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GUAR GUM:
ITS EFFECTS AND MECHANISM OF ACTION IN HUMANS

by

Jacki A. Tredger B.Sc., S.R.D.

A thesis submitted in accordance with the requirements of the
University of Surrey for the degree of Doctor of Philosophy

Department of Biochemistry,
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Guildford, Surrey.

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SUMMARY

In acute studies guar flour, guar powder and guar granules reduced mean post prandial insulin levels in normal subjects following liquid glucose. The flour and powder also reduced mean plasma GIP levels compared to glucose alone. Guar granules had no effect on the mean plasma glucose, insulin or GIP responses of either normal or non insulin dependent diabetic (NIDDM) subjects to a solid mixed meal but guar powder reduced mean post prandial plasma glucose, insulin and GIP levels in both groups. Guar flour was not investigated.

The mean glycosylated haemoglobin level of seven NIDDM subjects taking 25g guar powder in bread daily for eight weeks was reduced after four weeks. Mean total serum cholesterol levels were also reduced whilst subjects were following the regimen but on termination returned to prettrial levels. In addition, the response to a meal immediately before and after supplementation was similar indicating guar powder has no carry over effect.

Guar flour prevented the hypoglycaemia caused in two of six subjects by consumption of a drink containing alcohol and sucrose and a high carbohydrate snack. However it also caused higher blood alcohol levels and they appeared more intoxicated for five hours after the meal.

When guar gum was given with a fat load it did not affect chylomicronaemia implying it is unlikely to impair bile salt reabsorption as has been suggested. Guar flour incorporation into a predominantly protein meal did not affect the rate of liquid or solid gastric emptying in
normal subjects but significantly increased mean post prandial gastrin secretion. This may be due to the significant reduction in mean post prandial GIP levels also seen or to the buffering capacity of guar flour.

None of the subjects enjoyed guar containing foods and it was difficult to prepare acceptable products. Therefore until a better formulation is available guar gum is of limited use in diabetic management.
I am indebted to Dr. L. Morgan and Dr. S. Hampton, without whom, this thesis would not have been submitted. They offered continuous support, advice and technical assistance. My thanks also to N. O'Connor, P. Kwasowski, D. Teale, K. Terry, L. Harding, J. Lewis and J. Peke for their technical assistance. I am grateful for the co-operation of Dr. Bown and his colleagues especially the dietitians at Frimley Park Hospital and the staff of the Clinical Investigation Units who ensured that the studies were suitable for approval by the relevant Ethical Committees. I would also like to thank the Surrey undergraduate students who provided technical assistance and to all those people who participated in the studies.

Norgine Ltd., Speywood Laboratories, The Boots Company and Wellcome Foundation Ltd. were of great assistance in providing the guar gum products used in the studies. I am most grateful to M. Whatley, J. Cole and A. Belcher for typing this manuscript and P. Kentish for his help with the diagrams. My supervisors Professor J.W.T. Dickerson and Professor V. Marks have also been very helpful. Last, but by no means least I would like to thank C.J. Powell and all my family for their support and encouragement which have been invaluable.
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>A.D.A.</td>
<td>American Dietetic Association.</td>
</tr>
<tr>
<td>APUD</td>
<td>Amine Precursor Uptake or Decarboxylation.</td>
</tr>
<tr>
<td>AUC</td>
<td>Area under the curve.</td>
</tr>
<tr>
<td>B.D.A.</td>
<td>British Diabetic Association.</td>
</tr>
<tr>
<td>CAD</td>
<td>Coronary artery disease.</td>
</tr>
<tr>
<td>CHD</td>
<td>Coronary heart disease.</td>
</tr>
<tr>
<td>CHO</td>
<td>Carbohydrate</td>
</tr>
<tr>
<td>$^{57}$Co</td>
<td>Radioactive cobalt.</td>
</tr>
<tr>
<td>CVD</td>
<td>Cardiovascular disease.</td>
</tr>
<tr>
<td>GIP</td>
<td>Gastric Inhibitory Polypeptide.</td>
</tr>
<tr>
<td>EDTA</td>
<td>Ethylene dianiminetra-acetic acid</td>
</tr>
<tr>
<td>GTT</td>
<td>Glucose tolerance test.</td>
</tr>
<tr>
<td>Hb</td>
<td>Haemoglobin.</td>
</tr>
<tr>
<td>HDL</td>
<td>High density lipoprotein.</td>
</tr>
<tr>
<td>HLA</td>
<td>Human leucocyte antigen.</td>
</tr>
<tr>
<td>IDDM</td>
<td>Insulin dependent diabetes mellitus.</td>
</tr>
<tr>
<td>$^{113}$In DTPA</td>
<td>Radioactive diethyl amino penta acetic acid.</td>
</tr>
<tr>
<td>kcal</td>
<td>Kilocalories.</td>
</tr>
<tr>
<td>keV</td>
<td>Kilo-electronvolt</td>
</tr>
<tr>
<td>kJ</td>
<td>Kilojoules.</td>
</tr>
<tr>
<td>LDL</td>
<td>Low density lipoproteins.</td>
</tr>
<tr>
<td>LSI</td>
<td>Light scattering index.</td>
</tr>
<tr>
<td>MBq</td>
<td>Megabecquerels.</td>
</tr>
<tr>
<td>Na$_2$$^{51}$Cr$_2$O$_7$</td>
<td>Radioactive sodium chromate.</td>
</tr>
<tr>
<td>NIDDM</td>
<td>Non insulin dependent diabetes mellitus.</td>
</tr>
<tr>
<td>n.s.</td>
<td>Non significant.</td>
</tr>
<tr>
<td>NSB</td>
<td>Non specific binding.</td>
</tr>
<tr>
<td>N.T.</td>
<td>Not detectable.</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Definition</td>
</tr>
<tr>
<td>--------------</td>
<td>------------------------------------------------</td>
</tr>
<tr>
<td>PEG</td>
<td>Polyethylene glycol</td>
</tr>
<tr>
<td>rev/min</td>
<td>Revolutions per minute.</td>
</tr>
<tr>
<td>ROI</td>
<td>Region of interest.</td>
</tr>
<tr>
<td>SEM</td>
<td>Standard error of the mean.</td>
</tr>
<tr>
<td>$^{99m}\text{Tc}$</td>
<td>Radioactive technetium.</td>
</tr>
<tr>
<td>tsp</td>
<td>Teaspoon.</td>
</tr>
<tr>
<td>VLDL</td>
<td>Very low density lipoproteins.</td>
</tr>
<tr>
<td>$w/v$</td>
<td>Weight per volume.</td>
</tr>
<tr>
<td>$w/w$</td>
<td>Weight per weight.</td>
</tr>
</tbody>
</table>
LIST OF SUPPLIERS

All antisera was supplied by Guildhay Antisera, Biochemistry Department, University of Surrey, Guildford, Surrey.

All chemicals were supplied by BDH Ltd., Poole, Dorset, unless otherwise stated.

Bovine Serum Albumin: Sigma London, Poole, Dorset.
Cholesterol Kit: Boehringer, Lewes, Sussex.
Glucose Kit: Boehringer, Lewes, Sussex.
Glucotest: Boehringer, Lewes, Sussex.
Guar bread: The Boots Company Ltd., Nottingham.
Guar flour and granules: Norgine Ltd., 59/62 High Holborn, London (since moved to 116-120 London Road, Headington, Oxford).
Guar + gelation inhibitor: The Wellcome Foundation Ltd., 183-193 Euston Road, London.
Human insulin standard: Wellcome Reagents Ltd., Beckenham, Kent.
Human serum albumin: Blood Products Laboratory, Lister Institute, Elstree.
Hycal: Beecham Products, Brentford, Middlesex.
Iodinated insulin: Amersham International plc, Amersham, Buckinghamshire.
Paracetamol: Winthrop Laboratories, Surbiton, Surrey.
$^{99m}$Tc: Amersham International plc, Amersham, Buckinghamshire.
Triglyceride Kit: Boehringer, Lewes, Sussex.
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CHAPTER ONE

General Introduction
Although descriptions of the symptoms of Diabetes Mellitus date back to 1500 BC in Egypt, it was only 100 years ago that it was recognised that the disease appeared in at least two forms: one form mainly affected obese adults and the other, younger people who were usually thin at the time of diagnosis.

The need for a standard classification of diabetes was realised in 1978 when the National Diabetes Data Group brought together a working party to rationalise the classification of idiopathic diabetes mellitus (West, 1979). This classification is presented in Table 1.1 and will be used throughout this text.

In the United Kingdom approximately one percent of the population are diagnosed as diabetic (600,000). By far the majority (70%) are NIDDM (B.D.A. 1980). While IDDM and NIDDM appear to be two distinct clinical entities the concept of genetic and environmental interaction in their causation is equally applicable. The susceptibility to IDDM is inherited and this may also be the case for NIDDM, but whether or not diabetes develops appears to depend on the interaction of hereditary and environmental influences (Zimmit, 1983). In IDDM the inherited factors are HLA linked and one of the environmental factors is thought to be viral infections. NIDDM shows no HLA association although the inherited element is strong (Pyke, 1979). The environmental factors implicated include age, sex, obesity, lack of exercise and diet.

The advent of insulin therapy meant that diabetes was no longer a life-threatening disease and long term survival of children and young adults with IDDM is now the norm (Ross et al. 1983). As a result, the problems of microvascular disease, that is retinopathy and nephropathy as
TABLE 1.1

Idiopathic diabetes mellitus (DM)

1. Insulin-dependent type (IDDM).

2. Not insulin dependent type (NIDDM).
   (a) Non obese NIDDM,
       (i) Insulin treated for hyperglycaemia;
       (ii) Not insulin treated;
   (b) Obese NIDDM,
       (i) Insulin treated for hyperglycaemia;
       (ii) Not insulin treated.

Adapted from West (1979)
well as the sequelae of macrovascular disease (myocardial infarction, cerebral infarction and the loss of limbs due to gangrene) have assumed primary importance in clinical management and means of preventing them should be a matter of priority.

COMPLICATIONS OF DIABETES

Microvascular disease

The geographical variations in the prevalence of microvascular disease amongst diabetics are small and the complications are associated more with the duration of diabetes (B.D.A. 1982) and therefore, by implication to the length and degree of exposure to hyperglycaemia. Thus it would appear prudent to make one of the aims of diabetic management maintenance of blood glucose levels within the normal range. Recent attention has, however, been drawn to the short-term risks of meticulous control of blood glucose, particularly that of hypoglycaemic encephalopathy (Ross et al. 1983). Such a problem is not, however, pertinent to NIDDM subjects maintained on drug therapy and/or diet, only to IDDM subjects. For the NIDDM subject, maintenance of blood glucose levels within the normal range, achieved without the danger of frequent hypoglycaemic attacks is advocated to help prevent or delay the vascular complications of diabetes (Knowles 1964 and Siperstein et al. 1977).

Macrovacular disease

Arteriosclerosis is a general term used to describe the degeneration of the arteries resulting in thickening and hardening of the arterial wall. Atherosclerosis, one type of arteriosclerosis underlies most coronary artery disease (CAD) and coronary heart disease (CHD). It is characterised by accumulation of lipid (primarily cholesterol) in the vessel walls and has been described as the greatest killer of civilised man (Le Compte, 1955).
Diabetes has a major impact on the prevalence of CAD. The reported prevalence of CAD in diabetic patients ranges from 9.5-56% while prevalences of 1.6-4.1% are given for the general population (Fein and Schauer, 1983). In contrast to microvascular disease the geographical variation in the prevalence of CAD is marked (Greenwood and Taylor, 1968, and Brunner et al. 1964). Although genetic influences, either protective or detrimental may be partially responsible they fail to explain why Japanese diabetics who migrate to Hawaii develop the same risk of dying from heart disease as Caucasians in Hawaii (Kawate et al. 1979). Thus it seems likely that some environmental factor plays a part in the aetiology of CAD and changes in dietary habits have been implicated (Thomas, 1979).

One of the major differences in the management of diabetics in countries with a low prevalence of CAD is the composition of the diet. It may provide up to 80% of the energy as carbohydrate. This higher percentage, compared with the 40% typical of westernised diabetic diets, means that the percentage energy derived from fat is considerably lower. The lower fat intake may be of significance in relation to serum cholesterol levels, since the serum cholesterol level of westernised individuals tends to be much higher than those of non-westernised populations. Evidence to support this comes from O'Dea et al. (1982) who have shown that Australian aborigines living in rural conditions have significantly lower serum cholesterol levels than those living in urban surroundings.

**Influence of serum lipid levels on macrovascular disease**

One of the major risk factors for subsequent development of CAD is an elevated serum cholesterol level (Kannel et al. 1976). The argument in favour of a direct causal relationship between raised serum cholesterol
levels and atherosclerotic lesions is based on the finding that a large proportion of the fatty material of the lesion is cholesterol (Davidson et al., 1979) and that disorders resembling human atherosclerosis can be produced in many animal species by dietary manipulations which raise the serum cholesterol level (Moore and Williams, 1964). In addition, epidemiological studies have shown symptomatic atherosclerosis to be rare where the mean concentration of serum cholesterol is less than 4.1 mmol/L, even when predisposing factors such as diabetes, cigarette smoking and hypertension are present (Taylor and Betteridge, 1979).

By virtue of its water insolubility cholesterol is carried in the blood from its site of production to sites of removal, or alteration, by lipoproteins. These are formed in the gut and liver. There are four major lipoproteins, classified according to their density; chylomicrons, VLDL, LDL and HDL.

HDL cholesterol concentration has been widely accepted as being inversely correlated to the risk of acquiring CHD (Barr et al., 1951; Nikkila 1953; Castelli et al., 1977 and Miller et al., 1977). Miller and Miller (1975) have suggested that HDL facilitates the uptake of cholesterol from peripheral tissues and its transport to the liver for catabolism and excretion. The anti-atherogenic effect of HDL may also be connected with its ability to inhibit the uptake of cholesterol rich LDL by arterial smooth muscle cells (Carew et al., 1976). Since LDL cholesterol levels have been found to predict the risk of acquiring CHD more strongly than total serum cholesterol levels (Walton and Williamson, 1968) the ratio LDL/HDL may well be a more useful index for CHD risk than total serum cholesterol levels alone.
Effect of diet on circulating cholesterol levels

Total and fractional serum cholesterol levels can be affected by dietary manipulation. Keys *et al.* (1965) showed that a reduced intake of total or saturated dietary fat reduced total and LDL serum cholesterol levels. It does not necessarily follow that a reduction in serum cholesterol level will reduce the risk of CHD as emphasized by intervention studies (Leren, 1966; Medical Research Council, 1965 and 1968). However, several primary prevention trials lend support to the theory that a reduced fat intake may lead to a reduction in morbidity (Dayton *et al.* 1968; Rinzler 1968 and Miettinen *et al.* 1972).

Serum cholesterol levels are usually elevated in diabetics (Saudek and Young, 1981) and non-human primates and rats made diabetic also have hypercholesterolaemia. These findings would imply that dietary manipulation should aim to reduce serum cholesterol levels in the diabetic population as well as to achieve glucose homeostasis.

Diet in diabetes

Diet is the basis of management in both IDDM and NIDDM. It aims to:

1) Maintain the general condition of the patient by providing adequate amounts of all essential nutrients.
2) Achieve and/or maintain ideal body weight.
3) Maintain plasma glucose levels within the normal range.
4) Prevent or retard the development of vascular changes and related complications.

In IDDM the main concern is to prevent hypoglycaemia and ketoacidosis but in both IDDM and NIDDM the contribution that dietary modification can make to the prevention and retardation of vascular changes is increasingly appreciated.
In 1971 the USA Committee on Food and Nutrition published their report on the Principles of Nutrition and Dietary Recommendations for patients with diabetes (A.D.A. Special Report, 1971). They tentatively questioned the long held concept concerning carbohydrate restriction and suggested a reduction in dietary fat intake. The follow up report (A.D.A. Special Report, 1979) was more specific. It suggested an increased consumption of foods containing unrefined carbohydrate and fibre at the expense of foods high in refined carbohydrate and low in fibre.

Other countries have also published, or are producing, guidelines encouraging an increased consumption of foods containing unrefined carbohydrate (Canada, Germany, Finland, Germany, Japan, Switzerland) (B.D.A. personal communication).

The Nutrition Sub-Committee of the British Diabetic Association's Medical Advisory Committee have reconsidered dietary policy as it relates to the diabetic (B.D.A. 1982). They too have favoured the liberalisation of carbohydrate intake with the proviso that it be complex carbohydrate. They stressed the importance of a high fibre intake.

The studies on which these recommendations are based date back to 1935 when Himsworth showed that in normal man glucose tolerance can be improved by increasing the proportion of energy derived from carbohydrate, but that the mechanism capable of stimulating the utilisation of carbohydrate cannot exert its action in the absence of an adequate supply of insulin (Himsworth, 1935).
Confirmation comes from later studies. Treated diabetics showed a decrease in fasting blood glucose when given a diet providing 85% of the energy as carbohydrate (Brunzell et al. 1971) but when untreated diabetics were switched from 45% to 85% carbohydrate they exhibited increased glycosuria (Brunzell et al. 1974). Likewise, Weinsier et al. (1974) achieved control in 16 out of 18 NIDDM subjects, by giving a diet providing 60% energy as carbohydrate. The two patients whose symptoms were uncontrolled were described as severe diabetics.

More recently it has been shown that newly diagnosed diabetics can achieve reduction in serum cholesterol levels in addition to glucose homeostasis when following a 56% compared to a 40% carbohydrate diet (Hockaday et al. 1978). More impressive results have been achieved when both the carbohydrate and fibre content have been increased. Kiehm et al. (1976) were able to discontinue insulin or oral hypoglycaemic therapy in 10 of 13 hyperglycaemic men by giving a diet providing 75% of the energy as carbohydrate and approximately three times as much dietary fibre as the conventional American diabetic diet.

These diets differ from conventional diabetic diets in several ways as the type and proportion of carbohydrate, fat and fibre were all modified. The success of the diets has generally been attributed to their high fibre content but little work has been done on the effect of modifying only the fibre content of the diabetic diet.
Dietary Fibre

Epidemiological and experimental observations have attributed several major disease states to the low fibre content of foods consumed in western societies (Eastwood 1969; Burkitt 1971 and Trowell 1975). These disorders include coronary heart disease, diabetes mellitus, diverticular disease, carcinoma of the colon, appendicitis, gall bladder disease and dental caries.

Kellogg (1923) was amongst the first to show an interest in whole or unrefined foods and relate their intake to health. In 1929 McCance and Lawrence used the term unavailable carbohydrate to define all the polysaccharides not hydrolysed by the intestinal secretions of man. This included pectic substances, hemicelluloses, cellulose and some storage polysaccharides such as inulin. It also encompassed the non carbohydrate lignin and so, in order to avoid confusion, in 1953 Hipsley suggested the term dietary fibre which was redefined by Trowell (1972) to include indigestible plant cell wall material.

Biologically plant fibres can be divided into three groups; structural fibres which compose the plant cell walls (cellulose, lignin, hemicellulose and pectin), gums and mucilages which have precise roles, such as injury repair and storage polysaccharides which provide an energy store (Anderson and Chen 1979). They are thought to effect physiological changes in the gastro-intestinal tract, primarily because of their physiochemical properties (Table 1.2). The fibre content of foods is due primarily to that found in the original plant but some fibres may be used in small amounts as additives to improve and stabilise food products. The daily dietary fibre intake in Britain is variable but small. Bingham et al. (1979) assessed the average intake of a small population in Cambridgeshire to be 20g/day.
<table>
<thead>
<tr>
<th>Physiochemical properties</th>
<th>Type of fibre</th>
<th>Suggested modifying action</th>
</tr>
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<tbody>
<tr>
<td>Gel formation</td>
<td>Pectin</td>
<td>gastric emptying</td>
</tr>
<tr>
<td></td>
<td>Gums (guar)</td>
<td>mouth/caecum transit time</td>
</tr>
<tr>
<td></td>
<td></td>
<td>small intestinal absorption</td>
</tr>
<tr>
<td>Water holding capacity</td>
<td>Polysaccharides</td>
<td>mouth/caecum transit time</td>
</tr>
<tr>
<td></td>
<td>Lignin</td>
<td>faecal weight</td>
</tr>
<tr>
<td></td>
<td></td>
<td>intraluminal pressure</td>
</tr>
<tr>
<td>Bile acid adsorption</td>
<td>Lignin</td>
<td>cholesterol turnover</td>
</tr>
<tr>
<td></td>
<td>Pectin</td>
<td>faecal steroids</td>
</tr>
</tbody>
</table>

Adapted from Caspary et al. (1980)
Dietary fibre is closely associated with sources of carbohydrate and therefore it is likely that diabetics, prescribed a low (40%) carbohydrate diet will have a low dietary fibre intake. Thomas (1982) in her study of 292 British diabetics and 141 age and sex matched controls found, however, that IDDM subjects consumed more fibre than controls. Much of the difference was due to the diabetics higher bread consumption. When expressed in relation to total energy consumption NIDDM subjects had a greater fibre intake than their controls, but, since they consumed less energy their total fibre intake was similar.

Not only the quantity but the type of fibre consumed may be important in determining its effect upon blood glucose and cholesterol levels. Various sources of dietary fibre have already been investigated. Monnier et al. (1981) found that pectin and cellulose phosphate, but not cellulose, reduced peak post prandial plasma glucose levels in patients with chemical diabetes given a liquid glucose load. Munoz et al. (1979) confirmed that the source of fibre is important by giving healthy subjects 26g/day of hard red spring wheat bran (HRS), soft white wheat (SWW), corn bran (CB), soy hulls (SH), freeze dried apple powder (AP) and carrot powder (CP) for 30 days. Although SH, AP and CP provided less fibre than either HRS or SWW, they caused an improved response to a 75g oral glucose load, as did CB. The two ineffective wheat brans were similar in composition but differed markedly from the other sources of fibre. More evidence that the type of fibre is at least as important as the quantity used was supplied by Tredger et al. (1981) who showed that in normal subjects sugar beet pulp equivalent to 19g fibre had no effect on the post prandial plasma glucose response to a solid mixed meal providing 85g carbohydrate.
Diabetes mellitus has been defined as a fibre deficiency disorder (Trowell, 1972). Jenkins et al. (1978a) therefore attempted to define the type of dietary fibre or fibre analogue with the greatest potential use in diabetic treatment. In normal volunteers after a 50g liquid GTT with and without the addition of one of several substances equivalent to 12g fibre they found the greatest flattening of the post prandial plasma glucose curve was seen with the addition of guar gum but this effect was abolished when hydrolysed non-viscous guar gum was used. Since the reduction in the mean peak rise in blood glucose concentration for each substance correlated positively with its viscosity they concluded that viscous types of dietary fibre, particularly guar gum, are most likely to be therapeutically useful in modifying post prandial hyperglycaemia.

They had previously reported that hydrolysed guar gum was ineffective in normal subjects in reducing post prandial glucose and insulin levels following a liquid GTT (Jenkins et al. 1977a), but later concluded that the viscosity of guar gum within the gastrointestinal tract was probably the most important factor in flattening glucose tolerance and thus its viscosity when ingested was immaterial, provided it was taken with sufficient fluid to allow hydration within the stomach (Wolever et al. 1978b).

The earlier work investigating the effect on post prandial glycaemia of addition of guar gum to a solid mixed meal given to normal (Jenkins et al. 1977b), NIDDM (Gassull et al. 1976) and IDDM subjects (Jenkins et al. 1976a) also incorporated 10g pectin, another viscous fibre, and thus results cannot be attributed entirely to the presence of guar gum.
Guar gum, pectin, gum tragacanth, methyl cellulose, wheat bran and cholestyramine however, when added to a 50g oral glucose load in quantities equivalent to 12g fibre each significantly decreased plasma glucose concentration at one or more investigation points during the first two hours after the meal (Wolever et al. 1978a). Guar gum was the most effective and since it appeared effective at a relatively low concentration, thus making it easier to consume, it warrants further attention.

Guar gum

Guar gum is derived from the seed of the nitrogen fixing legume CYAMOPSIS TETRAGONOLOBA. The seed comes from a robust erect plant 3-10 feet high which bears clusters of thick fleshy pods between two and four inches long, each containing five to 12 seeds. The plant is probably indigenous to India and Pakistan where the seeds are eaten by cattle and humans (Goldstein et al. 1973). The plant was introduced as a cover crop to the United States of America in 1903. It was only produced on a commercial scale during World War II when its potential as a source of galactomannan gum was realised. Prior to this the carob seed, from which locust bean gum is derived was used as a source of gum for the paper, textile and food industries (Urquidi, 1978). During the war the USA supplies of carob seed from the Mediterranean were cut off and thus, finding that the endosperm of home grown guar contained approximately 50% usable form of galactomannan was opportune.

Guar gum is a galactomannan of molecular weight approximately 220,000. It consists of linear chains of (1-4)-β-D mannopyranosyl units with α-D-galactopyranosyl units attached by (1-6) linkages. The ratio of D-galactose to D-mannose is 1:2 with single D galactopyranosyl unit side
chains attached to every other D-mannopyranosyl unit (Figure 1.1).

Guar gum is used in the paper, mining, explosive, pharmaceutical, tobacco, textile and oil industries. In the food industry it acts as a binder of free water in sauces and salad dressings, a stabiliser and binder in cream and frozen desserts, and a binder and lubricant in sausages. It improves the yield of curds in soft cheese and its water holding capacity serves to increase the yield of dough and baked products (Goldstein et al. 1973).

Guar gum was initially studied for therapeutic use as a hypcholesterolemic agent (Fahrenbach et al. 1965) and as a bulking agent in the treatment of obesity. When mixed with water it remains as a fluid, colloidal material, unlike methyl cellulose, the commonly used bulking agent which under the same conditions tends to aggregate into conglomerates with resultant loss of fluidity and the possibility of causing intestinal obstruction. In addition, in vitro it expands more than methylcellulose when mixed with simulated gastric juice, making it more likely to cause satiety. However, both substances have been shown to be equally ineffective in reducing the food intake of obese subjects (Evans and Miller, 1975).

Later, Leeds et al. (1975) showed, in normal subjects, that addition of guar gum to a liquid mixed meal caused a flattening of the post prandial glycaemic curve and also prevented the fall in blood glucose level to below fasting seen 120 minutes after the guar gum free meal. Thus, the importance of guar gum in helping to achieve glycaemic control in diabetics could be hypothesized.
Figure 1.1  Macromolecular structure of guar gum.

Galactose  CH₂OH

Manose  CH₂OH
Other workers have achieved beneficial results adding guar gum, alone to a solid test meal given to diabetic (Gould et al. 1978) and/or to normal (Wahlqvist et al. 1979) subjects. These beneficial results have, however, been disputed by Williams et al. (1980) who found that supplementation of a breakfast with 10g guar gum had no effect on post prandial glycaemia when given to NIDDM.

The effect in diabetics of supplementing meals with guar gum for a week or more is also controversial. Jenkins et al. (1976b and 1977c) achieved positive results based on reduced urinary glucose excretion and reduction in insulin dosage (Jenkins et al. 1978b) but others (Cohen et al. 1980; Botha et al. 1981 and Carroll et al. 1981) found no improvement in diabetic control after periods of up to three months.

Although the mechanism of action of guar gum has not been elucidated there are two hypotheses; that it delays gastric emptying, and/or delays the absorption of glucose from the small intestine (Jenkins et al. 1978a). These mechanisms would depend upon the form in which, and time of the meal, that guar gum was given and could help to explain the conflicting results.

It is well established that liquids leave the stomach faster than solids (Alvarez, 1948 and Thomas, 1957). Therefore it is reasonable to assume that guar gum, which will solidify a liquid meal may delay the rate of liquid gastric emptying. Further studies are required to clarify the circumstances in which guar gum reduces post prandial glycaemia and its mode of action. It has already been confirmed (Leeds et al. 1978) using breath hydrogen studies as described by Bond and Levitt (1977) and the excretion pattern of xylose (Cochet et al. 1978 and Jenkins et al. 1978a)
that guar gum does not cause carbohydrate malabsorption and thus this can be discounted as a possible mode of action.

The effect of guar gum on serum cholesterol levels is more widely accepted than its effect on blood glucose levels.

After three weeks rats fed diets containing sucrose, cholesterol and guar gum had lower fasting serum cholesterol and liver cholesterol values than rats fed a guar gum free diet (Lin and Anderson, 1978). Results in humans have been equally encouraging. Diets containing guar gum have been found to reduce serum cholesterol levels, in particular the LDL fraction, in normal volunteers (Fahrenbach et al. 1965; Jenkins et al. 1975 and 1976c) patients with hypercholesterolemia (Jenkins et al. 1976d; 1977d; 1979a and 1980a) and diabetics (Botha et al. 1981 and Kyllastinen et al. 1981).

Although reduced absorption has been discounted as a mechanism for reducing blood glucose levels it has been suggested that guar gum lowers serum cholesterol levels by sequestering intraluminal bile salts, thus increasing their faecal loss and stimulating cholesterol metabolism in order to replace them (Jenkins, 1978). The loss of bile salts could reduce dietary fat absorption since they are required for micelle formation and further studies are required to verify this.

If guar gum does improve diabetic control and also reduce serum cholesterol levels its therapeutic value to diabetics both short and long term is enormous. The general public, diabetics and general practitioners have already been advised of its potential benefits (Gillie, 1977; Editorial, 1978 and Tredger, 1978) but the feasibility of its use needs to be investigated further.
Practical problems of the use of guar gum

A therapeutic agent can only be of benefit if patients find the planned regimen acceptable. As previously reported the usual dietary fibre intake of the British population is low and problems in consuming guar gum have already been encountered (Dewar et al. 1979 and Wahlqvist et al. 1979). There is no evidence that guar gum has to be incorporated into food but its bulk excludes administration in tablet form.

Guar gum has been incorporated into bread and soup (Wolever et al. 1979a) and fruit juice (Jenkins et al. 1976b). Apling et al. (1977) have produced guidelines for the preparation of guar bread (8g guar gum/100g bread) which they devised because bread is a common food which can be eaten at any meal. Such a useful vehicle could be used in future studies on the effect of long term guar gum dietary supplementation in diabetics but if possible, other alternatives should be made available and more work is needed to produce a range of acceptable guar gum containing foods.

If this proves difficult then the mechanism of action of guar gum should be further explored so that a more palatable alternative can be sought.
Aims

Guar gum has received considerable attention in the medical and nutritional fields because many workers have claimed it can contribute to the management of hyperglycaemia and hypercholesterolaemia. Conflicting results have been published however, and in the light of these, further studies using guar gum were needed to confirm its therapeutic effect and elucidate its mechanisms of action.

The aims of this study were therefore to provide insight in the following areas:-

1. **Acceptability of guar gum**

   Any clinical benefit to be gained by guar gum consumption can only be maintained on a long term basis if guar gum can be given in a form acceptable to the patients. Studies were carried out to ascertain whether amounts of guar gum, sufficient to provide a physiological effect could be disguised in a variety of baked goods. The ability of diabetic patients to distinguish between different concentrations of guar gum in a food was also assessed.

2. **Effectiveness of various formulations of guar gum in acute feeding trials**

   Guar gum formulations have been developed by a number of companies in an attempt to improve its palatability without adversely affecting its suggested therapeutic action.

   The ability of three formulations to modify post prandial blood glucose levels following a liquid glucose load was assessed and compared to their hydration properties as measured by viscosity development.
Much of the discrepancy between published results is probably due to failure to differentiate clearly between liquid and solid test meals. The formulations were therefore also given to normal and NIDDM subjects in conjunction with a solid mixed meal and theireffect on post prandial plasma glucose, insulin and GIP levels measured.

3. **Effectiveness of guar gum supplementation in NIDDM in the long term**

It has been claimed that guar gum can only be effective in modifying the blood glucose profile in diabetics and reducing serum cholesterol levels if it is consumed regularly for a long period. A trial was therefore carried out to see what effect daily guar gum supplementation of NIDDM subjects for eight weeks would have on:

(a) Fasting plasma glucose, insulin and GIP levels and glycosylated haemoglobin.

(b) Fasting serum triglyceride and cholesterol levels.

(c) Twenty-four hour urinary glucose concentration immediately prior to initiation and termination of supplementation.

d) Plasma glucose, insulin and GIP response to a solid mixed meal prior to initiation and immediately after termination of the supplementation period. The subjects were assessed at two weekly intervals with the intention of withdrawing or reducing hypoglycaemic therapy should their metabolic improvement justify it.

4. **The effect of guar gum on alcohol absorption and hypoglycaemia**

It has been reported that guar gum can 'smooth' the post prandial blood glucose curve, reducing hypoglycaemia as well as hyperglycaemia. It has also been suggested that it reduces the rate
of nutrient absorption. Guar gum was, therefore, incorporated into a
snack and consumed at lunchtime with an alcoholic beverage and
sucrose containing 'mixer' to establish its effect on the rate of
absorption of alcohol, the development of intoxication and of
reactive hypoglycaemia which often follows the ingestion of alcohol
and readily assimilable carbohydrate.

5. **Effect of guar gum on fat absorption**

   In order to provide indirect evidence on the effect of guar gum
   on micelle formation, the rate of absorption of an oral fat load given
   with and without guar gum was assessed by measuring the serum light
   scattering index and post prandial serum triglyceride levels.

6. **Effect of guar gum on gut hormone secretion**

   It has been suggested that guar gum delays gastric emptying
   and increases mouth to caecum transit times. In order to ascertain
   whether these effects were associated with altered patterns of
gastro-intestinal hormone secretion, a primarily protein meal was
   given with and without the addition of guar gum and plasma post
   prandial, motilin, gastrin and GIP levels compared.

7. **Effect of guar gum on gastric emptying**

   The rate of solid and liquid gastric emptying when 5g guar gum
   was incorporated into the solid part of a primarily protein meal was
   compared to the rate of emptying of the same meal eaten without
   guar gum.
CHAPTER TWO

Materials and Methods
2.1 Guar gum preparations

Guar flour - guar flour was a non brand product kindly donated by Norgine Ltd.

Guar powder - guar powder was prepared from guar flour by Speywood Laboratories, using a spray drying process.

Guar granules - guar granules were prepared and provided by Norgine Ltd. The composition of the coated granules was 80% w/w guar gum, 5% w/w liquid paraffin and 15% w/w paraffin wax. They were mildly peppermint flavoured.

Guar gum bread - bread containing guar gum was provided in the form of a bread mix by The Boots Company Ltd. It was produced and packed in the consumer products development section. One mix was sufficient to make two loaves and required the addition of 1130ml water. The yeast was packed separately and mixed with 200ml warm water and 2tsp. sugar 15 minutes before addition to the bread mix. Each bread mix was prepared individually to ensure, as near as possible, even distribution of guar gum between loaves. They were prepared in the kitchens of the Hotel Catering and Tourism Management department of the University of Surrey, Guildford. After baking, the loaves were stored frozen, prior to use by the patients. Each loaf provided 860 kcal, 180g carbohydrate and 50g guar gum.

Guar and gellation inhibitor - a formulation was kindly donated by the Wellcome Foundation Ltd. It contained guar gum and a gelatin hydrolysate
in the ratio 1:2, sodium glycinate 1% w/w and artificial mango flavouring. In an alkaline medium the gelatin hydrolysate acted as a gellation inhibitor but its effect was reversed by pH change in the stomach, allowing gellation of guar gum to occur.

The formulation was a powder, and was packed into sachets which each provided 6g guar gum.

2.2 Subjects

Those taking part in the tasting sessions were IDDM and NIDDM patients attending the diabetic clinic at St. Luke's Hospital, Guildford, Surrey.

The long term guar gum supplementation trial involved NIDDM patients attending the diabetic clinic at Frimley Park Hospital, Frimley, Surrey. Further details are provided in Chapter 5.

Normal subjects were volunteers from the University of Surrey and staff of St. Luke's Hospital.

All those participating were given a full description of the study and told what their contribution would involve. It was made clear to them that they could decline to take part or withdraw from the study at any time. The informed consent of the patient's consultant and general practitioner were obtained before any diabetic was approached and the informed consent of all volunteers was also obtained.

Each study was approved by the appropriate Ethical Committee.
2.3 Blood sampling

2.3.1. Blood sampling during test meals

Subjects fasted for 12 hours prior to each test meal unless otherwise stated. Diabetic patients took their oral medication as usual on the morning of each test.

A cannula was inserted into their antecubital vein and kept patent with 0.12M sodium citrate. Two basal venous blood samples were taken and sampling continued 20, 40, 60, 80, 100, 120, 150 and 180 minutes after the start of the meal unless otherwise indicated.

2.3.2. Blood sampling during guar gum supplementation period

At two weekly intervals a fasting venous blood sample was taken from each NIDDM subject but those requiring it, took their oral hypoglycaemic agents prior to sampling.

2.4 Blood Processing

Depending on the analyses to be carried out aliquots of each blood sample were stored at 4°C prior to analysis the same day. Plasma and serum samples were separated, aliquoted, frozen as quickly as possible and stored at -20°C prior to analysis.

2.5 Plasma and urinary glucose

Plasma glucose was determined by a hexokinase method in an automatic glucose analyser (Cobas Bio Centrifugal Analyser).

The approximate glucose content of each urine sample was determined using Glukotest (Boehringer) and then the sample was diluted and analysed using a Boehringer Test-Combination Glucose Kit which employs the hexokinase method.
2.6 Insulin Radioimmunoassay

Plasma immunoreactive insulin was measured by a double antibody technique using an antiserum raised in guinea pig against porcine insulin (Guildhay Antisera). Iodinated bovine insulin (Amersham International plc) was used as the label and human insulin as the standard (NISBC 66/3004). The sensitivity of the assay was 2.5 mU/L. The interassay coefficient of variation was 15% at 6.5 mU/L and 7.4% at a mean plasma insulin level of 46 mU/L.

Plasma immunoreactive insulin assay reagents and procedure

2.6.1. Assay Reagents

Buffer 0.04M phosphate, pH 7.4 containing 0.01M EDTA.

Solution X 14.2g disodium hydrogen phosphate (Na₂HPO₄) and 0.117g merthiolate added to 1L glass distilled water.

Solution Y 13.61g potassium dihydrogen phosphate (KH₂PO₄) and 0.468g merthiolate added to 1L glass distilled water.

400 ml solution X and 100ml solution Y were mixed and 4.65g disodium EDTA added. The pH was adjusted to 7.4 with solution X or Y and made up to 1.25L.

Assay diluent 0.5% bovine serum albumin was added to the required volume of buffer.

Charcoal stripped serum Pooled serum was obtained from volunteers after an overnight fast. The serum was mixed with activated charcoal (20g per 100ml serum) overnight at 4°C prior to centrifugation at 2000g for 15 minutes in a MSE Mistral 4L refrigerated centrifuge. The supernatant was then filtered through a Seitz filter (GradeAP/EKS). The resulting charcoal stripped serum was aliquoted and stored at -20°C until required.
Insulin antiserum  Guildhay guinea pig anti insulin serum was used. Each vial contained 1ml freeze dried antiserum at a dilution 1:60. It was reconstituted to 1ml with distilled water and diluted a further 1:400 prior to use.

Label  The $^{125}$I insulin label with a specific activity > 1.85 MBq/μg was stored at 4°C until required.

Standards  The standard was supplied by the National Institute of biological standard and controls. Each vial was diluted with assay diluent to produce a 1000 mU/L solution and stored frozen in 0.2ml aliquots. For use 0.8ml charcoal stripped serum was added to each aliquot to produce a top standard of 200mU/L. This was double diluted with stripped serum to produce standards containing 100, 50, 25, 12.5, 6.25 and 3.13mU/L.

Second antiserum  Guildhay donkey anti-guinea pig (H/D/4/PE) was diluted 1:20 with assay diluent prior to use.

Normal guinea-pig serum  Normal guinea pig serum was heated at 56°C for 30 minutes in a waterbath and then stored at 4°C until required. This was diluted 1:100 prior to use.

Quality controls  Serum was collected from normal subjects after an overnight fast and 30 minutes, one and two hours after a meal to give four serum pools covering the insulin concentration range of the assay. Aliquots were freeze dried, stored at 4°C and reconstituted with 500μl deionised water prior to use.
2.6.2 Method - see protocol sheet.

Day 1
50μl each of standards, quality control and samples were dispensed in LP3 tubes in duplicate and 350μl of antiserum added. In order to estimate the non specific binding 350μl of buffer diluent only was dispensed into duplicate tubes for each assay.

Tubes were mixed on a Vortex and incubated for 24 hours at 4°C.

Day 2
125I insulin was diluted with assay diluent 1:40. 100μl were added to all tubes which were then mixed on a Vortex and incubated for 24 hours at 4°C.

Day 3
Normal guinea pig serum was diluted with assay buffer 1:100 and 50μl added to all except total counts tubes. The second antiserum was diluted 1:20, with assay diluent and 100μl added to all tubes except totals, 550μl of 4% polyethylene glycol was added to all tubes. They were mixed and incubated at 4°C for 2 hours. All except the total tubes were then centrifuged at 2,500 rpm at 4°C for 25 minutes in a MSE Mistral 4L centrifuge. The supernatant was then aspirated and the protein precipitate which contained the antibody bound 125I insulin counted in an automatic gamma counter. The quantity of immunologically active insulin in standards and samples was determined by subtracting the number of counts in the non specific tubes. These corrected values were used in the construction of a standard curve and in calculating the results.
Protocol for insulin assay

### DAY 1

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<th>Standard/Sample(μl)</th>
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<td>-</td>
<td>-</td>
</tr>
<tr>
<td>NSB</td>
<td>350</td>
<td>50</td>
<td></td>
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<tr>
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<td>50</td>
<td>350 1:10K</td>
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<tr>
<td>Zero</td>
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<td>50</td>
<td>350 1:80K</td>
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<tr>
<td>Standards</td>
<td>-</td>
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<td>350 1:80K</td>
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<tr>
<td>Samples</td>
<td>-</td>
<td>50</td>
<td>350 1:80K</td>
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### DAY 2

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<tr>
<td>NSB</td>
<td>100</td>
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<tr>
<td>Maximum binding</td>
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<tr>
<td>Zero</td>
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<tr>
<td>Standards</td>
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<tr>
<td>Samples</td>
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</table>

### DAY 3

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<th>Double Antibody(μl)</th>
<th>4% PEG(μl)</th>
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</tr>
<tr>
<td>NSB</td>
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<td>Samples</td>
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<td>100</td>
<td>650</td>
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</tbody>
</table>
2.7 **Gastric inhibitory polypeptide**

Plasma immunoreactive gastric inhibitory polypeptide was measured in plasma samples by radioimmunoassay using the method described by Morgan et al. (1978). The sensitivity of the assay was 100 ng/L and the interassay coefficient of variation was 4.1% at 2677 ng/L and 22.5% at 138 ng/L.

2.8 **Plasma motilin**

Plasma immunoreactive motilin was measured using a double antibody method developed by Kwasowski (unpublished data). The method employs an antiserum raised against synthetic motilin. Iodinated synthetic motilin was used as the label and synthetic motilin as the standard. The sensitivity of the assay was 15 ng/L.

2.9 **Plasma gastrin**

Plasma immunoreactive gastrin was measured by a double antibody technique (Hansky and Sovery, 1977).

The sensitivity of the assay was 15 mU/L and the interassay coefficient of variation was 12% at 100 mU/L and 10% at 300 mU/L. The antiserum cross reacts 100% with gastrin-17 and 50% with gastrin-34.

2.10 **Glycosylated haemoglobin**

Blood samples were collected in lithium heparin tubes, stored at 4°C and analysed on the same day by column chromatography according to the method of Kynoch and Lehmann (1977).

2.11 **Serum lipids**

Total serum cholesterol was measured using a fully enzymatic colorimetric method (Boehringer No. 148-393) and total serum triglycerides using a fully enzymatic determination of triglycerides as glycerol (Boehringer No. 126-012).
HDL cholesterol was measured using a Bio-Rad Technique Kit (No: 182-5001). LDL cholesterol was calculated using the equations developed by Myers et al. (1976).

2.12 Blood alcohol

Blood alcohol levels were measured by gas chromatography according to the method of Curry et al. (1966).

2.13 Serum Paracetamol

Serum paracetamol was measured by high pressure liquid chromatography (HPLC) using a Varian HPLC machine equipped with a variable wavelength spectrophotometer. (Starkey unpublished data).

Reagents - Organic solvents used were HPLC grade. Buffers and salt solutions were made up with Analar or Aristar reagents using double glass distilled water.

Precipitant solution - (to precipitate serum proteins in the sample).
Zinc sulphate (1g) and benzoic acid as internal standard (12mg) dissolved in 100ml methanol.

HPLC running solvent - Sodium acetate (0.05M) adjusted to pH 4.6 using glacial acetic acid.

The solvent consisted of 70% sodium acetate buffer and 30% methanol.

Standards - These were prepared in the range 0-200μmol/L paracetamol using paracetamol-free serum.
Column - The column was 10 x 0.5cm O.D.S. packed with (C_{18}) Hypersil (5μ) and operated at ambient temperature. The flow rate was 1.5ml/min and the chart speed 600nm/h.

The eluate was monitored at λ 249nm and the paracetamol concentration calculated by correction of peak height paracetamol for peak height internal standard and reference to the standard curve.

2.14 Presentation of results and analysis data

Parameter measurements were recorded as the mean ± standard error of the mean (SEM).

The area under the post prandial curves was calculated using the trapezoidal rule on a Commodore PET computer 8032 using a program written by P. Kwasowski.

Results were compared using students t-test for paired data unless otherwise specified.
CHAPTER THREE

The effectiveness of guar gum preparations in relation to their hydration properties and conditions of study.
3.1 INTRODUCTION

Supplementation of food with a therapeutic agent can have adverse effects on the palatability of the food and require a modification in lifestyle so, if given the choice, some people would opt to eat unadulterated food and take a separate form of medication.

Guar gum, when mixed intimately with a meal has been shown to reduce post prandial hyperglycaemia in both normal and diabetic subjects (Wolever et al. 1979a; Goulder et al. 1978 and Morgan et al. 1979) but not without a deleterious effect on the acceptability of the food into which it is incorporated. Norgine Limited have developed paraffin coated guar granules designed for consumption immediately prior to a meal. Their ability to modify post prandial glycaemia had to be investigated, in the light of reports suggesting that guar gum can only be effective when intimately mixed with each meal (Jenkins et al. 1979b and Wolever et al. 1979b).

Not all reports in the literature have shown guar gum to be efficacious in reducing post prandial glycaemia and improving metabolic homeostasis. The discrepancies may be due to the different conditions under which studies have been carried out.

Jenkins et al. (1977a and 1978a) reported reduced post prandial plasma glucose and insulin levels in normal subjects following a 50g liquid glucose load to which 14.5g guar gum had been added. These results confirmed others (Leeds et al. 1975 and Jenkins et al. 1977b). Wolever et al. (1979a) also showed, in normal subjects, that 5g guar gum mixed with the liquid part of a meal (soup) was more effective in reducing the peak post prandial plasma glucose and insulin levels than 5g guar gum in bread.
However, 5g in each was the most effective in both normal and IDDM subjects. This was confirmed by Gould et al. (1978), who subsequently showed that the post prandial GIP response was significantly reduced by the addition of guar gum to the meal in both groups of subjects.

GIP is an acronym for Gastric Inhibitory Polypeptide. It was initially named for its ability to inhibit gastric acid secretion but has since been shown to stimulate insulin secretion (Brown et al. 1975). It has been known for many years that the gut is able to modify insulin secretion and in 1964 McIntyre et al. and Elrick et al. showed that oral administration of glucose caused much higher circulating insulin levels than either the same amount of glucose administered intravenously or the intravenous administration of glucose in amounts sufficient to produce the same degree of hyperglycaemia. Unger and Eisentraut (1969) proposed the term entero-insular axis to describe a regulatory system in which insulin secretion from the pancreatic islets is modified, in part, by gastrointestinal hormones. The gastrointestinal hormone GIP appears to be one of the major hormones involved in this regulation.

GIP cells are predominantly localised in the middle cells of the glands of the duodenum and to a lesser extent in the jejunum. They have been recognised as the K cells of the APUD series of endocrine polypeptide cells (Brown and Otte, 1979). The secretion of GIP itself is stimulated by both oral glucose and fat but GIP only stimulates insulin secretion in the presence of hyperglycaemia (Brown et al. 1978). This provides a safeguard against inappropriate insulin secretion as insulin secretion in response to oral fat stimulated GIP, in the absence of carbohydrate ingestion, would result in hypoglycaemia.
Abnormalities of GIP secretion have been reported in the pathogenesis of NIDDM (Creutzfeldt et al. 1978) but there remains a great deal of disagreement. Ebert et al. (1976) showed NIDDM subjects had an exaggerated GIP response to a test meal compared to normal subjects but others have shown no difference (May and Williams, 1978) or a diminished response (Alam and Buchanan, 1980).

The release of GIP from the gut in response to food may be regulated by the rate of absorption of glucose (Ebert and Creutzfeldt, 1978) and/or the rate of gastric emptying (Creutzfeldt, 1981). Since it has been hypothesized that guar gum may modify both the rate of gastric emptying and nutrient absorption (Jenkins et al. 1976a) the addition of guar gum to a meal may affect GIP secretion. Therefore, in any study involving the incorporation of guar gum into the diet, especially of diabetics, plasma GIP levels may provide valuable information, but they have rarely been measured (Morgan et al. 1979).

The importance of reporting fully the composition and consistency of the test meal as well as the type of subjects consuming it has been highlighted by the variable results of the effectiveness of guar gum. Some workers (Gassull et al. 1976; Jenkins et al. 1976a and 1977b) used a typical breakfast meal of bread and marmalade but their positive findings of the effectiveness of fibre supplementation in reducing post prandial glycaemia cannot be attributed to guar gum alone because the marmalade was supplemented with 10g of pectin, shown to reduce post prandial glycaemia (Jenkins et al. 1978a).

Williams et al. (1980) found no effect on post prandial plasma glucose and insulin levels in NIDDM subjects given 5g guar gum in diabetic squash
and 5g sprinkled on to bread and butter, subsequently spread with marmalade. Their initial report of this work (Williams and James, 1979a) and their criticism of the validity of using a liquid test meal caused heated correspondence in the Lancet. Jenkins et al. (1979b) stressed the importance of mixing guar gum with the predominantly carbohydrate portion which should be distributed throughout the meal. They also criticised the meal chosen by Williams' group because of its high sucrose load, atypical of the prescribed diabetic diet. Williams and James (1979b) quickly responded regarding the addition of guar gum to orange juice and crispbread by Jenkins' group (not the predominant carbohydrate portion of the meal) and the use of marmalade which is a source of readily assimilable carbohydrate, comparable to sugar.

Another important factor which arose from the correspondence was the importance of taking guar gum in a prehydrated form. Jenkins and his colleagues (1978a) had previously provided evidence that the ability of an unabsorbable carbohydrate to increase the viscosity of an aqueous solution directly related to its pharmacological effects. They showed that the greater effectiveness of guar gum, compared with pectin, gum tragacanth, methylcellulose, wheat bran or cholestyramine in reducing mean peak post prandial plasma glucose concentrations after a 50g liquid glucose load was abolished when hydrolysed non viscous guar gum was used. In addition the effectiveness of the other fibres was positively correlated with their viscosity.

However, viscosity development prior to consumption may not be vital. Wolever et al. (1978b and 1979b) found that when unhydrated guar gum was given with a liquid glucose load it was as effective in reducing the post prandial blood glucose response in normal subjects as when it was
given hydrated and concluded that viscosity prior to consumption was unimportant provided guar gum was taken well dispersed with sufficient water to allow hydration within the stomach. Williams and James (1979a) found no effect whether the guar gum was given prehydrated or not and refuted (1979b) that their method of incorporating gelled guar gum into the meal had caused it to form lumps which could not disperse or hydrate as suggested by others (Jenkins et al. 1979b).

It emerges from this confusion that several factors must be considered when designing a trial to study the effect of guar gum on the plasma glucose response to a meal. These are:

1) The ability of the guar gum preparation to form a viscous solution before consumption, or whilst in the stomach.
2) The consistency of the meal - liquid or liquid and solid.
3) The composition of the meal - predominantly carbohydrate or mixed.
4) The nutritional component to which the guar gum is added - carbohydrate or mixed throughout.
5) The stage of the meal that the guar gum is eaten.
6) The subjects - non-diabetic, IDDM or NIDDM volunteers.
7) The blood parameters measured as an indication of effectiveness.

The relative importance of each factor is still not clear and therefore the following study was conducted.

3.2 AIMS

Three guar gum formulations were available, guar powder and flour which could be intimately mixed with a meal and guar granules which were
designed to be taken as medication immediately prior to the meal but their effectiveness was unknown.

The aims of this study were initially to measure the viscosity of solutions of each guar gum formulation under either standard or stomach conditions and relate that to their ability to modify post prandial hyperglycaemia when -

(a) given to normal subjects with an oral liquid glucose test meal,
(b) given in conjunction with a solid mixed meal to normal and NIDDM subjects.

Finally, the results of this study were used to help choose the most effective and palatable form of providing guar gum in the diet of NIDDM subjects for a long term study.

3.3 MATERIALS AND METHODS

3.3.1. Measurement of viscosity of guar gum suspensions

One percent guar gum suspensions of guar flour, guar powder and guar granules were made up with either tap water (pH 4.0) or water acidified to pH 1.0 with hydrochloric acid and maintained at either 22°C or 32°C. Stomach conditions were mimicked at a temperature of 32°C and pH 1.0. Each suspension was prepared by whisking the guar gum preparation vigorously into the water. The point at which the addition began was taken as the start of the hydration. Viscosity measurements were made with a Brookfield Synchro-Lectic Viscometer (model RVF-100, spindle No.4) at 20 rev/min at 2, 5, 10, 20, 30, 45 or 60 minutes from the start of hydration. Each suspension was prepared and measured on two separate occasions.
3.3.2. Clinical studies in normal subjects using a liquid glucose test meal

Five healthy volunteers all within 10% ideal body weight for height (Metropolitan Life Insurance Company) and aged between 22-25 years participated. They each attended on four separate occasions at least a week apart. After an overnight fast they consumed, in two equal portions, one minute apart, 80ml Hycal (Beechams) diluted to 250ml with water. This provided 50g glucose and was the control glucose load. Guar gum was incorporated into the test loads in the following manner -

1. Guar flour and guar powder (5g) was vigorously whisked into each portion immediately prior to consumption.
2. Guar granules (6.25g, equivalent to 5g guar gum) were chewed immediately before consumption of each portion.

Venous blood was collected and plasma was separated and subsequently analysed for glucose, immunoreactive insulin and immunoreactive GIP. All methods are described in Chapter 2.

3.3.3. Clinical studies using a solid mixed test meal

Guar powder and guar granules were used in this study.

Six normal healthy volunteers aged between 25-45 years and within 10% ideal body weight for height (Metropolitan Life Insurance Company) and seven NIDDM subjects (see Table 3.1) participated. Each volunteer attended on three separate occasions at least a week apart. The study was always carried out after an overnight fast but those subjects maintained on oral hypoglycaemic agents took their usual medication in the morning.

The composition of the test meal is shown in Table 3.2. The 'bread' was made of flour and water and baked at 180°C for 20 minutes. It was given as part of the control meal and meal in which guar granules were
<table>
<thead>
<tr>
<th>Patient</th>
<th>Sex</th>
<th>Age (yrs)</th>
<th>Weight (Kg)</th>
<th>Height (cm)</th>
<th>Medication (mg)</th>
<th>Energy kJ (kcal)</th>
<th>Carbohydrate (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>J.B.</td>
<td>M</td>
<td>76</td>
<td>67</td>
<td>168</td>
<td>Glibenclamide (5) Metformin (850)</td>
<td>7530 (1800)</td>
<td>180</td>
</tr>
<tr>
<td>P.B.C.</td>
<td>M</td>
<td>49</td>
<td>85</td>
<td>186</td>
<td>Glibenclamide (10)</td>
<td>9205 (2200)</td>
<td>180</td>
</tr>
<tr>
<td>K.E.</td>
<td>F</td>
<td>70</td>
<td>54</td>
<td>157</td>
<td>Chlorpropramide (250) Phenformin (5)</td>
<td>5020 (1200)</td>
<td>120</td>
</tr>
<tr>
<td>D.M.</td>
<td>F</td>
<td>45</td>
<td>59</td>
<td>161</td>
<td>None</td>
<td>5020 (1200)</td>
<td>120</td>
</tr>
<tr>
<td>KM</td>
<td>F</td>
<td>70</td>
<td>67</td>
<td>157</td>
<td>Glibenclamide (5) Metformin (850)</td>
<td>9205 (2200)</td>
<td>140</td>
</tr>
<tr>
<td>W.P.</td>
<td>M</td>
<td>34</td>
<td>64</td>
<td>173</td>
<td>Glibenclamide (10)</td>
<td>6275 (1500)</td>
<td>150</td>
</tr>
<tr>
<td>J.S.</td>
<td>M</td>
<td>30</td>
<td>90</td>
<td>175</td>
<td>Glibenclamide (10)</td>
<td>9205 (2200)</td>
<td>150</td>
</tr>
</tbody>
</table>
## TABLE 3.2

Composition of Mixed Meal

<table>
<thead>
<tr>
<th></th>
<th>Wt (g)</th>
<th>CHO (g)</th>
<th>kcal</th>
<th>kJ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tomato soup</td>
<td>298</td>
<td>28.3</td>
<td>220</td>
<td>920</td>
</tr>
<tr>
<td>Egg</td>
<td>50</td>
<td>-</td>
<td>74</td>
<td>309</td>
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<tr>
<td>Milk</td>
<td>100</td>
<td>4.7</td>
<td>65</td>
<td>272</td>
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<td>'Bread'</td>
<td>60</td>
<td>31.8</td>
<td>145</td>
<td>606</td>
</tr>
<tr>
<td>Butter</td>
<td>10</td>
<td>-</td>
<td>74</td>
<td>309</td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td><strong>64.8</strong></td>
<td><strong>578</strong></td>
<td><strong>2416</strong></td>
<td></td>
</tr>
</tbody>
</table>
consumed. The guar powder was mixed with the flour prior to the addition of water and the 'bread' was baked for 30 minutes.

Following an overnight fast subjects were asked to consume the meal at 0900h within 20 minutes. The meals were given in a random order but the study could not be conducted blind. On one occasion 18.5g guar granules were taken with 250ml water 10 minutes before the meal. During the other test meal the bread providing 10g guar powder was substituted for the control bread and 5g guar powder was whisked vigorously into the soup immediately prior to consumption.

Venous blood was collected and processed as previously described in Chapter 2. The post prandial plasma glucose curves of the NIDDM subjects were calculated as the change in plasma levels from fasting for each subject, due to the large variations between them in fasting levels. All other results were processed and analysed using the methods described in Chapter 2.

3.4 RESULTS

3.4.1 Viscosity measurements

There were considerable differences between the ability of the three guar gum preparations under study to develop a viscous suspension.

**Guar granules**: It was impossible to obtain reproducible viscosity measurements on the wax coated guar granules. They swelled slightly but did not form a uniform suspension. Visual assessment showed that they formed a much less viscous suspension than the other two preparations.
Guar flour and guar powder: The effect of pH and temperature on viscosity development of the guar flour and guar powder suspensions is shown in Figures 3.1 and 3.2. The guar powder suspension was less viscous at 32°C than at the lower temperature (22°C) at both pH 1.0 and pH 4.0. At both temperatures the viscosity was higher at pH 4.0 than 1.0. The guar flour suspension was little affected by pH, especially at 22°C. At 32°C and pH 1.0 a higher viscosity was initially achieved than at pH 4.0 but within 40 minutes it was similar regardless of pH.

3.4.2. Clinical studies in normal subjects using a liquid glucose test meal

The guar granules were well tolerated. The flavour and consistency were acceptable but the subjects disliked the tendency of the granules to stick between the teeth.

It was easier to incorporate the guar powder uniformly into the glucose drinks than the guar flour which formed lumps which were difficult to disperse.

The results of the clinical studies are shown in Figures 3.3, 3.4 and 3.5. When guar gum was taken in addition to the glucose drink there was no statistically significant effect upon mean peak post prandial plasma glucose levels, although in each case they were lower than the control (Figure 3.3). During the latter part of the sampling time (90-180 minutes) supplementation with guar flour and powder prevented a fall in plasma glucose levels below fasting, thus smoothing the post prandial plasma glucose curve compared with the curves following the control and guar granule supplemented meals. At no time, however, was there any significant difference from the control situation, irrespective of the guar gum preparation given.
Figure 3.1
Effect of temperature on the viscosity development during hydration of two guar gum preparations at pH 4.0.

- - 32°C  o--o--o  22°C.
Figure 3.2
Effect of temperature on the viscosity development of two guar gum preparations at pH 1.0.

- - - - - $32^\circ$C  o-o-o-o $22^\circ$C
Figure 3.3  Effect of three guar gum preparations on mean (± SEM) post prandial plasma glucose levels following a 50g liquid glucose load in normal subjects (n=5)

- - 50g glucose control; 50g glucose + guar granules; 50g glucose + guar powder; 50g glucose + guar flour.
In contrast, as shown in Figure 3.4, post prandial insulin levels were significantly reduced by addition of each of the preparations. The area under the curve 0-180 minutes was significantly reduced by consumption of the granules (p < 0.025), flour (p < 0.01) and powder (p < 0.01). Guar flour and powder were equally effective in reducing post prandial insulin compared with the control but guar granules were significantly less effective. The area under the curve 0-180 minutes was greater after the guar granules than after either of the other guar supplemented liquid meals (p < 0.05).

Similarly, as shown in Figure 3.5 the area under the post prandial plasma GIP curve 0-180 min (903.6 ± 138.8 pg. ml\(^{-1}\) min) was significantly reduced when guar powder (586.9 ± 85.6 pg. ml\(^{-1}\) min) or guar flour (554.4 ± 145.2 pg. ml\(^{-1}\) min) were incorporated into the liquid meal (p < 0.01).

GIP levels following consumption of the liquid meal and guar granules were not measured but based on the data obtained a smaller reduction in the area under the curve than that achieved with either guar flour or powder would be expected.

3.4.3. Clinical studies using a solid mixed test meal
The work was not conducted as a blind study because the subjects were aware of the differing circumstances i.e. chewing guar granules and the presence of guar powder in the soup and 'bread'. They did not know, however, that the substance under investigation was guar gum in different forms. The guar granules were consumed with relative ease although all the subjects complained that they stuck between their teeth. Guar powder was easily incorporated into the soup and did not form lumps. If the soup
Figure 3.4 Effect of three guar gum preparations on mean (± SEM) post prandial plasma insulin levels following a 50g liquid glucose load in normal subjects (n=5)

- - 50g glucose (control); 50g glucose + guar granules; 50g glucose + guar powder; 50g glucose + guar flour
Figure 3.5  Effect of three guar gum preparations on mean (± SEM) post prandial plasma GIP levels following a 50g liquid glucose load in normal subjects (n=5).

- - - - - 50g glucose (control);
- - - - - 50g glucose + guar powder;
- - - - - 50g glucose + guar flour.

* P < 0.025  ** P < 0.01
was not consumed quickly it became very thick and three NIDDM subjects found the texture objectionable although the taste of the soup was unaffected. The guar bread was easily made and despite being a heavier texture than the control bread it was quite acceptable.

The effects of incorporating guar gum into the mixed meal on normal subject's post prandial plasma glucose, insulin and GIP levels are shown in Figures 3.6, 3.7 and 3.8 respectively. Post prandial plasma glucose and insulin levels were unaffected by either preparation, however the area under the post prandial plasma GIP curve was significantly reduced by the addition of guar powder (1791.6 ± 132.2 pg. ml\(^{-1}\) min and 1356.7 ± 100.3 pg. ml\(^{-1}\) min after the control and guar powder supplemented meals respectively, p < 0.025).

The effect of incorporating the two guar gum preparations into the meal of NIDDM subjects on mean post prandial plasma glucose, insulin and GIP levels is shown in Figures 3.9, 3.10 and 3.11 respectively.

In seven NIDDM subjects guar granules, equivalent to 15g guar gum, taken as a pre-meal medication had no effect on the metabolic response to the mixed meal. The incorporation of guar powder into the meal, however, caused a reduction in the mean post prandial rise of the three parameters measured as determined by comparison of areas under the post prandial curves 0-180 minutes.

The area under the curve of the mean change in plasma glucose levels from fasting (3.02 ± 0.56 mmol. L\(^{-1}\) min) was reduced to (2.07 ± 0.47 mmol. L\(^{-1}\) min), by the addition of guar powder (p < 0.0125). The area under the post prandial plasma GIP curve was similarly reduced (from 1158 ± 206 pg.
ml\(^{-1}\) min to 1034 ± 79 pg. ml\(^{-1}\) min) (p < 0.0125). The effect of inclusion of guar powder on the mean post prandial insulin responses was also significant (AUC reduced from 29.4 ± 5.5 mU.L\(^{-1}\) min to 26.7 ± 5.3 mU.L\(^{-1}\) min) (p < 0.05).

3.4.4. **GIP response to a mixed meal in normal and NIDDM subjects**

The fasting mean plasma GIP levels of the normal and NIDDM subjects were similar (438 ± 34pg/ml and 346 ± 71 pg/ml respectively) (p = ns). The rise in mean plasma GIP levels following the mixed meal was lower in NIDDM subjects at 80 and 120 minutes (p < 0.05) and at 40 and 150 minutes (p < 0.02) than in normal subjects but there was no significant difference between the area under each mean post prandial plasma GIP curve 0-180 minutes.

3.5 **DISCUSSION.**

3.5.1. **Viscosity of guar gum preparations**

The three guar gum preparations under study exhibited remarkably different abilities to hydrate in solution to form a viscous suspension.

Guar granules were unable to produce a viscous suspension either at room temperature and pH 4.0 or in a situation chosen to mimic stomach conditions. Guar powder was consistently less viscous than guar flour and the differences between the preparations were more marked than the changes brought about by altering pH or temperature. If, as Jenkins et al. (1978a) suggest the ability to form a viscous suspension is critical for pharmacological effect then these differences in rates of viscosity development and maximum viscosity attained should be mimicked by the physiological effect of the preparation. This was subsequently investigated.
Figure 3.6  Effect of two guar gum preparations on mean (± SEM) post prandial plasma glucose levels following a solid mixed meal in normal subjects (n=6).

- - - meal alone (control); o--o--o meal + guar powder;
△ △ △ meal + guar flour.
Figure 3.7 Effect of two guar gum preparations on mean (± SEM) post prandial plasma insulin levels following a solid mixed meal in normal subjects (n=6).

- meal alone (control); meal + guar powder; meal + guar flour.
Figure 3.8

Effect of two guar gum preparations on mean (± SEM) post-prandial plasma GIP levels following a solid mixed meal in normal subjects (n=6)

- - - - meal alone (control); - - - - meal + guar powder;
△ - - - - meal + guar flour. * P < 0.05
Figure 3.9  Effect of two guar gum preparations on mean (± SEM) incremental change in plasma post prandial glucose levels from fasting following a solid mixed meal in NIDDM subjects (n=7).

- - - meal alone (control); o--o--o meal + guar powder; ▲▲▲▲ ▲ meal + guar flour.  ** P < 0.01.
Figure 3.10  Effect of two guar gum preparations on mean (± SEM) post prandial plasma insulin levels following a solid mixed meal in NIDDM subjects (n=7).

- - - meal alone (control); o--o--o--o meal + guar powder; ΔΔΔΔΔ meal + guar flour.
Figure 3.11
Effect of two guar gum preparations on mean (± SEM) post prandial plasma GIP levels following a solid mixed meal in NIDDM subjects (n=7).

- - - meal alone (control); O--O--O meal + guar powder;
△△△△△ meal + guar flour. * P < 0.05.
3.5.2. **Clinical studies in normal subjects using a liquid glucose test meal**

All the guar gum preparations were effective in slowing down the rate of glucose absorption as indicated by the reduction in mean post prandial plasma insulin levels. Mean plasma GIP levels were also significantly lower when guar flour and guar powder were taken with the oral glucose load. Jenkins et al. (1979b) showed that the timing of consumption of the guar gum in relation to the glucose solution was vital and a few minutes interval reduced the effectiveness of guar gum. They were however using guar powder which quickly became viscous. In the present study the guar granules were taken before the glucose solution to allow time for the coating to dissolve and if possible some hydration to occur within the stomach before the glucose reached it. It is unlikely that the limited action of the guar granules can in any way be accredited to their consumption in advance of the liquid load and is more likely attributable to their inability to increase the viscosity of an aqueous medium.

Guar flour and guar powder were significantly more effective than guar granules in reducing the mean post prandial plasma insulin response. They were equally effective in reducing that and the mean post prandial plasma GIP response despite their widely different viscosities under stomach conditions. This, unlike the findings with guar granules, is contrary to the hypothesis of Wolever et al. (1978b) that the effectiveness of a guar gum preparation given ungelled depends upon its ability to become viscous under stomach conditions.

If efficacy of the three guar gum preparations in normals had been judged solely on post prandial plasma glucose measurements they would all
have been shown to be ineffective, even when given with a liquid glucose load, the situation most likely to reveal a physiological action. By measuring post prandial insulin and GIP levels it was clear that addition of each guar gum preparation to the liquid glucose load caused a reduced insulin response, probably mediated by the reduced GIP response. The reason for the reduced stimulation of GIP release is unknown but could be caused by guar gum delaying the rate of gastric emptying.

It has been suggested that guar gum reduces the rate of gastric emptying by virtue of its ability to hydrate (Jenkins et al. 1976a). According to the results of the present study this cannot be the only factor involved because guar powder and guar flour were pharmacologically similar in action despite their differing hydration properties.

Guar powder is a much easier preparation to incorporate into foods and is far more acceptable to the consumer than guar flour. It was essential therefore to establish whether, despite a relatively poor ability to increase viscosity compared with guar flour, it was as effective when incorporated into a mixed meal as it was with a liquid glucose meal.

3.5.3. Clinical studies using a solid mixed meal.

The addition of 15g guar powder to a solid mixed meal modified the post prandial GIP response in normal subjects and reduced the rise in plasma glucose and the plasma insulin and GIP response in NIDDM subjects. Thus in normal subjects, adding three times the quantity of guar powder incorporated into the liquid glucose load to a solid mixed meal had less effect. NIDDM subjects were more sensitive to the addition of guar gum to the solid meal than normals. Normal subjects have an efficient regulatory system for maintaining plasma glucose levels within narrow limits and
these results confirm that discrepancies concerning effectiveness may be due, at least in part, to the type of subject participating.

The lack of effect of guar granules when given to normal subjects with a mixed meal was partially expected because they were the least effective when taken with a liquid glucose load. However, they were not even effective enough to modify the response of the diabetics.

One purpose of the present study was to establish the most effective and palatable means of incorporating guar gum into the diabetic diet in order to carry out an eight-week trial. The meal was chosen to provide the same food items as one previously used (Morgan et al. 1979). They had shown that incorporation of 10g guar flour into the meal significantly reduced post prandial plasma glucose and GIP levels in both normal and IDDM subjects and post prandial plasma insulin levels in normal subjects. Guar flour was the most difficult of the tested preparations to incorporate into the meal because it tended to form lumps when sprinkled into the soup and when water was added to the flour to make bread. In order to inconvenience the NIDDM volunteers as little as possible and hopefully obtain their cooperation for a long term trial they were only asked to attend on three occasions. The addition of the two most palatable preparations were therefore compared with the control situation. It was assumed from the previous results of Morgan et al. (1979) that guar flour was effective.

The reductions observed in the post prandial parameters measured in this study were achieved by adding 15g guar powder (5g in soup and 10g in
bread) to a meal providing 65g carbohydrate. Wolever et al. (1979a) achieved an effect using just 5g guar gum in a meal providing 45g carbohydrate. The effect was greater when the guar was added to the liquid phase (soup) than the solid phase (bread). The subjects in the present study found the bread palatable whether guar flour or guar powder was incorporated but had difficulty in consuming the soup as the viscosity was unexpected (Figure 3.12). It was, therefore, considered likely that greater compliance would be achieved in a long term trial if more guar gum was incorporated into bread or any other solid food found to be acceptable when supplemented with guar gum.

The significant reduction which occurred in the area under the incremental post prandial plasma glucose curve due to the addition of guar powder to the meal is in agreement with the findings of Gassull et al. (1976) and Jenkins et al. (1977b) who added 16g guar gum and 10g pectin to a 100g carbohydrate breakfast given to normal subjects and diabetic subjects (NIDDM and IDDM) (Jenkins et al. 1976a). It is also in agreement with the findings of Wolever et al. (1979a) and Morgan et al. (1979) who, as in this present study achieved an effect with guar gum alone. Williams et al. (1980) however, giving a meal which provided 85g carbohydrate to 13 NIDDM subjects found the addition of 10g guar gum, 5g sprinkled on bread and 5g in squash did not significantly affect post prandial plasma glucose or insulin levels. These negative findings, which contradict those of the other workers quoted, show the importance of giving guar gum intimately mixed with the meal and this is confirmed by the present findings.

The results of this study support the hypothesis that guar gum when incorporated into a mixed meal can favourably modify the post prandial plasma glucose, insulin and GIP response of NIDDM subjects. It is justified
Figure 3.12. An accurate assessment of the effect of guar gum on soup (taken from the Sun, 1979).
therefore to find a variety of foods to which guar gum can be added and establish whether it is as feasible to include them in addition to, or in place of guar gum supplemented bread, shown during this study to be acceptable, into the diet of NIDDM subjects on a regular long-term basis, in an attempt to improve metabolic control.

3.5.4. GIP response to a mixed meal in normal and NIDDM subjects

The fasting mean plasma GIP levels of the normal and NIDDM subjects were similar and although the GIP response to the meal was lower in NIDDM than normal subjects the difference was not significant. In addition the samples were assayed on separate occasions and therefore little emphasis can be put on these differences.
CHAPTER FOUR

The effect of addition of guar gum to foods on their
preparation and acceptability to diabetic subjects
4.1 INTRODUCTION

There are theoretically two methods available for incorporating guar gum into the therapeutic regimen of diabetics. It can be taken as a pre-meal medication as already discussed (Chapter 3) or incorporated into foods. The results reported in the previous chapter however, showed that the granular formulation, available as a pre-meal medication, was ineffective in reducing post prandial hyperglycaemia and highlighted the importance of finding an acceptable method for incorporating guar gum into each meal on a long term basis.

Many workers who have tried to incorporate guar gum into the diet and/or a test meal have reported difficulty in retaining the palatability of the food (Dewar et al. 1979; Wahlquist et al. 1979 and Williams et al. 1980). Hill and Leeds (1979) added varying concentrations of guar gum to 30 recipes but subjects only found the foods acceptable when very small quantities of guar gum were used (0.05g - 2.3g/100g finished product). This concentration may well not be sufficient to cause a therapeutic effect since many studies have been reported using test meals providing 10 - 16g guar gum (Gassull et al. 1976; Jenkins et al. 1976a; 1977b; Goulder et al. 1978; Morgan et al. 1979 and Williams et al. 1980). The incorporation of guar gum into a meal at this level obviously requires the development of food items which retain their palatability when guar gum is added at higher concentrations and/or can be eaten in sufficiently large quantities to provide enough guar gum to be therapeutically effective.

4.2 AIMS

The aim of this study was therefore to assess the effect on preparation and palatability of addition of 5g guar gum to 10g or 20g
carbohydrate portions of a variety of baked products in order to assess the feasibility of including them regularly in a diabetic dietary regimen.

4.3 MATERIALS AND METHODS

4.3.1. Incorporation of guar gum into food

Three recipes were chosen which could be made in large quantities and stored frozen in portions providing 10g and 20g carbohydrate. Ingredients were purchased from a local supermarket and guar flour was used. The carbohydrate and energy content of the recipes were calculated using the food tables of McCance and Widdowson edited by Paul and Southgate (1978). The recipes and nutrient content per portion are shown in Table 4.1.

When preparing cheese scones guar flour was mixed with the plain flour. Half the guar flour was mixed with the plain flour used to make the base of both the flan and pizza and half was sprinkled over the prepared base through a fine mesh strainer before the filling/topping was added.

Twenty two diabetic patients participated in the tasting session and 20 other diabetics compared the three cheese biscuits. Their age range was 15 - 78 years and they were treated with either insulin or oral hypoglycaemic agents in conjunction with a modified diet. They were asked to taste each item and assess them according to their appearance, taste, texture and overall acceptability, including size of a 10g or 20g carbohydrate portion. They used a scale 1-5 as shown in Table 4.2. They were asked to say if the food was one they liked and usually ate. At no time did they know that the foods contained guar gum.
### TABLE 4.1

**Cheese biscuits (20 biscuits 7.5 x 3 cm)**

90g grated red Leicester cheese  
60g butter-softened  
60g flour  
20g guar flour  
**Seasoning**

1. Liquidise cheese  
2. Blend cheese with butter  
3. Sieve together flour, guar flour and seasoning  
4. Blend cheese/butter with dry ingredients  
5. Roll into a firm ball  
6. Roll out thinly and cut into rectangles (20)  
7. Place on an ungreased baking tray and bake at gas mark 6 (400°F) until brown (5-10 min)

Total recipe provides 40g carbohydrate, 4200 kJ (1020 kcal) and 20g guar gum.  
One portion (5 biscuits) provides 10g carbohydrate, 1050 kJ (255 kcal) and 5g guar gum.

**Pizza (13 x 11.5 cm rectangle)**

**Base**

105g flour  
175ml milk  
15g butter  
10g guar flour  
1. Sieve together flour and guar flour  
2. Rub in fat until mixture resembles fine breadcrumbs.  
3. Mix milk in quickly  
4. Knead mixture until it forms a firm ball of dough  
5. Roll out to a rectangle (13 x 11.5cm) and place on a greased baking tray

**Topping**

60g onion  
50g tomato  
10g tomato puree  
200g grated cheddar cheese  
10g guar flour  
Oregano and basil to taste  
6. Chop onions finely  
7. Blanch and skin tomatoes, chop roughly  
8. Spread tomato puree over dough base and sprinkle onions, tomato, guar flour and cheese evenly over the surface of the pizza base  
9. Bake at gas mark 6 (450°F) for approximately 20 min

Total recipe provides approximately 80g carbohydrate, 6200 kJ (1480 kcal) and 20g guar gum.  
One portion (6.5 x 5.7 cm) provides 20g carbohydrate, 1550 kJ (370 kcal) and 5g guar gum.
Egg and bacon flan (Round flan - diameter 22 cm)

**Base**
- 90g flour
- 22.5g margarine
- 22.5g lard
- 7.5g guar flour
- 10 ml cold water

1. Sieve together flour and guar flour
2. Rub in the fat until mixture resembles fine breadcrumbs
3. Add 10ml chilled water and mix quickly.

**Filling**
- 90g lean gammon, cooked and chopped
- 100g egg
- 75 ml single cream
- 100 ml water
- 100g chopped onion
- 60g grated cheddar cheese
- 7.5g guar flour
- Seasoning and mixed herbs

4. Knead mixture firmly to form a compact ball
5. Roll out carefully and use to line greased 22cm loose bottomed flan dish
6. Mix eggs, cream and water
7. Sprinkle guar flour evenly over pastry base
8. Cover with chopped gammon and onion
9. Pour egg mixture over this sprinkle grated cheese and mixed herbs on top
10. Bake at gas mark 6 (400°F) for 30-40 min.

Total recipe provides approximately 60g carbohydrate, 6700 kJ (1600 kcal) and 15g guar gum.

One portion (1/3 of total) provides 20g carbohydrate, 2200 kJ (533 kcal) and 5g guar gum.
### TABLE 4.2

**Scale used for product assessment**

<table>
<thead>
<tr>
<th>Mark Awarded</th>
<th>Range for mean values</th>
<th>Assessment</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>5 - 4.6</td>
<td>Excellent</td>
</tr>
<tr>
<td>4</td>
<td>4.5 - 3.6</td>
<td>Good</td>
</tr>
<tr>
<td>3</td>
<td>3.5 - 2.6</td>
<td>Moderate</td>
</tr>
<tr>
<td>2</td>
<td>2.5 - 1.6</td>
<td>Poor</td>
</tr>
<tr>
<td>1</td>
<td>1.5 - 1</td>
<td>Unacceptable</td>
</tr>
</tbody>
</table>
4.3.2. Detection of guar flour in food

The recipe for cheese biscuits was modified so that a 10g carbohydrate portion provided either 4g (biscuit 4G) 5g (biscuit 5G) or 6g (biscuit 6G) guar gum.

Another group of diabetic patients assessed the overall acceptability of each biscuit. They then tasted biscuits A1, A2 and A3 (equivalent to 4G, 5G and 6G respectively) and indicated which one they preferred.
4.4 RESULTS

4.4.1. Palatability and acceptability of foods incorporating guar gum flour

The cheese biscuits, pizza and egg and bacon flan were evaluated using the scale shown in Table 4.2. The frequency with which a grade was allocated to each aspect of each product is shown in Table 4.3 and the mean scores in Table 4.4.

Cheese biscuits - The biscuits were easy to make and the resulting texture was short and crumbly. Thirteen of the 22 on the panel thought the overall acceptability of the biscuits was good. Only one person found any aspect unacceptable and that was taste. Seventeen had eaten cheese biscuits before and liked them. They tended to give lower scores than those who had not eaten them before (Table 4.4). Two said that the biscuits tended to stick to their teeth.

Pizza - When making the scone base for the pizza it was essential to mix the milk in quickly. The dough had to be handled firmly and kneaded more than usual for a scone mix.

The panel as a whole considered the pizza to be moderate. This judgement was unaltered when the group was subdivided according to whether or not they had eaten pizza before. None thought the taste excellent but only one found it unacceptable. Sixteen thought the texture was moderate/poor compared to eight who gave these grades to cheese biscuits and nine who gave them to egg and bacon flan.
TABLE 4.3

Frequency with which a grade was allocated to each aspect of the foods assessed by 22 patients

<table>
<thead>
<tr>
<th>Grade</th>
<th>Cheese biscuits</th>
<th>Pizza</th>
<th>Egg &amp; bacon flan*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>3</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>3</td>
<td>4</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>4</td>
<td>5</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
</tbody>
</table>

| Appearance | 0 | 2 | 8 | 10 | 2 |
| Taste      | 1 | 1 | 5 | 11 | 4 |
| Texture    | 0 | 1 | 7 | 10 | 4 |
|            | 0 | 6 | 6 | 8  | 2 |
|            | 0 | 0 | 14| 7  | 0 |
|            | 0 | 3 | 6 | 10 | 2 |

TABLE 4.4

Mean value for acceptability score of each food. A = All volunteers, F = Those to whom product was familiar, N = Those who had never tasted the product before.

<table>
<thead>
<tr>
<th></th>
<th>Cheese biscuits</th>
<th>Pizza</th>
<th>Egg &amp; Bacon flan*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
<td>F</td>
<td>N</td>
</tr>
<tr>
<td>Number of volunteers</td>
<td>22</td>
<td>17</td>
<td>5</td>
</tr>
<tr>
<td>Appearance</td>
<td>3.7</td>
<td>3.4</td>
<td>4.0</td>
</tr>
<tr>
<td>Taste</td>
<td>3.7</td>
<td>3.6</td>
<td>4.2</td>
</tr>
<tr>
<td>Texture</td>
<td>3.8</td>
<td>3.7</td>
<td>4.0</td>
</tr>
<tr>
<td>Overall acceptability</td>
<td>3.8</td>
<td>3.6</td>
<td>4.2</td>
</tr>
</tbody>
</table>

* 21 assessments only vegetarian abstained
Egg and bacon flan - The pastry was extremely difficult to make. Once the water had been added to the flour and fat mixture the dough became very sticky and adhered to the hands, rolling pin and work surface.

None of the participants found any aspect of the flan unacceptable but one was vegetarian and therefore declined to assess it because of its bacon content.

The overall acceptability according to the rest of the panel was moderate but the 16 who had eaten the product before classed texture and overall acceptability as good.

4.4.2. Ability of diabetics to distinguish between cheese biscuits containing different quantities of guar gum

Biscuit 4G (4g guar gum) was classified as good and biscuits 5G and 6G as moderate. When asked to indicate which of the three biscuits identical to 4G, 5G and 6G they preferred 11 out of 20 chose the one with the lowest concentration of guar gum (4g). Five out of 20 preferred the one containing 5g guar gum and the remainder (four) preferred the biscuit providing the most guar gum (6g).

Fourteen of the 20 subjects chose as their favourite biscuit the one with the same concentration of guar gum as the one they had previously judged to be most acceptable.
4.5 DISCUSSION

4.5.1. Palatability and acceptability of guar gum containing foods

Incorporation of 5g guar gum into a 10g or 20g carbohydrate portion resulted in an acceptable product in all three foods considered. The amount of guar gum in the final product was much higher than that achieved by Hill and Leeds (1979), 5g guar gum being incorporated into a portion of cheese biscuits providing 10g carbohydrate.

Furthermore, the cheese biscuits were the most acceptable of the three products made. This could be because they were the most familiar of the foods but subdivision of the group showed that those who had not eaten them before tended to give a higher score than those who had, possibly because they had no basis for comparison. The inclusion of guar gum in this recipe caused no culinary problems, primarily because no liquid was needed. Hill and Leeds (1979) found that baked products which could have extra water incorporated produced a more acceptable product than drier biscuits which caused a cumulative 'sticky' mouth-feel. The cheese biscuits tended to do this and two of the panel commented that they stuck to their teeth.

Hill and Leeds (1979) also suggested that highly seasoned products may be more acceptable since strong flavouring may distract attention from impaired flavour. In the present study this was not so. Pizza, which was liberally seasoned with oregano and basil was the least popular product. It may have been too highly seasoned for some of the panel. There are, however, other possibilities. Pizza should ideally be served whole and piping hot. Circumstances dictated that the panel had to assess 3cm square portions which were lukewarm. Even the base of a normal pizza often becomes soggy when allowed to cool! Under different
conditions both appearance and texture would have been better. Pizza has only relatively recently become a food item readily available in the United Kingdom. The majority of the taste panel were over the age of 55 years and, in general, people become more conservative about food with advancing age. Indeed 12 out of 22 had not eaten pizza before. Subdivision of the group into those who had and had not eaten pizza before did not however alter the score.

The egg and bacon flan was more popular amongst those who had eaten it before. It is possible that the unfamiliarity of the flan and not the presence of guar gum caused the group eating it for the first time to give it a low score. The precaution was taken of calling the item by the more familiar term, egg and bacon flan and not quiche.

In this study the pastry case of the flan was not cooked before the filling was added. Several of the panel commented on the soggy base and later the recipe was modified and the pastry case baked blind prior to sprinkling on the guar flour and adding the filling. This procedure as judged by ten members of staff at the University of Surrey resulted in a better product.

The viscosity of the guar flour once it was mixed with liquid made both the scone and pastry bases difficult to prepare. Speed was of the essence, once liquid had been added, to prevent the dough adhering tenaciously to all work surfaces. Since these products are intended for the consumption of diabetics and should contain a known amount of carbohydrate it is not desirable to use excess flour to coat the working surfaces in an attempt to prevent adherence. In order to retain a workable dough only half the guar flour was added. The remainder was sprinkled
onto the prepared base through a fine mesh strainer. It was essential that
the strainer be kept near the surface to be covered to prevent dispersion of
the fine guar flour and be kept absolutely dry to prevent the guar flour
sticking to the strainer. These procedures required extreme dexterity, not
always found in elderly people. Many of the panel and indeed many NIDDM
patients would have found it very difficult to prepare these products.

There were two other main problems encountered. If individual
portions were being prepared kitchen scales are not sensitive enough to
accurately weigh 5g guar flour. However, if some degree of freedom is
acceptable it would be possible to measure the guar on a regular basis using
household measures i.e. 2 tsp = 5g guar flour.

Once guar flour is wet it becomes very sticky and this made washing
up an arduous task.

4.5.2. Guar gum detection

Eleven of the twenty who sampled the cheese biscuits containing
different quantities of guar gum preferred the one with the lowest
concentration (4g guar gum in a 10g carbohydrate portion). Nine of these
correctly matched their favourite biscuit. This tends to indicate that the
less guar gum incorporated the better from the point of view of
palatability. However, there is obviously some individual variation because
five preferred the intermediate concentration. Three of these matched it
correctly and two of the four preferring the highest concentration matched
it correctly. It is difficult, on the opinion of so few, to confirm whether
such a small difference in concentration as 1g or 2g can be accurately
detected but since 70% of the sample chose as their favourite biscuit the one with the same guar gum concentration on two occasions it is not unreasonable to suggest that it is possible.

4.6 CONCLUSION

Although these trials have shown that guar gum can be added to baked foods without making them universally unacceptable it has highlighted the practical problems of preparing such foods containing guar gum. Subjects interested in cooking and motivated to take guar gum on a regular basis could find this mode of incorporating guar gum into the diet useful. However, in order to achieve compliance in a long term trial it would appear that the most likely means of success is to provide the subjects with a ready to eat food which has already been supplemented with guar gum. Guar gum supplemented bread is easy to make, store and provide to the subjects and was therefore chosen for use in the long term study of the effect of guar gum supplementation in NIDDM subjects.
CHAPTER FIVE

The effect of eight weeks daily dietary supplementation with 25g guar gum on the metabolic profile of non insulin dependent diabetics.
5.1 INTRODUCTION

Non insulin dependent diabetics may be controlled by dietary modification, or require in addition oral hypoglycaemic therapy. Oral hypoglycaemic drugs fall into two categories; sulphonylureas and biguanides.

The sulphonylurea drugs, tolbutamide, chlorpropramide and the more recently developed glibenclamide, cause, on acute administration an increased secretion of insulin and degranulation of the β cells of the pancreas. Chronic administration however, causes decreased insulin synthesis and secretion and it has been suggested that chronic sulphonylurea therapy alters the plasma membranes of cells to increase their responsiveness to insulin action, perhaps by increasing the number of insulin receptors (Lebovitz and Feinglos, 1978). The hypoglycaemic action of all sulphonylureas requires the presence of endogenous insulin because no effect is seen on administration to pancreatectomised animals or insulin dependent diabetics. The efficacy and safety of sulphonylureas has been a matter of widespread concern since 1970 when the University Group Diabetes Programme (U.G.D.P.) published a report suggesting that tolbutamide therapy as well as being no more effective than diet alone in the treatment of diabetes maybe associated with an increased cardiovascular mortality (U.G.D.P., 1970). Although the conclusions are not in accordance with other workers findings (Drury and Timoney, 1972), they have apparently affected the prescribing patterns in some diabetic clinics (Timoney, 1979).

The mechanism for the hypoglycaemic action of the biguanides is unknown. It is not caused by an increase in insulin secretion although a certain quantity of endogenous insulin is a prerequisite for their
therapeutic effect (Hermann, 1979). The mode of action is probably multifactorial and the following mechanisms are considered important: increased muscular glucose uptake, decreased gluconeogenesis and decreased intestinal glucose absorption.

There is an association between phenformin and lactic acidosis and this has led to its withdrawal in many countries. In Finland, neither phenformin nor metformin are prescribed unless other treatments are unsatisfactory (Siitonen et al. 1980). Metformin associated lactic acidosis is rare. Although it can cause gastrointestinal problems in some patients the advantages of metformin include the tendency to promote weight reduction and its seeming inability to cause significant hypoglycaemia. In appropriate doses it reduces plasma glucose levels to within but not below, the normal range (Hermann, 1979).

Whatever the degree of benefit of oral hypoglycaemic agents in management of NIDDM it is obvious that the doubts surrounding their safety and effectiveness make it worthwhile to try and find alternative means of achieving glucose homeostasis.

Kiehm et al. (1976) were able to discontinue sulphonylurea treatment in five men by increasing their carbohydrate intake to provide 75% total energy and providing 15g crude fibre/day. Anderson and Ward (1978), treating 10 patients, were able to withdraw insulin therapy from five and sulphonylurea therapy from three following initiation of a high carbohydrate, high fibre diet. Jenkins et al. (1979c) also reduced insulin or oral hypoglycaemic requirements in 11 patients by giving an average of 18g guar gum a day. The major change occurred during the first month but small reductions in insulin dose were possible up to 12 weeks after the start
of guar gum consumption. They suggest the effect of guar gum is progressive, substantiated by their findings that urinary glucose concentration was only significantly reduced after three days of guar gum administration (Jenkins et al. 1978b).

These same workers have also shown a 'carry-over' phenomenon associated with guar gum treatment. They found significantly lower urinary glucose levels on the two days immediately after the end of guar gum therapy compared to the following three days. The effect of combining guar gum with one meal also affected the metabolic response to a guar free meal taken four hours later (Jenkins et al. 1979d and 1980b). Such an effect would be of great benefit as it would obviate the need to take guar gum intimately mixed with each meal.

The assessment of metabolic control of NIDDM subjects can be carried out in several ways. Some methods are convenient but unreliable, such as measurement of random plasma glucose, fasting plasma glucose and urinary glucose levels. Other methods are less convenient and are time consuming but provide more detail of the metabolic state. These methods include measurement of plasma glucose, insulin and GIP response to a glucose load or mixed meal and measurement of 24h urinary glucose output.

More recently glycosylated haemoglobin level estimation has received considerable attention. The percentage of glycosylated haemoglobin (HbA) has been shown to reflect the integrated plasma glucose level over the preceding eight weeks (Albutt et al. 1981). Glycosylated haemoglobin refers to a series of components of haemoglobin A which are formed by its combination with glucose or glucose phosphates at the N
terminus of the β chain. They include HbA_{1a} HbA_{1b} and HbA_{1c}, although HbA_{1c} is the major component. The reaction is a slow one which occurs throughout the lifespan of the erythrocyte and the quantity formed is governed by the plasma glucose concentration (Gonen et al. 1977). In normal individuals it is present as 3-5% total haemoglobin but patients with diabetes have been reported to have levels within the range 6-15% (Cerami and Koenig 1978).

Although long term trials have been carried out incorporating guar gum into the diet of diabetics the results are not in agreement. These discrepancies may, in part, be due to the various types of diabetics involved in the study or, more likely, to the parameters measured to ascertain metabolic control. Maintenance of plasma glucose within the normal range is an important aim in diabetic therapy and can now be monitored more efficiently by the measurement of glycosylated haemoglobin over at least a four week period.

5.2 AIMS

Part of the aim of this study was therefore to provide guar gum at each meal for an eight week period to a group of NIDDM subjects and monitor its effect on glucose homeostasis by measuring fasting plasma and urinary glucose levels and to gain a more accurate picture of long term effects by measuring glycosylated haemoglobin. If results indicated an improvement, oral hypoglycaemic therapy was to be reduced or withdrawn.

In NIDDM subjects assessment of serum lipid profile may be as important as assessment of plasma glucose profile because these patients are particularly prone to cardiovascular disease.
The ability of guar gum to reduce risk factors for CVD, namely serum cholesterol levels has been studied but serum LDL cholesterol levels, considered one of the most important indicators of risk, have received little attention in studies involving NIDDM subjects and were therefore measured and compared to HDL and total serum cholesterol level changes.

Because there is no information on the carry over effect of a period of guar gum supplementation a guar free meal was given before and after the supplementation period and the plasma glucose, immunoreactive insulin and immunoreactive GIP response compared.

5.3 MATERIALS AND METHODS

Seven NIDDM subjects (five male and two female) participated in the study (see Table 5.1). They were aged between 30-76 years and their mean weight was 104 ± 3% (mean ± SEM) ideal body weight for height (Metropolitan Life Insurance Company). The subjects were all receiving oral hypoglycaemic therapy and the need for any modification in dose was monitored throughout the study.

5.3.1 Long term study

A detailed diet history was taken from each subject and their usual energy and carbohydrate intake assessed using McCance and Widdowson's food tables edited by Paul and Southgate (1978). The diet history was also used to provide each subject with guidelines for incorporating the guar gum supplemented bread into their diet without altering either their total carbohydrate and energy intake or the distribution of carbohydrate throughout the day. Subjects were asked to eat daily half a loaf of the guar gum supplemented bread which is described in Chapter 2. The supplementation continued for an eight week period. On the first day, at
### TABLE 5.1

<table>
<thead>
<tr>
<th>Patient</th>
<th>Sex</th>
<th>Age (yrs)</th>
<th>Weight (Kg)</th>
<th>Height (cm)</th>
<th>Medication (mg)</th>
<th>Energy kJ (kcal)</th>
<th>Carbohydrate (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>J.B.</td>
<td>M</td>
<td>76</td>
<td>67</td>
<td>168</td>
<td>Glibenclamide (5) Metformin (850)</td>
<td>7530 (1800)</td>
<td>180</td>
</tr>
<tr>
<td>P.B.C.</td>
<td>M</td>
<td>49</td>
<td>85</td>
<td>186</td>
<td>Glibenclamide (10)</td>
<td>9205 (2200)</td>
<td>180</td>
</tr>
<tr>
<td>K.E.</td>
<td>F</td>
<td>70</td>
<td>54</td>
<td>157</td>
<td>Chlorpropramide (250) Phenformin (5)</td>
<td>5020 (1200)</td>
<td>120</td>
</tr>
<tr>
<td>D.G.</td>
<td>M</td>
<td>43</td>
<td>70</td>
<td>183</td>
<td>Glibenclamide (5)</td>
<td>7110 (1700)</td>
<td>170</td>
</tr>
<tr>
<td>L.G.</td>
<td>M</td>
<td>58</td>
<td>66</td>
<td>175</td>
<td>Glibenclamide (5)</td>
<td>10450 (2500)</td>
<td>220</td>
</tr>
<tr>
<td>J.S.*</td>
<td>M</td>
<td>30</td>
<td>90</td>
<td>175</td>
<td>Glibenclamide (10)</td>
<td>9205 (2200)</td>
<td>150</td>
</tr>
<tr>
<td>E.T.</td>
<td>F</td>
<td>59</td>
<td>60</td>
<td>162</td>
<td>Glibenclamide (15) Metformin (500)</td>
<td>5020 (1200)</td>
<td>125</td>
</tr>
</tbody>
</table>

*Withdrew after 4 weeks
two weekly intervals and two weeks after terminating the guar gum supplemented regimen blood was taken from each subject following an overnight fast. Their body weight was also recorded at these times. Immediately before they started and on completion of guar gum supplementation subjects provided a total 24h urine collection which was collected into a plastic container into which 10ml concentrated hydrochloric acid had been added to act as a preservative.

5.3.2. Test meal

On the first and last day of the study period each subject fasted overnight. They came to the investigation unit and were given a test meal. The composition of the meal is given in Table 5.2. It was identical on both occasions and did not contain guar gum.

Blood was taken before and after the meal as described in Chapter 2.

5.3.3. Sample Analysis

All fasting blood samples, including those taken prior to the test meals were analysed for glycosylated haemoglobin, plasma glucose, immunoreactive GIP and serum cholesterol and triglycerides by the methods described in Chapter 2. The samples taken before and after each test meal were analysed for plasma glucose, immunoreactive insulin and immunoreactive GIP.

Statistical analysis was carried out using the methods described in Chapter 2.
<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Wt (g)</th>
<th>CHO (g)</th>
<th>kcal</th>
<th>kJ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tomato soup</td>
<td>298</td>
<td>28.3</td>
<td>220</td>
<td>920</td>
</tr>
<tr>
<td>Egg</td>
<td>50</td>
<td>-</td>
<td>74</td>
<td>309</td>
</tr>
<tr>
<td>Milk</td>
<td>100</td>
<td>4.7</td>
<td>65</td>
<td>272</td>
</tr>
<tr>
<td>Bread</td>
<td>60</td>
<td>31.8</td>
<td>145</td>
<td>606</td>
</tr>
<tr>
<td>Butter</td>
<td>10</td>
<td>-</td>
<td>74</td>
<td>309</td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td></td>
<td>64.8</td>
<td>578</td>
<td>2416</td>
</tr>
</tbody>
</table>
5.4 RESULTS

5.4.1. Making and distributing the guar gum supplemented bread

When the yeast solution had been added to the flour mixture a very sticky dough formed which was difficult to knead. To ensure even distribution of guar gum between the two loaves care had to be taken that the dry ingredients were thoroughly mixed before the yeast solution was added.

The taste and texture of the bread were unaffected by freezing.

5.4.2. Acceptance and tolerance

Seven NIDDM subjects embarked upon this study. One withdrew after four weeks due to distressing abdominal distension and flatulence. He underwent a test meal at this time and returned two weeks later to provide a fasting blood sample and his results are included. The remaining six subjects initially suffered flatulence and some abdominal distension but these effects were transient. They did however find the bread filling. One subject found it difficult to eat her daily quota as she had a small appetite but another was delighted because she normally often felt hungry. Throughout the trial the subjects were able to consume the specified half a loaf daily and maintain a constant body weight.

5.4.3. Effect of guar gum supplementation on fasting blood levels

The effect of the regimen on mean fasting plasma glucose levels is shown in Figure 5.1.

The pre-trial mean fasting plasma glucose concentration of the seven subjects was $7.9 \pm 1.74$ mmol/L and ranged between $8.1 \pm 1.6$ - $8.8 \pm 2.0$ mmol/L during the trial period ($p = \text{n.s}$). Two weeks after the
Figure 5.1  Effect of guar gum supplementation on fasting plasma glucose (mean ± SEM) in NIDDM subjects (n=7)
supplementation ceased the fasting mean plasma glucose concentration was 
7.7 ± 1.5 mmol/L.

The mean fasting plasma GIP concentrations are shown in Figure 5.2. 
Prior to the guar gum supplementation it was 307 ± 70 pg/ml and did not 
alter significantly during or in the two weeks following the trial.

The pre-trial mean glycosylated haemoglobin level was 12.5 ± 2% of 
total haemoglobin. In Figure 5.3 this has been taken to represent 100% to 
illustrate the percentage fall in mean glycosylated haemoglobin throughout 
the study period. The mean percentage decrease became significant after 
four weeks on the guar gum supplemented regimen (p < 0.05). A further 
four weeks caused a greater decrease (p < 0.01) and two weeks after 
supplementation ceased the lowest concentration was achieved (85% of 
starting value p < 0.01).

The effect of the regimen on mean serum lipid levels is shown in 
Figure 5.4. The mean total serum cholesterol level (initially 5.08 ± 0.35 
mmol/L) fell throughout the trial. This was significant after two and eight 
weeks (p < 0.05). When the subjects had been without guar gum for two 
weeks the mean serum cholesterol rose above presupplementation levels 
(5.4 ± 0.5 mmol/L). The reduction in mean total serum cholesterol levels 
during the trial was due, almost entirely, to a reduction in the LDL 
fraction. The mean LDL cholesterol level fell gradually from 3.1 ± 0.34 
mmol/L to 2.3 ± 0.15 mmol/L (p < 0.05) but two weeks later had returned 
to the pre-trial concentration.

Although the mean HDL cholesterol concentration was not 
significantly altered throughout the study the relative amount of total
Figure 5.2
Effect of guar gum supplementation on fasting plasma GIP (mean ± SEM) in NIDDM subjects (n=7)
Figure 5.3. Effect of guar gum on fasting glycosylated haemoglobin levels (mean ± SEM) in NIDDM subjects (n=7).

* P < 0.05; ** P < 0.01.
Figure 5.4. Effect of guar gum on fasting serum lipid levels (mean ± SEM) in NIDDM subjects (n=7)

- Total serum cholesterol
- LDL cholesterol
- HDL cholesterol
- Total triglycerides

* P < 0.05; ** P < 0.02.
cholesterol present in the HDL fraction rose from $27.3 \pm 3.5\%$ to $32.2 \pm 4.1\%$. In addition the ratio of LDL to HDL cholesterol and total to HDL cholesterol were reduced (Table 5.3). Mean serum triglyceride levels were unchanged throughout the study period.

Individual 24h urinary glucose outputs before and after the study are shown in Table 5.4. Three subjects showed no urinary glucose loss before or after guar gum supplementation. One had a 40% increase and the other three showed decreases between 17 - 75% following guar gum supplementation.

5.4.4. Response to test meals

The effect of guar gum supplementation on the incremental changes in the mean plasma glucose response to a guar free mixed meal is shown in Figure 5.5. Test meal 1 refers to the meal prior to supplementation and test meal 2 to the same meal taken immediately after the supplementation period.

The initial effect on mean plasma glucose levels was similar but three hours after the meal the incremental change was significantly closer to fasting levels following test meal 2 ($p < 0.05$) at 180 minutes.

Mean post prandial plasma insulin levels were similar for the first hour after each meal. They were lower for the next two hours after test meal 2 but this was only significant 80 minutes after the meal ($p < 0.05$) (Figure 5.6).

The effect of the meals on mean post prandial GIP levels is shown in Figure 5.7. The response was increased during the first hour after test meal 2 and the area under the mean plasma GIP response curve for the
TABLE 5.3

The effect of 8 weeks guar gum supplementation (25g/day) on mean serum HDL, LDL and total cholesterol levels in six NIDDM subjects

<table>
<thead>
<tr>
<th></th>
<th>Mean serum cholesterol mmol/L</th>
<th>HDL/Total %</th>
<th>Total/HDL Ratio</th>
<th>LDL/HDL Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total</td>
<td>HDL</td>
<td>LDL</td>
<td></td>
</tr>
<tr>
<td>Presupplementation</td>
<td>5.1</td>
<td>1.3</td>
<td>3.1</td>
<td>27.3 + 3.5</td>
</tr>
<tr>
<td>8 weeks supplementation</td>
<td>4.1</td>
<td>1.3</td>
<td>2.3</td>
<td>32.2 + 4.1</td>
</tr>
</tbody>
</table>
TABLE 5.4
The effect of guar gum supplementation (25g/day) on 24h urinary glucose excretion in seven NIDDM subjects

<table>
<thead>
<tr>
<th>Subject</th>
<th>Presupplementation urinary glucose g/24 hr</th>
<th>Immediately following supplementation urinary glucose g/24 hr</th>
<th>% change</th>
</tr>
</thead>
<tbody>
<tr>
<td>J.B.</td>
<td>61</td>
<td>85</td>
<td>+40</td>
</tr>
<tr>
<td>P.B.C.</td>
<td>55</td>
<td>23</td>
<td>-59</td>
</tr>
<tr>
<td>K.E.</td>
<td>114</td>
<td>95</td>
<td>-17</td>
</tr>
<tr>
<td>D.G.</td>
<td>N.T.</td>
<td>N.T.</td>
<td>-</td>
</tr>
<tr>
<td>L.G.</td>
<td>N.T.</td>
<td>N.T.</td>
<td>-</td>
</tr>
<tr>
<td>J.S.</td>
<td>29</td>
<td>7</td>
<td>-75</td>
</tr>
<tr>
<td>E.T.</td>
<td>N.T.</td>
<td>N.T.</td>
<td>-</td>
</tr>
</tbody>
</table>

N.T. - not detectable
Figure 5.5. Effect of eight weeks guar gum consumption on incremental post-prandial plasma glucose levels in NIDDM subjects following a solid mixed meal (n=7). * P < 0.05.

0---0 pre guar; 0---0 post guar.
Figure 5.7. Effect of eight weeks guar gum consumption on post prandial plasma GIP levels in NIDDM subjects following a solid mixed meal (n=7). o-o-o pre guar; •-•-• post guar.
first 80 minutes was significantly greater compared with test meal 1 (p < 0.01).

The results were not considered to show sufficiently improved metabolic control in any subject to warrant discontinuation of, or reduction in, oral hypoglycaemic therapy.

5.5 DISCUSSION

5.5.1. Feasibility of using guar gum in the diabetic diet

There are several methods available for incorporating guar gum into the dietary regimen of diabetics as described in Chapters 3 and 4. On the basis of those findings it was decided to use bread as the vehicle for guar gum supplementation in this trial. Making the bread was relatively easy but time consuming and although it froze well subjects found that it went mouldy quickly and it had to be refrigerated. This is contrary to the experience of Apling et al. (1977) who found guar gum containing bread went stale much less rapidly than ordinary bread.

Despite recent trends in favour of homemade bread few people routinely bake their own and the subjects in this study were unwilling/unable to do so. However because the bread could be frozen they were able to collect a two week supply at each hospital visit. This was a viable procedure for the eight week trial but it is unlikely that such a routine could be continued indefinitely. This casts doubt on the practicality of providing guar gum to the general diabetic population in the form of a bread mix.

A further drawback encountered was the need to consume 90g carbohydrate and 430 kcal a day in the form of bread. This limited the
subject's choice of other foods. The bread provided between 50 - 75% of the total daily carbohydrate allowance of the subjects and they found bread with every meal and snack monotonous. In order to avoid monotony it would be desirable to provide a variety of foods containing guar gum.

While the problem of incorporating guar gum into the diet has to some extent been overcome the adverse side effects persist. They are reported in every trial although the degree of emphasis placed upon these varies. In the present study six subjects initially experienced minimal flatulence and abdominal distension, which ceased to be troublesome after a few days. This is in accordance with other reports (Aro et al. 1981; Jenkins et al. 1978b and Stokholm et al. 1981). One of the subjects in this trial had side effects which were severe enough for him to withdraw after four weeks. This is not uncommon. Cohen et al. (1980) had three subjects withdraw immediately. Two others suffered nausea, flatulence and feelings of satiety and were only able to continue on a reduced intake of 15g guar gum a day. Flatulence is reported as an adverse side effect in every study but there appears to be an individual variation as to whether diarrhoea or constipation is caused by taking guar gum. None of the subjects in the present study were troubled by either.

If the side effects of taking guar gum are transient then the patient can be warned what to expect and reassured that this is normal and will soon pass. Those who appear particularly sensitive to the side effects of guar gum consumption should not consider it a viable therapy whatever the possible benefits.

During the eight weeks that the subjects took 25g guar gum daily their weight remained stable. Botha et al. (1981) reported weight gain in three subjects and Smith et al. (1982) achieved significant weight reduction
in their subjects. They were however IDDM subjects and no report of whether or not they modified their food intake is given. Guar gum has been reported to delay, not impair nutrient absorption (Jenkins et al. 1978a; Cocke et al. 1978 and Leeds et al. 1978) and thus a weight loss should not be expected, provided energy intake remains constant. However in those subjects who experience feelings of fullness and early satiety when taking guar gum a reduced food intake and accompanying weight loss could occur. Evans and Miller (1975) showed that 10g guar gum taken in two equal doses during the day for one week was as effective as methyl cellulose in reducing appetite and achieving weight loss in obese and non-obese subjects. However the weight loss achieved with either substance was not dramatic and guar gum would be of dubious value as part of a weight reduction regimen.

5.5.2. Response to long term guar gum supplementation

The reports in the literature of the studies of the effect of long term guar gum supplementation on fasting plasma glucose levels initially seem conflicting. Some discrepancy can be accounted for by the variation in regimen followed and the type of diabetic participating (NIDDM or IDDM).

In the present study the subjects were all NIDDM receiving oral hypoglycaemic therapy. Guar gum supplementation had no significant effect on mean fasting plasma glucose levels throughout the study which substantiates other results (Kyllöstinen and Lahikainen 1981). Aro et al. (1981) however achieved a significant reduction in fasting plasma glucose concentrations in nine NIDDM after three months (21g guar gum a day) and Smith et al. (1982) after two weeks on 9.15g guar gum a day. After one week on 12g guar gum a day 10 NIDDM had non significantly lower fasting plasma glucose concentrations (Stokholm et al. 1981). These subjects had
however been taken off their oral hypoglycaemic therapy prior to the study in order to establish glycosuria and adaptation to this may well have affected their response to guar gum. It is obvious that methods must be carefully studied when interpreting reported results. The subjects taking part in the present study were maintained on their usual drug regimen throughout because there was no reduction in plasma glucose levels as shown in Figure 5.1. Hockaday et al. (1979) pointed out that the principle postulated action of guar gum is to effect a reduced absorption rate of nutrients from the small bowel without causing malabsorption and therefore it is unreasonable to expect a reduced fasting plasma glucose level. However, if due to delayed absorption after each meal the pancreas was less stressed, it is possible that it could secrete sufficient insulin to reduce the fasting plasma glucose level and allow withdrawal of oral hypoglycaemic therapy. Hockaday et al. (1979) also defended the use of urinary glucose concentration as a means of assessing diabetic control. They maintained that it accurately reflects alterations in post prandial plasma glucose concentration. Carroll et al. (1981) disagreed because they consider comparisons between subjects to be of little value due to large individual variation in renal threshold. In the present study 24h urinary glucose excretion before and after supplementation was compared in each subject and therefore this problem does not arise. Three subjects did not exhibit glycosuria. Although Stockholm et al. (1981) withdrew oral hypoglycaemic therapy from their subjects in order to initiate glycosuria prior to this study, it was not considered justified here. The changes found in urinary glucose output before and after supplementation are inconclusive. In retrospect more frequent 24h urinary collections would have been useful in determining individual variation in urinary glucose excretion and establishing the existence of a trend towards reduced glucose excretion.
Even though fasting plasma glucose levels were unchanged and changes in 24h urinary glucose outputs were inconclusive there may well have been an effect on plasma glucose concentration throughout the day. It has been shown that following a mixed meal containing guar gum the post prandial plasma glucose curve is flatter (Morgan et al. 1979). It is possible therefore that when diabetics take guar gum regularly with all meals there is less fluctuation in plasma glucose levels throughout the day. Some indication that this occurred is provided by the glycosylated haemoglobin levels. The measurement of HbA\textsubscript{lc} provides an indirect means of assessing the mean plasma glucose concentration over the life span of the red blood cell. The subjects in this trial initially had a mean percentage HbA\textsubscript{lc} of 12.5 ± 2% with a range 9.5 - 21.7%. The percentage decrease was significant from the fourth week of the trial onwards. This could be because the more recently produced red blood cells were exposed to lower post prandial plasma glucose levels and therefore glycosylation was reduced. Such an effect would only show when a considerable number of the existing erythrocyte population reached the end of their life span. This hypothesis is consistent with the present findings that mean HbA\textsubscript{lc} levels continued to fall as the study progressed and for two weeks after guar gum supplementation ceased.

Kyllståtin and Lahikainen (1981) have since carried out an eight week trial in which they found no change in mean HbA\textsubscript{lc} levels. They were only providing 16g guar gum a day. However on a smaller dose (9.15g) Smith et al. (1982) have achieved a significant reduction in HbA\textsubscript{lc} in NIDDM subjects (p < 0.05) in only two weeks. These differences could be attributable to the form in which guar gum was administered (granules and biscuits respectively). It was shown in Chapter 3 that a particular granule formulation of guar gum had little therapeutic effect. Other formulations
may be similarly less potent than guar gum incorporated into foods. The reduction in mean percentage HbA\textsubscript{lc} seen in this study after four weeks of guar gum supplementation lends support to the hypothesis that the rise in post prandial plasma glucose levels is lower following meals containing guar gum than after meals without its presence.

It has been suggested that measuring the partition of total serum cholesterol between the LDL and HDL lipoproteins is of more importance in determining the risk for coronary heart disease than relying on total serum cholesterol measurements and Kannel and Castelli (1979) suggest the most favourable response to any therapy is an improvement in LDL/HDL and total/HDL cholesterol ratios, the latter being ideally below 3.5.

Although the mean total serum cholesterol level was within the normal range (3.9 - 7.8 mmol/L) prior to the trial, a significant decrease was still achieved. This was associated with lowering of the LDL/HDL ratio and the total/HDL cholesterol level which fell from 4.0 to 3.3. This brought the latter below the recommended level suggested by Kannel and Castelli (1979). In accordance with Kyllöläinen and Lahikainen (1981) the relative amount of cholesterol present in the HDL fraction was increased.

It is unlikely that the reduced mean serum total and LDL cholesterol levels are secondary to reduced energy intake and/or weight reduction since the subjects in this study did not lose weight. Although their dietary intake was not accurately measured dietary recall showed that they did not reduce their daily energy consumption.

Three possible mechanisms to explain the action of dietary fibre on blood cholesterol levels have been proposed by Anderson and Chen (1978);
altered intestinal handling of cholesterol, altered hepatic metabolism of cholesterol and/or altered peripheral metabolism of lipoproteins. Miettinen and Tarpila (1977) support the hypothesis of increased excretion of cholesterol in bile acids.

The mechanisms by which guar gum is able to exert an effect on serum cholesterol levels needs further investigation since it is clear that the effect is a beneficial one.

5.5.3. Response to a mixed meal before and after guar gum supplementation

After two months supplementation with guar gum the mean post prandial glucose response to a test meal, eaten 12h after the last guar gum dose was not significantly improved for two hours after the meal. During the third hour mean plasma glucose and insulin levels were lower (Figure 5.5) compared to the pre-supplementation meal but this was unaccompanied by differences in plasma GIP levels. It is unlikely this effect could be due to guar gum remaining in the gut as this would have affected absorption rate and thus the initial post prandial glucose level. This is in accordance with Jenkins et al. (1979b) who showed guar gum, taken just two minutes before a glucose drink was ineffective compared to its administration in the drink. However they also showed (Jenkins et al. 1980b) that when two glucose drinks were taken four hours apart addition of guar gum to the first markedly reduced the rise in blood glucose levels after the subsequent guar free drink (0-120 mins). In the present study this carry over effect was not evident 12h after guar gum.

Since there was no significant increase in insulin secretion the beneficial effect seen in the present study is probably due to enhanced
glucose uptake by peripheral tissue caused by increased sensitivity to insulin. This is more likely to be caused by the progressive effect of guar gum supplementation than a carry over phenomenon since Smith et al. (1982) have since found a weeks supplementation with guar gum had no effect on the mean plasma glucose response to a guar free test meal.

The differences seen in post prandial mean plasma GIP levels two hours after the meal before and after the supplementation period are difficult to explain in relation to the mean plasma glucose and insulin levels. Guar gum incorporated into a meal has been shown to reduce post prandial GIP levels in both normal, IDDM (Morgan et al. 1979) and NIDDM subjects (Chapter 3). Following two months guar gum supplementation, the initial GIP response to a guar free meal was significantly increased. It is possible that during the supplementation period the rate of absorption of nutrients after each meal was reduced and since GIP release is dependent upon absorption of nutrients the need for the K cells to release GIP was diminished. This allowed time for accumulation of GIP within the cells and when the first guar free meal was eaten, being more rapidly absorbed than guar gum containing meals the K cells were able to respond to the stimulus by secreting significantly more GIP. However there appeared to be no benefit in terms of increased insulin secretion.

The results of this study indicate that regular consumption of guar gum can be of some benefit to the metabolic profile of NIDDM subjects as assessed by their glycosylated haemoglobin level and serum lipid levels, but insufficient to allow withdrawal of oral hypoglycaemic agents. In addition, the response to a guar free meal taken 12 hours after supplementation ceased and measurement of serum lipid levels two weeks later emphasise the lack of carry over effect and the need for chronic consumption of guar gum for efficacy.
CHAPTER SIX

The effect of guar gum on alcohol absorption
and alcohol induced hypoglycaemia
6.1 INTRODUCTION

The addition of guar gum to a meal has been shown to smooth the post prandial plasma glucose curve by reducing peak values and preventing the fall below fasting often seen when a predominantly carbohydrate meal is eaten (Jenkins et al., 1978a). The ingestion of large amounts of glucose is often followed by a transient fall in plasma glucose levels below fasting (Folin and Berglund, 1922) and in some otherwise healthy individuals the nadir coincides with, and is responsible for, acute neuroglycopenic symptoms. These symptoms, due to rebound hypoglycaemia, are generally mild and of short duration (10-15 minutes) and rebound hypoglycaemia is the normal physiological response to glucose and starch ingestion. It represents the brief period during the absorptive and post-absorptive phase when glucose assimilation by all the body tissues exceeds the rate of glucose input into the body pool from both the gastrointestinal tract and the liver (Marks and Rose, 1981).

Alcohol has the capacity to increase insulin secretion in response to an oral glucose load and so enhance the natural tendency to develop rebound hypoglycaemia. This effect has been shown in normal subjects who have consumed a mixture of alcohol and sucrose, such as gin and tonic, on an empty stomach and then refrained from eating for a few hours afterwards (O'Keefe and Marks, 1977). Between 10-15% of healthy subjects develop rebound hypoglycaemia and acute neuroglycopenic symptoms under these circumstances. Food does not necessarily protect against the hypoglycaemia induced by alcohol because consumption of a meal comprised mainly of easily digestible carbohydrate such as cooked starch e.g. bread may hasten and exacerbate the hypoglycaemia.

Some symptoms of hypoglycaemia, namely lack of coordination and difficulty in speaking articulately can easily be confused with the
symptoms of alcohol intoxication. They can only be measured on a subjective scale but can be correlated with blood alcohol and plasma glucose levels to provide some indication of their cause. Other symptoms such as sleepiness, coldness and a feeling of misery are more likely to be attributable to hypoglycaemia.

The inclusion of guar gum in a high carbohydrate meal given with alcohol may, by smoothing the post prandial plasma glucose curve, ameliorate the rebound hypoglycaemia in susceptible subjects. In addition, since it reduces the post prandial plasma glucose levels following a liquid meal (Chapter 3) it may similarly reduce post prandial blood alcohol levels.

6.2 AIMS
1) To record the occurrence of hypoglycaemia following the consumption of alcohol and a sucrose containing drink i.e. gin and tonic, in conjunction with a high carbohydrate snack, similar to many bar snacks.
2) To measure the effect of inclusion of guar gum in the snack on post prandial plasma glucose and blood alcohol levels.
3) To record symptoms attributable to raised blood alcohol levels and/or hypoglycaemia, already described, and correlate them with blood alcohol and plasma glucose levels.

6.3 MATERIALS AND METHODS
Six healthy volunteers aged between 24-34 years, none of whom were known to be heavy drinkers, participated in the study.

The composition of the meal and drink is shown in Table 6.1. Each subject consumed the meal (8 cheese scones each weighing 15g) and drink
### TABLE 6.1

**Composition of Cocktail and Scones**

<table>
<thead>
<tr>
<th></th>
<th>Wt (g)</th>
<th>CHO (g)</th>
<th>Energy (kcal)</th>
<th>kJ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethyl alcohol</td>
<td>50</td>
<td>-</td>
<td>(350)</td>
<td>1470</td>
</tr>
<tr>
<td>Schweppes tonic water</td>
<td>700</td>
<td>40</td>
<td>(160)</td>
<td>670</td>
</tr>
<tr>
<td>*cheese scones (8)</td>
<td>120</td>
<td>73</td>
<td>(530)</td>
<td>2220</td>
</tr>
<tr>
<td>TOTAL</td>
<td>113</td>
<td>(1040)</td>
<td>4360</td>
<td></td>
</tr>
</tbody>
</table>

*Scones used in the test meal provided, in addition, 14.5g guar flour*
(50ml ethyl alcohol made up to 750ml with tonic water (Schweppes), twice, with an interval of a week between each occasion. On one of these occasions the scones were supplemented with 14.5g guar flour.

On study days the subjects ate breakfast between 0700h-0800h and thereafter fasted and performed their usual morning activities. The procedure immediately before and during the test meal is described in Chapter 2.

At 1300h subjects consumed one scone and approximately 110ml alcohol and tonic every eight minutes for an hour. These conditions were chosen to mimic those of a lunchtime drink. Blood was collected immediately prior to the study and at 20 minute intervals for the following five hours. Plasma glucose, blood alcohol and plasma immunoreactive insulin were measured. The procedure and methods are described in Chapter 2. The behaviour of each subject was monitored and any symptoms they experienced recorded.

As none of the subjects knew that guar gum adversely affected the consistency of the scones they attributed the heaviness of the guar gum containing scones to poor cooking and thus this trial could be carried out blind.

Results were analysed using the methods described in Chapter 2.

6.4 RESULTS

Mean baseline plasma glucose levels were similar on both occasions (3.5 ± 0.17 mmol/L before plain scones and alcohol (PS) and 3.46 ± 0.27 mmol/L before guar scones and alcohol (GS)).
Following GS the area under the plasma glucose curve was less than following PS (4.18 ± 0.15 mmol.L\(^{-1}\)min and 4.52 ± 0.17 mmol.L\(^{-1}\)min respectively). The mean peak plasma glucose level was lower after GS than PS, but not significantly (Figure 6.1). At no time after eating GS however, did plasma glucose levels fall below baseline values, whereas, after PS they did, during the final hour of the study. In two of the subjects the plasma glucose concentration reached a nadir of 2.6 mmol/L after PS (Figure 6.2). At this time both complained of a headache and feeling cold, and they became sleepy and morose.

Mean plasma insulin levels were consistently lower after GS than after PS (Figure 6.3). The area under the post prandial plasma insulin curve was 28.3 ± 5.4 mU.L\(^{-1}\)min after GS compared to 53.9 ± 11.3 mU.L\(^{-1}\)min after PS (p < 0.02).

Mean blood alcohol levels rose more rapidly and to a higher level after GS than PS but the differences were not significant (Figure 6.4).

Clear cut behavioural changes were observed in two subjects. After GS both appeared markedly more intoxicated than after PS i.e. cheerful and speaking loudly but not coherently. Their blood alcohol levels were higher during the first two hours after GS compared to the same time after PS (Figure 6.5). They remained cheerful throughout the five hour period following GS. After PS one subject became miserable, cold and sleepy approximately two hours after the beginning of the meal and this corresponded to his lowest plasma glucose level. The other subject developed similar symptoms three and a half hours after the meal, again corresponding to her lowest measured plasma glucose level (Figure 6.2). The blood alcohol level was falling at this time in both subjects (Figure 6.5).
Figure 6.1.

Effect of guar gum on plasma glucose levels following cocktail and scones (n=6).

○-○-○ plain scones (control) ▼▼▼▼ guar scones. * P < 0.05; ** P < 0.01.
Figure 6.2 The effect of guar gum on plasma glucose levels in two subjects given cocktail and scones.

- --- plain scones; v---v guar scones.
Figure 5.3  Effect of guar gum on plasma insulin levels following cocktail and scones (n=6)
-.- plain scones (control); ▲▲▲ guar scones. * P < 0.05; ** P < 0.01.
Figure 6.4 Effect of guar gum on blood alcohol levels (mean ± SEM) following cocktail and scones (n=6).

-plain scones (control); - guar scones. * P < 0.05
Figure 6.5  The effect of guar gum on blood alcohol levels in two subjects given cocktail and scones.

- - - - - plain scones; ▼——▼—— guar scones.
The remaining subjects felt mildly intoxicated (i.e. cheerful) during the first two hours of each study but this wore off as they became sleepy and progressively more hungry but not uncomfortable. There was no difference in behaviour of these four subjects on the two occasions.

6.5 DISCUSSION

In studies similar to the present one symptoms attributable to hypoglycaemia have been found in approximately 15% of normal volunteers between three to five hours after ingestion of alcohol and readily assimilable carbohydrate (O'Keefe and Marks, 1977), whereas in this study two out of six volunteers (33%) exhibited symptoms of hypoglycaemia. In these two subjects, guar gum had a marked effect. Incorporated into the scones it reduced the rebound hypoglycaemia they experienced during the control study (Figure 6.2) and prevented the symptoms attributable to hypoglycaemia they exhibited concurrently. The blood alcohol levels were higher in these subjects after eating the guar scones and in accordance with their more intoxicated behaviour. The assessment of symptoms of hypoglycaemia and intoxication were only subjective but they were made prior to any blood analyses and therefore unbiased.

This effect of guar gum on post prandial blood alcohol levels was unexpected. The mean blood alcohol level was initially higher, and significantly so, four hours after the meal when guar gum was added to the scones. This indicates that guar gum facilitated rather than delayed or reduced the absorption of alcohol. There is no obvious explanation for these findings. Guar gum has been hypothesized to delay the rate of gastric emptying but it has been shown that rapid emptying of the stomach promotes ethanol absorption (Kricka and Clark, 1979) since the ethanol then reaches the highly absorptive jejunem and duodenum more quickly.
These findings cast doubt on the hypothesis of delayed gastric emptying being a mechanism of guar gum action and so this has been investigated (Chapters 7 and 8).

Only 10-15% of the population have been reported to suffer from symptomatic rebound hypoglycaemia following ingestion of alcohol with a high carbohydrate load. In this study the figure was higher (33%). The use of guar gum containing nibbles is still however not a viable proposition. They are not enjoyable and the people who suffer with alcohol induced hypoglycaemia can easily be told that if they eat a meal providing a variety of nutrients soon before their intake of alcohol and sucrose they can counteract the unpleasant symptoms of rebound/reactive hypoglycaemia. Therefore when considering the general public there can be no advantage to promoting the use of guar gum supplemented foods prior to, or in conjunction with imbibing.
CHAPTER SEVEN

The effect of guar gum on the response to oral protein and/or fat
7.1 INTRODUCTION

We have previously shown that guar gum reduces post prandial hyperglycaemia and plasma insulin and GIP levels when incorporated into a mixed meal (Morgan et al. 1979 and Chapter 3) and have postulated that the reduction in plasma GIP levels is partially responsible for the reduction in insulin secretion. In addition to its insulin stimulating properties GIP has the ability to inhibit gastric acid secretion (Brown et al. 1975) but the effect of guar gum on other gastrointestinal hormones which affect gastric acid secretion (gastrin) and motility (motilin) has not been studied. It has been suggested that guar gum may exert its action by delaying the rate of gastric emptying (Leeds et al. 1975 and Jenkins et al. 1978a) and thus, it may modify the rate of motilin secretion. In addition because guar gum reduces GIP secretion and GIP inhibits gastric acid secretion it may have an indirect effect on gastrin release. These two hormones are therefore worthy of study.

The rate of gastric emptying can be measured by a variety of techniques involving the use of radioisotopes which will be described in Chapter 8. Holt et al. (1979) have shown, however, that because paracetamol is absorbed only in the small intestine and not the stomach, measurement of post prandial serum paracetamol levels provides an assessment of the rate of liquid gastric emptying, and they showed that this method correlated well with sequential scintiscanning techniques using $^{113}$In DTPA.

Guar gum has long been known to lower serum cholesterol levels when given daily both to patients with hypercholesterolaemia and subjects
with plasma cholesterol levels within the normal range (Fahrenbach et al. 1965). These findings have been confirmed many times (Jenkins et al. 1975; 1976c; 1977; 1979a; 1980a; Aro et al. 1981; Botha et al. 1981 and Chapter 5), but the mechanism whereby it exerts this effect is not established. Three principal mechanisms have been suggested; altered intestinal absorption, altered hepatic metabolism and release of cholesterol or altered peripheral metabolism of lipoproteins (Anderson and Chen, 1979). The formation of a gel in the intestine and colon, due to the presence of guar gum may partition cholesterol in such a way that its absorption is reduced. In addition, if bile salts are sequestered by guar gum and subsequently excreted in the faeces, they are unavailable for micelle formation and therefore triglyceride absorption will be impaired.

The loss of bile salts from the enterohepatic circulation would require diversion of cholesterol for bile acid production and therefore less would be available for incorporation into lipoproteins and release into the venous circulation. If guar gum lowers serum cholesterol levels by sequestering intraluminal bile salts then chylomicron synthesis should be diminished and post prandial serum chylomicron levels reduced. Jenkins (1978) has suggested that the rise in serum triglyceride levels after a high fat meal parallels chylomicron absorption and therefore measurement of post prandial serum triglyceride levels can be used to give an indication of chylomicron absorption. Chylomicron absorption can also be assessed by measurement of the light scattering index (LSI) in plasma which indicates the degree of turbidity of the sample.

7.2 AIMS

Previous investigations have centred primarily on the effect of guar gum on carbohydrate absorption and metabolism and little work has been
done on its effect when given with a predominantly protein or fat meal.
The aims of this study were therefore twofold:-

(1) To study the effect on gastric activity and secretion of hormones known to affect it (GIP, gastrin and motilin) of addition of guar gum to a predominantly protein meal.

(2) To establish whether guar gum causes sequestering of bile salts and therefore loss of cholesterol from the body by assessing absorption of triglycerides following an oral fat load taken with and without guar gum.

7.3 METHODS

7.3.1.1. Effect of guar gum on response to a predominantly protein meal

Seven healthy volunteers age range 25-40 years consumed, within 20 minutes on one occasion, 150g lean minced beef providing 30g protein and 7g fat, and on a similar occasion the same meal with 5g guar flour intimately incorporated prior to cooking. The meals were taken in conjunction with 300ml water and 1.5g paracetamol (Panadol, Winthrop). The subjects fasted overnight prior to each study and were then treated as described in Chapter 2. Blood samples were withdrawn and plasma aliquoted, frozen and stored at -20°C prior to plasma immunoreactive gastrin, GIP and motilin analysis. Serum was collected and stored for paracetamol analysis (see Chapter 2). Statistical analysis was carried out using the methods described in Chapter 2.
7.3.1.2. Buffering capacity of guar gum

Suspensions containing 0.5% and 1.0% w/v guar flour in 50ml distilled water were individually titrated in triplicate against a solution of 0.1M hydrochloric acid and compared with a control of 50ml water.

7.3.2. Effect of guar gum on response to an oral fat load

Seven healthy volunteers aged between 19-26 years participated. They each consumed, on two occasions, an oral fat load of 200g double cream providing 96g fat. It was flavoured with 5g Kool Aid (General Foods Company, New York) a soft drink flavouring with no nutritional value.

On one occasion the fat load contained in addition, 6g guar gum mixed with gellation inhibitors. To keep the consistency of each fat load similar, the control, (cream only), was whisked prior to consumption. The presence of guar gum could not be masked and so the study could not be carried out blind. Paracetamol (1.5g) and 300ml water were consumed simultaneously with each meal. The subjects fasted overnight prior to each study and were treated as described in Chapter 2. The cream was consumed within 20 minutes.

Blood samples were withdrawn and plasma aliquoted frozen and stored at -20°C prior to plasma immunoreactive GIP and motilin analysis. Serum was stored frozen prior to measurement of triglyceride and paracetamol levels (see Chapter 2).

Light scattering index

The light scattering index of plasma was measured as an indicator of chylomicronaemia using a Thorpe Nephelometer (Thorpe Instruments, Cheshire).
7.4 RESULTS

7.4.1. Effect of guar gum on response to a predominantly protein meal.

Guar flour affected the consistency of the minced beef and was detected by all subjects. They found it unpleasant, not only because it made the meat very dry but because it stuck between their teeth. However, no abdominal side effects were experienced by any of the subjects.

Mean plasma GIP levels are shown in Figure 7.1 and mean plasma gastrin levels following the protein meal in Figure 7.2.

The addition of guar flour to the protein meal caused a significantly diminished mean post prandial GIP secretion compared to the control meal (area under the curve 0-120 min 1744 \pm 110 pg.ml^{-1}min (control) and 1265 \pm 100 pg.ml^{-1}min (test) (p < 0.05). In contrast, mean post prandial gastrin secretion was significantly enhanced by the presence of guar flour in the meal (area under the curve 0-120 min 43 \pm 11 mU.ml^{-1}min and 68 \pm 14 mU.ml^{-1}min for control and test meals respectively (p < 0.05).

Plasma motilin levels (Figure 7.3) rose post prandially but mean levels were similar after both meals (basal mean motilin levels were 108 \pm 18ng/L and the peak mean level after the control meal was 148 \pm 32 ng/L and after the test meal 140 \pm 22 ng/L (p = n.s.).

Peak circulating levels of paracetamol occurred 20 minutes after the start of each meal (Figure 7.4) and mean post prandial levels were similar after each meal indicating that the rate of gastric emptying was unaffected by the addition of guar flour to the protein meal.
Figure 7.1. Mean (± SEM) post prandial plasma GIP levels following a predominantly protein meal with and without guar gum in normal subjects (n = 7).

- - - meal alone (control); o-o-o meal and guar gum;
* P < 0.05
Figure 7.2 Mean (± SEM) post prandial plasma gastrin levels following a predominantly protein meal with and without guar gum in normal subjects (n=7).

- - - meal alone (control); o-o-o meal + guar flour.
Figure 7.3  Mean (± SEM) post prandial plasma motilin levels following a predominantly protein meal with and without guar gum in normal subjects (n=7).

- meal alone (control); o-o-o meal + guar flour.
Figure 7.4 Mean (± SEM) post prandial serum paracetamol levels following a predominantly protein meal with and without guar gum in normal subjects (n=7).

- • • • meal alone (control); ○ ○ ○ meal + guar flour.
7.4.1.2. Buffering capacity of guar flour

The titration curves of a 0.5% and 1.0% solution of guar flour in water are shown in Figure 7.5. At both concentrations guar flour exhibited a buffering capacity between pH 5.5 and 2.75.

7.4.2 Effect of guar gum on response to an oral fat load

The double cream taken without guar gum (control) was tolerated by all subjects but made four of them feel slightly nauseated. Three subjects suffered abdominal discomfort during the afternoon of the study day. The fat load supplemented with guar gum (test) was disliked by all subjects. One failed to consume the entire meal and her data has been excluded. Within an hour of consuming the test meal another subject experienced diarrhoea which persisted for ten hours. Three other subjects had mild diarrhoea later on the same day.

Mean plasma GIP levels (Figure 7.6) rose following oral fat but were similar after both meals (area under the curve 0-150 min 1775 ± 240 pg.ml⁻¹.min and 1707 ± 169 pg.ml⁻¹.min after test and control meals respectively (p = n.s.).

Mean plasma motilin levels (Figure 7.7) were similarly unaffected. They rose from a mean basal level of 287 ± 86ng/L to 356 ± 141ng/L after the control and from 364 ± 104ng/L to 463 ± 159ng/L after the test meal (p = n.s.).

Mean serum triglyceride levels did not rise significantly from basal levels until an hour after the fat load (Figure 7.8) and the area under the mean serum triglyceride curve from 60-150 minutes was not significantly
Figure 7.5 Titration curves of water ——, 0.5% guar gum; —— and 1.0% guar gum suspension; —— against 0.1M HCl.
Figure 7.6  Mean (± SEM) post prandial plasma GIP levels following a 200g fat load with and without guar gum in normal subjects (n=6).

- - - fat alone (control); o-o-o fat + guar gum.
* P < 0.05.
Figure 7.7  Mean (± SEM) post prandial plasma motilin levels following a 200g fat load with and without guar gum in normal subjects (n=7).

- fat alone (control); o-o-o fat and guar gum.
Figure 7.8  Mean (± SEM) post prandial serum triglyceride levels following a 200g fat load with and without guar gum in normal subjects (n=6).

- - - fat alone; o-o-o fat + guar gum.
different after control or test occasions (47 ± 36 mmol.L⁻¹.min and 16.4 ± 15 mmol.L⁻¹.min respectively) (p = n.s.).

The mean light scattering index, measured in plasma following each meal was similarly unaffected by the presence of guar gum (Figure 7.9).

The gastric emptying rate, as assessed by mean serum paracetamol levels (Figure 7.10) was also unaffected by the addition of guar gum to the fat load.

7.5 DISCUSSION

Addition of guar gum to the protein meal resulted in diminished post prandial GIP secretion but enhanced gastrin secretion. The mechanism by which guar gum exerts this effect is not clear. Gastrin secretion, which is mainly stimulated by the products of partial protein digestion can be affected by the rate of gastric emptying and the pH of the gastric contents. The rate of gastric emptying of the liquid phase of the protein meal, as assessed by the appearance of paracetamol in the circulation, was not affected by guar gum. The differences in gastrin secretion cannot therefore be explained in terms of the length of time the stomach contents were in contact with the gastrin producing (G) cells. Suspensions of guar gum in water exhibited a significant buffering capacity above pH 2.75 suggesting the effect of guar gum on gastrin secretion could be due to its buffering capacity. Gastrin release is inhibited by acid acting directly on the antral G cells and a luminal pH of 2.5 suppresses 80% of amino acid stimulated gastrin release in men (Walsh and Grossman, 1975). Fasting antral pH is between 1-3 and the pH rise after a meal depends on the diluting and buffering capacity of the food, gastric acid secretion and the rate of gastric emptying. Guar gum may therefore raise the post prandial
Figure 7.9 Mean (± SEM) post prandial light scattering indices following a 200g fat load with and without guar gum in normal subjects.

- - - fat alone; o-o-o fat + guar gum.
Figure 7.10: Mean (± SEM) post prandial serum paracetamol levels following a 200g fat load with and without guar gum in normal subjects (n=6).

- - - fat alone; o-o-o fat + guar gum.
pH thus diminishing the contribution of the negative feedback loop in inhibiting gastrin release. The modulation of GIP secretion by guar gum could provide an alternative explanation for the observed differences in gastrin secretion. GIP was initially named for its capacity to inhibit gastric acid secretion (Gastric Inhibitory Polypeptide) (Brown and Dryburgh 1971) and exogenous GIP infusions have been shown to inhibit gastrin release (Villare et al. 1976 and Arnold et al. 1978). It is possible that the attenuation of the GIP response caused by addition of guar gum to the meal led to an augmented gastrin secretion.

Following consumption of the fat meal the absorption of fat as chylomicrons was unaffected by the presence of guar gum as shown by measurement of LSI. Mean serum triglyceride levels were also similar after each meal. This is not in accordance with Jenkins (1978) who found that 14.5g guar gum added to a lunch test meal resulted in serum triglyceride levels significantly higher than the control during the first three hours after the meal. He suggested that guar gum may act by facilitating the action of bile salts enhancing lymphatic chylomicron flow thus protecting the liver from direct exposure to free fatty acids for hepatic cholesterol synthesis and reducing the synthesis of cholesterol. Although this hypothesis is feasible, using post prandial triglyceride level measurement alone, is a very crude and indirect means of assessing one of many factors affecting cholesterol metabolism, namely, reabsorption of bile salts. The mechanisms of action of guar gum in reducing serum cholesterol are still unclear and further work is required to elucidate them.

Mean serum paracetamol levels were similar after the fat meal irrespective of the presence of guar gum. The mean peak levels occurred earlier (20 minutes post prandially) after the protein meal than the fat
meal (40 minutes post prandially). This suggests the gastric emptying rate of the fat meal was slower than the protein meal and is consistent with previous studies (Hunt and Stubbs, 1975).

Motilin is released in response to oral fat and protein (Christofides et al. 1979a), and is implicated in altering the rate of gastric emptying (Christofides et al. 1979b). In this study guar gum did not affect the rate of secretion of motilin in response to fat or protein and neither were any changes in gastric emptying rate detected.

This work has indicated that guar gum, in amounts commonly used in human studies, affects the secretion of GIP and gastrin, both of which are implicated in the control of gastric acidity. It does not appear to alter the rate of liquid gastric emptying but its effect on gastric emptying of solids should be ascertained.
CHAPTER EIGHT

The effect of guar gum on the rate of gastric emptying in healthy human subjects
8.1 Introduction

It has been suggested that the reduction in post prandial hyperglycaemia following a test meal containing guar gum could be due to delayed gastric emptying, slower movement of glucose to the absorptive mucosal surfaces, or both (Jenkins et al. 1978a). Although the hypothesis that guar gum delays gastric emptying has been widely accepted, the evidence is controversial. The study frequently quoted in support of this mechanism of action involved giving pectin (10g) as well as guar gum (16g) with a liquid glucose load (Holt et al. 1979). Although addition of the fibre mixture significantly delayed liquid gastric emptying, it is uncertain whether the effect was due to guar gum, pectin or a synergistic action.

The addition of guar gum to an intragastric liquid glucose load has been shown to reduce the rate of gastric emptying in rats (Leeds et al. 1979 and Daumerie and Henquin 1982) and one study has shown liquid gastric emptying can be delayed in humans by the addition of guar gum alone to a liquid meal (Wilmshurst and Crawley, 1980). However, when the same meal was given to pigs, guar gum did not affect the gastric emptying rate of the dry matter content (Rainbird et al. 1984). Other investigations using pigs have shown that guar gum delays the emptying of a glucose solution but has no effect on the rate of gastric emptying of a solid mixed meal (Rainbird and Low 1983 and Rainbird et al. 1983).

Nothing has been reported on the effect of guar gum on solid gastric emptying in humans and the reports on liquid gastric emptying need clarification. One of the reasons for the scarcity of such investigations is undoubtedly the lack of convenient methods for measuring gastric emptying in humans.
Invasive techniques can be employed in animal studies (Leeds et al. 1979 and Low and Rainbird, 1983) but these are undesirable and frequently unsuitable for human gastric emptying rate measurement. Nonetheless, a common method used in human studies is aspiration of the stomach contents (Nutrition Reviews, 1982). It can be used to study liquid gastric emptying but the stress it causes is likely to modify gastric motility and secretion (Bennett and Venables, 1920 and Nagamachi and Taniquichi, 1975).

Isotopic methods have now been employed in human gastric emptying studies (Heading et al. 1971; Meyer et al. 1976; MacGregor et al. 1977; Tothill et al. 1978 and Moore et al. 1981). The principal advantage of isotopic over intubation methods is the ability to non-invasively study gastric emptying of both solid and liquid components of normal meals.

The equipment and radioisotopes necessary to employ a dual liquid and solid phase isotopic method are expensive and not readily available. However, Holt et al. (1979) found a significant positive correlation between paracetamol absorption and isotopic measurements of the rate of liquid gastric emptying. Thus, by giving paracetamol with a meal both solid and liquid phase emptying can be assessed using only one radioisotope.

Griffiths et al. (1968) developed the first isotopic method which was claimed to be capable of quantifying gastric emptying of solid food. It depended upon external gamma counting over the stomach after subjects were fed scrambled eggs into which Na$_2$$^{51}$Cr$_2$O$_7$ had been added during cooking. The $^{51}$Cr tag has since been shown to dissociate from the egg and so the validity of counting $^{51}$Cr is unsuitable for successive tests on the same subject because of its long half-life (28 days).
Technetium has become a popular radionuclide marker for studying gastric emptying of solids because of its labelling efficiency and short half-life (6h). The latter makes it safer than $^{51}$Cr for human studies (Christian et al. 1983). It has been used to label two solids which can be mixed with a test meal: chicken liver and paper particles. Meyer et al. (1976) prepared $^{99m}$Tc-tagged chicken liver by injecting $^{99m}$Tc sulphur colloid into the wing vein of a live chicken 30 minutes prior to sacrifice. The liver was then removed, cooked and mixed with minced beef or a beef stew. Although this material was an ideal solid food marker, most laboratories cannot cope with live chickens and a method using small inert paper particles impregnated with $^{99m}$Tc has since been described (Heading et al. 1976 and Holt et al. 1981). The emptying patterns of the labelled liver and paper particles are similar in normal subjects (Holt et al. 1982). Thus labelling a meal with $^{99m}$Tc by incorporating paper particles would appear to be a simple and effective method for measuring the effect of guar gum on gastric emptying of a solid meal.

8.2 AIMS

Due to the widespread belief that a mechanism of action of guar gum is its ability to delay gastric emptying, despite the paucity of evidence to support it, this study was undertaken. The aim was to measure liquid and solid gastric emptying rates in humans following a meal containing both liquids and solids. Having reviewed the literature and the facilities available, solid phase emptying was measured isotopically by incorporating $^{99m}$Tc labelled paper particles into the meal, and liquid emptying assessed from paracetamol absorption studies.
8.3. MATERIALS AND METHODS

The effect on gastric emptying rate of guar gum incorporation into a solid meal was measured using a gamma camera and $^{99m}$Tc as a solid phase marker. Serum paracetamol levels were measured as an assessment of liquid gastric emptying.

8.3.1. Isotope

$^{99m}$Tc was eluted from a Technetium 99m Sterile Generator supplied weekly to St. Luke's Hospital, Guildford, by Amersham International plc, and used to label stannous fluoride. The resulting $^{99m}$Tc tin colloid solution was diluted with saline to a concentration of approximately 750 MBq/ml on the afternoon prior to each study.

Filter paper particles, 3mm in diameter were individually immersed in this solution, dried and coated three times in a 3% w/v solution perspex in chloroform. They were dried thoroughly between each coating.

8.3.2. Meal Preparation

Lean beef was purchased in bulk and after all visible fat had been removed it was minced, divided into 150g portions and frozen prior to use.

Eighteen hours before each study sufficient filter paper particles (30 - 40), to provide approximately 7MBq $^{99m}$Tc at the time of consumption, were uniformly mixed with each defrosted meat portion. This was achieved by incorporating approximately 50 MBq radioactivity, since $^{99m}$Tc has a half-life of six hours. The test meat portions also had 5g guar flour evenly mixed into them at this time. The meat portions were refrigerated overnight and cooked on the morning of the study.
8.3.3. Validation

In order to verify that there was no significant elution from the filter paper particles a labelled meat portion was chopped into small pieces and mixed with 0.1M HCl pH 1.0 for six hours. The supernatant was decanted and the eluted radioactivity measured in a gamma scintillation counter (Wallac 80,000, LKB Instruments Ltd). The percentage radioactivity eluted was calculated, making allowance for radioactive decay and counting efficiency.

8.3.4. Clinical Trial

Following an overnight fast, five male and two female non-obese subjects (Metropolitan Life Insurance Company) age range 21-36 years each underwent two studies of gastric emptying. The meal consisted of 150g lean minced steak, radioactively labelled as described above and 300ml water. In 200ml of the water 1.5g paracetamol had been dissolved. The meal was identical each time apart from the addition of 5g guar flour intimately mixed with the beef on one occasion. Subjects were randomly selected to receive the plain or guar flour containing meat during their first study. The presence of guar flour could not be disguised and thus the study was not blind.

On the morning of the study the beef was cooked at 180°C for 20 minutes and given to the subjects, who were asked to consume the paracetamol solution and water evenly throughout the 10 minute period of eating.

Gastric emptying was examined using an Elscint CEI gamma camera with a field view of 280mm diameter, set to accept the photons emitted by $^{99m}\text{Tc}$ with a 140keV centre-line and a 35keV window width. A general
purpose low energy parallel hole collimator was employed. The gamma camera was linked to an Elscint Dycomette data processor which was used for data acquisition and image analysis. A radioactive cobalt spot marker \((^{57}\text{Co}=2\text{MBq})\) was affixed to the base of the xiphisternum prior to each anterior reading and to an equivalent posterior position prior to each posterior reading. The position of this activity was marked on the visual display unit. Each subject was in close contact with the collimator face during data acquisition and it was possible to ensure that they did not move by lining up the \(^{57}\text{Co} \) spot marker with the cross on the screen and observing the visual display unit throughout the recording time.

Counts were collected for two minutes both anteriorly and posteriorly, five minutes after the end of the meal and at intervals for up to three and a half hours. The images were recorded on to floppy discs for future analysis and photography (see Appendix 1).

Subjects stood in front of the collimator during imaging and remained seated at other times. They did not consume anything after the specified meal and did not smoke throughout the study period.

Blood was withdrawn before and 10, 20, 40, 60, 90 and 120 minutes after the meal and serum separated for paracetamol analysis (see Chapter 2).

The time course of solid gastric emptying was measured by drawing two rectangular regions of interest. The size and position of the rectangular boxes used to define the stomach were chosen after visual examination of the early images and kept constant for both anterior and posterior counting and for test and control meals for each subject.
Anterior and posterior counts were corrected for radioactive decay and the geometric mean of each pair of anterior and posterior counts calculated because the geometric mean of counts from opposing views is less sensitive to changing source depth. It has been successfully applied to many gastric emptying studies and is easily determined (Christian et al. 1983).

It was unnecessary to correct the data for the very low radiation background levels on the images since all the activity was present within the solid markers and not diffused in blood and tissue as in conventional nuclear medicine imaging.

The counts observed at each imaging interval were normalised to percent of the counts obtained at mid-meal times, which was assigned a 100% value. Graphs were plotted for each subject following the test and control meal. From these graphs the percent of counts remaining at set intervals after the mid-meal time was read and the mean for all subjects calculated and plotted. Thus mean half-emptying time ($T_{1/2}$) of the solid phase, taken to be the time at which 50% of the counts remained in the stomach was obtained following both the control and test meals.

Mean serum paracetamol levels were calculated and compared to provide information on the effect of guar gum on liquid phase gastric emptying as previously described (Holt et al. 1979).
8.4 RESULTS

8.4.1. Validation

Following a six hour incubation period of a labelled meat portion in 0.1M HCl pH 1.0, less than 4% of the $^{99m}$Tc counts had entered the liquid phase.

8.4.2. Meal acceptability

These subjects, like those involved in the previous study (Chapter 7), detected the addition of 5g guar flour to 150g lean beef steak and commented on the unpleasant texture of the meal during eating and the film left on their teeth following consumption. In contrast, the $^{99m}$Tc labelled paper particles were undetectable in either meal.

8.4.3. Solid phase emptying

The mean gastric emptying time, expressed as the half-life ($T_{1/2}$) of the solid phase of the meal was not significantly affected by the addition of 5g guar flour to the meat (Figure 8.1). The mean half-life after the control meal was 100 minutes and after the meal containing guar gum 103 minutes.

8.4.4. Liquid phase emptying

The rate of liquid phase gastric emptying indicated by the rate of paracetamol absorption was not affected by the presence of 5g guar flour in the meal.

Mean serum paracetamol levels following control and test meals were not significantly different during the two hour post prandial period (Figure 8.2).
Figure 8.1  The effect of addition of guar gum to a solid meal on the mean solid gastric emptying curve using $^{99m}$Tc as a solid phase marker in normal subjects (n=7). $\times$--$\times$ meal without guar gum (control); ++++++ meal + guar gum
Figure 8.2  Mean (± SEM) post prandial serum paracetamol levels following a predominantly protein meal with and without guar gum in normal subjects (n=7).

- - - - meal alone (control); ○○○○ meal + guar flour.
8.5. **DISCUSSION**

In conjunction with the findings of Meyer *et al.* (1976) *in vitro* studies show that less than 4% of the $^{99m}$Tc counts entered the liquid phase. This confirms that adding $^{99m}$Tc labelled-paper particles to a meal is a valid means of measuring solid phase gastric emptying. In addition, gastric emptying times, expressed as the half-life ($T_1/2$) of meal in the stomach were within the range of values calculated by other workers for similar meals (Harvey *et al.* 1970 and Meyer *et al.* 1983). Therefore the results reported can be considered as reliable measurements of the gastric emptying rate of solid food.

In order to accurately quantitate isotopic gastric emptying it has been found necessary to correct for changes in depth activity due to anterior movement of the stomach following ingestion of a meal. Correction can be achieved by calculating the geometric mean of counts from anterior and posterior images (Tothill *et al.* 1979). This required little extra work or subject cooperation and in order to minimise the effect of posture all images were recorded with the subject in a standard upright posture, already shown to be less sensitive to depth activity changes (Tothill *et al.* 1980).

Low levels of psychological stress have recently been found not to affect gastric emptying (Cann *et al.* 1983). Nonetheless, to minimise the overall effect of any stress caused by novelty of the procedure during the initial study, the subjects receiving guar gum on the first occasion were randomly selected. There was, however, no apparent effect of stress on either occasion.
The previously validated methods used in this study for measuring solid and liquid phase emptying showed that addition of 5g guar gum to 150g lean meat consumed in conjunction with 300 ml water had no effect on the rate of emptying of either phase. Although 5g is a smaller quantity than has been added to many test meals (Gassull et al. 1976; Jenkins et al. 1976a and Morgan et al. 1979) it was detected by the subjects. They would have been unable to tolerate a larger quantity and such a dose is representative of that taken with a meal by subjects on chronic guar gum therapy (25g a day divided between 3 meals and 3 snacks). In addition this amount has previously been shown to reduce post prandial glycaemia when added to the liquid or solid part of a mixed meal (Wolever et al. 1979a) and to modify gastrointestinal hormone secretion (Chapter 7).

Smaller doses (2g) when added to a liquid meal have been shown to significantly delay gastric emptying (Wilmshurst and Crawley, 1980). This is not surprising because solids leave the stomach more slowly than liquids (Thomas, 1957) and addition of just 2g guar gum to a liquid meal would increase its viscosity considerably. These present results, however, suggest that if solids, as well as liquids, form part of a meal, their presence overrides any effect of guar gum on the rate of liquid phase emptying. It could be that when guar gum is given as part of the solid phase it is entrapped in the solid matrix until gastric secretion and motility liquify the solid phase. It is therefore not able to combine with the liquid phase and make it viscous until the majority of the liquid phase has left the stomach in its normal exponential pattern (Heading et al. 1976). Thus guar gum may only be effective in delaying the gastric emptying of a liquid meal and this has been substantiated by animal studies (Rainbird et al. 1984). Delayed gastric emptying cannot be the only mechanism of action of guar gum because it still modifies post prandial
glycaemia (Leeds et al. 1980) and gastrointestinal hormone secretion (Chapter 7) in situations where it does not affect gastric emptying. Thus some other mechanism must be of major importance for its action. Malabsorption has been discounted as indicated in Chapter 1 and therefore it is likely that slower movement of nutrients to the absorptive surfaces of the small intestine plays a major role in the acute metabolic effect of guar gum.
CHAPTER NINE

Final Discussion
9.1 Introduction

This study was initiated because reports of the ability of guar gum to modify post prandial glycaemia, that is, to reduce the peak levels and smooth the post prandial plasma glucose curve as well as to achieve long term improvement in metabolic control in diabetics were conflicting. It was therefore important to find a palatable means of providing guar gum and establishing its efficacy in both modifying post prandial plasma glucose levels and reducing serum lipid levels on long term administration.

The major problem in further studying the effects of guar gum was the difficulty in providing a palatable means of incorporating it into the diet. No medication or form of therapy can be successful if patients do not comply with instructions and therefore one of the first objectives of this work was to provide guar gum either as a convenient medication or in a form which when added to food caused little or no deterioration to appearance, texture or palatability.

9.2 Patient acceptability

Five different forms of guar gum have been assessed for palatability and/or effectiveness in the described studies; flour, powder, granules, guar bread, and guar gum with a gellation inhibitor.

Guar flour and guar powder were intended to be taken intimately mixed with each meal. When they were used in acute studies involving liquid glucose or solid mixed meals they were mixed with the solid and/or liquid portion. Neither product adversely affected the acceptability of the liquid glucose drink provided it was consumed quickly but the guar flour was the more difficult to mix into the drink. When the mixed meal was given both forms, but especially the flour, made the tomato soup
distasteful to the subjects for reasons already illustrated (Figure 3.12). However, when mixed with flour and water to make 'bread' both guar flour and powder resulted in a heavy but acceptable product.

The effect of guar flour on the palatability of several baked products proved encouraging (Chapter 4). Without adversely affecting flavour and with only a minor detrimental effect on texture, 5g guar flour was added to 10g or 20g carbohydrate portions of cheese biscuits, pizza and egg and bacon flan. These foods are not, however, suitable to serve at each meal and such a limited selection would not be adequate in the long term. Carroll et al. (1981) provided their subjects (6 IDDM) with guar gum and instructions on how to incorporate it into foods. The subjects added it to a large number of foods resulting in intakes of guar gum between 6-60g/day.

There are however, problems in obtaining patient cooperation in respect to adding guar gum to their food regularly, as discussed previously (Chapter 4), and in these studies it was considered that providing products already containing guar gum would be a more realistic means of incorporating it into the diet.

In the long term study (Chapter 5) 25g guar gum was taken daily by each subject in the form of ready made bread. This caused little extra work for the subjects but the bread was originally provided as a bread mix and had it not been made up prior to distribution it is doubtful whether the subjects would have complied for the whole study period.

A limited number of prepared foods containing guar gum are now available. Jenkins et al. (1978a) have provided crispsbreads each containing 1g guar gum. Their subjects have eaten up to 26/day. Although published
results of their acceptability are favourable we have found that the crispbreads are dry and flavourless and eating them at each meal proved monotonous for the patient (unpublished observation).

Recently two snack bars containing guar gum have been produced by a Scandinavian Company and their effectiveness in modifying the post prandial plasma glucose, insulin and GIP levels studied in normal healthy volunteers (Appendix 2). Neither of these snack bars significantly altered the post prandial profile compared with the control bar and although the chocolate coated one was judged tolerable by the subjects they found the appearance and texture of the non-chocolate coated bar unpleasant. There is, therefore, still no palatable, effective ready to eat food containing guar gum available.

As well as detracting from the enjoyment of food if guar gum is provided in only one food which also has a high carbohydrate content (bread) or must be eaten in large quantities (crispbread) patients food choice may become limited leading to a monotonous diet to which they have difficulty adhering. The formulation and production of one guar containing food is therefore of little value.

Providing guar gum as a pre-meal medication appears to be the best means from the point of palatability. Guar granules, stuck between the teeth but were well tolerated by all subjects. The guar gum formulation containing the gellation inhibitor was only available towards the end of the study period and was used in just one study, mixed with 200g double cream. Under these conditions it was detected and disliked by the subjects but it can be administered as a pre-meal medication mixed with water and drunk. Due to the presence of the gellation inhibitor the
solution does not become viscous until after it is drunk and is well accepted by subjects. In addition it has been shown to reduce post prandial hyperglycaemia in healthy subjects following an oral glucose tolerance test (personal communication). It therefore deserves to be further studied; initially given prior to a mixed solid meal to diabetic subjects, and, depending on results, being used in a long term trial, given to diabetics before all main meals.

9.3 Effectiveness and mechanisms of action of guar gum.

9.3.1. Short term studies.

The action of guar gum when mixed with a variety of test meals has been examined here in an attempt to ascertain its effectiveness, initially in the short term, in view of the controversy surrounding existing reports. The main features which can be differentiated between various studies are the viscosity of the preparation, whether the test meal was liquid or solid and whether the subjects were normal or diabetic. It is clear from these results (Chapter 3) that the effect of guar gum is more marked in the diabetic than normal population. This is to be expected because normal subjects are more capable of regulating their response to a meal than diabetics. It does, however, highlight the fact that if any product shows a tendency to reduce post prandial hyperglycaemia in normal subjects it is worthy of study in trials involving diabetic subjects.

The present studies have shown that viscosity plays a role in acute effectiveness of guar gum because guar granules which did not form a viscous solution were ineffective in reducing post prandial hyperglycaemia. However, guar powder and guar flour both modified post prandial hyperglycaemia despite widely differing viscosity properties (Chapter 3). This implies that although a critical viscosity attainment may be
important, viscosity may not be the only factor contributing to the action of guar gum in modifying the post prandial response. The ability of guar gum to reduce post prandial hyperglycaemia has previously been shown to be related to some extent to its hydration properties (Jenkins et al. 1977 and Wolever et al. 1978a). It has been shown in the present studies (Chapter 3) that guar gum is more effective in modifying post prandial hyperglycaemia when mixed with a liquid than a solid meal, which also suggests its effectiveness may be due to its ability to increase the viscosity of liquids. However, its effectiveness when mixed with the solid meal could also be explained by its ability to increase the viscosity of the liquid portion and perhaps delay gastric emptying. The role of gastric emptying as a mechanism of guar gum action had not been fully investigated prior to the present studies but it has already been shown that solids leave the stomach more slowly than liquids (Meyer et al. 1981).

The meal used in the present short term study and that of Morgan et al. (1979) provided 51% of the carbohydrate in a liquid form (soup and milk). Guar gum, by increasing the viscosity of the liquids could therefore have delayed the gastric emptying of half the carbohydrate load. Similarly, in the study providing alcohol (Chapter 6) although only 35% of the carbohydrate was in a liquid form, seven of the eight scones were each eaten in conjunction with 110ml liquid. The guar gum present could have hydrated and increased the viscosity of the stomach contents sufficiently to delay gastric emptying.

Wilmshurst and Crawley (1980) have shown that addition of guar gum to a milky drink delays gastric emptying in obese humans and Leeds et al. (1979) also delayed gastric emptying in rats by adding guar gum to a glucose solution given by orogastric intubation. Daumerie and Henquin
(1982) achieved similar results, also in rats, and correlated the delay with a reduction in post prandial hyperglycaemia.

The theory of delayed gastric emptying has been substantiated by the findings of Levitte et al. (1980) that improved glucose tolerance following a fibre containing meal (5g guar gum and 5g pectin) only occurred in NIDDM subjects who did not have autonomic neuropathy (AN). Since AN produces gastric atony, and thus delayed gastric emptying, the effect of addition of fibre to the meal could have been too small to cause any further delay. It is not clear, however, whether the delayed gastric emptying in NIDDM subjects without AN was due to guar gum, pectin or a synergistic action of the two fibres. The same problem pertains to the work of Holt et al. (1979) who used a mixture of guar gum and pectin.

The lack of effect of guar gum on the rate of both liquid and solid gastric emptying found in the present studies can be partly explained by the composition of the meals. The first meal was an oral fat load given in the form of double cream. Gastric emptying is known to be delayed by the presence of fat (Hunt and Knox, 1968) and therefore the presence of 6g guar gum may not have been sufficient to further delay the gastric emptying of 100g fat.

The second meal was a solid, predominantly protein meal, providing 300ml liquid which did not contribute to the nutritional value. The guar gum present in the meal was probably unavailable for hydration in time to delay the gastric emptying of the liquid portion and had no effect on the longer time which the solid portion took to empty.
Very little work has been done on the effect of guar gum on the rate of gastric emptying of mixed liquid and solid meals in humans. Subsequent to the findings reported here, Ray et al. (1983) have attempted to study the effect of addition of dietary fibre to a mixed meal. Their results, however, fail to clarify the picture. In 12 NIDDM subjects they compared the response to a non-fibre containing meal given before treatment with the response to the same meal supplemented with 6g guar gum and 3g wheat bran given after eight weeks on a diet supplemented with 10g wheat bran and 20g guar gum/day. They found a significantly delayed rate of both liquid and solid gastric emptying of the fibre supplemented meal. The delayed gastric emptying did not, however, significantly affect post prandial plasma glucose levels. Although the authors suggest the lack of effect was due to administration of guar gum in a non-hydrated form insufficiently mixed with the meal, which was a method previously shown to be ineffective by Williams and James (1979a), they fail to explain the association between delayed gastric emptying and lack of effect on post prandial plasma glucose levels. In addition, they make no allowance for the effect of eight weeks dietary supplementation with fibre on the results. If gastric emptying can be affected with no effect on post prandial plasma glucose levels the significance of delayed gastric emptying as a mechanism of action of guar gum must be questioned.

The lack of effect of guar gum on the rate of gastric emptying, found in the present studies is supported by the work of Rainbird et al. (1983 and 1984) and Rainbird and Low (1983) described in the previous chapter. However, before delayed gastric emptying can be entirely ruled out a further study is required involving comparison of the effect on rate of gastric emptying of addition of guar gum to an oral liquid mixed meal and a solid meal of similar nutritional composition. Post prandial plasma glucose
and insulin levels should be measured after the four meals and correlated. This would provide sufficient evidence to establish the importance of increasing the viscosity of the liquid portion of a mixed meal. If this proves to be the main mechanism of guar gum action on post prandial plasma glucose levels then its value in diabetic therapy is doubtful since few meals are predominantly liquid or provide a significant carbohydrate portion in liquid form.

Another mechanism for the action of guar gum, that of increasing the resistance of the mucosal diffusion barrier to nutrients and thus delaying absorption, has been proposed (Jenkins et al. 1976a) and has been elegantly substantiated by in vitro work. Johnson and Gee (1980) using everted gut sacs showed that intestinal transport of glucose is reduced in the presence of guar gum, but not if the incubation media is shaken causing mucosal stirring. Thus they suggest that guar gum in the fluid film surrounding the villi increases its viscosity thereby thickening it and slowing nutrient diffusion. This data has been confirmed in pigs (Rainbird et al. 1982). In vivo however the influence of intestinal motility probably diminishes the contribution of such an effect.

Many reports in the literature and the present studies suggest that the effect of guar gum on the response to a meal is predominantly due to its ability to increase the viscosity of the liquid portion and delay its gastric emptying. Its effect when given with a predominantly solid meal is less obvious. It is more apparent in diabetic subjects than normals for reasons previously discussed and therefore deserves further study in diabetic subjects. It is, of course, the long term effect of guar gum on metabolic profile which is of relevance to the general diabetic population.
9.3.2. Long term studies

(a) Effect of guar gum on glycaemic control

The benefit to NIDDM subjects of consuming guar gum in the long term needs careful evaluation. In the present study (Chapter 5) the effect of guar gum on glucose homeostasis in NIDDM subjects was partially favourable in that the mean percentage glycosylated haemoglobin fell, however fasting plasma glucose levels were unaffected. The patients would not have willingly continued to consume the guar bread on a regular basis and thus any beneficial effect would not have been maintained.

In Chapter 3, guar granules taken as a pre-meal medication were ineffective in reducing post prandial hyperglycaemia but were the form in which guar gum consumption was easiest. Recent reports have come from Scandinavia on the effects of a granulated slow gelling guar gum preparation taken before meals. The formulation is said to be palatable but reports of its effectiveness are again conflicting.

Stokholm et al. (1981) withdrew oral hypoglycaemic therapy from their NIDDM subjects and showed that one week's supplementation of 4g three times a day significantly reduced urinary glucose excretion. Kyllösten and Lahikainen (1981) gave 4-6g three to four times a day to 14 NIDDM's for two months. They achieved a moderate improvement in glycaemic control in some of their subjects. Aro et al. (1981) showed that 21g/day in three doses significantly improved glycaemic control in nine NIDDM's over three months. In a crossover trial the subjects who began on guar gum had a mean plasma glucose level of 9.4 mmol/L before and after the guar gum supplementation period and 11.1 mmol/L after placebo. Those who began on placebo first had a mean plasma glucose level of 11.5 mmol/L which rose to 13.5 mmol/L after placebo and then fell to 10.7 mmol/L after guar gum supplementation.
Although the authors were very enthusiastic about their results this data suggests that they should have reached the same conclusion as Kyllästinen and Lahikainen (1981), that is, that dietary supplementation with guar gum improves the glycaemic control of some diabetics but not impressively, and the response varies from one individual to another.

Although long term studies involving dietary supplementation with guar gum have not been impressive with regard to improving glycaemic control in NIDDM subjects this is not the case when the blood lipid profile is studied.

(b) Effect of guar gum on serum lipids

In the present study (Chapter 5) total and LDL serum cholesterol levels were significantly reduced by the presence of guar gum in the diet and this is consistent with other studies (Aro et al. 1981; Botha et al. 1981; Carroll et al. 1981 and Kyllästinen and Lahikainen, 1981). The effect has been shown to be continued when patients are maintained on guar gum for more than six months (Jenkins et al. 1980c).

More recently the granular formulation, which can be taken as a medication, has also been shown to reduce blood cholesterol levels in hypercholesterolaemic patients (Tuomilehto et al. 1983).

The mechanisms of action of guar gum in reducing the blood cholesterol level are still not clear.

In the present study the addition of guar gum to a fat load did not cause a reduction in mean post prandial serum triglyceride levels, taken as an indirect index of assessing bile salt reabsorption. It could, however, be
argued that because guar gum is a stabiliser of emulsions (Chapter 1) it facilitated triglyceride absorption with a lower requirement for bile salts. This would reduce the reabsorption of bile salts, thus increasing their faecal loss and the excretion of cholesterol. In support of this, in studies where faecal bile salt output has been measured it has been shown to be increased by 70% by the addition of guar gum to a meal (Jenkins, 1978). In order to test this hypothesis, faecal bile salt output would need to be measured in conjunction with serum triglyceride levels following a test meal with and without the addition of guar gum.

9.4. The future of guar gum.

The Scandinavian product shown to be successful in reducing blood cholesterol levels when taken as a premeal medication, is now available in Great Britain (Rybar Company) and may be worthy of investigation. However, the side effects of guar gum consumption appear to be common to all preparations. These include abdominal distension and flatulence, which are embarrassing to patients and in many studies cause them to withdraw from the trial.

Throughout this discussion ideas have been put forward for further work involving guar gum. Although the use of the slow gelling Scandinavian product may be viable, it causes side effects in some patients and has not been shown to improve glycaemic control in all diabetics (Kyllästinen and Lahikainen, 1981). It may therefore be of more benefit to the diabetic population to study their present dietary management and how best that can be improved.
9.5. Improved diabetic control by dietary management.

At present glucose homeostasis in diabetics is attempted using a 10g carbohydrate exchange list of foods, the total carbohydrate content of each food being used to devise the exchange list. Evidence is now accumulating that equivalent amounts of carbohydrate from different foods have different effects on plasma glucose and insulin levels. For example, test meals consisting of different types of complex carbohydrate (potato, rice and corn) have been shown to differ widely in their effects on post prandial plasma glucose and insulin levels (Crapo et al. 1977). Potato causes a rise in plasma glucose and insulin levels as great as an equivalent glucose load, in contrast to rice, which elicits a much smaller response. The same effect is also seen when the foods are given as part of a mixed meal (Coulston et al. 1980). It is not only the chemical composition of the food but the physical state in which it is consumed that appears to be important. Apple juice elicits a much greater plasma insulin response than the equivalent amount of carbohydrate as apple puree or intact apple (Haber et al. 1977). In both normal and diabetic subjects carbohydrate eaten as cooked ground rice elicits a plasma glucose, insulin and GIP response similar to that produced by glucose and significantly higher than that produced by an equivalent amount of carbohydrate as cooked rice grains (Collier and O'Dea 1982). The post prandial glucose and insulin responses to these starches following oral ingestion has been shown to be proportional to the rate of hydrolysis by pancreatic amylase \textit{in vitro} (O'Dea et al. 1981) and a similar \textit{in vivo} and \textit{in vitro} correlation has been found using a variety of legumes and cereals (Jenkins et al. 1980d and 1982).

Schauberger et al. (1977) and Jenkins et al. (1981) have attempted to define a physiological basis for a carbohydrate exchange by devising a
glycaemic index of foods expressed as a percentage of the area under the plasma glucose response curve when the same amount of carbohydrate is taken as glucose. Evidence that data based on measurement of plasma glucose levels alone, however, may not provide reliable information about the insulin response necessary to maintain glucose homeostasis, is provided by Haber et al. (1977). They found that consuming apple juice and intact apples had a similar effect on plasma glucose levels but as previously mentioned elicited a significantly different insulin response.

The glycaemic effect of foods is not well documented. Much work is required not just on the effect of foods taken in isolation but more importantly on the effect of combinations of foods since that is how they are usually eaten, it appears therefore that composition of the diabetic diet i.e. the mixture of foods consumed, needs careful appraisal.

The quantity and quality of carbohydrate has received much attention recently from the British Diabetic Association and their dietary guidelines (1982) have advocated that diabetics should increase the percentage of energy derived from carbohydrate at the expense of fat, especially saturated fat. Thus the guidelines are directed towards decreasing serum cholesterol levels as well as improving glucose homeostasis. The guidelines also stress the importance of increasing both cereal and vegetable fibre intake from natural sources by taking carbohydrate in an unrefined form.

In geographical areas where the guidelines have been enthusiastically followed (even before they were official) results have been encouraging. Hockaday et al. (1978) showed that a high carbohydrate low fat diet reduced serum cholesterol levels in newly diagnosed NIDDM subjects and Simpson et al. (1979) showed that a change from their normal diabetic diet
to this new regimen caused a fall in basal plasma glucose, mean post prandial plasma glucose and percentage of glycosylated haemoglobin over six weeks in 14 NIDDM subjects. Fasting serum cholesterol levels also fell. Similar success was achieved in 18 NIDDM and 9 IDDM subjects (Simpson et al. 1981). The diet used was rich in legumes and in the latter study provided 60% energy as carbohydrate and 104g dietary fibre/day. Despite encouragement and help, including publication of a recipe book (Mann, 1983) it is unlikely that patients would follow this diet in the long term, due primarily to its monotony. The diet recommended by the British Diabetic Association is, however, not so extreme and they suggest that half or more of the energy should be derived from carbohydrate.

These dietary guidelines devised by the British Diabetic Association have supposedly been accepted by the whole country but a recent survey indicated that they have not been successfully implemented (Davies and Tredger, 1983; unpublished data). This small pilot study, carried out in conjunction with the British Diabetic Association, centred on patients from hospitals in Scotland (Edinburgh), N. England (Bolton), the Midlands (Birmingham), S.E. England (Cambridge), S.England (Bristol), and Wales (West Glamorgan), with a view to comparing the nutrient intake of those on the new style regimen with sex and age matched patients on the old style regimen. The most striking observation was that although patients on the new style regimen tended to have a higher fibre intake (19-42g fibre/day compared with 13-30g fibre/day) none achieved more than 47% intake of energy as carbohydrate and 63% of the sample were taking 40% or less energy as carbohydrate thus following the old style recommended proportion.
Despite the publication of dietary guidelines for diabetics by the British Diabetic Association there is still insufficient scientific evidence to state conclusively what comprises the best dietary regimen for diabetics. However, it is obvious that the diet should not spoil the patients enjoyment of food and for psychological, as well as physical well being, should aim to deviate as little as possible from the dietary advice given to the rest of the population. The recently published discussion paper on proposals for nutritional guidelines for health education in Great Britain (James, 1983) is in accordance with the dietary recommendations for diabetics (B.D.A., 1982) and future work should centre on the effect of implementing such a diet on the metabolic homeostasis of diabetics.

The data presented in this thesis suggest that the use of guar gum in the dietary management of diabetics is of little practical importance and further work should be directed towards using natural foodstuffs which are palatable and available to the whole population.
REFERENCES


MacGregor, I.L., Martin, P. and Mayer, J.H. (1977) Gastric emptying of solid food in normal man and after subtotal gastrectomy and truncal vagotomy with pyloroplasty. Gastroenterology, 72, 2, 206-211.


APPENDIX ONE

Anterior and posterior scans of the abdominal region of one subject after consuming a $^{99m}$Te labelled hamburger (see Chapter Eight)
10 MINUTES AFTER CONSUMPTION

ANTERIOR

R01

REPRESENTS 28cm

POSTERIOR

57Co MARKER SOURCE FOR POSITIONING SUBJECT

R01

INTENSITY OF RADIATION

R01 = REGION OF INTEREST
2 HOURS AFTER CONSUMPTION

ANTERIOR

POSTERIOR
APPENDIX TWO

Evaluation of the effectiveness of two guar gum containing snack bars in modifying the post prandial plasma glucose, insulin and GIP response in healthy human subjects
SUBJECTS

Five healthy male volunteers aged 22 years within 10% of their ideal body weight for height (Metropolitan Life Insurance Company).

SNACK BARS

Each snack pack contained two bars. The subjects consumed three bars on each occasion i.e. one and a half packs. The composition of the total snack is provided in Table A2.1.

PROCEDURE

The study was not blind as the subjects could distinguish between the bars. They each underwent the study on three separate occasions, at least a week apart and received a different type of snack bar each time.

The subjects ate their usual breakfast before 0800h and received the snack at 1300h. Blood was collected according to the procedure and at the times indicated in Chapter 2. Plasma glucose, immunoreactive insulin and immunoreactive GIP were measured and statistical analyses carried out as previously described in Chapter 2.
Table A2.1

Composition of the snack taken on each occasion by 5 healthy volunteers

<table>
<thead>
<tr>
<th>Amount consumed</th>
<th>Control choc bars</th>
<th>Guar containing choc bars</th>
<th>Guar containing non choc bars</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight (g)</td>
<td>60</td>
<td>60</td>
<td>67</td>
</tr>
<tr>
<td>Total carbohydrate (g)</td>
<td>36</td>
<td>36</td>
<td>44</td>
</tr>
<tr>
<td>Total available energy kJ (kcal)</td>
<td>954 (228)</td>
<td>978 (236)</td>
<td>978 (236)</td>
</tr>
<tr>
<td>Guar gum (g)</td>
<td>-</td>
<td>1.5</td>
<td>1.5</td>
</tr>
</tbody>
</table>

*choc = chocolate coated
RESULTS

**Plasma glucose.**

Mean baseline plasma glucose levels were similar on each occasion (Figures A2.1 and A2.2). The mean peak plasma glucose level occurred 40 minutes after consuming the control chocolate coated bars (6.3 mmol/L) and the guar containing chocolate coated bars (5.9 mmol/L) and 20 minutes after consuming the guar containing non-chocolate bars (5.4 mmol/L).

Mean plasma glucose levels were lower up to 100 minutes after the guar containing non-chocolate bars compared to the control bars, and then higher. This resulted in a smoother post-prandial plasma glucose curve but differences were not significant.

**Plasma insulin**

The mean peak plasma insulin level occurred 40 minutes after eating the snack on each occasion (Figure A2.3) and there was no significant difference between them.

Mean plasma insulin levels were consistently lower after guar containing non-chocolate bars than after both control bars and guar containing chocolate bars but the differences were not significant.

**Plasma GIP**

The mean plasma GIP levels following each snack are shown in Figures A2.4 and A2.5. The presence of guar gum had no significant effect.
Figure A:2.1 Mean (± SEM) post prandial plasma glucose levels following consumption of snack bars in normal subjects (n=5).

- - control chocolate coated bars; ▲▲▲ guar containing chocolate coated bars.
Figure A2.2 Post prandial plasma glucose levels following consumption of snack bars in normal subjects (n=5) Mean (± SEM).

- - - control chocolate coated bars; ××× guar containing non-chocolate coated bars.
Figure A2.3 Mean (± SEM) post prandial plasma insulin levels following consumption of snack bars in normal subjects (n=5).

- - - control chocolate coated;
- - - guar containing chocolate coated;
- - - - - guar containing non-chocolate coated.
Figure A2.4 Mean (± SEM) post prandial plasma GIP levels following consumption of snack bars in normal subjects (n=5).
- - - control chocolate coated bars; --- guar containing chocolate coated bars
Figure A2.5  Mean (± SEM) post prandial plasma GIP levels following consumption of snack bars.

- control chocolate coated bars; x-x-x guar containing non-chocolate coated bars.
In healthy human subjects the addition of 0.5g guar gum to a snack bar had no significant effect on post prandial plasma glucose, insulin and GIP levels when three bars were consumed.

Post prandial plasma glucose and insulin levels were lower following the non-chocolate coated guar containing bars than the control bars but this was probably due as much to the effect of the chocolate in the control bar as the guar gum in the other. Chocolate contains a mixture of disaccharides which are rapidly absorbed and therefore likely to increase the initial post-prandial rise in plasma glucose more than the oligosaccharides present in the non-chocolate containing bar. The smaller differences between the chocolate coated guar and non-guar containing bars confirms this.

Although both types of bar containing guar gum were edible the subjects did not like the appearance of the non-chocolate coated one and would not have eaten either on a regular basis.

This study shows that, from the point of view of patient acceptability and effectiveness in diabetic therapy, there is no justification for further study of these snack bars.