyperactivity and/or learning disability". Two years later a National Institute of Health, Consensus Development Conference (1982) reached generally similar conclusions, although it was pointed out that in a small proportion of children the Feingold diet did appear to reduce hyperactivity, and published challenge studies were criticized for not having addressed the role of diet sufficently broadly.

Until recently the weight of scientific evidence seemed to have definitively refuted Feingold's hypothesis (Kavale & Forness, 1983), but the controversy was rekindled by a carefully controlled study published by Egger et al. (1985). Sixty two of 76 selected children with hyperactivity improved objectively when placed on an "oligoantigenic" diet, and of these 28 completed a double-blind, cross-over placebo-controlled trial in which the suspected foods were re-introduced. The study showed a clear association between deterioration in behaviour and exposure to the suspect food, but not placebo, with 45 different foods and additives being incriminated overall. The authors interpreted these findings as indicating that allergic mechanisms rather than pharmacological idiosyncracies were involved, a view which we have disputed on the basis of our own findings (Swain et al., 1985).

Of the 301 children evaluated in the present study, 45 were given a modified diet without formal testing (for the reasons outlined above), and the remainder were given instructions for following the elimination diet. One hundred and eighteen were lost to follow-up (Table 6.4), two failed to improve, and 136 appeared to improve and proceeded to double-blind challenge testing. Because of the subjective bias inherent in parental evaluation, no firm conclusions can be drawn from the reported benefits of dietary elimination. However, it is significant that of those challenged, all but 4 children were judged by the parents to have reacted to at least one challenge compound, and in only 5 instances was this a placebo ($P < 0.0005$) (Figure 6.2 and Table 6.8). Somatic symptoms such as headache, abdominal pain or urticaria occurred together with behavioural changes in two thirds of the children tested, although 73% of the children were reported as manifesting an isolated behavioural change after at least one of the active challenge compounds, and in one-third behavioural change was the only manifestation of challenge reactions. In all, positive challenge reactions were recorded on 522 occasions in 136 children. Behavioural symptoms occurred on 397 occasions, 247 of which (62%) were in the absence of any accompanying physical symptoms.

The commonest compound responsible for positive challenge reactions was aspirin, which was reported as causing behavioural changes, with or without somatic symptoms, in 68% of the children tested (Table 6.9). Half the children reacted to tartrazine and 51% to preservatives, but interestingly over one third also reacted to amines

and/or MSG. No child reacted to tartrazine alone. As with the RIU/AO patients, those sensitive to aspirin had a higher probability of reacting to other substances (Figure 6.3), but the pattern in each child was individual (Loblay & Swain, 1985).

Our findings tend to support the original hypothesis put forward by Feingold (1975), although the range of food substances involved is broader than he had suspected. Clearly, therefore, it is not possible to devise a standard diet suitable for all hyperactive children, and in this regard we are in agreement with Egger et al. (1985). In retrospect, some of the reasons for the failure of previous studies to support Feingolds' claims can now be appreciated. Of critical importance is the need for rigorous elimination of both salicylate and amine containing foods from the baseline diet in order to reduce the "background noise" of symptoms, as well as to lower the dose threshold for challenge reactions. A careful reading of published studies indicates that in no case has this been done adequately, with the possible exception of Egger et al., where an "oligoantigenic" diet was arrived at empirically for each child. Furthermore, as pointed out by Weiss (1986) and Egger (1987), some authors disguised the active and placebo ingredients in chocolate cookies, apparently without realizing that some children may react to chocolate (Williams et al., 1978; Swanson & Kinsbourne, 1980; Thorley, 1983, 1984). Other methodological problems include the fact that some studies used inadequate doses (Swanson and Kinsbourne, 1980; Weiss et al., 1980), and others did not insert washout periods between the test periods, with likely carry-over effects (Williams et al., 1978; Harley et al., 1978; Swanson and Kinsbourne, 1980; Weiss et al.,

1980; Thorley, 1984). Finally, no account was taken in any study of the possible effects of MSG or nitrates, which in our hands provoked reactions in 39% and 44% of the children challenged, respectively.

Adults with "Systemic" Symptoms

Once the RPAH Allergy Clinic became known for its interest in food intolerance, the proportion of patients presenting with various non-cutaneous disorders increased substantially (Figure 1.1). In many cases symptoms were restricted to a single organ system, and these have been discussed in detail above. In other cases, however, there were symptoms referable to multiple organ systems, and such patients were designated as having a "systemic" syndrome. Although specific problems such as abdominal pains, diarrhoea or headache sometimes led patients to present for investigation, the most characteristic clinical feature of this group was the presence of multiple somatic complaints of a "functional" nature, which are often accompanied by vague neuropsychiatric symptoms. The latter were commonly described as a feeling of being "drugged", "fuzzy" or "thick" in the head, or an inability to think clearly, variously associated with poor concentration, loss of short-term memory, impairment of cognitive tasks, blurring of vision, fatiguability, physical lassitude, and unexplained bouts of irritability or depression. Two-thirds of the patients were women, and the majority were in their third or fourth decade (Figure 6.1), with an average duration of symptoms of nine years. One third dated the onset of symptoms to a documented viral infection, often glandular fever, from which they felt they had never fully recovered, and in this group the clinical picture corresponds to that described as the post-viral

fatigue syndrome (Behan et al., 1985). In some cases the onset appeared to follow a pregnancy, adverse drug reaction, or a sudden change of diet, but in others the onset was insidious or episodic, with no obvious triggering factor, often heralded by a feeling of being "run down". Though many were under a degree of stress in the home and/or work environment, this generally appeared to be a consequence of trying to cope with the demands of daily life in the presence of chronic ill-health.

Altogether, 809 patients were classified at presentation as having "systemic" symptoms. Of these, 62 were not considered suitable for formal testing, 35 reported no improvement on the elimination diet and did not undergo challenge, and 309 were lost to follow-up (Table 6.4). The remaining 403 patients either became asymptomatic or were substantially improved on the elimination diet, often for the first time in many years. Because of the subjective nature of patients' self-evaluation it is inappropiate to attach much significance to this observation alone. However, almost all those patients reporting subjective improvement on the elimination diet experienced a recurrence of symptoms in response to one or more of the active double-blind challenges, whereas reactions to placebo occurred in less than 10% of patients ($P < 0.0005$, Table 6.8).

As in the other patient groups studied, aspirin was the compound most frequently responsible for provoking symptoms, yielding positive results in three-quarters of those challenged (Table 6.8). Generally, responsiveness to all active challenge compounds was higher in this group than in patients with symptoms confined to a single organ

system, reflecting the fact that "systemic" patients behave clinically as the most sensitive of all. Not only did each patient, on average, react to a greater number of substances within the challenge battery (Figure 6.2), but their reactions tended to be more severe and longer lasting, and successful long-term dietary control required more stringent avoidance (Chapter 8). The symptoms provoked by double-blind challenge generally corresponded to the presenting symptoms in this group of patients. The commonest were headache, lethargy and malaise, followed by myalgia, mood disturbances, gastrointestinal symptoms, urticaria, rhinitis and asthma (Table 6.10). Aspirin was more often responsible for provoking lethargy ($P < 0.05$), but reactions were otherwise idiosyncratic, with no particular symptoms being associated with specific challenge compounds. It is interesting to note that amongst those in whom urticaria was provoked there was usually a past history of RIU/AO, but many had not experienced symptoms for several years prior to the challenge reaction.

Psychological symptoms such as irritability, moodiness or depression, mental "fuzziness" and lack of concentration were reported in 63% of positive challenge reactions, usually in conjunction with lethargy, malaise and myalgia and other physical symptoms. On 101 occasions, 62 patients (15%) experienced isolated mood changes with a challenge in the absence of any accompanying somatic symptoms, but in only four instances did this occur following a placebo (sucrose in all four, none after starch).

A review of the literature shows that "systemic" reactions to foods involving neuropsychiatric as well as physical symptoms have been described often in the past, although we were not aware of this when the present study began. Indeed, as Speer pointed out when surveying the literature in his monograph (1983), "... the tension-fatigue syndrome is often discovered independently by those who have never heard of it". As outlined above, reports of this kind have generally been met with scepticism by orthodox physicians (May, 1986), although it is not difficult to see why this has been the case. To begin with, many of the symptoms described are typical of psychoneurosis, and are not recognized features of classical allergic reactions.

Characteristically laboratory investigations do not show evidence of organic pathology, and such patients are frequently labelled as being neurotic or hypochrondriacal. On top of this, the prejudices of orthodox physicians tend to be reinforced by the over-enthusiastic claims of "clinical ecologists" and other fringe practitioners whose assertions usually go far beyond the available evidence. Rigorous testing procedures are almost never employed and sweeping generalizations are often made on the basis of anecdotal *evidence; their* observations are rarely published in the scientific literature, usually appearing in popular books accompanied by misguided or outdated mechanistic explanations (see, for example, Mackarness, 1976; Randolph & Moss, 1980, 1981). Such claims are often seized upon by an uncritical media anxious to sensationalize any percieved shortcomings of orthodox medicine, and pandering to the popular belief that a corrupt alliance between government and industry is responsible for adulterating the food supply and environment with toxic substances.

Probably the most important source of confusion, however, is the failure by most investigators to appreciate the pharmacological basis of the great majority of adverse food reactions, making clinical recognition difficult. As emphasized previously (Loblay & Swain, 1986), symptoms can be provoked by a variety of chemical substances, both natural and artificial, common to many different foods. The effects of these compounds are dose-related, and in susceptible people they exhibit pharmacological properties such as withdrawal, supersensitivity, tachyphylaxis and tolerance. Thus, for each chemical the dose threshold for triggering symptoms varies depending on the individual's recent intake from a variety of food sources, so that a particular food need not necessarily produce the same reaction on different occasions. This, together with the fact that reactions may be delayed by many hours (or even a day or two), means that patients can become easily confused or mistaken about which foods cause symptoms, if indeed they are able to recognize the relationship at all. Similarly, the physician may be misled by negative challenge tests with individual foods which contain only small doses of the relevant chemicals, particularly if the latter have not been adequately eliminated from the diet beforehand.

These problems are well illustrated in the recent studies of Pearson, Rix and Bentley (Bentley et al., 1983; Pearson et al., 1983; Rix et al., 1984; Pearson & Rix, 1987). Citing the popularity of a number of books which claim that psychological as well as physical symptoms are commonly the result of "food allergy", and expressing their own scepticism, these authors set out to examine the validity of such claims. They studied 23 adult outpatients who suspected food allergy as a cause of various symptoms, and were only able to confirm this in

four cases who did not manifest psychological symptoms. Criteria for confirmation of the diagnosis were a positive immediate skin test, prevention of open challenge reactions by pretreatment with oral cromoglycate, and the double-blind challenge with encapsulated dried foods or foods disguised in a bland "milk-shake".

The protocol used in these investigations was designed only to identify patients with true, IgE-mediated food allergy, and is likely to have overlooked the occurrence of geniune food intolerance due to pharmacological idiosyncracies. To begin with, a baseline diet of lamb, pears and rice is difficult to follow for more than a week or so, which we believe to be insufficient time for background symptoms to settle in most patients with pharmacological food intolerance. The authors do not specify the duration of their baseline diet, nor do they indicate which foods were subsequently reintroduced, the timing and the amounts used for open challenges, or the clinical responses of the patients. Similarly, there is no indication of the composition of the blind challenges and placebos, of the dosages in which they were administered. In a reply to the criticisms of Alun Jones et al. (1983) it was stated that the dose was "up to 40g" (Pearson et al., 1983), but it is difficult to imagine this being possible with encapsulated challenges; disguising the taste of the foods themselves raises even more difficulties, since even natural flavourings are likely to be rich in salicylates and/or amines. Finally, the amounts used in food challenges of this kind would often be inadequate to elicit an acute reaction in our experience, although when eaten on a regular basis their cumulative effects may result in the insidious reappearance of symptoms after several days, or even longer. This is likely to confound the interpretation of challenges

by producing both false negative and false positive (including placebo) reactions. Such complexities highlight the need for a testing protocol specifically designed for the investigation of pharmacological food intolerance, even though patients themselves may mistakenly claim to be "allergic" to specific foods.

It is evident from the results presented here that even though RIU/AO commonly occurs as an isolated clinical manifestation of food intolerance, it can also be regarded as forming part of a broad clinical spectrum involving several disorders which are not generally thought to be directly related to one another. This is most clearly illustrated by the data shown in Table 6.8. In each of the clinical syndromes studied, not only are the same challenge substances commonly implicated in provoking a recurrence of symptoms, but the frequency with which they do so is also very similar. Thus, as a rule, aspirin is the single commonest challenge to produce a positive reaction in almost every syndrome, followed closely by preservatives, amines, MSG and nitrates. Other challenges such as colourings, anti-oxidants, lactose and gluten provoke reactions less commonly, in some instances approaching the placebo rate of between 5 and 10%.

These observations are even more striking if all symptoms provoked by each of the challenges are included, rather then only those constituting the main presenting complaints (Table 6.9). Indeed, many patients admit to having had symptoms they themselves did not connect with their presenting problem when questioned initially, but the relationship usually became quite obvious when a number of diverse symptoms would occur together in response to particular challenges. Categorizing patients into specific syndrome groups was, therefore,

not always completely clear-cut. As a rule, symptoms were judged to be significant if they had recently prompted consultation with a doctor or other health professional, or had interfered significantly with the patients' day-to-day life and/or work.

In general, each patient tended to react to challenges in an idiosyncratic, though reproducible manner. Thus, even though certain *compounds more frequently provoked reactions, any permutation or* combination could occur in individual cases, emphasizing the importance of systematic investigation and individualized dietary management. Furthermore, when the reactions to a particular challenge compound were compared in the various patient groups it became evident that a single substance such as aspirin could provoke many different symptoms, and that these were generally the same as a the patients' presenting complaint (Table 6.10). Thus, each patient could be regarded not only as having an individual cluster of chemical idiosyncracies, but also a specific pattern of target organ susceptibility which may involve one or more organ systems.

From the pattern of challenge reactions it can also be seen that patients with migraine, irritable bowel and "systemic" symptoms were a more sensitive group than those with urticaria or asthma (Figure 6.2), reacting on average to a significantly greater number of challenges within the test battery. Those with systemic symptoms were the most sensitive of all, with a strong tendency to react adversely to multiple drugs and other environmental substances as well (Chapter 8). In these patients aspirin-sensitivity appears to be a good marker of heightened reactivity to other challenge substances (Figure 6.3).

CHAPTER 7

FAMILY STUDIES

INTRODUCTION

Once the full spectrum of clinical manifestations of food intolerance was appreciated it became apparent that a family history of related symptoms was common. In 1982 a paediatrician, Dr. V. Soutter, joined the Allergy Clinic at RPAH, and it was then possible to test symptomatic children as well as their parents. In a three-year period (from late 1982 to September 1985) 198 families presented with at least one child and one parent suffering from symptoms suspected of being due to food intolerance. In 126 families all affected members underwent dietary elimination and double blind challenge testing, providing an opportunity to determine whether idiosyncratic reactions to each particular food substance might have a genetic basis.

PATIENTS AND METHODS

Of the 198 families presenting to the Allergy Clinic, 72 were not included in the present analysis since at least one member did not complete the elimination and challenge protocol. The remaining 126 families were made up of 350 individuals, as summarized in Table 7.1.

The distribution of presenting symptoms amongst the 350 individuals tested is shown in Table 7.2. Because of the paediatric referral pattern at the clinic there was a selection bias towards children presenting with behaviour disturbances (34% of those under the age of 15). A majority (50%) of parents presented with symptoms referrable to two or more organ systems, with or without constitutional symptoms such as lethargy and malaise, and were therefore classified in the

"systemic" group. There was a strong tendency for similar symptoms to cluster within families, although this was not universal (data not shown). In families where two or more members were classified as having "systemic" manifestations the dominant symptoms often varied between individuals.

TABLE 7.1

FAMILY MEMBERS TESTED

Family Members Tested	Number of Families
Mother and 1 child	52
Mother and 2 children	12
Mother and 3 or more children	4
Father and 1 child	1
Father and 2 or more children	0
Both parents and 1 child	6
Both parents and 2 children	11
Both parents and 3 or more children	5
Neither parent and 2 children	23
Neither parent and 3 or more children	6

* Two families included one grandchild and one family included two grandchildren.

TABLE 7.2

PRESENTING SYMPTOMS

Syndrome	Number of Patients	
	Children	Parents
Urticaria	4	11
Eczema	19	3
Asthma	7	4
Rhinitis	16	6
Irritable bowel syndrome	22	11
Mouth ulcers	0	3
Migraine	15	17
Hyperactivity	77	7
Systemic	66	62
TOTAL (350)	226	124

Challenges

Double-blind challenges were administered as outlined in Chapter 6. Care was taken to ensure that each member of a family was given challenges in a different order so as to minimize the likelihood of them developing reactions in "sympathy" with one another. Since the time of onset and duration of reactions between individuals was

extremely variable, challenges within a family rapidly became out-of-step after two or three reactions, further reducing the likelihood of the results being influenced by expectations.

Statistical Analysis

Reactions to each test substance were tabulated separately for each individual. To determine whether reactions were random, or whether there was a significant tendency for members of the same family to react to the same substances, the results were re-tabulated as follows. If every member tested within a family reacted to a particular challenge compound, that family was classified as +/+ for that compound; if none of the members tested showed any reaction, the family was classified as -/- for the compound; if some members reacted and others did not the outcome was classified as discordant (+/-) for the challenge in question. This procedure was repeated for each challenge compound. The random chance of any two or more unrelated individuals having +/+, -/- or +/- reactions to each of the challenges was determined from the frequency of reactions in the overall patient population described in Chapter 6, from whom the families were drawn, and expressed as binomial probabilities. The number of families reacting as +/+, -/- or +/- for each challenge compound was then calculated by multiplying the number of families in which all members were tested with that compound, by the relevant binomial probabilty. These were compared with the number of families observed in each category (+/+, -/- or +/-) for each compound, using the Chi-squared goodness-of-fit test (Chapter 3).

RESULTS

The expected and observed reaction patterns for each challenge substance are shown in Tables 7.3, 7.4, 7.5 and 7.6. In each table, the number of families tested with each challenge substance is indicated, along with the observed number of families where none of the members reacted (-/-), all members tested developed a reaction (+/+), or there were discordant results within the family (+/-). For comparison, the number of families "expected" in each category is tabulated alongside the observed results, together with the Chi-square statistic and P-value.

Table 7.3 shows the observed and expected patterns with each of the challenges, pooling all positive reactions regardless of the nature of the symptoms provoked in each case. With the exception of propionate, there was a significant tendency for members of the same family to react in the same way to each of the challenges.

Since the use of pooled data might obscure a more significant pattern within one or more sub-groups, the reactions of each individual were re-tabulated according to the particular symptom complex provoked by each challenge, the most common of which were headache, gastro-intestinal, and neuropsychiatric symptoms. The latter included lethargy, cerebral symptoms such as impairment of concentration and

memory, hyperactive behaviour, or changes in mood such as depression and irritability, with or without concurrent physical symptoms (Chapter 6).

Comparisons of observed and expected patterns for each of these, using the Chi-squared goodness-of-fit test, are shown in Tables 7.4, 7.5 and 7.6, respectively. Although asthma, rhinitis, eczema and urticaria also occurred in some individuals, the numbers were too small for valid statistical analysis.

Among family members who experienced headache or gastrointestinal reactions there were only a few significantly shared patterns, a finding which could be due to chance given the relatively small number of individuals involved. However, amongst those who experienced neuropsychiatric symptoms there was a striking concordance of reaction pattern with all challenge compounds (Table 7.6), suggesting that this sub-group is largely responsible for the significant figures shown in Table 7.3. A closer examination of the data in Table 7.6 shows that the magnitude of the Chi-square statistic is almost entirely accounted for by the +/+ component of the reaction pattern with each challenge. Thus, at least with respect to symptoms involving the central nervous system, there appears to be a significant familial tendency to react to the same test substances.

TABLE 7.3

OBSERVED AND EXPECTED RESPONSE PATTERNS IN 126 FAMILIES

Challenge	Number of families tested	Observed			Expected			X^2	P value
		-/-	+/+	+/-	-/-	+/+	+/-		
Aspirin	126	11	62	53	6.3	54.2	65.5	7.0	<0.01
Amine	106	21	37	48	12.7	27.6	65.7	13.4	<0.005
MSG	95	15	39	41	10.5	26.6	58.9	13.2	<0.005
Preservative	97	13	38	46	9.7	28.1	59.2	7.6	<0.01
Antioxidant	91	30	26	35	19.1	14.6	58.2	24.4	<0.005
Propionate	35	11	6	18	8.1	4.6	22.1	2.2	ns
Nitrate	93	15	32	46	8.4	28.8	58.6	8.3	<0.005
Tartrazine	117	21	34	62	18.7	23.4	74.9	7.3	<0.005
Erythrosine	31	9	9	13	5.0	6.2	19.8	6.8	<0.005

TABLE 7.4

HEADACHE RESPONSE PATTERNS IN 126 FAMILIES

Challenge	Number of families tested	Observed			Expected			X^2	P value
		-/-	+/+	+/-	-/-	+/+	+/-		
Aspirin	126	71	7	48	60.2	4.8	61.0	5.7	<0.05
Amines	106	61	6	39	49.9	4.0	52.0	6.7	<0.01
MSG	95	48	6	41	43.6	4.0	47.4	2.3	ns
Preservatives	97	67	7	40	55.2	4.1	54.7	8.5	<0.01
Antioxidant	91	57	3	30	51.3	2.0	36.7	2.4	ns
Propionate	35	22	0	13	22.5	0.5	12.0	0.6	ns
Nitrate	93	40	6	45	36.6	5.2	49.2	0.8	ns
Tartrazine	117	72	5	40	65.8	2.8	48.4	3.8	ns
Erythrosine	31	23	1	7	19.1	0.5	10.5	2.6	ns

TABLE 7.5

GASTROINTESTINAL RESPONSE PATTERNS IN 126 FAMILIES

Challenge	Number of Patients Tested	Observed			Expected			X^2	P value
		-/-	+/+	+/-	-/-	+/+	+/-		
Aspirin	126	54	10	61	44.5	9.0	71.3	3.6	ns
Amines	106	52	6	47	57.5	3.8	43.7	2.1	ns
MSG	95	38	6	51	41.7	4.5	48.8	0.9	ns
Preservatives	97	59	14	41	51.8	4.9	57.3	22.5	<0.005
Antioxidant	91	55	5	30	46.6	2.7	40.7	6.2	<0.05
Propionate	35	20	2	13	17.6	1.2	16.3	1.6	ns
Nitrate	93	37	9	46	34.1	6.2	51.7	2.1	ns
Tartrazine	117	67	8	42	65.8	3.0	50.2	9.8	<0.005
Erythrosine	31	15	5	11	14.6	1.2	15.2	12.7	<0.005

TABLE 7.6

NEUROPSYCHIATRIC RESPONSE PATTERNS IN 126 FAMILIES

Challenge	Number of Families Tested	Observed			Expected			X^2	P value
		-/-	+/+	+/-	-/-	+/+	+/-		
Aspirin	126	54	19	53	64.1	3.9	57.0	60.3	<0.0001
Amines	106	62	8	35	67.8	1.5	35.7	29.5	<0.0001
MSG	95	50	9	37	60.5	1.5	34.1	39.6	<0.0001
Preservatives	97	61	12	42	68.1	2.3	44.6	41.8	<0.0001
Antioxidant	91	62	5	24	61.2	1.1	28.7	14.6	<0.0005
Propionate	35	28	2	4	25.0	0.2	8.8	19.2	<0.0001
Nitrate	93	53	6	34	60.5	1.2	31.3	18.6	<0.0001
Tartrazine	117	63	14	40	72.4	1.9	42.7	77.4	<0.0001
Erythrosine	31	16	3	12	19.6	0.5	11.0	14.4	<0.0005

DISCUSSION

The data presented here, derived from analysis of the challenge reaction patterns in 126 families, provides evidence of a tendency for chemical idiosyncrasies to exhibit a familial pattern. This is most marked with neuropsychiatric symptoms (Table 7.6), which may be a result of the fact that in over half of the families were referred for investigation of a child with behavioural symptoms. Although frequently labelled "hyperactive", such children often also manifested systemic symptoms such as lethargy, malaise, limb pains, abdominal pain, headaches and irritability. The difficulties associated with evaluating such children have already been discussed (Chapter 6).

With subjective symptoms of this kind the obvious question arises whether similar challenge reactions within a family might be due to psychological factors, such that children, parents and/or siblings may have reacted "in sympathy" with one another. This is unlikely to have occurred, however, since each family member was given a different set of challenge capsules in which the order of the test compounds was randomized, and since the variable duration of the challenge reactions usually resulted in the challenges becoming out of step within a family. Thus, two members of the same family would rarely, if ever, have taken the same challenge on the same day.

Thus the question of whether "learned illness behaviour" might result in family members developing similar symptoms (Whitehead et al., 1982), regardless of the trigger, is more difficult to answer. However, the finding of significant familial clustering of reactivity using a randomized series of challenge tests suggests that the specific chemical idiosyncracies are genetically determined, even though the particular symptoms expressed may in some way have been psychologically conditioned.

It is well recognized that recurrent abdominal pain, headache and limb pains, sometimes referred to as "periodic" syndromes, are common in childhood, and tend to cluster in "painful families" (Wylie & Schlessinger, 1933; Cullen & MacDonald, 1963; Oster, 1972; Apley, 1975). In the absence of organic pathology such symptoms are often assumed to be psychogenic, although there is evidence that this may not be the case (McGrath et al., 1983). Some authors have suggested that such families may have a "constitutionally low pain threshold" (Oster, 1972). The role of food intolerance in the periodic syndromes of childhood is controversial (Chapter 6). Most authorities consider this to be a rare cause (Apley, 1975), although it is acknowledged that patients themselves frequently implicate specific foods (Stone & Barbero, 1970). Coca (1953) noted the occurrence of "familial non-reaginic food allergy" in 40 families, but his diagnostic criteria are of doubtful validity, and his notion of "idioblaptic" allergy has fallen by the wayside (Chapter 9).

CHAPTER 8

FOLLOW-UP

INTRODUCTION

From Chapter 6 it is evident that amongst patients with a number of apparently diverse clinical syndromes there is a high prevalence of food intolerance as judged by the ability of double-blind challenges to provoke a recurrence of symptoms. However, the challenge data alone do not allow any conclusions to be drawn about the effectiveness of long-term dietary management in such individuals. In an attempt to address this issue, a retrospective follow-up study of patients presenting for dietary investigation was carried out by questionnaire, the results of which are outlined in this chapter.

MATERIALS AND METHODS

Patients surveyed were those described in Chapter 6, falling into the diagnostic groups listed in Table 8.1. Up to December 1985, a total of 1,859 patients were evaluated, 1,158 of whom completed the strict elimination and challenge protocol. Those who improved on the elimination diet and subsquently completed the challenge protocol were surveyed separately from those who did not return for follow-up (Appendix 15 and Appendix 16).

Patients were asked to indicate the degree to which their diet was still restricted, and to rate their current state of health compared with when the elimination diet was first prescribed. They were also asked whether there were recurrences of symptoms, and if so to identify suspected triggering factors.

TABLE 8.1

PATIENTS COMPLETING ELIMINATION DIET AND CHALLENGE PROTOCOL

Presenting Syndrome	Total No. Presenting	No. Completing Protocol	No. Available * For Follow-Up
Eczema	213	114	107
Asthma	191	140	121
IBS	309	156	143
Mouth ulcers	14	13	12
Migraine	195	111	99
Hyperactive	244	163	143
Systemic	696	461	414

* Patients who had not changed their address or died.

TABLE 8.2

QUESTIONNAIRE REPLIES (%)

Presenting Syndrome	Patients Completing Protocol	Patients Not Completing
Eczema	51	43
Asthma	40	40
IBS	66	38
Migraine	69	37
Hyperactivity	46	37
Systemic	67	42

Ten percent of the patients had changed address, and could not be contacted for follow-up. Of the remainder, 53% of patients who completed the challenges, replied to the questionnaires, and 39% replied of those who were started on the elimination diet but failed to return for follow-up and did not undergo double-blind challenge (Table 9.2).

RESULTS

Overall, of those who completed the elimination diet and challenge protocol 76% continued to maintain a restricted diet six months to five years after initial presentation (Table 8.3). The majority (81%) experienced recurrences related to foods, and as in patients with RIU/AO the foods identified by each individual corresponded closely to those incriminated from the results of double-blind challenge (data not shown).

In addition, 35% of patients noted that certain smells also precipitated symptoms, most often headache, nausea and malaise. Stress was felt to be a triggering factor by 10% of patients, and other miscellaneous factors (e.g. infection, hormonal changes and exertion) were incriminated by 31%. Three-quarters regarded themselves as being either completely well, or considerably better than when they had first presented for investigation.

TABLE 8.3

SYMPTOMS ON FINAL DIET AT FOLLOW-UP

	Eczema	Asthma	IBS	Migraine	Hyperactive Children	Systemic
Number of patients	55	48	95	68	66	278
Continuing restriction (%)	73	73	84	77	77	75
Recurrences (%)						
Foods	95	79	71	79	86	82
Smells	20	48	22	38	20	44
Stress	15	10	7	9	5	12
Other	40	38	26	41	21	29
Symptoms at follow-up (%)						
Completely well	13	8	23	9	11	17
Much better	69	50	58	54	71	58
A little better	15	29	15	25	15	14
No change	4	10	3	7	3	8
Worse	0	2	1	2	0	4

Although the number of questionnaire replies from those who failed to complete the elimination and challenge programme was rather low (39%), it was interesting to note that 63% had continued to restrict

their diet (Table 8.4). The foods incriminated as triggering recurrences in this group were very similar to those identified by patients who had completed the challenges. Half this group of patients considered themselves to be either completely well or much better than they had been at the time of presentation, but a significant minority reported their symptoms as being "no better".

TABLE 8.4

NON STARTERS/COMPLETERS

	Eczema	Asthma	IBS	Migraine	Hyperactive Children	Systemic
No. of patients	35	18	55	29	25	85
Continuing restriction (%)	63	72	56	55	64	67
Symptoms at follow-up: (%)						
Completely well	14	11	13	17	0	8
Much better	37	44	33	35	48	45
A little better	14	17	29	17	28	21
No better	29	22	18	24	24	22
Worse	6	6	7	7	24	4

DISCUSSION

At initial presentation patients suspected of having food intolerance were given all the necessary instruction for dietary testing. Those whose symptoms improved on the elimination diet were asked to contact the dietitian and were sent a set of double-blind challenge capsules, with instructions to return for follow-up once these were completed. Overall, of the 1,859 patients commenced on the elimination diet 62% successfully completed the elimination and challenge protocol. The remainder were lost to follow-up, and it was initially assumed that most of these patients had experienced no improvement on the elimination diet. However, when surveyed by questionnaire it was found that of the 39% who replied a significant proportion had indeed restricted their diet, although thy did not proceed with the challenges. The most common reasons for failure to complete the protocol had to do with the stringency and inconvenience of the elimination diet, but many of these patients had arrived at a modified diet by trial and error, based on the information recieved at the Allergy Clinic. It therefore seems reasonable to conclude that the prevalence of food intolerance amongst our patients, as judged by the proportion completing the elimination diet and challenge programme (Chapter 6, Table 6.4), is likely to be a conservative estimate.

The fact that the demanding nature of the elimination diet and challenge protocol was such that nearly half the patients presenting dropped out might be taken to indicate that this is an unsatisfactory

means of dietary investigation. In general, however, it was found that those who were most highly motivated to comply with the program were patients with the most disabling and chronic symptoms, in whom the elimination diet had resulted in substantial (and sometimes dramatic) improvement. A stringent initial diet can therefore be regarded as a useful means of screening out those patients with relatively mild symptoms who would be unlikely to comply with a restricted diet in the long-term, even if some of their symptoms were found to be food-related.

It is difficult to accurately estimate the long-term success-rate of dietary management, since only slightly more than half the patients surveyed replied to the questionnaires. The figures in Table 8.3 are therefore likely to be significantly biased towards those patients who improved after dietary modification. Nevertheless, it is evident that amongst these individuals a majority experienced food-related exacerbations and had continued to restrict their diet. In many patients other factors such as strong smells, hormonal changes, stress and infections were also incriminated in causing recurrences, emphasizing that food is not always the sole triggering factor in such cases. Indeed, at a clinical level it was noted that the threshold for adverse food reactions could sometimes be lowered premenstrually, during an acute infection, or when a patient was under severe emotional stress. Adverse reactions to strong smells and fumes usually consisted of headache, nausea and malaise, and were more of a problem when the diet was highly restricted; conversely as

the diet was liberalized such reactions tended to become less marked. The most sensitive patients, particularly those in the "systemic" group, often found it impossible to liberalize their diet significantly since even minor lapses would lead to the recurrence of disabling symptoms. In such individuals successful long-term management often required a multidisciplinary approach, with attention being paid to dietary, as well as environmental, physical and emotional triggering factors. In order to maintain adequate nutrition patients were asked to keep a check on their weight and were also prescribed appropriate vitamin and mineral supplements. If indicated, dietary adequacy was formally assessed by asking the patients to keep a 5-day food diary. This was analysed using the "Soda" computor programme (Veraion 1.2, R.J. Hartley, 1982; Computer Models, P.O. Box 280, Bentley, Western Australia), which provides an estimate of the daily intake of all macronutrients, vitamins, calcium and iron.

CHAPTER 9

HISTORICAL PERSPECTIVE

Although food idiosyncrasy has been known since Hippocratic times, modern interest in the subject began around the turn of the century with the rapid expansion in knowledge of immunological reactions to toxins. Progress since that time can be divided into three phases: (i) a scientific phase during the decade after the first description of anaphylaxis by Portier and Richet in 1902, in which most of the basic allergic phenomena were described in both animals and man; (ii) a clinical descriptive period, spanning half a century, during which little scientific progress was made; and (iii) a period of renewed scientific interest beginning with the identification of IgE as the "reaginic" antibody by Ishizaka and colleagues in 1965 (Ishizaka et al., 1966; Ishizaka & Ishizaka, 1967).

Early Scientific Studies

The discovery of anaphylaxis is generally attributed to Richet and his associates Hericourt and Portier in 1902, arising out of Richet's earlier studies on the toxic or urticating substance of the Portugese man-of-war. Initially, it was believed that anaphylaxis represented the loss of protection from a toxin, but it was soon shown that non-toxic substances could produce similar reactions (Arthus, 1903), and that sensitization resulted in the formation of an anaphylactogenic substance which could be passively transferred with serum (Otto, 1907; Friedman, 1909; Doerr & Russ, 1909). In 1906 Von Pirquet coined the term "allergy", although at the time it was still not clearly appreciated that this was an immunological phenomenon. The suggestion that anaphylaxis and immunity were different manifestations of the same underlying processes was first made by Vaughan

(1907) after he discovered that animals could be sensitized not only to bacterial toxins, but also proteins in horse serum and egg white. By 1910 it was recognized that urticaria, angioedema and asthma in humans could occur as manifestations of anaphylaxis, and histamine had been identified as the principal mediator (Dale and Laidlaw, 1910).

Rosenau and Anderson (1906) first demonstrated the occurence of sensitization via the gastrointestinal tract, and the idea that human food idiosyncrasies might be allergic reactions was put forward soon after by Horwitz (1908) and Hutinel (1908). Subsequently Doerr (1909) quoted the case of a 13 year-old boy with urticaria and asthma triggered by egg, and this was followed by similar reports the following year by Barbier (1910) and Castaigne and Gourmand (1910) in the French literature. Schloss (1912), an American paediatrician, described allergy to egg white, oatmeal and nuts, and drew attention to the occurrence of urticaria, angioedema and eczema in such cases. He also showed that serum from such patients could passively transfer sensitivity to guinea pigs, and adapted the scarcification technique of Von Pirquet for use as a diagnostic test.

The phenomenon of desensitization, termed "antianaphylaxis", was observed by Rosenau and Anderson (1906) and Otto (1907), as well as by Richet and others. By 1911, Noon and Freeman had reported the successful use of this procedure in patients with hay fever (Noon, 1911), and soon after this Schloss (1912) reported the oral desensitization of a boy with egg allergy.

Thus, by the outbreak of World War I all the basic phenomena associated with allergic reactions had been documented both in experimental animals and humans. The subsequent history of food allergy is one of increasing confusion. For nearly half a century there was little further scientific progress in the understanding of the basic mechanisms of allergic reactions, although much descriptive work was carried out by practicing clinicians.

Clinical Descriptive Period

Those in clinical practice were hampered by the lack of appreciation that symptoms such as asthma, urticaria and angioedema, which had by then come to be regarded as typical of allergic reactions, could also be triggered by non-immunological mechanisms. There was clearly a strong temptation amongst clinicians to attribute all adverse reactions to "allergy", due no doubt to their new-found ability to explain so many previously mysterious clinical phenomena in immunological terms. This included reactions to foods, so that a wide variety of associated symptoms such as indigestion, colitis, migraine, Meniere's disease, canker sores and chronic head colds also came to be regarded as manifestations of food allergy, as did chronic fatigue, "allergic toxaemia", and other ill-defined constitutional symptoms (Hoobler, 1916; Shannon, 1922; Duke, 1923; Rowe, 1928).

The interpretation of skin tests was another major problem. Many authors considered the frequent absence of cutaneous reactivity to food extracts in patients with clinical symptoms to be a "false negative", due either to failure of observers to pay attention to delayed positive reactions (Vaughan, 1927), or to the fact that

patients were likely to be sensitive to digested fragments rather than the intact food as used in the test solutions (Duke, 1923). The introduction of intradermal testing only added to the confusion (Cooke, 1921). This method proved to be 10 - 100 times more sensitive than the traditional "scratch" test, resulting in fewer "false negatives", but more "false positive" reactions.

It is interesting to observe that a number of early allergists began with a sound scientific background of animal experimentation, but extrapolation to humans often led them far from the path they had originally embarked upon. For example, Coca began his career with passive transfer studies in the guinea pig (Coca, 1919) and was later responsible for establishing the standard method for preparing allergen extracts (Coca 1922), and for coining the widely used terms "reaginic antibody" and "atopy" (Coca and Grove, 1925).

Subsequently, he published a monograph describing a clinical syndrome of familial non-reaginic food allergy, the symptoms of which included headache, tiredness, indigestion, constipation, nervousness, eczema, dizziness, neuralgia, canker sores, heartburn, chronic rhinitis, urticaria, hypertension, asthma, and a variety of other non-specific symptoms. He called this "idioblaptic allergy" to distinguish it from reaginic allergy (Coca, 1942), but fortunately the term never gained widespread acceptance.

Another case in point is Richet's son, Charles Jr., who started working in his father's laboratory and was among the first to study the experimental induction of food allergy. Later however, he, like many

of his contemporaries in the 1920's, was led astray when confronted with patients complaining of a wide variety of subjective symptoms associated with the ingestion of specific foods (Rowe and Richet, 1930). Richet Sr., who was Professor of Physiology at the University of Paris, and was awarded the Nobel Prize in 1913 for the discovery of anaphylaxis, ended his career in obvious frustation, as described by Vaughan (1944):

> "He took up study of the occult. He visited mediums. He became versed in the lore of their profession. It is said that what he wanted most was to produce a specimen of 'ectoplasm' so that he might examine it under the microscope. This, of course, he never accomplished."

During the 1930's there was much lively debate amongst the clinical allergists struggling to comprehend the various types of allergic disorders, particularly those attributed to food. Although there has been considerable criticism of clinicians during this era (particularly by May, 1982), it must be remembered that they were working at a time when basic knowledge of immunopathological mechanisms was still rudimentary, and there were no reliable laboratory methods for identifying immunological reactions. A good example of the difficulties encountered is that of urticaria and angioedema. The clinical features had long been recognized as typical of anaphylaxis, and there was no doubt in the minds of most that they were allergic in origin. However, skin tests were found to be positive in only a minority of cases, and as pointed out by Schloss (1920), the relation ship of the symptoms to specific allergies was impossible to prove in

practice. The degree of confusion caused by such observations can be appreciated by perusing the transcripts of debates which took place during conferences at the time (e.g. discussion following Balyeat and Rusten, 1933). One popular hypothesis to account for the occurrence of "false negative" skin tests was that many patients might have become sensitized to the digestive fragments of certain foods within the gastrointestinal tract, or following absorption into the blood-stream, so that tests with the intact foods would often fail to react with the reaginic antibodies. Based on this idea, experiments were sometimes carried out as described in the following passage by Bernton (1933):

> "I have had the opportunity to study a patient who was subject to giant hives of the most aggravated type. I tested this patient intracutaneously with her whole blood and with her blood serum - both of which were collected at the height of an eruption. I also performed a skin test with extracts of her stools and of urine, with the hope of reproducing wheals, but with negative results".

Based on similar reasoning, "autohemotherapy" (Balyeat and Rusten, 1933) and "autogenous urine therapy" (Plesch, 1947) were tried as desensitizing techniques. Although these procedures now seem almost comically bizarre, they were based on hypotheses which appeared plausible in the intellectual climate of the time.

Another puzzling example was that of aspirin, which by the 1930's was recognized as capable of causing acute urticaria, angioedema and/or

asthma. These symptoms were widely regarded as allergic manifestations despite the almost invariable finding of negative skin tests (Cooke, 1919; Duke, 1923), a misconception which has persisted to the present day, at least in the minds of some authors (Speer, 1983).

Reviewing the situation at that time, May (1982) comments as follows:

> "By 1930 the stage was set for overwhelming confusion: the usefulness of skin tests had been discounted, confidence in clinical impressions was high, the distinction between asymptomatic and symptomatic sensitization was not generally made, the placebo effect was not considered in evaluation of therapeutic manipulations, basic immunologic knowledge was limited, and no one resorted to blind challenges to eliminate bias and the power of the imagination as well as the mimicry of neuroses".

In his historical reviews, May (1982, 1986) describes the emergence of two schools of thought during the 1940's and 1950's. The "orthodox" school were said to have adopted a rigorously scientific approach, accepting as geniune only those reactions which could be shown to have an immunological basis. By contrast, the "unorthodox" school, which evolved into the Clinical Ecology movement, were said to rely on unsubstantiated clinical impressions, and to attribute a wide array of ill-defined symptoms to "multiple food allergies". Whilst May's criticism of today's fringe practitioners is largely justified (discussed in Chapter 6), the historical picture he paints is over-simplified. For example, commenting on the article by Rowe

and Richet (1930) he states:

> "....bearing the reputable and illustrious name of Richet amounted to opening a Pandora's Box which has not yet been closed."

Yet, a careful reading of Rowe's later work (Rowe and Rowe, 1972) shows that he had adopted a sound empirical approach. He tested, but eventually rejected, most of the bizarre methods of investigation and treatment proposed by some of his less critical colleagues. Furthermore, he pioneered the systematic use of elimination diets, most of which are still in use today (Chapter 3). He ultimately concluded that individual food challenges were the only reliable means of testing, provided symptoms had settled on a suitable elimination diet.

Meanwhile, many other "orthodox" practitioners of the 1930's and 1940's searched unsuccessfully for a more objective means of diagnosing food allergies. For example, Vaughan (1939), who wrote one of the most authoritative and widely cited textbooks of the time on allergy, regarded leukopaenia as a characteristic feature of food allergy and promoted the "leukopaenic index" as a diagnostic test. Based on this, Black (1956) developed a more rapid <u>in vitro</u> test which was later modified by Bryan and Bryan (1960) and popularized as the "cytotoxic food test", which, despite evidence of its unreliability (Van Metre, 1983; Anderson, 1987), remains widely used today. Similarly, the "pulse test" was developed by Coca (1942) in order to detect non-reaginic or "idioblaptic" allergies (see above). This test has also fallen into disrepute in medical circles, although it

is still advocated by many alternative practitioners. Another method, still popular amongst Clinical Ecologists, is intracutaneous and/or sublingual "provocation-neutralization" testing, based on the skin-test end-point titration method popularized by Hansel and Rinkel in the 1940's and later modified by Lee (1961), and Dickey and Pfeiffer (1964). As with the other tests described above, the current view is that this technique has no valid scientific basis (Van Metre, 1983; Anderson, 1987).

Recent Scientific Studies and Current Views

Thus, for nearly half a century little scientific progress was made in the understanding of allergic disorders, creating an intellectual void which was filled with a variety of fanciful theories and practices advanced by clinical allergists. As a result, the field of allergy in general, and food allergy in particular, came to be regarded as "unscientific" by many orthodox physicians. The situation began to change during the 1960's, particularly after the discovery that mast cells were the source of histamine (Riley & West, 1953) and the identification of IgE as the reaginic antibody (Ishizaka et al., 1966). This opened the way for a detailed understanding of mast cell function and the identification of a wide array of inflammatory mediators, including the prostaglandins and leukotrienes, and more recently the central role of T cells in regulation of IgE production (reviewed by Goetzl & Kay, 1982; Ishizaka, 1984). At a clinical level, it became possible to classify hypersensitivity reactions on a more rational basis according to the underlying immunopathology (Coombs & Gell, 1968), more sophisticated diagnostic tests were

introduced (reviewed by Freed, 1987), and a range of powerful pharmacological agents became available for the treatment of allergic disease. As a consequence there has recently been renewed interest in food allergy, as witnessed by the increasing volume of literature on the subject (Crook, 1975; May, 1979; Bock, 1980; Denman, 1983; Lessof, 1983; Moneret Vautrin, 1983; Anderson, 1984; Atkins & Metcalfe, 1984; Lessof et al., 1984; Metcalfe, 1984; Truswell, 1985; Brostoff & Challacombe, 1987).

It has gradually become clear that reactions to food may be of two broad types:

(i) True allergic reactions, with a demonstrable immunological mechanism and characteristic clinical features

(ii) Non-immunological reactions, which may have a metabolic or pharmacological basis, and the symptoms of which may or may not resemble those of the classical allergic disorders.

There appears to be a general consensus about the nature of true food allergy. This occurs predominantly in young children with an atopic family background and a history of eczema. Staple foods such as eggs, milk or wheat, and less commonly peanuts or fish are usually responsible, and in many cases the allergenic components have been well characterized (Aas, 1987; Langeland & Aas, 1987). Unrecognized food allergy may be responsible for chronic eczema (Sampson, 1983), gastrointestinal symptoms and asthma (Lessof, 1983). Acute reactions

can cause contact urticaria and angioedema around the lips and mouth, vomiting, abdominal pain and diarrhoea, and rarely generalized urticaria, wheezing and anaphylaxis. Reactions of this kind are well recognized manifestations of food allergy and are usually accompanied by positive skin prick tests with the offending foods (Lessof et al., 1980; May & Bock, 1983). Most children with clinical food allergy are eventually able to tolerate the relevant foods without a recurrence of symptoms, although life-long avoidance of a particular food is sometimes necessary (May, 1982). It is rare for an individual to present for the first time in adult life with symptomatic food allergy.

An important unresolved issue is the question of why many atopic individuals with IgE antibodies to various foods never develop symptoms, yet others do so readily, particularly in early childhood. Clearly, the presence of IgE antibodies alone is insufficient to account for the presence or absence of symptoms. No doubt, the elucidation of other factors involved in the development of clinical symptoms (and in the subsequent development of tolerance) will serve to clear up much of the confusion which has bedevilled this subject since skin testing was introduced in the 1920's.

The situation with non-immunological food reactions is less well understood, as reflected by the inconsistency in terminology used by different authors. The umbrella term "food intolerance" is used to describe any adverse food reaction, usually excluding those mediated immunologically and those of psychological origin. Under this broad category are included idiosyncratic, metabolic, pharmacological, irritant and toxic reactions, as well as symptoms produced by foods

which are said to release histamine and other chemical mediators (Barnetson & Lessof, 1983; Lessof et al., 1984; Anderson, 1986; Sampson, 1986). Most classifications also include a variety of gastrointestinal disorders in which symptoms may be related to meals as a result of abnormal fermentation of food residues, enzyme deficiences (e.g. phenylketonuria, galactosaemia and lactase deficiency), and food poisoning with bacterial or other toxins, chemical contaminants, or micro-organisms. Many of these conditions are included for the sake of completeness, but, since they rarely present as food intolerance in clinical practice, can be ignored for the purpose of the present discussion.

Terms such as "idiosyncratic", "pharmacological", "irritant" and "toxic" are often used with a mechanistic connotation in the literature, but are rarely defined in relation to the underlying pathophysiology, and their use tends to be rather haphazard. There is generally little discussion of the basis of individual susceptibility beyond the listing of a few of the known enzyme deficiencies, and no attempt to address questions such as the variability of target organ responsiveness in different individuals (Chapter 10). More importantly, it has not previously been recognized that most patients with non-immunological food intolerance tend to react idiosyncratically to a range of substances in the diet rather than having an isolated problem with, for example, aspirin, MSG, metabisulphite, etc (Chapter 6).

Present knowledge is still largely in a descriptive phase, and a perusal of current literature reveals that there are almost as many viewpoints as there are authorities in the field. Some authors still

believe that IgE-mediated allergy is the explanation of most food reactions (Speer, 1983), some focus mainly on toxic reactions (Jelliffe & Jelliffe, 1982), some take a rather narrow organ-based perspective (Chandra, 1984), and others take a broader and more balanced point of view (e.g. Lessoff, 1983; Breneman, 1987), but adopt a sceptical attitude to unproven ideas and practices. The most recently published textbook on food allergy and intolerance, edited by Brostoff and Challacombe (1987), embraces the full spectrum of orthodox and unorthodox views from mainstream immunology to the more extreme fringes of clinical ecology.

Unorthodox Views

Amongst the various unorthodox ideas about food allergy the most widespread are those advocated by clinical ecologists, who have attracted increasing attention from physicians, insurance carriers, governments, the scientific community, the media and the public (Terr, 1987). In the USA and Britain this movement has polarized medical opinion to the point where rational debate is often not possible (Check, 1980). Advocates believe that orthodox medicine has failed to recognize a wide range of environmentally induced illness (Mackarness, 1976, 1980; Randolph & Moss, 1980), whereas orthodox physicians reject these ideas as unsubstantiated, regarding such patients as part of a "medical subculture" of malingerers and otherwise psychologically disturbed individuals (Brodsky, 1983). The theoretical and practical basis of clinical ecology has recently been criticized by the American Academy of Allergy and Immunology in a position statement (Anderson et al., 1986), and in Canada it was the subject of a recent Government inquiry (The Report of the Ad Hoc

Committee on Environmental Hyperactivity Disorders, Thompson et al., 1985).

The historical roots of the Clinical Ecology movement can be traced back to the 1940's when, as outlined above, the conceptual vacuum which existed was filled with theories which, though plausible at the time, now seem naive. The most influential of these was originally put forward by Rinkel (1944) who suggested that cyclic fluctuation in sensitivity governs the clinical manifestations of food allergy, and that "masking" could occur with foods eaten regularly. This concept was taken up by Randolph (1950) who, together with Rinkel and Zeller (1951), popularized the idea that "masked food allergy" could be responsible for a wide range of vague, ill-defined symptoms often attributed to psychoneurosis. Like Coca (1942), they contrasted this with the reaginic, or "fixed" food allergies, in which typically atopic symptoms were regularly provoked by the same offending foods. Randolph and his colleagues developed an elaborate hypothesis, based partly on Selye's theory of physiological adaption to stress, in which cyclic food allergy represented alternating adaption and mal-adaption to foods in susceptible people. This later formed the basis of the Clinical Ecology movement (Dickey, 1976), the focus of which has since shifted to the harmful effects of toxic environmental chemicals which are said to produce a form of "immune deficiency", resulting in multiple allergies to foods and other substances (reviewed by Terr, 1987).

The issues involved in the ongoing debate between orthodox allergists and clinical ecologists are complex, and have been discussed extens-

ively by others (May, 1982, Thompson et al., 1985; May, 1986; Terr, 1987). The present study points towards the possibility that in some areas the truth may lie somewhere in the middle, although as pointed out in Chapter 6, the issues are clouded by a lack of scientific rigour, sweeping generalizations and exaggerated claims by those who subscribe to the clinical ecology school of thought.

Common Misconceptions

There are a number of popular misconceptions worthy of discussion since they are encountered so commonly in clinical practice. One is the idea that many vague symptoms which appear to be food related are due to "reactive hypoglycaemia", resulting from the intake of sugar (sucrose) and sugar-containing foods. This concept was first put forward in the 1940's as an explanation for various "psychosomatic" symptoms (Portis & Zitman, 1943; Alexander & Portis, 1946) and has resurfaced repeatedly since then in popular books and amongst "alternative" practitioners. There is very little evidence for the existence of this form of hypoglycaemia as a clinical entity (Cahill & Soeldner, 1974; Yager & Young, 1974; Hofeldt et al., 1975; Ford et al., 1976; Merimee, 1977; Johnson et al, 1980; Charles et al., 1981; Lev-Ran & Anderson, 1981; American Diabetes Association, 1982; Hogan et al., 1983; Lev-Ran, 1983; Best, 1984; Anderson & Lev-Ran, 1985; Australian Diabetes Society, 1987; Truswell, 1987), although one recent study suggests that there may be a small subgroup of patients in whom excessive adrenaline release after hypoglycaemia correlates with post-prandial symptoms (Chalew et al., 1984).

Another misconception currently in vogue is the notion that "candida

hypersensitivity" is responsible for many of the clinical manifestations of food intolerance. Fungal infections have long been incriminated as a cause of allergic disease on an anecdotal basis (Vaughan & Black, 1954), and some authors have claimed that a relationship exists between Candida sensitivity and urticarial reactions to foods containing yeast (Holti, 1966; James & Warin, 1971). In recent years this concept has been extended and elaborated into a comprehensive theory to account for the occurrence of an extensive array of physical and psychological symptoms. It has been widely publicized both in the medical literature and the popular press (Truss, 1978; Crook, 1980; Saifer & Becker, 1987; Kroker, 1987), and is currently embraced enthusiastically by many fringe practitioners.

In the present study, patients who presented with a diagnosis of "hypoglycaemia" or "candida hypersensitivity" almost invariably proved to have pharmacological food intolerance when investigated systematically. Blood sugar levels rarely correlated with symptoms when measured, and symptoms occurring during a glucose tolerance test were usually due to the colouring and/or preservative present in the oral glucose preparations routinely used because such patients were almost always able to consume sugar without difficulty once the other relevant dietary chemicals had been eliminated. Indeed, sucrose and starch were useful placebos because they were so well tolerated generally. Although brewer's yeast contributed to symptoms in some patients with food intolerance (chapters 3 and 6), we found no evidence of the extensive problems supposedly due to yeast as claimed by many fringe practitioners.

Although misconceptions about food allergy are common among lay people, alternative practitioners and medical fringe groups, they may also be found among orthodox allergists. One, which has been perpetuated for several decades, is illustrated by the tables of "food families" published in many monographs and textbooks on the subject (Coca, 1942; Rowe & Rowe, 1972; Speer, 1978; Workman et al., 1984; Buist, 1984; Chandra, 1984; Brostoff & Challacombe, 1987; Breneman, 1987). The idea of grouping foods according to their botanical families probably dates back to the early work of Schloss (1920), but it was Vaughan (1930) who first introduced this as a systematic means of identifying foods likely to show allergenic cross-reactivity. Although based on anecdotal evidence, this approach obviously captured the imagination of clinicians who are accustomed to thinking of inhalant allergens such as pollens in terms of botanical taxonomic groups. However, this classification is based mainly on flower morphology rather than biochemical relationships, and is therefore unlikely to be a useful means of predicting pharmacological cross-reactivity in patients with food intolerance. In a typical list of 52 food families (Collins-Williams & Levy, 1984), 40 include at least one member which contains moderate or high salicylate levels, and 20 include one or more amine-rich foods. Furthermore the pattern is quite haphazard within families. For example, five of the six common members of the potato family are salicylate-rich, potato itself being the sole exception, and two of the six (tomato and eggplant) contain amines. Of the ten members of the grain family corn alone contains salicylates; refined cane sugar is free of detectable salicylate, but molasses is not. In the apple family, apple and quince are salicylate-rich, but pears only contain

significant amounts in the skin. Chemotaxonomy of plants is a rapidly developing field which may eventually prove to be relevant (Singleton, 1981), although apart from the present study there is as yet insufficient information on the chemical composition of commonly eaten foods to be clinically useful.

From a practical point of view, the main use of traditional food families appears to be for the design of rotation diets for patients with multiple food "allergies" (Monro, 1987), but as pointed out recently (Lessof et al., 1984) there is little objective evidence to justify the use of this approach. Patients with true food allergy are almost always clinically sensitive to only one or two foods which are easily avoided, whereas those who react adversely to multiple foods usually prove to have chemical idiosyncrasies (Chapter 6). In our experience rotation diets eliminate additives, and may also reduce the daily intake of natural chemicals by limiting the range of food choices, so that some patients do indeed improve, at least temporarily. More sensitive patients, however, often do not respond and require systematic control of natural salicylate, amine and/or MSG intake, depending on the individual's idiosyncrasies.

Practising allergists often also have particular prejudices about the dangers of a specific food. For example, it is commonplace for the patients to be prescribed a milk-free or wheat-free diet in the mistaken belief that these foods frequently produce "mucus" or gastrointestinal symptoms in allergic patients. Atopic patients often have positive skin tests to these foods, regardless of whether they cause symptoms, and this is sometimes taken as evidence of an "allergy",

indicating that they should be eliminated from the diet. In a recent review of food-induced migraine, Monro (1987) focuses heavily on milk and wheat as the culprits, as do many of the other authors she cites, and the same is true of Alun Jones and Hunter in their review of food intolerance in the irritable bowel syndrome (1987). In our own experience, however, these foods are only responsible for provoking reactions in a minority of patients overall (Chapter 6), and even then they are rarely the sole problem.

It is interesting to speculate as to why patients and practitioners have been so easily led astray, whether it be in relation to hypoglycaemia, yeast hypersensitivity, or food "allergies" generally. It is often suggested that misattribution is due to psychological factors (May, 1982; Pearson and Rix, 1985), but in our experience this is not usually the case. With the benefit of hindsight it can readily be appreciated how this situation comes about. Most patients have very limited knowledge of food composition, and tend to blame the most obvious things. For example, patients who believe in "hypoglycaemia" focus on the sugar in chocolates, lollies, biscuits, sweet desserts, cakes, fruit, sweet drinks etc., and their assumptions are often reinforced when elimination of these foods results in clinical improvement. However, removing these foods from the daily diet not only reduces the intake of sugar, but also of salicylates, amines, preservatives and colourings, and these latter changes are more likely to be the relevant ones. Similarly, an apparently simple change such as the elimination of bread on a wheat-free diet can have a profound effect on the intake of other foods such as honey, jams, Vegemite, cheese, tomato, processed meats and antioxidants (in

margarine), all of which contain significant quantities of the substances commonly responsible for symptoms in patients with food intolerance. Even more extensive changes occur with most "yeast-free" diets currently recommended by fringe practitioners. Not only are bread, yeast extracts, beer and wine excluded, but so too are all food additives, and all naturally "mouldy" foods such as cheese, mushrooms, many fruits and vegetables (including processed fruits and fruit juices), malt, vinegar, sauces, pickles, spices, peanuts, tea, coffee and alcohol, sugar, sweets and all other high carbohydrate foods. These foods are incriminated on the basis that they supposedly encourage the growth of yeast in the gastrointestinal and genitourinary tracts (Crook, 1980; Turner and Simonsen, 1985), although evidence of candida overgrowth is rarely found when sought.

Patients with symptomatic food intolerance often benefit, even if only temporarily, from a major change of diet of the kind outlined above. Clinical improvement can be a dramatic occurrence, sometimes after many years of chronic ill-health where all other therapeutic measures have failed, and both the patient and therapist can thus become deeply convinced that their rationale for dietary change must have been correct. This probably also accounts for some of the sweeping claims of naturopaths and other alternative practitioners who dabble in dietary modification although, as in any other clinical situation, the therapeutic relationship and placebo effect play an important part as well.

CHAPTER 10

CONCLUSIONS

As outlined in Chapter 1, the present study began in 1977 when it was decided to test the hypothesis that RIU/AO is often caused by adverse reactions to food additives and salicylates (Warin & Smith, 1976). The inadequacy of available data on the occurrence of dietary salicylates led to the laboratory analysis of a wide range of commonly eaten foods (Chapter 2), and this information greatly simplified the dietary investigation and management of patients with RIU/AO (Chapter 3 and Chapter 4). Once the importance of natural salicylates was recognized, it became easier to identify a number of other compounds, found in apparently unrelated foods, capable of provoking a similar range of clinical symptoms (Chapters 6 and 7). These observations made it possible to gain a broader understanding of the nature of non-immunological food reactions, as well as providing a useful conceptual framework within which the investigation and management of individual patients could be rationally planned.

Clinical Observations

The majority of patients presenting to the RPAH Allergy Clinic with recognized or unrecognized adverse food reactions were shown to be sensitive to a range of natural and artificial food chemicals. True allergic reactions to foods were sometimes a contributory factor, mainly in children with eczema, but even in the latter group pharmacological intolerance often appeared to be the more dominant problem. Most patients reacted idiosyncratically to several compounds when challenged double-blind, and these results generally correlated well with clinical food reactions. Symptoms could involve the skin, gastrointestinal tract, respiratory tract, or nervous system, each patient manifesting an individual pattern of target organ reponsiveness, independent of the chemical compounds involved.

In approximately 10% of cases, "supersensitivity" was a clinical problem. Such patients found that, as they restricted their diet and their dose-response threshold fell, they became sensitive to foods which were previously tolerated. This sometimes led patients to believe that they had developed "new allergies", without realizing that the same component, found in smaller doses in a variety of foods, was responsible. In most individuals, this process was reversed when symptoms were controlled and the diet was systematically liberalized. However, the most sensitive patients found it necessary to keep to a highly restricted diet in order to maintain well-being. Other, non-dietary factors could also be a complicating problem in such patients, and dietary management was sometimes only one of several therapeutic modalities required, including psychological support.

The clinical behaviour of these reactions strongly suggested an underlying pharmacological mechanism. Thus, abrupt dietary restriction was often followed by withdrawal symptoms, and a fall in the dose-response threshold. Acute reactions behaved in a dose-dependent fashion, and could be followed by tachyphylaxis, i.e. a refractory period, lasting up to 48 hours. Chronic or recurrent symptoms were often more insidious, and appeared to depend on the cumulative dose ingested from a variety of food sources over several days, or over two to three weeks in some cases. Most of these phenomena have also been documented by other investigators, including dose dependence, tachyphylaxis, tolerance, and withdrawal reactions (Pleskow et al., 1982; Asad et al., 1983, 1984; Slepian et al., 1985). Lowering of the dose-threshold with abstinence (supersensitivity), has not been

previously reported, however, probably because most other workers have not excluded salicylate-containing foods to the same extent (Chapter 2).

The use of a pharmacological "paradigm" made it possible to resolve much of the conflicting information in the literature, as well as providing a valuable conceptual framework to assist in day-to-day dietary management. For example, patients often reported that a particular food caused symptoms on some occasions but not others. The explanation became clear once it was appreciated that reactions depended on: (i) the cumulative dose of the relevant chemicals from a variety of food sources, and (ii) the patient's dose-threshold, which may fluctuate depending on which foods had been eaten recently. A commonly encountered example of this was the amine-sensitive patient with migraine who had noticed that chocolate sometimes, but not always, triggered a headache. The diagrams shown in Figures 10.1 and 10.2 were used to illustrate to such patients how a combination of foods, eaten in varying amounts and at varying intervals, could determine whether or not a reaction might likely to occur after a "bolus" dose.

Similarly, charts showing the salicylate, amine and MSG content of commonly eaten foods (Appendices 10, 11, 12) enabled sensitive patients to introduce more variety into their daily diet, at the same time ensuring that they avoided the cumulative effects which could lead to a relapse of symptoms over several days or weeks.

FIGURE 10.1

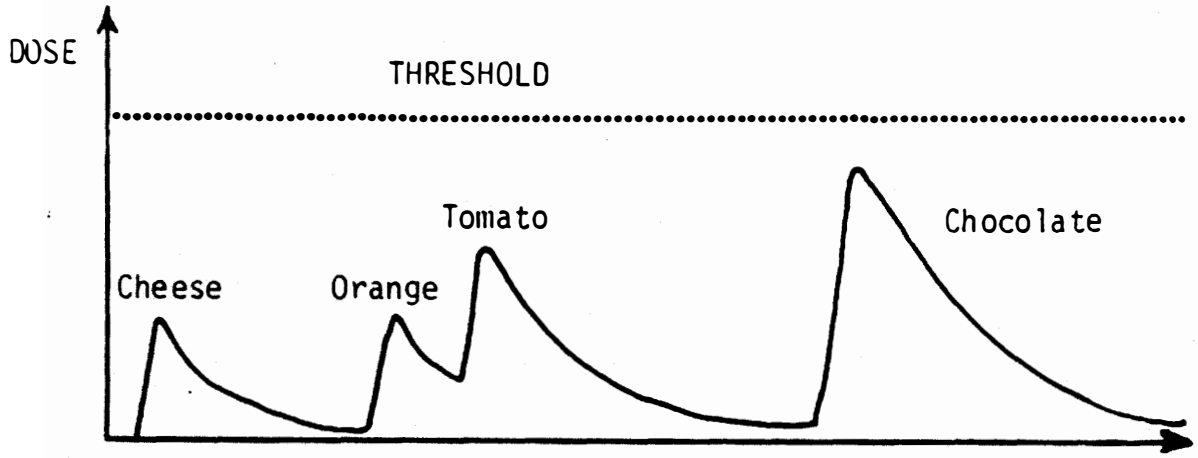
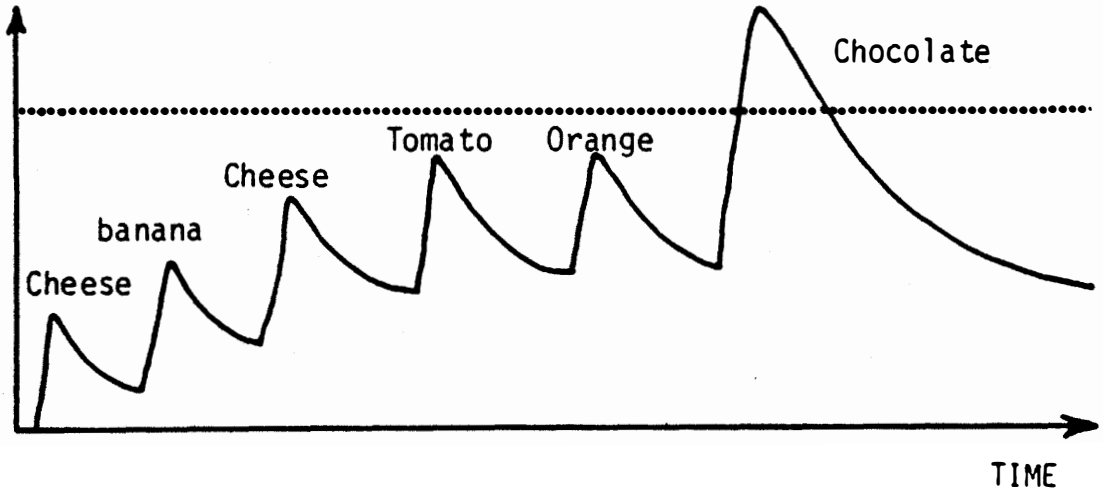


FIGURE 10.2



Interpretation of Challenge Results

The interpretation of positive of challenge reactions in the present study warrants further discussion. Most of the challenge dosages were selected on the basis of those used in previously published

studies, taking into account the amounts likely to be consumed in a day on an average diet. The doses used for metabisulphite and MSG are two to three times those which might be consumed under normal circumstances, and that of aspirin is approximately 10-20 times the average daily intake. It could therefore be argued that the challenges represent an unphysiological exposure to these food substances, and that some healthy individuals may also react if tested. On the other hand, no single challenge given as a bolus dose can accurately reproduce the cumulative effects of long-term low-dose salicylate ingestion in the daily diet of a sensitive individual. In the clinical context, the objective of challenge testing is to reliably identify the food substances responsible for provoking symptoms in patients selected because of a favourable response to dietary elimination.

Ideally, normal control subjects should have been included in the present study in order to estimate the sensitivity, specificity and predictive value of the challenge tests in the diagnosis of food intolerance (Galen & Gambino, 1975). However, the practical and ethical difficulties associated with placing normal individulas on a highly restricted diet for up to six weeks precluded this. The only available information is our own retrospective analysis of 27 "healthy" relatives who went through the elimination diet and challenges in order to provide moral support for their children who were undergoing investigation. Of these, 15 had no reactions whatsoever whereas 12 developed symptoms in response to at least one active challenge (data not shown). Interestingly, each of those who reacted could identify the symptoms as having occurred previously,

even though they had not generally been attributed to food, whereas those who did not react to any challenge had been previously asymptomatic.

Clearly, this question requires further analysis, and steps have been taken to carry out a prospective study of normal individuals. It may eventually emerge that a proportion of asymptomatic people are susceptible to the challenge doses employed here, and that, as with allergen skin prick tests, a positive result can only be interpreted in the appropriate clinical context. Nevertheless, in the present study challenge testing did appear to reliably identify the relevant foods symptomatic patients as judged by the follow-up results (Chapters 4 and 7).

Possible Underlying Mechanisms

For the purposes of the present discussion it is useful to begin by examining the literature dealing with two overlapping issues: the pathogenesis of RIU/AO, and the mechanism of aspirin intolerance. In both cases it is evident that, despite a plethora of information and the existence of many plausible hypotheses, the nature of the underlying abnormalities remains unknown.

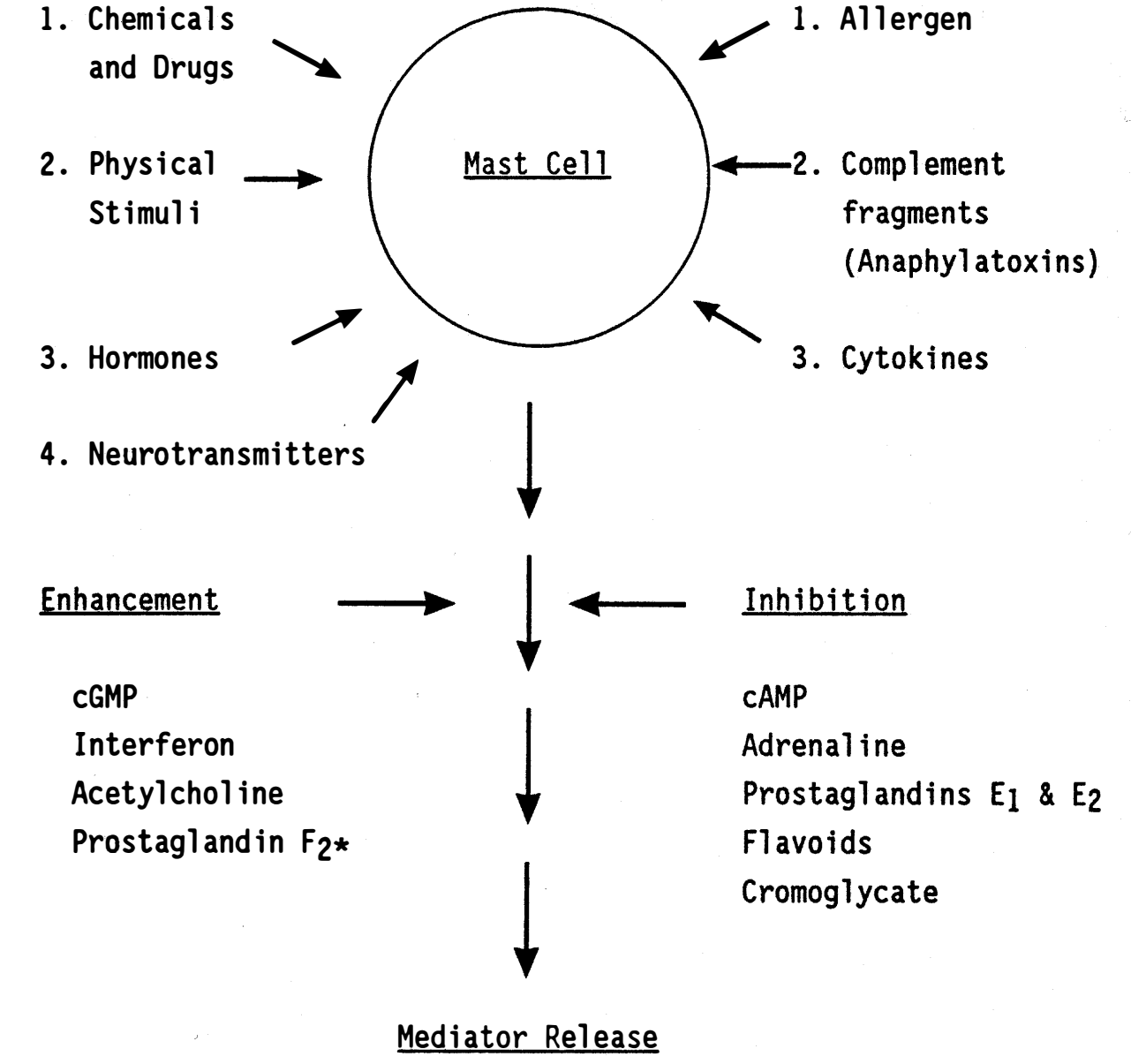
It is widely acknowledged that the final common pathway in urticaria involves the release of histamine and other mediators from skin mast cells (reviewed by Kaplan, 1981; Czarnetzki, 1986). Mast cells may release mediators in response to immunological as well as in response to non-immunological stimuli, as illustrated in Figure 10.3. It is now generally recognized that RIU/AO is not an IgE mediated reaction,

by contrast with acute urticaria. Many mechanisms have been proposed, including deposition of immune complexes, vasculitis, complement activation and kinin formation (reviewed by Lessof, 1983, and Czarnetzki, 1986) but none has proved capable of explaining all the clinical and laboratory findings in patients with RIU/AO. In a recent review of the subject, Rosenstreich (1986) points out that any hypothesis concerning the pathogenesis of chronic urticaria must account for three observations: (i) the presence of activated T-lymphocytes in lesions, (ii) increased dermal mast cell histamine release with a variety of stimuli, and (iii) the decreased releasability of peripheral blood basophils. He argues that there is strong evidence to link increased mast cell releasability to T-cell derived lymphokines, but points out that the T-cell infiltrate is itself likely to be a late-phase reaction secondary to mast cell degranulation, concluding that "... the major unresolved question in this disease is the nature of the inciting agent" (Rosenstreich, 1986).

The other relevant issue, about which there has been a great deal of recent speculation, concerns the underlying mechanism of aspirin intolerance. Urticaria, asthma and anaphylaxis due to aspirin have been known since the early part of the century (Hirschberg, 1902) and were assumed to be allergic phenomena until recently, mainly on clinical grounds (Duke, 1923; Vaughan and Black, 1954; Speer et al., 1981). However, extensive investigation has shown little evidence of an IgE mediated mechanism and most authors now consider aspirin intolerance to be a non-immunological phenomenon (reviewed by Spector & Farr, 1983; Rainsford, 1984).

FIGURE 10.3

MAJOR TYPES OF STIMULI WHICH CAUSE MAST CELL MEDIATOR RELEASE



* Adapted from Czarnetzki, 1986

Stevenson (1984), who has written extensively on the subject, discusses four main hypotheses: (1) cyclooxygenase blockade with diversion of arachidonate into the lipoxygenase pathway, (2) non-IgE mediated mast cell degranulation, (3) excess production of inflammatory complement fragments, and (4) activation of the contact system with excess kinin formation. The first of these is currently the most favoured mechanism, partly because of the known effects of aspirin on arachidonic acid metabolism (Vane, 1971; Smith & Willis, 1971; Ferreira et al., 1971), and partly because of the cross reactivity with other non-steriodal anti-inflammatory drugs (NSAID's) (Lancet Editorial, 1981; Spector et al., 1981; Szezeklik, 1983; Szezeklik & Gryglewski, 1983; Asad et al., 1983, 1984; reviewed by Rainsford, 1984; Stevenson, 1984; Slepian et al., 1985).

One interesting aspect of aspirin idiosyncrasy is the observation that regular ingestion can lead to desensitization which wanes after cessation of exposure (Widal et al., 1922; Zeiss & Lockey, 1976; Pleskow et al., 1982; Asad et al, 1983; reviewed by Stevenson et al., 1982, 1984; Slepian et al., 1985). Possible explanations include: (1) saturation of aspirin binding sites, (2) depletion of mediators, (3) increased clearance and/or degradation of mediators, (4) tachyphylaxis to mediators, (5) feedback inhibition of mediator release, or (6) a non-specific decrease in airway irritability. Present evidence suggests that mediator depletion and reduced bronchial reactivity are unlikely explanations (Slepian et al., 1985).

Despite the attraction of the idea that aspirin idiosyncrasy is due to an abnormality of arachidonic acid metabolism there are a number of inconsistencies and anomalies to be found in the literature, leading at least two reviewers to conclude that there is currently no satisfactory explanation for the underlying mechanism of asthma and/or urticaria in such patients (Stevenson, 1984; VanArsdel, 1984; Stevenson & Lewis, 1987). One important problem is the observation that tartrazine and benzoate appear to cross-react with aspirin, yet neither are known to interfere with prostaglandin synthesis (Gerber et al., 1979). To this may be added the finding from the present study that a range of other substances may also provoke reactions in aspirin-sensitive patients, including sodium salicylate, amines, MSG, and a number of food additives (Chapters 3 and 6). It might be argued that different mechanisms operate in different clinical syndromes (Settipane et al., 1974; Stevenson et al., 1982), but like Asad et al. (1984) we have found it difficult to draw clear dividing lines in individual patients, suggesting a common underlying abnormality.

In considering alternative hypotheses it may be helpful to examine the known pharmacological actions of the various compounds capable of provoking adverse reactions. The actions of sodium salicylate have been the subject of some debate, but it now seems clear that this is due to differences between in vitro and in vivo findings (Higgs and Vane, 1983; Rainsford, 1984). Aspirin is a much stronger inhibitor of prostaglandin production in vitro, but is rapidly converted to salicylate after absorption (Chapter 4), and in vivo these two

compounds have similar anti-inflammatory properties. Salicylate may also paradoxically increase the formation of prostaglandins by acting as a free radical scavenger, partly over-riding the effects of inhibition of cyclooxygenase (Rainsford, 1984). Free radical scavenging could also account for reactions to benzoic acid and other phenolic compounds which have no direct effect on prostaglandin metabolism (Rainsford, 1984). Interestingly, a role for free radicals has been suggested in aspirin intolerance (Moneret-Vautrin and Martin, 1985), although the evidence was rather indirect. Another possibility is that salicylates and other lipophilic phenols may act by uncoupling oxidative phosphorylation (Wainio, 1970). Indeed, before the discovery of prostaglandins in the early 1970's this was considered one of the most important actions of aspirin (Rainsford, 1984).

Another important group of compounds to consider are food additives, in particular the colourings and preservatives, which often cross-react with aspirin in sensitive patients. Little is known of the precise mode of action of most preservatives, but they are thought to inhibit microbial enzyme systems, including those responsible for basic metabolism and for synthesis of proteins, nucleic acids and cell wall constituents (Lueck, 1980). It is acknowledged that similar enzyme systems may be inhibited in humans, although factors such as absorption, distribution, metabolism and excretion are likely to lower the effective tissue concentration of these substances far below those required to inhibit microbial growth in foods. Many mechanisms have been proposed to account for the adverse effects of tartrazine, but the evidence so far available is inconclusive

(reviewed in detail by Hesser, 1984; Murdoch et al., 1987a,b). Tartrazine itself is not structurally related to salicylates (which are 2-OH benzoic acid derivatives) or to the commonly used preservatives, but its major metabolite, sulphanilic acid, is an analogue of para-aminobenzoic acid (PABA). Indeed, the family of sulphonamide drugs was discovered accidently through tests of azo dyes (Schreiber, 1985), raising the possibility that antimicrobial activity might be a common property of compounds which are prone to trigger adverse reactions. It is interesting to note that erythrosine has been found to alter gut microflora, even though its principal use is as a food colour (Drs. R. Adams and K. Murray, CSIRO Division of Food Research, personal communication). The salicylates are also known to possess antimicrobial activity, and were used as preservatives in some countries until the 1950's (Lueck, 1980).

A major difficulty with the various mechanisms described above is the problem of how to account for the variable pattern of individual idiosyncrasies and target organ susceptibility seen in patients with food intolerance. One possibility is that differences in absorption, metabolism and/or excretion of particular compounds could determine the individual's idiosyncrasies (Glover et al., 1983), with the final common pathway being via a non-specific mechanism. The occurrence of desensitization might then be seen as a reflection of substrate-dependent enzyme induction in the relevant metabolic pathways (Guengerich, 1984). However, the absence of any significant difference in salicylate pharmacokinetics between aspirin-sensitive RIU/AO patients and controls argues against this as the principal abnormality (Chapter 5).

An Alternative Working Hypothesis

As outlined above, no single hypothesis has thus far satisfactorily explained the protean clinical manifestations of food intolerance, the relationship between aspirin and various other substances capable of provoking idiosyncratic reactions, and the associated pharmacological phenomena. However, an important clue may lie in the finding of a high incidence of reactivity to biogenic amines and MSG amongst our patients (Chapter 6). These substances are not recognized as antimicrobials, inhibitors of prostaglandin production or uncoupling agents in oxidative phosphorylation, and are more likely to act via neurogenic mechanisms. The biogenic amines have well-recognized neuro-humoral actions (Jones, 1983), and glutamate is an excitatory neurotransmitter in the central nervous system (CNS)(Filer et al., 1979; Fagg, 1985), suggesting the possibility of an underlying neuropharmacological abnormality involving regulatory neuropeptides (Loblay & Swain, 1986).

As discussed already, RIU/AO is characterized by abnormal dermal mast cell releasability with a variety of stimuli, for reasons which are not understood (Rosenstreich, 1986). One pathway of mediator release is via peptidergic nerve fibres which are involved in the axon reflex, first described by Sir Thomas Lewis in 1937 (reviewed by Lembeck, 1983; Foreman & Jordan, 1983). The afferent C-type nerve fibres in this pathway mediate pain and itch, the wheal and flare response, and the vascular response to cold, and in recent years have come to be regarded as the mediators of "neurogenic inflammation" (Hartschuh et al., 1983; Foreman & Jordan, 1984; Payan et al.,

1984; Pernow, 1985). They comprise several populations of neurons containing substance P, somatostatin, CCK-8, bombesin, VIP and/or neurotensin (Lembeck, 1983; Hartschuh et al., 1983), and recent evidence shows that some fibres terminate in direct contact with cutaneous mast cells (Professor S. Holgate, personal communication). Primary involvement of peripheral nerves in RIU/AO is also suggested by the size and distribution of the lesions, the occasional occurrence of prodomal tingling, and the interesting observation that angioedema of the lip often does not spread beyond the midline (Czarnetzki, 1986; and unpublished personal observations). A mechanism involving abnormal sensitivity of the axon reflex could also account for the tendency in some patients for urticaria to be triggered by physical factors such as cold, sunshine, vibration and exercise, as well as the common observation that local pressure or scratching can induce lesions.

Similarly, it has recently been suggested that asthma can be regarded as a disorder of the axon reflex (Barnes, 1986) and that neurogenic inflammation may play an important role in the pathogenesis of bronchial hyperreactivity (Barnes, 1987a,b). Migraine also is now thought to be triggered primarily by neuronal mechanisms (Rose, 1983), with vascular dilatation occuring secondary to release of VIP and substance P (Moskowitz, 1984; Goadsby & Macdonald, 1985). In irritable bowel syndrome, too, it has been shown that motor control mechanisms are defective (Kumar & Wingate, 1985), raising the possibility of an abnormality in the structure or function of the enteric nervous system in such patients (Calam et al., 1983; Makhlouf, 1985; Cervero & Sharkey, 1985; Furness & Costa, 1987). A neuropharmacological abnormality can also readily be envisaged in children with

food-related behaviour disturbances (Shaywitz et al., 1977, 1978; Swanson & Kinsbourne, 1980; Weiss et al., 1980; Augustine & Levitan, 1980; Kaplita & Triggle, 1982; Gualtieri & Hicks, 1985), as well as in adults with ill-defined neuropsychiatric manifestations (Chapter 6).

With the existence of ten classical neurotransmitters, and the ever-increasing number of newly discovered regulatory neuropeptides, each acting on multiple receptors, there is ample scope to envisage a wide range of individual idiosyncrasies to substances which may act as agonists or antagonists at multiple sites (Krieger & Martin, 1981; Polak and Bloom, 1983; Schmitt, 1984; Bloom, 1985; Quirion, 1985; Altman, 1985; Snyder, 1986). Phenomena such as withdrawal effects, desensitization and supersensitivity could then be interpreted as manifestations of ligand-induced modulation of receptor function (Iversen & Iversen, 1981; Creese & Sibley, 1981; Fleming, 1981; Hollenberg, 1985b; Sibley & Lefkowitz, 1985). Desensitization is a well-recognized occurrence under these circumstances, involving down-regulation of receptor numbers and/or their uncoupling from regulatory G-proteins and the second messenger system, whereas supersensitivity is the reverse of these processes (Hollenberg, 1985a; Sibley & Lefkowitz, 1985; Dunlap et al., 1987). Receptor "cross-talk" (Hollenberg, 1985a; Sibley & Lefkowitz, 1985) could also account for the apparent cross-reactivity of a wide range of apparently unrelated substances, as well as for the phenomenon of cross-desensitization between aspirin, other NSAID's and tartrazine (Pleskow et al., 1982; Lumry et al., 1983; Michel et al., 1984; reviewed by Slepian et al., 1985).

The Basis of Individual Susceptibility

As yet nothing is known of the individual variability of neuronal receptor structure or control mechanisms. However, given the enormous range of variation in the human genome, amounting to approximately one base change per 100 nucleotides (Lancet Editorial., 1987), it is likely that polymorphisms will eventually be found in each receptor system. If these occur, for example, at allosteric sites which can bind chemicals of the kind discussed here (Changeux & Revah, 1987), this would result in genetically determined variations in sensitivity to the ligand in question. Variability of this kind in tissue-specific receptors might then explain the pattern of target organ susceptibility seen in individual patients (Chapter 6).

Metabolic variations are also likely to modify the expression of food intolerance in some individuals. For example, it has been shown that some patients with diet-related migraine have lower than normal levels of the enzyme phenol-sulphotransferase which is responsible for conjugating tyramine and other related phenols (Glover et al., 1983; Ishikawa et al., 1986; Gibb et al., 1987). In women hormonal factors such as pregnancy, menarche, menopause, oral contraceptives or the menstrual cycle can influence the clinical expression of food intolerance (Chapter 6), and it is tempting to speculate that this may be due to the effects of steroid hormones on neuroregulatory mechanisms (McEwen & Pfaff, 1985).

Idiosyncrasies, Side-effects and Toxicity

Before concluding this discussion, it is relevant to consider the toxic side-effects of salicylates, and their relationship to the idiosyncrasies documented in the present study. Typical symptoms of chronic salicylate toxicity include headache, nausea, vomiting, diarrhoea, blurred vision, tinnitus, vertigo, and central nervous system (CNS) symptoms such as lassitude, drowsiness, confusion, restlessness, excitement, tremor, progressing in severe cases to hallucinations, delirium, convulsions, and eventually depression and coma (Rainsford, 1984; Koller & Cohen, 1979). The mechanism(s) of salicylate side effects are not well understood. Some symptoms, including many of those involving the CNS, are due to the direct effects of salicylates on neuronal function (reviewed by Rainsford, 1984). Known mechanisms include reduced synthesis of gamma-aminobutyric acid (GABA), an inhibitory neurotransmitter (Koller & Cohen, 1986), increased serotonin production, and altered membrane permeability to K^+ and Cl^- ions, resulting in impaired impulse conduction and synaptic activity (Rainsford, 1984). The CNS effects of salicylates also occur at therapeutic doses, and are responsible for their analgesic and antipyretic properties independent of any anti-inflammatory action.

Although salicylate intoxication is usually associated with chronic high-dose ingestion, it is known that there is a wide range of individual variation (Smith & Smith, 1966). Indeed, the differences between "toxicity", "side-effects" and "idiosyncrasy" may simply be one of degree, depending on cumulative dosage and a multitude of

genetic and environmental factors which determine individual responsiveness (Vesell & Penno, 1983; Klaassen, 1985). The results of the present study suggest that similar symptoms may occur at much lower doses than hitherto suspected in a small proportion of the population, presumably comprising individuals who are at the tail-end of the normal distribution curve (Ariens & Simons, 1982).

Food Intolerance - A Biological Perspective

It is of some interest to consider the origins of dietary salicylates, and to speculate about the biological significance of the tendency for some humans to react adversely to such compounds. Salicylates, along with several thousand other phenolic and related compounds, are secondary metabolites synthesized via the Shikimic acid pathway in plants (Singleton, 1981; Torssell, 1983) and have been a source of medicinal agents probably throughout human history. The functions of phenols in plants have been a long-standing puzzle, but it is now generally thought that their principal role is an ecological one, i.e. controlling the biology of other species in the environment (Torssell, 1983). Secondary metabolites are involved in protection from micro-organisms, insects and predators via their toxic effects, as well as in the reproductive cycle, repair and healing mechanisms, and interaction with other plants in the environment. Insects, herbivorous animals, and man, have evolved various means of adaptation to these toxic substances, including detoxification and excretion (Caldwell & Jakoby, 1983), tissue-specific resistance (Cohen, 1986), and behavioural mechanisms (Logue, 1986; reviewed by Swain T., 1978; Singleton, 1981; Futuyma & Slatkin, 1983).

Each of the general types of natural phenols can be subdivided into those of common, uncommon, or botanically rare occurrence. Most of the phenols noted for toxicity or physiological potency in animals are not only extremely limited in botanical distribution, but are also unique or highly unusual in their chemical structure when compared with common plant phenols (Singleton & Kratzer, 1973). By contrast, salicylates and benzoates may be regarded as botanically common, and of relatively low toxicity. It should be remembered, however, that toxicity is dependent not only on the pharmacological properties of a particular foreign substance, but also on the cumulative dose and the susceptibility of the individual (Ariens & Simons, 1982; Albert, 1987).

The total amounts of common dietary phenols can range from nearly zero in refined, plant-derived foods like table sugar, to 20% or so of the dry weight of the diet of some herbivorous animals (Singleton, 1981). When given a free choice, animals in general, and man in particular, select food plant parts with a low phenol content. Particularly with agriculture, man has not only selected and modified by breeding the plants he cultivates for food, but in a great part of the most fertile land he has completely changed the environment in terms of the diversity of individual plant species and the resultant occurrence of various phenols. We therefore may have a warped and limited view of the possible risk of plant toxins. When domesticated animals were introduced by Europeans to the unfamiliar flora in Australia, about one plant species in twenty was found capable of causing death (Culvenor, 1970). The ratio is a little more favorable

in native North American or African flora, but allowing for unrecognized, slow-developing, or subacute toxicity, it is clear that many plants are likely to be harmful. With the apparent exception of the grasses and grains of the Gramineae, few plants allow predation by animals without offering some form of active resistance, such as thorns or toxins (Culvenor, 1970). Fruit may be sacrificed to an animal in return for seed dispersal, but often the seeds themselves may be toxic or may at least resist digestion.

Whilst plants have evolved toxins to help protect them from pests, parasites, disease organisms, or predators, animals and man have evidently evolved and survived by "learning" to avoid or withstand these toxins (Swain T., 1978). One of the most important mechanisms of avoidance is the sensing of noxious substances by smell and taste, and the conditioning of aversive behaviour by foods which provoke unpleasant reactions (Riley & Tuck, 1985; Logue, 1986). The pharmacological and neurological basis of behavioural aversion are only beginning to be unravelled, and are likely to be exceedingly complex (Braveman & Bronstein, 1985). In the context of clinical food intolerance, "aversion" is a term sometimes applied to psychological reactions in which the patient mistakenly attributes symptoms to a particular food, which is then avoided (Lessof et al., 1984). Although conditioned aversion can to some extent be modified by psychological and social factors (Logue, 1986), it is essentially a physiological phenomenon which should be clearly distinguished from phobias and delusions relating to foods. The strongest aversive stimuli are those which provoke nausea, vomiting or other gastrointestinal symptoms via the CNS (Kiefer, 1985), whereas symptoms such

as urticaria or mouth ulceration appear to be less potent (Grill, 1985).

The existence in the human population of polymorphisms involving central and peripheral nociceptive mechanisms, and predisposing certain individuals to various food and chemical idiosyncrasies, may be a significant evolutionary advantage. For example, in times of famine or drought, or when exploring new habitats, a population of hunter-gatherers would be more likely to adapt to new food sources containing potentially toxic substances. It is also tempting to speculate that the higher frequency of adverse food reactions amongst females of reproductive age (Chapter 6; Young et al., 1987) may be of similar biological significance. Many food phenols are known to be teratogenic (Singleton, 1981), possibly including salicylates (Rainsford, 1984; Zierler & Rothman, 1985). It seems reasonable to suppose that women who are more sensitive to such compounds would be more likely to avoid them, and thus might enjoy a reproductive advantage (Loblay & Swain, 1986).

Conclusion

Much remains to be learned about the clinical features as well as the underlying mechanisms of food intolerance. The major findings of the present study have been recognition of the widespread distribution of natural salicylates in common foods, the clinical spectrum of adverse reactions in salicylate-sensitive patients, and the idiosyncratic cross-reactivity between a wide range of natural and artificial

substances in the diet. These findings led to the successful development of a systematic and reliable method for the routine investigation and management of food-sensitive patients, suitable for use in outpatient clinics or private practice.

The findings have also raised many questions for future study. For example, how common is unrecognized food intolerance in the general community? Which patients require formal investigation? How many normal, asymptomatic individuals would be likely to react to some of the challenges used here? Which other food substances, not yet studied might also be contributing to symptoms in some patients? What is the most appropriate challenge protocol for routine diagnostic use? How effective is long-term dietary modification in controlling symptoms? What role do psychological factors play in patient subgroups such as those with migraine, irritable bowel, hyperactivity or neuropsychiatric manifestations? A double-blind cross-over clinical trial in adults with migraine is already underway, and a detailed study of hyperactive children is in the planning stages to answer some of these questions.

Elucidation of underlying mechanisms is likely to be more difficult, and outside the scope of the present study. However, as discussed above, careful clinical investigation can provide valuable clues for generating plausible hypotheses worthy of closer examination.

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APPENDICES

APPENDICES

APPENDIX 1

ELIMINATION DIET I

DIETITIAN: _____ TELEPHONE: _____

The aim of this diet is to exclude certain additives and natural chemicals from your daily food intake. These include : natural salicylates, amines, brewers yeast, monosodium glutamate (MSG), antioxidants, preservatives and colourings. Once excluded, deliberate reintroduction of each of these substances ("challenge") can help identify the cause of your problem.

DIET RULES :

1. Use only those foods allowed
2. Do not use any foods not listed
3. Avoid all non-essential medications

DIET NOTES :

Withdrawal symptoms may start to occur during the first two weeks on the elimination diet. Some or all of your symptoms may increase temporarily, but these withdrawal symptoms usually disappear within a few days to two weeks.

CHALLENGE RULES :

1. Commence challenges after a minimum of two weeks on the elimination diet, and only after at least five days free of symptoms. If, after six weeks, there is no improvement, contact your dietitian and doctor.

2. Challenge with chemical capsules as outlined.
   a. Challenge capsules should be taken in the morning (1/2 hour before, or two hours after breakfast)
   b. Reactions may occur anytime up to 48 hours after taking a challenge capsule.
   c. If you do develop symptoms during this time, wait until they have gone completely, and then a further three days free of symptoms, before going on to the next challenge.
   d. If you do not develop any reaction within 48 hours, go on to the next challenge.

Please read carefully :

ELIMINATION DIET

1. The diet should be followed for at least two weeks or until symptoms settle, which may take up to six to eight weeks.** Once you achieve a period of five days in a row free of symptoms, you are to be challenged with each of the chemicals which commonly cause symptoms. If symptoms are still present after six weeks, contact your dietitian or doctor.

2. On the chart provided, keep a detailed record of :
   a) foods eaten throughout the day
   b) symptoms - type and how long they last
   c) challenges and/or medications taken

3. Any non-essential medication should cease at least five days before starting the challenges (consult your doctor if uncertain). Should you have a headache during the challenge period, Panadol and/or codeine preparations may be used.
   - ALL ASPIRIN-CONTAINING DRUGS SHOULD BE AVOIDED
   - Coloured capsules (e.g. antibiotics) can be opened and emptied into clear gelatine capsules which can be bought from most pharmacies.
   - Coloured tablets can be gently rubbed under a running tap to remove the coloured coating.
   - Use only recommended vitamin preparations

** In some individuals, improvement may be gradual over four to six weeks, but if there is no change in symptoms after six to eight weeks of strict dieting, continued restriction is unlikely to be helpful and the dietitian should be contacted for instructions on how to resume a normal diet.

VITAMINS & MINERALS

It is recommended that patients on the elimination diet should take a vitamin and mineral supplement as the diet contains marginal amounts of some vitamins and minerals. Care should be taken to ensure that these supplements do not contain preservatives, colours or flavours as these will interfere with the challenge results.

Some preparations to avoid are those which contain :

A. PABA = Para amino benzoic acid, or ABA = Amino benzoic acid.
B. coloured capsules
C. syrups
D. alfalfa
E. kelp
F. rosehips

It is suggested that one of the following multivitamin supplements be used :

A. Elevit RDI (Roche)
B. any multivitamin in a capsule : take the powder only - discard the plastic coloured capsule coating, e.g. Myadec (Parke Davis)

CHALLENGES

1. Take the challenge capsules in the morning half an hour before or two hours after a meal, and note any effects over the following two days (a delayed reaction may begin up to 48 hours after taking the challenge capsule). On the sheets provided record the time each challenge was taken and, if a reaction occurs, record the time it began, type and severity of symptoms and how long they lasted. If the reaction is severe, contact your dietitian or doctor.

2. Some challenges may be labelled "A" and "B" (e.g. 3A, 3B), representing a small dose ("A") and a larger dose ("B") of the same (or similar) chemicals. These should be taken on the same day, the small dose ("A") first. If there is no reaction within two hours, take the second dose ("B"). However, if a noticeable reaction occurs to dose "A", dose "B" should not be taken.

3. Reactions to a challenge usually begin within a few hours, but may take up to 48 hours to develop. If a reaction occurs within two days of taking a challenge, wait until the symptoms have disappeared completely and then allow a further three days without symptoms before going on to the next challenge the following day, as illustrated in the example below :

Days	1	2	3	4	5	6	7	8
Challenge	3	-	-	-	-	4A 4B	-	5
Symptoms	++	+	0	0	0	0	0	0

Three full days free of symptoms before next challenge.

IN SOME INDIVIDUALS :

1. Withdrawal Symptoms may occur during the first week or two on the elimination diet. Some or all of your symptoms may increase for a short time, but these usually disappear within two weeks. Call your dietitian or doctor if symptoms persist.

2. Fumes may become more noticeable and can bring on symptoms. The commonest problems are petroleum products (petrol, gas, oil, kerosene), paints, perfumes, cigarette smoke, pressure-pack products and strong smelling cleaning agents. Try to avoid these fumes as much as possible during the elimination and challenge period. Once you include other foods in your diet at the end of the testing period, smells and fumes should become less of a problem.

FOOD LIST AND MEDICATIONS

FOODS ALLOWED	FOODS TO AVOID
MEAT	
Beef, lamb, veal and poultry (no skin or fat) chicken sausages by Summercross Smallgoods	Corned beef, ham, bacon, pork, offal, sausages fish and seafood, well browned meat.
EGGS	
Eggs	
FATS	
Cold pressed safflower or sunflower oil (NO ANTIOXIDANTS), butter and cream	Other oils and oils which contain antioxidants. Margarine, cheese and yoghurt.
VEGETABLES	
Iceberg lettuce (inside leaves only) Old white potatoes (no skin) Country Style plain potato crisps by Lips Parsley (fresh, sprinkle through food only)	All other lettuce New potatoes, Pontiac (red) potatoes Other commercial potato crisps and chips All other vegetables
FRUITS	
Pears - fresh, very ripe and thickly peeled Pears - canned in syrup not nectar or juice	Unripe pears, Goulburn Valley and SPC canned pears in juice or nectar, all other fruit including dried fruit
CEREALS	
Any plain unpreserved breads, rolls, muffins or crumpets. Plain homemade or commercial biscuits sponge, pikelets, pancakes, scones, pastry, etc. Brown rice cakes, San-Esu plain rice crackers White rice, rice flour, rice noodles, rice vermicelli, uncoloured pasta (spaghetti,macaroni), tapioca and sago. All wheat flours (wholemeal, white, self raising, plain), Fielders cornflour, Parsons cornflour, Kream cornflour. Arrowroot flour, potato flour. Rice Bubbles, Rolled Rice, Healtherie Rice Flakes, Weetbix, All Bran, Vita Brits, Bran Flakes, Puffed Wheat, Weeties, Weetflakes, Unprocessed bran.	Other cereals, bread and biscuits

Continued

FOODS ALLOWED	FOODS TO AVOID
SUGARS	
White sugar, brown sugar, Golden syrup	Honey, raw sugar, artificial sweeteners.
CONDIMENTS	
Salt, citric acid, fresh parsley (spinkle only), Homemade french dressing (cold pressed oil and citric acid to taste)	Herbs and spices Vinegar
COOKING AIDS	
Bicarbonate of soda, Cream of tartar, Baking Powder (McKenzies and Rite-Diet) Gelatine (boil before use)	Other cooking aids
BEVERAGES	
Decaffeinated instant and bean coffee, water, soda water, mineral water, homemade lemonade (two cups sugar dissolved in one cup of water, add citric acid to taste; dilute with soda water, mineral water or tap water), home made pear juice (blend a can of pears and syrup, then add water or mineral water to taste). Milk (homogenised, evaporated, condensed, UHT, skim, Hilo, Shape).	Tea, herbal tea, cereal coffee, any chocolate flavoured drinks, fruit juices and commercial pear juice, soft drinks and alcohol
MEDICATIONS	
Panadol, Panadeine, Codeine - only when necessary Antihistamines - only when necessary Savlon antiseptic cream	All medication not prescribed by your doctor e.g. Aspirin, Disprin, Alka Seltzer, Vincents. All medications which contain flavouring or colouring. Cough lozenges and syrups. Oil of Wintergreen e.g. Dencorub, Deep Heat, Tiger Balm, muscle balms etc.
TOILETRIES	
A mixture of salt and soda, ordinary salt, Floran HA or Soul Pattinson's unflavoured toothpaste and Sensodyne may be used as toothpaste substitutes. Nonallergenic or lightly perfumed cosmetics, toiletries and moisturisers can be used with with caution (e.g. Sunlight, Neutrogena).	Aerosols and pressure pack products should be avoided Toothpaste - avoid flavoured toothpaste. Perfumes and strongly perfumed cosmetics, toiletries and moisturisers are to be avoided e.g. Avon

SAMPLE MENU

BREAKFAST :

Rice Bubbles, milk and sugar
Boiled egg
Toast (unpreserved bread) and butter and Golden Syrup
Decaffeinated coffee

LUNCH :

Sandwich made with unpreserved bread
and fillings of cold roast meat, chicken, egg and lettuce
Fresh pear (no skin)
Glass of milk

TEA :

Grilled steak
mashed potatoes and lettuce
Baked custard and tinned pears in syrup
Decaffeinated coffee

BETWEEN MEALS

Decaffeinated coffee, milk or mineral water,
Homemade plain cake or plain biscuits,
Arnott's water biscuits or Thin Captain with butter,

DIET AND CHALLENGE SUMMARY

Diet Commenced : _____

CHALLENGE RESULTS

Capsule Challenges

	Date	Reaction (yes/no)
1	.....	.....
2	.....	.....
3	.....	.....
4	.....	.....
5	.....	.....
6	.....	.....
7	.....	.....
8	.....	.....
9	.....	.....
10	.....	.....
11	.....	.....
12	.....	.....
13	.....	.....
14	.....	.....

APPENDIX 2

ELIMINATION DIET II

DIETITIAN: _____ TELEPHONE: _____

The aim of this diet is to exclude certain additives and natural chemicals from your daily food intake. These include : natural salicylates, amines, yeast, monosodium glutamate (MSG), antioxidants, preservatives and colourings. Once excluded, deliberate reintroduction of each of these substances ("challenge") can help identify the cause of your problem.

DIET RULES :

1. Use only those foods allowed
2. Do not use any foods not listed
3. Avoid all non-essential medications

DIET NOTES :

Withdrawal symptoms may start to occur during the first two weeks on the elimination diet. Some or all of your symptoms may increase temporarily, but these withdrawal symptoms usually disappear within a few days to two weeks.

CHALLENGE RULES :

1. Commence challenges after a minimum of two weeks on the elimination diet, and only after at least five days free of symptoms. If, after six weeks, there is no improvement, contact your dietitian and doctor.

2. Challenge with wheat as outlined.

3. Challenge with milk as outlined.

4. Challenge with chemical capsules as outlined.
   a. Challenge capsules should be taken in the morning (1/2 hour before, or two hours after breakfast)
   b. Reactions may occur anytime up to 48 hours after taking a challenge capsule.
   c. If you do develop symptoms during this time, wait until they have gone completely, and then a further three days free of symptoms, before going on to the next challenge.
   d. If you do not develop any reaction within 48 hours, go on to the next challenge.

ELIMINATION DIET

1. The diet should be followed for at least two weeks or until symptoms settle, which may take up to six to eight weeks.** Once you achieve a period of five days in a row free of symptoms, you are to be challenged with wheat and milk and then each of the chemicals which commonly cause symptoms. If symptoms are still present after six weeks, contact your dietitian or doctor.

2. On the chart provided, keep a detailed record of
   a) foods eaten throughout the day
   b) symptoms - type and how long they last
   c) challenges and/or medications taken

3. Any non-essential medication should cease at least five days before starting the challenges (consult your doctor if uncertain). Should you have a headache during the challenge period, Panadol and/or codeine preparations may be used.
   - ALL ASPIRIN-CONTAINING DRUGS SHOULD BE AVOIDED
   - Coloured capsules (e.g. antibiotics) can be opened and emptied into clear gelatine capsules which can be bought from most pharmacies.
   - Coloured tablets can be gently rubbed under a running tap to remove the coloured coating.
   - Use only recommended vitamin preparations

** In some individuals, improvement may be gradual over four to six weeks, but if there is no change in symptoms after six to eight weeks of strict dieting, continued restriction is unlikely to be helpful and the dietitian should be contacted for instructions on how to resume a normal diet.

VITAMINS & MINERALS

It is recommended that patients on the elimination diet should take a vitamin and mineral supplement as the diet contains marginal amounts of some vitamins and minerals. Care should be taken to ensure that these supplements do not contain preservatives, colours or flavours as these will interfere with the challenge results.

Some preparations to avoid are those which contain :

A. PABA = Para amino benzoic acid, or ABA = Amino benzoic acid.
B. coloured capsules
C. syrups
D. alfalfa
E. kelp
F. rosehips

It is suggested that one of the following multivitamin supplements be used :

A. Elevit RDI (Roche)
B. any multivitamin in a capsule :
take the powder only - discard the plastic coloured capsule coating,
e.g. Myadec (Parke Davis)

and one of the following calcium supplements be used :

A. DCP 340 Powder (Parke Davis)
B. Sandocal 1000 (Sandoz)
C. Oyster Shell (Vitaglow)

CHALLENGES

1. Take the challenge capsules in the morning half an hour before or two hours after a meal, and note any effects over the following two days (a delayed reaction may begin up to 48 hours after taking the challenge capsule). On the sheets provided record the time each challenge was taken and, if a reaction occurs, record the time it began, type and severity of symptoms and how long they lasted. If the reaction is severe, contact your dietitian or doctor.

2. Some challenges may be labelled "A" and "B" (e.g. 3A, 3B), representing a small dose ("A") and a larger dose ("B") of the same (or similar) chemicals. These should be taken on the same day, the small dose ("A") first. If there is no reaction within two hours, take the second dose ("B"). However, if a noticeable reaction occurs to dose "A", dose "B" should not be taken.

3. Reactions to a challenge usually begin within a few hours, but may take up to 48 hours to develop. If a reaction occurs within two days of taking a challenge, wait until the symptoms have disappeared completely and then allow a further three days without symptoms before going on to the next challenge the following day, as illustrated in the example below :

Days	1	2	:	3	4	5	:	6	7	8
Challenge	3	-	:	-	-	-	:	4A	-	5
			:				:	4B		
			:				:			
Symptoms	++	+	:	0	0	0	:	0	0	0

Three full days free of symptoms before next challenge.

IN SOME INDIVIDUALS :

1. Withdrawal Symptoms may occur during the first week or two on the elimination diet. Some or all of your symptoms may increase for a short time, but these usually disappear within two weeks. Call your dietitian or doctor if symptoms persist.

2. Fumes may become more noticeable and can bring on symptoms. The commonest problems are petroleum products (petrol, gas, oil, kerosene), paints, perfumes, cigarette smoke, pressure-pack products and strong smelling cleaning agents. Try to avoid these fumes as much as possible during the elimination and challenge period. Once you include other foods in your diet at the end of the testing period, smells and fumes should become less of a problem.

FOOD LIST AND MEDICATIONS

FOODS ALLOWED	FOODS TO AVOID
MEAT	
Beef, lamb, veal and poultry (no skin or fat) chicken sausages by Summercross Smallgoods	Corned beef, ham, bacon, pork, offal, sausages fish and seafood, well browned meat.
EGGS	
Eggs	
FATS	
Cold pressed safflower and sunflower oil (NO ANTIOXIDANTS)	Other oils and oils which contain antioxidants. Butter, Margarine, cheese and yoghurt.
VEGETABLES	
Iceberg lettuce (inside leaves only) Old white potatoes (no skin) Country Style plain potato crisps by Lips Parsley (fresh, sprinkle through food only)	All other lettuce New potatoes, Pontiac (red) potatoes Other commercial potato crisps and chips All other vegetables
FRUITS	
Pears - fresh, very ripe and thickly peeled Pears - canned in syrup not nectar or juice	Unripe pears, Goulburn Valley and SPC canned pears in juice or nectar, All other fruit including dried fruit
CEREALS	
White rice, rice flour, rice noodles, rice vermicelli, tapioca and sago. Fielders cornflour, Parsons cornflour, Kream cornflour. Arrowroot flour, potato flour. Brown rice cakes. San-Esu plain rice crackers. Rice Bubbles, Rolled Rice, Healtherie Rice Flakes,	Other cereals, bread and biscuits

Continued

FOODS ALLOWED	FOODS TO AVOID
SUGARS	
White sugar, brown sugar, Golden syrup	Honey, raw sugar, artificial sweeteners.
CONDIMENTS	
Salt, citric acid, fresh parsley (spinkle only), Homemade french dressing (cold pressed oil and citric acid to taste)	Herbs and spices Vinegar
COOKING AIDS	
Bicarbonate of soda, Cream of tartar, Baking Powder (McKenzies and Rite-Diet) Gelatine (boil before use)	Other cooking aids
BEVERAGES	
Decaffeinated instant and bean coffee, water, soda water, mineral water, homemade lemonade (two cups sugar dissolved in one cup of water, add citric acid to taste; dilute with soda water, mineral water or tap water), home made pear juice (blend a can of pears and syrup, then add water or mineral water to taste).	Tea, herbal tea, cereal coffee, any chocolate flavoured drinks, fruit juices and commercial pear juice, soft drinks and alcohol
MEDICATIONS	
Panadol, Panadeine, Codeine - only when necessary	All medication not prescribed by your doctor e.g. Aspirin, Disprin, Alka Seltzer, Vincents.
Antihistamines - only when necessary	All medications which contain flavouring or colouring. Cough lozenges and syrups.
Savlon antiseptic cream	Oil of Wintergreen e.g. Dencorub, Deep Heat, Tiger Balm, muscle balms etc.
TOILETRIES	
A mixture of salt and soda, ordinary salt, Floran HA or Soul Pattinson's unflavoured toothpaste and Sensodyne may be used as toothpaste substitutes.	Aerosols and pressure pack products should be avoided Toothpaste - avoid flavoured toothpaste.
Nonallergenic or lightly perfumed cosmetics, toiletries and moisturisers can be used with with caution (e.g. Sunlight, Neutrogena).	Perfumes and stongly perfumed cosmetics, toiletries and moisturisers are to be avoided e.g. Avon

SAMPLE MENU

BREAKFAST :

Rice Bubbles and tinned pears and syrup
Boiled egg
Rice cake and Golden Syrup
Decaffeinated coffee

LUNCH :

Cold sliced chicken
Lettuce
Rice cakes
Fresh pear (no skin)
Mineral water

TEA :

Grilled steak
Boiled potatoes and saute lettuce
Tinned pears in syrup
Decaffeinated coffee

BETWEEN MEALS

Decaffeinated coffee, homemade pear juice, mineral water,
Rice cakes and golden syrup, fresh pears (NO SKIN).

DIET AND CHALLENGE SUMMARY

Diet Commenced : _____

A. Food Challenges :

1. Wheat Challenge - Six to 24 Arnotts Water Crackers or one to three cups of white pasta throughout the day for three days. If there is a reaction to the wheat challenge, stop eating the crackers or pasta immediately, wait until all symptoms have gone and a further three days free from symptoms before continuing on to the next challenge.

   If there is no reaction, include in the elimination diet immediately the following foods :
   - Any plain, unpreserved breads, rolls muffins or crumpets; homemade pastry;
   - Weetbix, All bran, Vita Brits, Bran Flakes, Puffed Wheat, Weeties, Weetflakes;
   - Uncoloured pasta (spaghetti, macaroni);
   - All wheat flours (white, wholemeal, self raising, plain);
   - Plain homemade or commercial biscuits, sponge, pikelets, pancakes, scones etc.

NOTE : LEAVE 48 HOURS BETWEEN THE WHEAT CHALLENGE AND THE MILK CHALLENGE.

2. Milk challenge - 1-3 glasses per day for three days. If there is a reaction, stop drinking the milk immediately, wait until all symptoms have gone and and further three days free from symptoms before going on to the next challenge.

   If there is no reaction, include in the elimination diet the following foods :
   - All plain milk (UHT, condensed, powdered and evaporated); all cream; all butter.

B. Capsule Challenges

	Date	Reaction (yes/no)
1	.....	.....
2	.....	.....
3	.....	.....
4	.....	.....
5	.....	.....
6	.....	.....
7	.....	.....
8	.....	.....
9	.....	.....
10	.....	.....
11	.....	.....
12	.....	.....

APPENDIX 3

URTICARIA CHALLENGE BATTERY

1.	Aspirin	300 mg
2.	Sodium salicylate	300 mg
3.	Sodium benzoate	500 mg
4.	40H benzoic acid	200 mg
5.	Sodium metabisulphite	500 mg
6.	Tartrazine	30 mg
7.	Brewer's yeast	500 mg
8.	Starch	700 mg

* Challenge capsules every 2nd day
* Reaction within 48 hours
* Allow for refractory period after positive response to challenge.
* If asthmatic, aspirin, metabisulphite and tartrazine taken under medical supervision.
* For children 0 - 2 years, only 1/4 of the dose of the metabisulphite challenge are to be taken.
* For children 2 - 7 years, only 1/2 of the dose of the metabisulphite challenge are to be taken.

APPENDIX 4

SYSTEMIC CHALLENGE BATTERY

1A.	Aspirin	600 mg
B.	Aspirin	600 mg
2A.	Sodium benzoate	500 mg
A.	Sorbic acid	200 mg
A.	40H benzoic acid	200 mg
B.	Sodium metabisulphite	500 mg
3.	Sodium nitrate	25 mg
	Sodium nitrite	25 mg
4.	Butylated hydroxy toluene	50 mg
	Butylated hydroxy anisole	50 mg
5.	Sodium propionate	500 mg
6.	Tartrazine	30 mg
7.	Erythrosine	30 mg
8.	B Phenylethylamine HCL	4 mg
	Tyramine HCL	140 mg
9.	Monosodium glutamate	2.5 g
	Monosodium glutamate	2.5 g
10.	Gluten	1.5 g
11.	Lactose	500 mg
12A.	Starch	500 mg
B.	Starch	500 mg
13.	Sucrose	1.0 g
14.	Brewers yeast	700 mg

* Food challenge with milk and wheat.
* Food challenge with egg in eczema patients only.
* Challenge capsules every 2nd day
* Reaction within 48 hours
* Allow for refractory period after positive response to challenge.
* If asthmatic, aspirin, metabisulphite, MSG, tartrazine taken under medical supervision and erythrosine not to be taken.
* For children 0 - 2 years, only 1/4 of the dose of aspirin, metabisulphite and MSG challenges are to be taken.
* For children 2 - 7 years, only 1/2 of the dose of aspirin, metabisulphite and MSG challenges are to be taken.

APPENDIX 5

ASTHMA CHALLENGE PROTOCOL

Assessment

All patients with a history of asthma should have their bronchial reactivity measured by histamine provocation before commencing dietary investigation. Supervision of challenge tests is arranged according to the following criteria:

1. Challenge in hospital:
   * patients with severe bronchial hyper-reactivity,
   * patients with a history of asthma provoked by aspirin, non steroidal anti inflammatory drugs (NSAID's), MSG, metabisulphite or specific foods,
   * patients with a history of laryngeal oedema or anaphylactoid reactions.

2. Challenge in outpatients:
   * patients with moderate bronchial hyper-reactivity.

3. Challenge under supervision of general practitioner:
   * patients with mild bronchial hyper-reactivity,
   * patients with a past history of asthma.

Before taking any challenges, patients should have been on the elimination diet for at least two weeks, and should be having optimal bronchodilator therapy according to their clinical state.

Monitoring

A baseline spirometry reading should be taken before each challenge and measurements repeated at 30 minute intervals for four hours. In severe asthmatics, lung function should be monitored at appropriate intervals for 24 hours after each challenge.

Challenges

Challenges should be taken at 48 hour intervals, half an hour before breakfast. A reaction is regarded as significant if there is a fall of 20% or more in FEV following a challenge.

Treatment of reactions

Appropriate resuscitation equipment should be available. Asthma provoked by a challenge should be treated promptly with appropriate bronchodilators. For patients with severe asthma, laryngeal oedema or anaphylactoid reactions, an IV cannula should be in place and loaded syringes containing adrenaline and an antihistamine, prepared before challenges are taken.

CHALLENGE DOSE SCHEDULE FOR PATIENTS IN HOSPITAL AND OUTPATIENTS

A. Metabisulphite

Dissolve one capsule containing 500 mg of metabisulphite in 250 ml (i.e. 2 mg/ml) of 0.5% citric acid and administer in divided doses as indicated below. Each dose is diluted to a final volume of 30 ml by addition of citric acid.

Dose 1: 10 mg; if no reaction after 30 minutes then give
Dose 2: 20 mg; if no reaction after a further 30 minutes give
Dose 3: 50 mg.

B. Tartrazine

Given in a single dose as a 30 mg capsule.

C. Monosodium Glutamate

Given in divided doses as capsules, each containing 0.5 g:

Dose 1: 1.0 g (2 capsules); if no reaction after one hour then give
Dose 2: 1.0 g

D. Aspirin

Given in divided doses removed from 2 capsules containing 300 mg each (or by breaking 300 mg tablets). Aspirin may be taken as the powder or dissolved in water. Moderate/severe asthmatics, and patients with laryngeal oedema or anaphylactoid reactions should begin with 25 mg, but mild asthmatics can begin with 75 mg:

Dose 1: 25 mg; if no reaction after one hour then give
Dose 2: 50 mg; if no reaction after a further hour
Dose 3: 75 mg; if no reaction after a further hour
Dose 4: 150 mg.

If there is no reaction to dose 4 after two hours, and the history is suspicious, a further challenge may be given if the patient is under hospital supervision:

Dose 5: 300 mg.

CHALLENGE DOSE SCHEDULE FOR PATIENTS WITH GENERAL PRACTITIONER

Dear Doctor,

Your patient is undergoing investigation for food intolerance. This involves adherence to a strict elimination diet followed by challenges with individual food chemicals.

Occasionally in patients with bronchial hyper-reactivity certain challenges can precipitate transient asthma within 1 - 2 hours of ingestion. Although the risk is remote, I would be grateful if you could supervise the following four relevant challenges at your surgery :

- salicylates
- MSG
- preservatives
- tartrazine

Each challenge should be taken as follows :

- 1/4 of the challenge* every hour, and remain at the doctor's surgery for 2 hours after the last dose
- If you develop a reaction at any stage, do not take any further doses of that challenge
- If you develop asthma, use your usual medications

*Example: If the challenge consists of:

1 capsule (dose B only)	take	1/4 capsule every hour.
2 capsules (dose A and B)	take	1/2 capsule every hour.
4 capsules (dose A and B)	take	1 capsule every hour.
10 capsules (dose A and B)	take	2 1/2 capsules every hour.

This will require the availability of means to monitor airway function and to administer bronchodilator by aerosols and for I.V. as required.

If you require further information please contact me or Dr. Yan at Royal Prince Alfred Hospital.

Yours sincerely,

Robert H. Loblay,
MB BS, PhD, FRACP
Allergy Clinic - R.P.A.H.

APPENDIX 6

FOOD CHALLENGE PROTOCOL

1. Record in the diary supplied the following information :

Foods & drinks	-	at each meal and as snacks;
Challenges	-	foods used, quantities, times
Symptoms	-	physical or behavioural
Other	-	e.g. parties, outings, infections, exposure to strong smells, domestic arguments, medications, etc.

2. Challenges may be commenced after a minimum of two weeks on the elimination diet and once there have been at least five days in a row free of symptoms.

3. Each chemical is tested by eating the foods listed for three consecutive days as a "challenge". The amounts of food/beverage listed for each challenge should be spread over the entire day. Reactions may occur within half an hour, but are often delayed by several hours or even a day or more.

4. If no symptoms occur during a challenge or within the following 48 hours, continue with the next food challenge.

5. If the challenge provokes a reaction, stop the food challenge, wait until symptoms have cleared and then allow a further three days before taking the next challenge.
Reactions will be no worse than previously experienced.

6. If any response is doubtful, repeat the challenge two or three times to confirm a reaction.

7. Once the challenges are completed contact your dietitian for further instructions. Foods belonging to those chemical groupings which did not appear to cause reactions will be reintroduced into the diet. If you remain well over the next four to six weeks, small amounts of foods containing the problem chemicals will then be tried, using the salicylate and/or amine charts.

FOOD CHALLENGES

FOOD CHEMICAL	FOOD CHALLENGES	MINIMUM DOSE PER DAY
SALICYLATE	apples, apricots, asparagus, beetroot, capsicum, carrot, corn, cucumber, herbs, honey, mango, onion, peppermint, pumpkin, rockmelon, spices, strawberries, sweet potato, tea, watermelon, zucchini.	6 serves *
AMINES	block cheese (tasty or cheddar) chocolate (plain, milk or dark) bananas	120 grams 120 grams 3 bananas
MSG	soy sauce (mix into fried rice or meatballs)	4 tablespoons
PRESERVATIVES	plain lemonade (preserved - check label)	1 litre
NITRATE	ham, or bacon, or corned beef	4 slices (120 gram)
ANTIOXIDANTS	margarine (antioxidants added - check label)	10 teaspoons (50 gram)
PROPIONATE	preserved bread (anti-mould inhibitor or preservative added - check label)	4 slices
COLOURINGS	coloured Icy Pole (If no reaction to salicylate) OR musk sticks	one 8 sticks

* 1 serve = 1 apple, 2-3 apricots, 6-8 asparagus spears, 3-4 slices beetroot, 1/2-1 capsicum, 1 carrot, 1 cob corn, 1/2-1 cucumber, 1 teaspoon of herbs, 1 tablespoon of honey, 1 mango, 1 onion, 1/2 packet of peppermint Lifesavers, 1 cup pumpkin, 1/4-1/2 rockmelon, 1 teaspoon spices, 1/2-1 cup strawberries, 1 cup sweet potato, 1 cup tea, 1 slice watermelon, 1 zucchini

APPENDIX 7

LOW SALICYLATE DIET

DIETITIAN:_____ TELEPHONE:_____

The aim of this diet is to keep the intake of salicylates, to a minimum.

SALICYLATES occur naturally in many fruits and vegetables, herbs and spices, and are frequently used in artificial flavourings, perfumes, toothpaste and medications (aspirin).

GUIDELINES

1. Use only foods listed as "allowed".
2. Avoid foods containing fruit or mint flavours. Check all package labels.
3. Be careful with medications, toiletries, pressure pack sprays and household cleaning agents.

NOTE

1. It is necessary to carefully check every food package or carton for mention of fruit or mint flavourings.

2. The salicylate content of the diet may be liberalised after four to six weeks. Follow the guidelines in the accompanying "Salicylate Chart" for introducing the moderate salicylate foods into the diet.

FOOD LIST AND MEDICATIONS

FOODS ALLOWED	FOODS TO AVOID
BEVERAGES	
Decaffeinated coffee, milk, cocoa, milo, malt mineral water, tonic water, soda water, water unpreserved lemonade, whisky, gin and vodka	Tea, fruit juices, commercial soft drinks and cordials, cider, wine, liqueurs and beer
BREAD	
Any plain breads, rolls, muffins or crumpets Pastry	Breads which contain dried fruits
FLOUR	
All flours except corn based flours, plain pasta (e.g. spaghetti, macaroni)	Cornmeal, polenta. Canned baked beans and spaghetti, Gravox
CEREALS	
All cereals except corn, commercial breakfast cereals which do not contain fruit or corn	Breakfast cereals which contain dried fruit or corn e.g. muesli, Sultana Bran, Froot Loops

Continued

FOODS ALLOWED	FOODS TO AVOID
BISCUITS	
Plain home made and commercial biscuits (using allowed ingredients)	Home made or commercial biscuits which contain fruit, spices or mint
CAKES	
Plain home made cakes and sponges (using allowed ingredients)	Home made and commercial cakes which contain fruit, spices or mint
MEAT	
Beef, lamb, veal, pork, mince, poultry rabbit, ham, corned beef and bacon	Meats containing herbs and spices e.g. sausages, sausage mince, sausage rolls, meat pies, frankfurts, devon, salami, processed chicken, meat paste, seasoned chicken
FISH	
Fresh, frozen or canned fish and seafood	
EGGS	
Eggs, custard powder	
DAIRY FOODS	
Butter, all cream, all plain milks including UHT, condensed, powdered and evaporated. Plain and vanilla yoghurt, all cheese	Fruit flavoured milks Fruit yoghurts
Ice cream - home made or commercial vanilla chocolate, pawpaw or banana flavours	Coloured or flavoured ice cream iceblocks and and gelato
FATS	
Butter, dripping, margarine and oils (safflower and sunflower), homemade salad dressings	All other oils, Copha, commercial salad dressings
FRUIT	
Pears, Golden Delicious apples (1 per day only), Paw Paw, Bananas. Fruit can be fresh, frozen or canned in syrup or water. Pick ripe fruit and do not eat skin	All other fruit Dried fruits (including pears) Canned fruits in natural juice or nectar (including pears)

Continued

FOODS ALLOWED	FOODS TO AVOID
VEGETABLES	
White potato (no skin), beans (French, string), brussel sprouts, cabbage, celery, chives, choko, lettuce, leeks, parsley, peas, Mung bean sprouts, swede, shallots, garlic, dried legumes	All other vegetables including broad beans, Pontiac (red) potatoes, New potato
SOUPS	
Home made soups from allowed ingredients	Commercial stock cubes, soups, gravies and sauces
DESSERTS	
Home made desserts from allowed ingredients e.g. steamed puddings, vanilla junket, egg custard	Commercial desserts
LOLLIES AND CHOCOLATES	
White jelly beans, white marshmellows, plain toffees (Callard & Bowser), milk chocolate dark chocolate and carob.	Liquorice, chewing gum, commercial, mint or fruit flavoured lollies and chocolates
NUTS AND CHIPS	
Cashews (raw, dry roasted) plain potato crisps, hot potato chips	Other nuts Snack foods and flavoured potato crisps
JAMS, SUGARS AND SWEETS	
Golden Syrup, malt, pear jam (home-made) sugar (white, brown, icing and castor)	Honey, all other jams, conserves and jellies (including lemon butter)
CONDIMENTS	
Parsley, garlic, salt, soy sauce, vanilla	Herbs, spices, mint, mustard, pickles, vinegar, tomato paste, tomato sauce etc., meat pastes, fish pastes, flavouring syrups, and essences, Bonox, Marmite, Promite, Vegemite

TOILETERIES

USE: Unscented soaps, shampoos and conditioners where possible and unflavoured toothpaste.

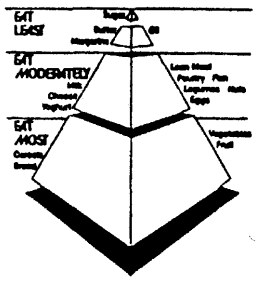
AVOID: Contact with perfumes, scented deodorants and cosmetics pressure pack sprays, household cleaning agents, cigarette smoke and other strong smells.

MEDICATIONS

USE: Paracetamol tablets (Panadol, Parapain, Panadeine) for pain relief.
Elevit RDI adult vitamin tablets (optional).

AVOID: All aspirin containing medications, and coloured, flavoured or preserved medications. Read the labels carefully. Most syrups and liquid preparations, lozenges, laxatives and antacids are unsuitable, as are menthol, eucalytus, Oil of Wintergreen, vitamins containing flavourings, herbal preparations.

BALANCING YOUR DIET



FOOD	SERVING SIZE	SERVES PER DAY
Bread and other cereal foods	1 slice bread 3/4 cup breakfast cereal 1/2 cup cooked rice	At least 4 serves
Vegetables & Fruit	1 potato 1/2 cup vegetables 1 piece fruit	At least 3 serves of vegetables and 1 pear (or an occasional Golden Delicious Apple)
Milk & Cheese	100 mls milk 60g fresh cottage cheese 100 mls plain yoghurt	At least 6 serves
Meat and Meat Substitutes	120g cooked meat 2 eggs 3/4 cup cooked lentils	At least 2 serves
Butter, margarine and oil	1 teaspoon	3 - 5 servings

NOTE

1. Try to vary the foods and recipes you use from day to day and week to week. This will help avoid a build up of salicylates.

2. If you are unable to follow these guidelines because of additional restrictions (e.g. dairy products or wheat), then the deficit must be made up from other food sources. An experienced dietitian can check your total energy intake (kilojoules), as well as making you meet your requirements for protein, vitamins, minerals and other essential nutrients.

BOOKS

FOOD FACTS - D. Briggs and M. Wahlqvist. Penguin Books. (general nutrition).
EATING MATTERS - D. Briggs and M. Wahlqvist. Methuen Haynes. (food additives).
COMMONSENSE COOKERY BOOK - N.S.W. Public School Cookery Teachers' Association. Angus and Robertson. (plain recipes).

APPENDIX 8

LOW SALICYLATE/LOW PRESERVATIVES/LOW ARTIFICIAL COLOURING/LOW BREWERS YEAST DIET

DIETITIAN:_____ TELEPHONE:_____

The aim of this diet is to keep the intake of salicylates, preservatives, artificial colours and brewers' yeast to a minimum.

SALICYLATES occur naturally in many fruits and vegetables, herbs and spices, and are frequently used in artificial flavourings, perfumes, toothpaste and medications (aspirin).

BREWERS' YEAST is present in Vegemite, Promite, Marmite, some wines and beers.

PRESERVATIVES are added to food to prolong shelf life. For example, benzoates are used in fruit juices; sorbate or sulphur dioxide in dried fruits, fruit salads and sausage mince; nitrites in processed meats; propionate in yeast products; and antioxidants in oils and margarines.

COLOURINGS such as tartrazine, erythrosine and sunset yellow are commonly used in commercial foods.

GUIDELINES

1. Use only foods listed as "allowed".
2. Avoid foods containing additives or flavours, except for vanilla. Check all package labels.
3. Be careful with medications, toiletries, pressure pack sprays and household cleaning agents.

NOTE

1. It is necessary to carefully check every food package or carton for mention of artificial flavourings, colours and preservatives. Use the accompanying "Food Additives" list to identify specific additives and their corresponding code numbers.

2. The salicylate content of the diet may be liberalised after four to six weeks. Follow the guidelines in the accompanying "Salicylate Chart" for introducing the moderate salicylate foods into the diet.

FOOD LIST AND MEDICATIONS

FOODS ALLOWED	FOODS TO AVOID
BEVERAGES	
Decaffeinated coffee, milk, cocoa, milo, malt mineral water, tonic water, soda water, water unpreserved lemonade, whisky, gin and vodka	Tea, fruit juices, commercial soft drinks and cordials, cider, wine, liqueurs and beer
BREAD	
Any plain breads, rolls, muffins or crumpets	Breads which contain dried fruits
Homemade pastry	Coloured and preserved pastry

Continued

FOODS ALLOWED	FOODS TO AVOID
FLOUR	
All flours except corn based flours, uncoloured pasta (e.g. spaghetti, macaroni)	Cornmeal, polenta. Canned baked beans and spaghetti, Gravox
CEREALS	
All cereals except corn, commercial breakfast cereals which do not contain fruit, corn or colouring	Breakfast cereals which contain dried fruit, corn or colouring e.g. muesli, Sultana Bran, Froot Loops
BISCUITS	
Plain home made and commercial biscuits (using allowed ingredients)	Home made or commercial biscuits which contain fruit, spices, mint and colouring
CAKES	
Plain home made cakes and sponges (using allowed ingredients)	Home made and commercial cakes which contain fruit, spices, mint and colouring
MEAT	
Beef, lamb, veal, pork, mince, poultry (no skin) rabbit	Meats containing preservatives, herbs and spices e.g. sausage mince, sausage rolls, meat pies, frankfurts, sausages, devon, salami, processed chicken, meat paste, seasoned chicken
FISH	
Fresh, frozen or canned fish and seafood	Coloured or preserved fish e.g. fish fingers
EGGS	
Eggs	Custard powder, custard mix
DAIRY FOODS	
Butter, all cream, all plain milks including UHT, condensed, powdered and evaporated Plain and vanilla yoghurt Ice cream - home made or commercial vanilla chocolate, pawpaw or banana flavours Cheese	Artificial "cream" in commercial cakes Flavoured milks Other flavoured and fruit yogurts Coloured or flavoured ice cream, iceblocks, gelato Cheese which contains preservatives or colouring e.g cheese slices and spreads, tubs of cottage cheese

Continued

FOODS ALLOWED	FOODS TO AVOID
FATS	
Butter, dripping, margarine and oils (safflower and sunflower)(no antioxidants), homemade salad dressings	All other oils, Copha, commercial salad dressings
FRUIT	
Pears, Golden Delicious apples (1 per day only), Paw Paw, Bananas. Fruit can be fresh, frozen or canned in syrup or water. Pick ripe fruit and do not eat skin	All other fruit Dried fruits (including pears) Canned fruits in natural juice or nectar (including pears)
VEGETABLES	
White potato (no skin), beans (French, string), brussel sprouts, cabbage, celery, chives, choko, lettuce, leeks, parsley, peas, Mung bean sprouts, swede, shallots, garlic. Dried legumes e.g. split peas, lentils, soybeans, chick peas	All other vegetables including broad beans, Pontiac (red) potatoes, New potatoes
SOUPS	
Home made soups from allowed ingredients	Commercial stock cubes, soups, gravies and sauces
DESSERTS	
Home made desserts from allowed ingredients e.g. steamed puddings, vanilla junket, egg custard	Commercial desserts
LOLLIES AND CHOCOLATES	
White jelly beans, white marshmellows, Plain toffees (Callard & Bowser), carob Plain chocolate	Liquorice, chewing gum, commercial, mint or fruit flavoured lollies and chocolates
NUTS AND CHIPS	
Cashews (raw, dry roasted)	Other nuts, snack foods, potato crisps and commercial hot potato chips

Continued

FOODS ALLOWED	FOODS TO AVOID
JAMS, SUGARS AND SWEETS	
Golden Syrup, malt, pear jam (home-made) Sugar (white, brown, icing and castor)	Honey, all other jams, conserves and jellies (including lemon butter)
CONDIMENTS	
Parsley, garlic, salt, soy sauce, vanilla	Herbs, spices, mint, mustard, pickles, vinegar, tomato paste, tomato sauce etc., meat pastes, fish pastes, flavouring syrups, and essences, Bonox, Marmite, Promite, Vegemite

TOILETERIES

USE: Unscented soaps, shampoos and conditioners where possible and unflavoured toothpaste.

AVOID: Contact with perfumes, scented deodorants and cosmetics pressure pack sprays, household cleaning agents, cigarette smoke and other strong smells.

MEDICATIONS

USE: Paracetamol tablets (Panadol, Parapain, Panadeine) for pain relief.
Elevit RDI adult vitamin tablets (optional).
White medications where possible. Colouring can be washed off the surface of tablets by rubbing gently under running water. Coloured capsules can be opened and the powdered contents taken in a spoonful of Golden Syrup.

AVOID: All aspirin containing medications, and coloured, flavoured or preserved medications. Read the labels carefully. Most syrups and liquid preparations, lozenges, laxatives and antacids are unsuitable, as are menthol, eucalytus, Oil of Wintergreen, vitamins containing PABA, colourings or flavourings, herbal preparations.

NOTE

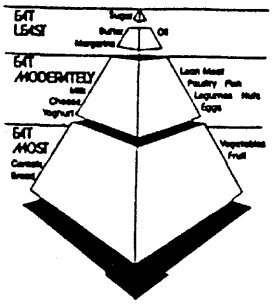
1. Try to vary the foods and recipes you use from day to day and week to week. This will help avoid a build up of salicylates.

2. If you are unable to follow these guidelines because of additional restrictions (e.g. dairy products or wheat), then the deficit must be made up from other food sources. An experienced dietitian can check your total energy intake (kilojoules), as well as making you meet your requirements for protein, vitamins, minerals and other essential nutrients.

BOOKS

FOOD FACTS - D. Briggs and M. Wahlqvist. Penguin Books. (general nutrition).
EATING MATTERS - D. Briggs and M. Wahlqvist. Methuen Haynes. (food additives).
COMMONSENSE COOKERY BOOK - N.S.W. Public School Cookery Teachers' Association. Angus and Robertson. (plain recipes).

BALANCING YOUR DIET



FOOD	SERVING SIZE	SERVES PER DAY
Bread and other cereal foods	1 slice bread 3/4 cup breakfast cereal 1/2 cup cooked rice	At least 4 serves
Vegetables & Fruit	1 potato 1/2 cup vegetables 1 piece fruit	At least 3 serves of vegetables and 1 pear (or an occasional Golden Delicious Apple)
Milk & Cheese	100 mls milk 60g fresh cottage cheese 100 mls plain yoghurt	At least 6 serves
Meat and Meat Substitutes	120g cooked meat 2 eggs 3/4 cup cooked lentils	At least 2 serves
Butter, margarine and oil	1 teaspoon	3 - 5 servings

FOOD ADDITIVES

Additive code numbers which MAY cause adverse reactions :

Benzoic Acids 210, 211, 212, 213
Colours 102, 107, 110, 122, 123, 124, 127, 132, 133, 142, 151, 155,160b
Propionic Acids 281, 282, 283
Sorbic Acids 200, 201, 202, 203
Sulphites 220, 221, 222, 223, 224
Nitrates 251, 252
Nitrites 249, 250
Anti-oxidants 310, 311, 312, 320, 321

Additive code numbers UNLIKELY to cause adverse reactions :

Anti-caking agents 536, 551, 554
Bleaching agents 925, 926
Emulsifiers 322, 433, 435, 436
Food acids and salts 260, 261, 262, 263, 270, 296, 297, 300, 325, 326, 327, 330, 331, 332, 333, 334, 335, 336, 350, 351, 352, 354, 380
Glutamates (including MSG) 620, 621, 622, 623
Mineral Salts 170, 339, 340, 341, 450, 500, 501, 503, 504, 508, 509, 529
Natural Colours 100, 101, 120, 140, 150, 153, 160a, 161, 162, 163, 172
Propellants 290
Sweeteners 420, 421, 422
Thickening agents ..... ---
Vegetable gums 400, 401, 402, 403, 404, 405, 406, 407, 410, 412, 413, 414, 415, 416, 440, 464
Vitamins 101, 160
Miscellaneous ..... 153, 353, 355, 460, 481, 482, 541, 553, 558, 559, 575, 578, 627, 631, 900, 901, 903, 904, 905, 920.

APPENDIX 9

LOW SALICYLATE/LOW PRESERVATIVES/LOW ARTIFICIAL COLOURING/LOW MSG/LOW AMINE/LOW BREWERS YEAST DIET

DIETITIAN:_____ TELEPHONE:_____

The aim of this diet is to keep the intake of salicylates, amines, preservatives, artificial colours, monosodium glutamate and brewers' yeast to a minimum.

SALICYLATES occur naturally in many fruits and vegetables, herbs and spices, and are frequently used in artificial flavourings, perfumes, toothpaste and medications (aspirin).

AMINES occur naturally in such foods as chocolate, cheese, wine, beer, liver, yeast extracts, dried and salted fish, bananas, avocados, broadbeans, tomatoes and fermented products.

MONOSODIUM GLUTAMATE occurs naturally in most sauces, tomato products, mushrooms, strong cheeses, yeast extracts, meat extracts, stock cubes and wines; it can also be added as a flavour enhancer in most soups, sauces, Chinese food and snack foods.

BREWERS' YEAST is present in Vegemite, Promite, Marmite, some wines and beers.

PRESERVATIVES are added to food to prolong shelf life. For example, benzoates are used in fruit juices; sorbate or sulphur dioxide in dried fruits, fruit salads and sausage mince; nitrites in processed meats; propionate in yeast products; and antioxidants in oils and margarines.

COLOURINGS such as tartrazine, erythrosine and sunset yellow are commonly used in commercial foods.

GUIDELINES

1. Use only foods listed as "allowed".
2. Avoid foods containing additives or flavours, except for vanilla. Check all package labels.
3. Be careful with medications, toiletries, pressure pack sprays and household cleaning agents.

NOTE

1. It is necessary to carefully check every food package or carton for mention of artificial flavourings, MSG, hydrolysed vegetable protein, colours and preservatives. Use the accompanying "Food Additives" list to identify specific additives and their corresponding code numbers.

2. The salicylate content of the diet may be liberalised after four to six weeks. Follow the guidelines in the accompanying "Salicylate Chart" for introducing the moderate salicylate foods into the diet.

3. Similarly, after establishing your salicylate threshold, the "Amine Chart" can be used to liberalise the amine content of the diet.

4. Try to vary the foods and recipes you use from day to day and week to week. This will help avoid a build up of salicylates.

5. If you are unable to follow these guidelines because of additional restrictions (e.g. dairy products or wheat), then the deficit must be made up from other food sources. An experienced dietitian can check your total energy intake (kilojoules), as well as making you meet your requirements for protein, vitamins, minerals and other essential nutrients.

BOOKS

FOOD FACTS - D. Briggs and M. Wahlqvist. Penguin Books. (general nutrition).
EATING MATTERS - D. Briggs and M. Wahlqvist. Methuen Haynes. (food additives).
COMMONSENSE COOKERY BOOK - N.S.W. Public School Cookery Teachers' Association. Angus and Robertson. (plain recipes).

FOOD LIST AND MEDICATIONS

FOODS ALLOWED	FOODS TO AVOID
BEVERAGES	
Decaffeinated coffee, milk, malt, mineral water tonic water, soda water, water, unpreserved lemonade, whisky, gin and vodka	Tea, fruit juices, commercial soft drinks and cordials, cider, wine, liqueurs, beer, cocoa
BREAD	
Any plain breads, rolls, muffins or crumpets Homemade pastry	Breads which contain dried fruits Coloured and preserved pastry
FLOUR	
All flours except corn based flours, uncoloured pasta (e.g. spaghetti, macaroni)	Cornmeal, polenta. Canned baked beans and spaghetti, Gravox
CEREALS	
All cereals except corn, commercial breakfast cereals which do not contain fruit, corn, cocoa or colouring	Breakfast cereals which contain dried fruit, corn, cocoa or colouring e.g. muesli, Sultana Bran, Froot Loops, Coco Pops
BISCUITS	
Plain home made and commercial biscuits (using allowed ingredients)	Home made or commercial biscuits which contain fruit, spices, mint, colouring, cocoa, MSG or savoury flavouring
CAKES	
Plain home made cakes and sponges (using allowed ingredients)	Home made and commercial cakes which contain fruit, spices, mint, cocoa and colouring
MEAT	
Beef, lamb, veal, rabbit, poultry (no skin)	Pork and meats containing preservatives, herbs and spices e.g. sausage mince, sausage rolls, meat pies, frankfurts, sausages, devon, salami, processed chicken, meat paste, seasoned chicken
FISH	
Fresh fish and seafood	Frozen, canned, coloured or preserved fish

Continued

FOODS ALLOWED	FOODS TO AVOID
EGGS	
Eggs	Custard powder, custard mix
DAIRY FOODS	
Butter, all cream, all plain milks including UHT, condensed, powdered and evaporated Plain and vanilla yoghurt Ice cream - home made or commercial vanilla Fresh cottage cheese, cream cheese and ricotta	Artificial "cream" in commercial cakes Flavoured milks Other flavoured and fruit yogurts Coloured or flavoured ice cream, iceblocks, gelato All other cheeses
FATS	
Butter, dripping, margarine and oils (safflower and sunflower)(no antioxidants), homemade salad dressings	All other oils, Copha, commercial salad dressings
FRUIT	
Pears, Golden Delicious apples (1 per day only), Fruit can be fresh, frozen or canned in syrup or water. Pick ripe fruit and do not eat skin	All other fruit Dried fruits (including pears) Canned fruits in natural juice or nectar (including pears)
VEGETABLES	
White potato (no skin), beans (French, string), brussel sprouts, cabbage, celery, chives, choko, lettuce, leeks, parsley, peas, Mung bean sprouts, swede, shallots, garlic. Dried legumes e.g. split peas, lentils, soybeans, chick peas	All other vegetables including broad beans, Pontiac (red) potatoes, New potato
SOUPS	
Home made soups from allowed ingredients	Commercial stock cubes, soups, gravies and sauces
DESSERTS	
Home made desserts from allowed ingredients e.g. steamed puddings, vanilla junket, egg custard	Commercial desserts
LOLLIES AND CHOCOLATES	
White jelly beans, white marshmellows, plain toffees (Callard & Bowser), carob	Liquorice, chewing gum, commercial, mint or fruit flavoured lollies and chocolates

Continued

FOODS ALLOWED	FOODS TO AVOID
NUTS AND CHIPS	
Cashews (raw)	Other nuts, snack foods, potato crisps and commercial hot potato chips
JAMS, SUGARS AND SWEETS	
Golden Syrup, malt, pear jam (home-made) Sugar (white, brown, icing and castor)	Honey, all other jams, conserves and jellies (including lemon butter)
CONDIMENTS	
Parsley, garlic, salt, vanilla	Herbs, spices, mint, mustard, pickles, vinegar, tomato paste, tomato sauce etc., meat pastes, fish pastes, flavouring syrups, and essences, Bonox, Marmite, Promite, Vegemite

TOILETERIES

USE: Unscented soaps, shampoos and conditioners where possible and unflavoured toothpaste.

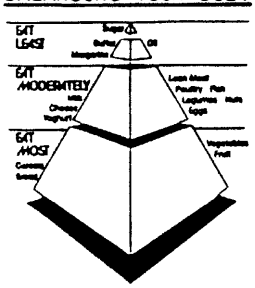
AVOID: Contact with perfumes, scented deodorants and cosmetics pressure pack sprays, household cleaning agents, cigarette smoke and other strong smells.

MEDICATIONS

USE: Paracetamol tablets (Panadol, Parapain, Panadeine) for pain relief.
Elevit RDI adult vitamin tablets (optional).
White medications where possible. Colouring can be washed off the surface of tablets by rubbing gently under running water. Coloured capsules can be opened and the powdered contents taken in a spoonful of Golden Syrup.

AVOID: All aspirin containing medications, and coloured, flavoured or preserved medications. Read the labels carefully. Most syrups and liquid preparations, lozenges, laxatives and antacids are unsuitable, as are menthol, eucalytus, Oil of Wintergreen, vitamins containing PABA, colourings or flavourings, herbal preparations.

BALANCING YOUR DIET



FOOD	SERVING SIZE	SERVES PER DAY
Bread and other cereal foods	1 slice bread 3/4 cup breakfast cereal 1/2 cup cooked rice	At least 4 serves
Vegetables & Fruit	1 potato 1/2 cup vegetables 1 piece fruit	At least 3 serves of vegetables and 1 pear (or an occasional Golden Delicious Apple)
Milk & Cheese	100 mls milk 60g fresh cottage cheese 100 mls plain yoghurt	At least 6 serves
Meat and Meat Substitutes	120g cooked meat 2 eggs 3/4 cup cooked lentils	At least 2 serves
Butter, margarine and oil	1 teaspoon	3 - 5 servings

FOOD ADDITIVES

Additive code numbers which MAY cause adverse reactions :

Benzoic Acids 210, 211, 212, 213
Colours 102, 107, 110, 122, 123, 124, 127, 132, 133, 142, 151, 155,160b
Propionic Acids 281, 282, 283
Sorbic Acids 200, 201, 202, 203
Sulphites 220, 221, 222, 223, 224
Nitrates 251, 252
Nitrites 249, 250
Anti-oxidants 310, 311, 312, 320, 321
Glutamates (including MSG) 620, 621, 622, 623

Additive code numbers UNLIKELY to cause adverse reactions :

Anti-caking agents 536, 551, 554
Bleaching agents 925, 926
Emulsifiers 322, 433, 435, 436
Food acids and salts 260, 261, 262, 263, 270, 296, 297, 300, 325, 326, 327, 330, 331, 332, 333, 334, 335, 336, 350, 351, 352, 354, 380
Mineral Salts 170, 339, 340, 341, 450, 500, 501, 503, 504, 508, 509, 529
Natural Colours 100, 101, 120, 140, 150, 153, 160a, 161, 162, 163, 172
Propellants 290
Sweeteners 420, 421, 422
Thickening agents ..... ---
Vegetable gums 400, 401, 402, 403, 404, 405, 406, 407, 410, 412, 413, 414, 415, 416, 440, 464
Vitamins 101, 160
Miscellaneous 153, 353, 355, 460, 481, 482, 541, 553, 558, 559, 575, 578, 627, 631, 900, 901, 903, 904, 905, 920

APPENDIX 10

GUIDELINES FOR THE USE OF THE SALICYLATE CHART

SALICYLATE CONTENT	AMOUNT USUALLY TOLERATED
NEGLIGIBLE	any amount
LOW	any amount
MODERATE	1 serve/day
HIGH	_ serve/day
VERY HIGH	none

* ONE SERVE:

Fruits:	One item (apple, orange, etc.)
	One slice (watermelon, rockmelon, pineapple, etc.)
	One cupful (150g)(sultanas, berries, grapes, etc.)
Vegetables:	Equivalent of one cupful (150g)
Nuts:	One half cupful (80g)
Sweets:	One tablespoon
Herbs/spices:	One teaspoon
Drinks:	One glass or cup (150ml)

* THE FOLLOWING AMOUNTS ARE APPROXIMATELY EQUIVALENT:

1 serve from the MODERATE group
_ serve from the HIGH group
_ serve or less from the VERY HIGH group

* AS A GENERAL RULE:
Those foods with the strongest flavour (tart, acid, spicy) and aroma have the highest content of salicylates.

ESTABLISHING YOUR THRESHOLD LEVEL

If you have just started your low salicylate diet you should not eat any foods from the moderate column for 4 - 6 weeks from commencement of the diet. After this period a gradual introduction of these foods can be allowed. You can have a 1/4 serve of any of the moderate foods every third day for a period of two weeks. If there is no adverse reaction you then have a 1/4 serve every second day for a further period of two weeks. Again if there is no adverse reaction you can have a 1/4 serve every day for another two weeks gradually building up to a 1/2 serve per day for two weeks and then one full serve per day for a further two weeks. After ten weeks of this gradual introduction you can try 1 - 3 serves per day provided there is no adverse reaction.

i.e.
First two weeks - 1/4 serve every third day
Second two weeks - 1/4 serve every second day
Third two weeks - 1/4 serve every day
Fourth two weeks - 1/2 serve every day
Fifth two weeks - 1 serve every day

USING THE SALICYLATE CHART

1. Foods from the NEGLIGIBLE and LOW columns can usually be combined freely, with one another and with foods from the MODERATE or HIGH column, as dictated by your threshold.
2. Foods from the MODERATE column may be combined with foods from the HIGH column, provided you do not exceed your daily threshold level.
3. Foods from the VERY HIGH column should only be taken very rarely and in small amounts, according to your threshold.

NOTE 1: THE EFFECTS OF SALICYLATES MAY BE CUMULATIVE, AND YOU MAY BEGIN TO REACT ADVERSELY ONLY AFTER SEVERAL DAYS OF EXCEEDING YOUR THRESHOLD.

NOTE 2: FOR THOSE WHO ARE ALSO SENSITIVE TO AMINES AND M.S.G.
Foods listed in CAPITALS contain SMALL amounts of AMINES
Foods listed in CAPITALS contain LARGE amounts of AMINES
Foods marked with * contain natural M.S.G.

FOOD SALICYLATE CONTENT

Negligible	Low	Moderate	High	Very High
FRUIT				
BANANA	golden delicious apple (peeled)	custard apple	AVOCADO	apricot
pear (peeled)	PAWPAW	FIG	grapefruit	blackberry
	pomegranate	LEMON	granny smith apple	blackcurrant
		loquat	jonathan apple	blueberry
		mango	KIWI FRUIT	boysenberry
		pear (with peel)	lychee	cherry
		persimmon	mandarin	cranberry
		red delicious apple	mulberry	currant
		rhurbarb	nectarine	DATE
		tamarillo	PASSIONFRUIT	GRAPE*
			peach	guava
			tangelo	loganberry
			watermelon	ORANGE
				PINEAPPLE
				PLUM
				PRUNE*
				raisin
				RASPBERRY
				redcurrant
				rockmelon
				strawberry
				sultana
				youngberry
VEGETABLES				
bamboo shoot	brussel sprout	asparagus	alfalfa sprout	capsicum
cabbage	chive	beetroot	broadbean	champignon
celery	choko	BROCCOLI*	cucumber	chicory
lettuce	green beans	carrot	EGGPLANT	endive
potato(peeled)	green peas	cauliflower	watercress	GHERKIN
swede	leek	marrow		hot pepper
	mungbean spout	MUSHROOM*		OLIVE
dried beans	red cabbage	onion		radish
dried peas	shallot	parsnip		TOMATO PRODUCTS*
brown lentils		pumpkin		
red lentils		SPINACH		zucchini
		sweetcorn*		
		sweet potato		
		turnip		
NUTS				
poppyseed	CASHEWS	BRAZIL		ALMOND
		COCONUT		waterchestnut
		HAZELNUTS		
		MACADAMIA		
		PEANUTS		
		PECANS		
		PINENUTS		
		PISTACHIO		
		SESAME SEEDS		
		SUNFLOWER SEEDS		
		WALNUTS		

Continued

Negligible	Low	Moderate	High	Very High
HERBS and SPICES				
	garlic		allspice	aniseed
	malt vinegar		bay leaf	canella
	parsley		cardamon	cayenne
	saffron		carraway	cumin
	SOY SAUCE*		cinnamon	curry
	TANDORI		cloves	dill
	vanilla		ginger	five spice
			nutmeg	garam masala
			pepper (black)	mace
			pepper (white)	MARMITE*
			pimiento	mint
			WHITE VINEGAR	mixed herbs
				mustard
				oregano
				paprika
				rosemary
				sage
				tarragon
				turmeric
				VEGEMITE*
				WORSTER SAUCE*
SWEETS				
carob	caramels	molasses		honey
COCOA	golden syrup			licorice
maple syrup				peppermints
white sugar				
BEVERAGES				
COFFEE	COFFEE	COFFEE		TEA
Andronicus	Harris instant	Harris Mocha		all brands
Pablo instant	Bushells instant	International		peppermint
decaffeinated	Bushells Turkish	Roast instant		
	Robert Timms	Moccona instant		CEREAL COFFEE
OTHER	instant	Nescafe instant		Nature's cuppa
Aktavite				
Milo	TEA	TEA		ALCOHOL
Ovaltine	camomille	decaffeinated		liqueur*
	rosehip	fruit		port*
ALCOHOL				RUM*
gin	CEREAL COFFEE	CEREAL COFFEE		WINE*
vodka	dandelion	Reform		
whisky	Ecco			
	Bambu	OTHER		
		coke		
		fruit juice		
		rosehip syrup		
		ALCOHOL		
		BEER		
		BRANDY*		
		cider		
		SHERRY*		

APPENDIX 11

GUIDELINES FOR THE USE OF THE AMINE CHART

AMINE CONTENT	AVERAGE AMOUNT TOLERATED*
NEGLIGIBLE	any amount
LOW	5 serves/day
MODERATE	1 serve/day
HIGH	1/10 serve/day
VERY HIGH	none

* Amounts equivalent to one serve:

Fruits	One item (apple, orange, etc.)
	One slice (rockmelon, pineapple, etc.)
	One cupful (150g) (berries, grapes, etc.)
Vegetables	Equivalent of one cupful (150g)
Nuts	One half cupful (80g)
Meats & fish	100 grams (NOTE: amine levels increase with ageing)
Cheese	60 grams (2 average slices)
Chocolate	30 grams (3 squares)
Sweets	One tablespoon
Herbs/spices & other condiments	One teaspoon
Drinks	One glass or cup (150ml)

AMINE CONTENT OF FOOD

Negligible	Low	Moderate	High	Very High
FRUITS				
APPLE	BLACKCURRANT	DATES	AVOCADO	
APRICOT	CHERRY	KIWI FRUIT	banana	
BLUEBERRY	GRAPEFRUIT	ORANGE	FIG	
GOOSEBERRY	HONEY DEW MELON	PASSIONFRUIT	GRAPES *	
LIME	MANDARIN	pawpaw	LEMON	
PEACH	REDCURRANT	TANGERINE	PINEAPPLE	
pear	ROCKMELON		PLUM	
RHUBARB			RASPBERRY	
STRAWBERRY				
VEGETABLES				
ASPARAGUS		BROCCOLI *	EGGPLANT	sauerkraut
cabbage		CAULIFLOWER	MUSHROOM *	SPINACH
CAPSICUM		DILL PICKLE	TOMATO *	
CARROT		OLIVES		
celery				
CORN *				
CUCUMBER				
french bean				
green pea				
lettuce				
lima bean				
ONION				
potato				
RADISH				
soy bean				
TURNIP				
ZUCCHINI				

Continued

Negligible	Low	Moderate	High	Very High
NUTS				
ACORN	ALMOND	BRAZIL NUT	ENGLISH WALNUT	BLACK WALNUT
AMERICAN CHESTNUT	BEECHNUT	FILBERT	MACKERNUT	BUTTERNUT
BUCKEYE NUT	cashew		PECAN	
HORSE CHESTNUT	COCONUT		SWEET PIGNUT	
SUNFLOWER	MACADAMIA			
PINENUT				
PISTACHIO				
MEATS, CHICKEN AND FISH				
	beef	chicken liver	bacon	beef liver
	chicken	ham	mackerel (canned)	caplin (salted)
	lamb	salami	pork	chicken skin
		salmon (canned)	sardines (canned)	fish marinades
		tuna (fresh)		fish meat
				herring (dried)
				herring (pickled)
				herring roe
				herring (salted)
				herring (smoked)
				mackerel (dried)
				sardines (dried)
				sausage
				tuna (canned)
CHEESE				
cottage cheese				brie*
				camembert *
				cheshire
				cheddar cheese
				cracker barrel
				danish blue
				dutch gloucester
				edam
				emmental
				english cheshire
				gouda *
				gruyere *
				jaalsberg
				leicester
				liederkratz
				limberger
				mozarella
				munster
				parmesan *
				processed cheese
				provolone
				roquefort *
				romano
				stilton
				swiss
				wensleydale

Continued

Negligible	Low	Moderate	High	Very High
SWEETS				
			milk chocolate	dark chocolate
			white chocolate	
CONDIMENTS				
			BONOX *	MARMITE *
			MEAT EXTRACTS *	
			soy sauce *	
			VEGEMITE *	
BEVERAGES				
		ALE	BEER	
		CHAMPAGNE	CHIANTI	
		SAKE	CLARET *	
		STOUT	drinking chocolate	
			FRUIT WINES	
			PORT *	
			RED WINE *	
			SHERRY *	
			WHITE WINE *	

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APPENDIX 12

MSG IDENTIFIED IN FOODS

Fruits	Meats/Fish	Beverages	Vegetables	Dairy
grape	beef	brandy	broccoli	buttermilk
grapefruit	codfish	gin	beetroot	camembert
nectarine	chicken	port	carrot	cow milk
orange	duck	whiskey	corn	danish blue
peach	egg	wine	mushroom	gruyere
plum	lamb		onion	human milk
prune	mackerel		peas	parmesan
strawberry	pork		potato	roquefort
	salmon		spinach	
			tomato	

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APPENDIX 13

FOLLOW-UP QUESTIONNAIRE FOR RIU/AO PATIENTS
WHO COMPLETED THE PROTOCOL

Have you had any recurrences of your symptoms? yes ☐ no ☐

IF NO:

How long have you been symptom free?
Have you relaxed your diet?
If so, which foods and how much?

IF YES:

How often?
How severe were these episodes?
What medication did you take?
Have they been related to food? yes ☐ no ☐

If yes:

Were they accidental?
Which foods were involved?
How much was eaten?
Have you noticed a combined effect with several foods?
Have you ever gone back to the elimination diet for relief?

APPENDIX 14

FOLLOW-UP QUESTIONNAIRE FOR RIU/AO PATIENTS WHO DID NOT COMPLETE
THE ELIMINATION DIET AND CHALLENGE PROTOCOL

Did you start the elimination diet?

If so, did it help your symptoms?

Did you remain on a modified diet or return to your normal diet?

If modified, what changes did you make to your diet?

Did you seek help or treatment elsewhere?

If so where?
What treatment did you have and what was the outcome?

Are you still getting recurrences of symptoms?

If so, how do you control them?

APPENDIX 15

FOLLOW-UP QUESTIONNAIRE FOR PATIENTS WHO COMPLETED THE PROTOCOL

At the beginning of your elimination diet (before you started the challenges):

Did you have a withdrawal reaction?	yes	no
Did you become more sensitive to smells?	yes	no

How much did your symptoms improve?

All symptoms disappeared ☐ Much better ☐ A little better ☐ No better at all ☐

Since finishing your challenges, are you?

Completely well ☐ Much better ☐ A little better ☐ No better at all ☐ Worse ☐

If you have had recurrences of symptoms, please list:

Symptoms (describe)	How often?	How severe?	Comments

Are your recurrences triggered by:

Foods ☐ Smells ☐ Other ☐ Don't know ☐

Please list:

Food/smell/other	Symptoms triggered

Have you been able to re-introduce any foods you were previously unable to eat?

Food	Amount tolerated per day	Food	Amount tolerated per day

APPENDIX 16

FOLLOW-UP QUESTIONNAIRE FOR PATIENTS WHO DID NOT COMPLETE THE ELIMINATION DIET AND CHALLENGE PROTOCOL

What were your reasons for not completing the elimination diet and challenge programme?

- No improvement on elimination diet
- Diet too strict or difficult
- Did not want to take challenges
- Other (state)

What diet are you currently following?

- An unrestricted (normal) diet
- A restricted (modified) diet

If you have modified your diet:

- Which foods have you restricted?
- Do any smells/fumes cause symptoms? (list)

How are you now, compared to when you were given the elimination diet?

Completely well	Much better	A little better	Just the same	A little worse	Much worse
☐	☐	☐	☐	☐	☐

Did you seek advice/therapy elsewhere?

- If so, where?
- What advice/therapy was given?
- Did this help?