Studies on Immuno-reactive Gastrin with special Reference to Rheumatoid Arthritis

By

Patrick Joseph Rooney

M.B. Ch.B. (Glasgow) M.R.C.P. U.K.

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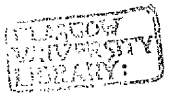

To

My wife, Katherine, and

my two children, Patrick

and Jennifer.

Amphora Coepit;
Institui Currenta rota cur urceus exit?
A wine jar is started ; why does it
Come out a pitcher as the wheel spins?

Horace 65-8 B.C.

Ars Poetica 21

PREFACE

Before taking up my post as Senior Registrar in Rheumatology at the Centre for Rheumatic Diseases, University Department of Medicine, Royal Infirmary, Glasgow, in January 1973 my clinical training had been in general medicine, but with experience in gastro-intestinal disease. The latter experience proved to be a happy one in the light of the work which forms the basis of this thesis. When I took up my clinical duties in rheumatology the high incidence of dyspepsia among patients with rheumatoid arthritis naturally stimulated my interest. Having read the literature on peptic ulceration and dyspepsia in patients with rheumatoid arthritis I appreciated how complex the problem was particularly since one of the most difficult aspects of therapy is the achievement of adequate anti-inflammatory action without causing gastro-intestinal upset. I appreciated that if I were to tackle this difficult problem with any hope of greater success than had been achieved by others using conventional gastro-enterological methods I would require some fresh approach. I decided therefore to study the gastric hormone, gastrin, and other foregut hormones, in patients with rheumatoid arthritis, and the possible relationship between changes in these hormones and

and dyspepsia, peptic ulceration and anti-rheumatic drug therapy. As discussed in the introduction of Chapter 3, I was fortunate in having facilities made available to carry out plasma gastrin radioimmunoassay in the laboratory of Dr. Keith D. Buchanan in the Department of Medicine, Queen's University of Belfast, Northern Ireland.

The thesis is set out in the conventional way. Chapter 1 reviews in considerable detail the background to the study. In Chapter 2, I review the method of immuno-assay of plasma gastrin. Chapter 3 describes my finding of marked elevation of plasma radioimmunoreactive gastrin in patients with rheumatoid arthritis. In Chapter 5, I report studies done in collaboration with Dr. Robert Imrie and Mr. David Turner of Beechams Research Laboratories, Brentford, Middlesex, England on the effects of adjuvant-induced arthritis in rats. The results of this experimental study suggest that gastrin may be involved in the cascade of events associated with the inflammatory reaction.

Naturally to someone who had previously been interested in gastro-intestinal disease the liquorice derivative, carbenoxolone, was particularly intriguing especially in view of its ulcer-healing effects and anti-inflammatory action. In Chapter 4 I describe the results of my studies of the effects of carbenoxolone on serum gastrin and secretin and

on the activity of rheumatoid arthritis. The results suggest for the first time how carbenoxolone may effect healing of peptic ulcers. Unfortunately I was unable to demonstrate any beneficial effect of carbenoxolone on joint inflammation in rheumatoid arthritis.

The relationship between gastrin and histamine has long perplexed those interested in gastric function. Histamine is both secretagoguic to the stomach and vasomotor to the microcirculation. Gastrin on the other hand, although a gastric secretagogue until now had not been shown to have any vascular effect. Using a canine model described in Chapter 6 I have convincingly shown in collaboration with Dr. David Grennan, that gastrin has no effect on synovial microcirculatory responses.

Pregnancy affects the symptomatology of both peptic ulceration and rheumatoid arthritis. I therefore decided to study the effects of pregnancy on serum gastrin, and in Chapter 7 I suggest on the basis of my investigations that gastrin rises sharply around parturition and in the early puerperium. The coincidental finding of hypergastrinaemia in neonates suggests a possible explanation for neonatal peptic ulceration.

But what could be the cause of hypergastrinaemia in

patients with rheumatoid arthritis ? This question continued to puzzle me, but the results of studies on serum calcium concentration in patients with rheumatoid arthritis presented in Chapter 8 suggest that the level of serum calcium may be a relevant variable.

I gratefully acknowledge the generous support of Professor Edward M. McGirr and the advice and encouragement of Professor W. Watson Buchanan and Dr. W.Carson Dick. As previously mentioned the work could not have been done without the technical facilities and advice available in Dr. Keith D. Buchanan's laboratory in Belfast. The Western Regional Hospital Board and the Glasgow Postgraduate Medical Board kindly gave permission and financial support for me to visit Belfast in order to carry out laboratory estimation of gastrin. I also owe a debt of gratitude to all my colleagues in Glasgow and to the many patients who freely gave of their time in order to participate in the studies. I am grateful to Miss Brenda Burns and her colleagues in the Department of Medical Illustration, University Department of Medicine, Royal Infirmary, Glasgow. I am also indebted to the patience of Miss Margaret Kernohan for typing the manuscript.

Much of the work contained in this thesis has been communicated to learned societies, including the Scottish

Society of Experimental Medicine, The Heberden Society, and the American Rheumatism Association and at International Conferences in Verona, Rotterdam, Tel-Aviv and Toronto. Much of the work is still in press, but some has already been published.

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Scottish Medical Journal	1973 18, 132
Nature	1973 246, 497
Scottish Medical Journal	1974 19, 233
Lancet	1974 1, 592
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Current Medical Research and Opinion	1974, 2, 295
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Summary of Thesis

The work presented in this thesis stems from the recurrent clinical problem of dyspepsia and peptic ulceration in patients with rheumatoid arthritis. Little light on the aetiology of this problem has been thrown by previous investigators using clinical techniques such as radiology, gastric function studies and gastro-intestinal endoscopy. A new approach using the modern technology of radioimmunoassay of small peptide hormones has been applied and the resultant findings are the major aspect of this work.

The first chapter deals extensively with the background to this work and includes detailed historical reviews of the incidence and possible aetiology of rheumatoid arthritis. In addition a detailed history of gastric function and of tests of gastric function are presented. Special emphasis is placed on those aspects known to influence gastrin secretion and action including the parietal cell functions of acid and intrinsic factor secretion. A shorter review of the background of pancreatic secretion and pancreatic endocrinology is also presented in view of its relevance to the work reported on immunoreactive secretin.

The technique of radioimmunoassay of gastrin which is the central investigative tool used in this work is presented in considerable detail in Chapter 2. The background to the development of radioimmunoassay and the limitations and advantages of the method are considered. Chapter 3 describes the results of the clinical significance of immunoreactive gastrin in rheumatoid arthritis. My original observation that immunoreactive gastrin is elevated in some patients with rheumatoid arthritis is reported in detail. Consideration is given to the extensive clinical and laboratory studies which this observation occasioned in an effort to uncover the aetiology of the phenomenon. It is reported that the distribution of values of immunoreactive gastrin in normal subjects is log/normal and the patients with rheumatoid arthritis seem to belong to two distinct populations in respect to immunoreactive gastrin status. No correlation could be found between this hypergastrinaemia and gastric acid output. Nor could it be attributed to anti-inflammatory drug therapy. No other clinical aspect of rheumatoid disease could be incriminated in this effect and it is of note that other chronic inflammatory arthritides failed to show a similar elevation of immunoreactive gastrin concentrations.

In Chapter 5 further evidence is presented that gastrin

may be implicated in the pathogenesis of chronic inflammation. In rats the induction of adjuvant arthritis is shown to cause an elevation of immunoreactive gastrin. Some evidence is also presented that the changes in hormone recorded by the immunoassay are biologically meaningful since a significant fall in gastric acid output in fistula rats was noted at the time of peak elevation of immunoreactive gastrin in intact animals.

The unusual combination of ulcer healing and anti-inflammatory actions of carbenoxolone sodium induced me to use this drug therapeutically in patients with rheumatoid arthritis. Chapter 4 records the failure of this drug to benefit the inflammatory joint disease but also reports the original observation that this drug may act on peptic ulcer via its action on immunoreactive secretin. How this accords with the observations of other investigators is considered in some detail.

The close association in terms of gastric physiology between gastrin and histamine is reviewed in Chapter 6. Histamine has long been associated with inflammation so that my implication of gastrin in inflammatory disease encouraged me to look at a possible role for gastrin in

micro-circulatory responses. The results obtained using a dog model are considered. There was no apparent effect of gastrin on this model which reflects well, the profound actions of histamine on blood vessels.

The major changes in symptomatology of both inflammatory joint disease and peptic ulceration which occurs in pregnancy induced me to consider the role of gastrin in pregnancy. Chapter 7 reports important new information which indicates that a significant rise occurs in immunoreactive gastrin towards the end of pregnancy and in the early puerperium. The very high level of immunoreactive gastrin in neonates is confirmed and the significance of these findings and their relationship to the known physiology of gastric acid secretion in pregnancy are considered.

In the final chapter a further clinical observation on rheumatoid arthritis is reported. The relationship between plasma calcium and serum albumin has been emphasised recently and using a correction factor for albumin it has been shown for the first time that serum calcium is elevated in rheumatoid arthritis. The relationship between calcium and gastrin is known to be a close one. While a correlation between this ion and immunoreactive gastrin in normogastrinaemic patients is noted the evidence

to date is insufficient for calcium to be causally implicated in elevation of plasma immunoreactive gastrin in rheumatoid arthritis, but this aspect is considered important in pursuing further studies in this field.

A History of Rheumatoid Arthritis and a History of Gastric and Pancreatic Function and of the Hormones Gastrin and Secretin.

"Not to know what has
transpired in former
times is to continue
always a child".

Cicero. 106-43 B.C.

History of Rheumatoid Arthritis

"Where agues chiefly abound"

Rev. Edward Stone (1763)

> "Rheumatoid arthritis is one of the great mysteries of medicine. Though considerable light has been thrown on this disease during the last few years, much work and study by many investigators have failed to disclose the true nature of the malady".

Although these words would form an eminently suitable introduction to any discourse on rheumatoid arthritis to-day, they are, in fact, taken from the American Journal of Medical Science almost half a century ago (Cecil, Nichol and Stainsby 1930) and emphasise the enigmatic nature of this disease.

Rheumatoid arthritis is a disorder unique to man (Boyle and Buchanan 1971) although Bywaters (1957) has described an arthritis in other primates bearing some clinical and histological similarities. Even more interestingly it appears to be a new disease there being no clear description of it before the nineteenth century (Parish 1963) although this may be more apparent than real as even as late as 1908 a standard student textbook used the terms rheumatoid arthritis and osteoarthritis as synonymous (Whitla 1908). Osteoarthritis certainly holds its place in antiquity having been clearly demonstrated in

the palaeopathology of the dinosaurs (Copeman 1970) and the mummified remains of the Pharoahs (Guthrie 1945). It is also demonstrated in the mediaeval classical paintings and is mentioned in Shakespeare (Ehrlich 1967). The failure to find any record or trace of rheumatoid arthritis in these sources nor in the medical writings of Hippocrates or Galen (Cohen 1951) or of Heberden has been interpreted as evidence of the recent arrival of this disorder in human pathology (Boyle and Buchanan 1971). Smith and Jones (1910), however, describe one prehistoric Egyptian skeleton showing fusion of one elbow joint suggestive of rheumatoid arthritis and May (1897) has given a convincing description of rheumatoid arthritis in a mummy found in a cemetery of the 5th Dynasty at Deshasheh in Egypt. Caughey (1974) has also attempted to attribute the illness of Emperor Constantine IX, described by Psellus (1063) as due to rheumatoid arthritis.

The first classical description of rheumatoid arthritis is commonly attributed to Landré-Beauvais in 1800 but Sydenham (1685) may well be referring to rheumatoid in the following quotation -

"Indeed it may happen that
where the said pains will
have harassed over many
days and very often they
may at length desist
spontaneously, and
meanwhile the sufferer may
be deprived of all movements
of his members until death,
with the joints of the
fingers as though reversed,
and with swellings as in
arthritis, knotted and
protruding on the inside
rather than on the dorsal
part of the fingers;
nevertheless he may have a
good stomach and tolerate
other aspects of life well".

Sydenham 1685.

The name 'rheumatoid arthritis' was first used by Sir Archibald Garrod in 1859. The title is an interesting etymological hybrid. The term 'arthritis' was used in Greek writings by Hippocrates for joint disorders, whereas Galen first introduced the term 'rheumatism' from the latin rheuma - a flux. This terminology was based on the humoral concept and was intended to signify that the disease arose from a flow of peccant humours. Interestingly the name "gout" is based on the same concept being considered due to drops of such humours entering the joints and tissues (Latin gutta - a drop)

Rheumatoid arthritis is now a very common disorder with an apparently world-wide distribution, the only community found free of the disorder to date being the islanders of Tristan da Cunha (Black et al 1963). There is a common conception, going back at least to the latter half of the nineteenth century, that rheumatoid arthritis is more prevalent in cold, wet climatic conditions. In 1876 McLagan, a Scottish orthopaedic surgeon, described the conditions under which -

> "the rheumatic miasma seemed, most to prevail - a low-lying, damp locality with a cold rather than a warm climate".

Considerable doubt on the validity of this concept has been cast by the demonstration that the incidence of rheumatoid arthritis in the Black Feet Indians, the inhabitants of a cold, semi-arid reservation in Montana at the foot of the Canadian Rocky Mountains was similar to that of the Pima Indians from the hot, dry Arizona desert (O'Brien et al 1968).

The aetiology of rheumatoid arthritis is unknown but in general three basic hypotheses prevail,

auto-immunity, infection and heredity.

The origin of the concept of auto-immunity in rheumatoid arthritis arose from the observation that serum from rheumatoid subjects would agglutinate many organisms, and even inert particles (Cecil, Nichols and Stainsby 1930; Wallis 1946; Ziff 1957). This was soon shown to be due to the presence of rheumatoid factor which acts as an antibody to gamma globulin and thus may be considered to be an auto antibody (Milgrom et al 1962). It is now apparent that rheumatoid factor is not the primary agent in the aetiology of rheumatoid arthritis. Its presence is not essential for the existence of the disorder (Dixon 1960). Rheumatoid factor may exist in normal subjects (Buchanan et al 1966) does not harm normal volunteers (Harris and Vaughan 1961) and about one third of children with agammaglobulinaemia develop a disease which has all the features of rheumatoid arthritis with no demonstrable rheumatoid factors being present in their blood (Good and Rotstein 1960). Rheumatoid factor may play a secondary pathogenic role in the disease. High titres are associated with severer disease (Ziff 1965) and a poor prognosis (Duthie et al 1964). Other auto antibodies have also been

demonstrated in rheumatoid arthritis (Hall et al (1960) but even less evidence is available to implicate them in the primary pathogenesis of the disease.

Infective agents have been sought in the joints of sufferers from rheumatoid arthritis for more than eighty years (Bouchard 1891; Strangeways 1907). An infective aetiology is an attractive possibility. A large number of infective diseases have arthritic manifestations on occasion, for example syphilis, tuberculosis, gonorrhoea, mumps and rubella. In addition many chronic infections, for example syphilis, may stimulate the production of rheumatoid factor although generally in low titres in comparison to those encountered in rheumatoid arthritis. To date no agent has proven to be a consistent or likely candidate for an aetiological role in rheumatoid arthritis including the mycoplasmata (Barnet et al 1966), the diphtheroids (Dubbie et al 1967) and viruses (Smith et al 1974).

Heredity has long been considered to be a major factor in the aetiology of rheumatoid arthritis but there is no evidence of a single dominant or recessive trait. The concept of variable penetrance has been

invoked but a mathematical model for estimating this in chronic disease fails to show that the penetrance of rheumatoid arthritis is different from zero (O'Brien et al 1965). Even the best of all the twin studies carried out in rheumatoid arthritis would suggest that the genetic component can account at best for only 30 per cent of the aetiological factors in rheumatoid arthritis (O'Brien 1967). It is also worth noting that no disease can ever be entirely environmental or entirely genetic and that infection and heredity may be inextricably mixed as in Aleutian disease of mink. This disease has been shown to be due to a filtrable agent yet breeds true as a Mendelian dominant trait (Boyle and Buchanan 1968).

The pathology of rheumatoid arthritis is primarily that of severe chronic inflammation. This occurs mainly in the synovial lining of diarthrodial joints although no tissue is exempt from the ravages of this disease (Copeman 1970). It is of note that while there is a good deal of evidence that the incidence of gastric peptic ulceration may be higher in rheumatoid arthritis than in a control population (Kerns et al 1957, Kammerer et al 1958, Taylor et al 1968)

no specific rheumatoid lesion of the stomach has to date been described (Crean 1960) although recently it has been suggested that antral gastric ulceration is especially liable to occur in rheumatoid subjects (Emmanuel and Montgomery 1971) and the gastric mucosa in rheumatoid arthritis with chronic salicylate ingestion responds in a very similar way to that in chronic atrophic gastritis (Ivey and Clifton 1974).

Historical Aspects of the Drug

Therapy of Rheumatoid Arthritis

"Excellent herbs had our fathers
of old, excellent herbs to ease
their pain".

R.Kipling. 1865-1936.

With the lack of any specific remedy for rheumatoid arthritis, management is based largely on the relief of symptoms especially pain and stiffness. Most of the therapeutic pharmacological agents in current use have some inhibitory effect on the complex mechanisms of the inflammatory process (Rooney et al 1973) but almost all these drugs share a tendency to produce gastro-intestinal intolerance.

Of the anti-inflammatory drugs in use to-day, the first to be introduced into therapeutics were the salicylate group of compounds. These were developed from the use of 'salicin' an extract of willow bark. Willow bark was first used in the rheumatic diseases in 1876 by McLagan although it had been used as an antipyretic and analgesic from very early times (Bayles 1966). Its use is mentioned in the hieratic script of the Edwin Smith Surgical papyrus. This document, which first came to light in Thebes in 1862 is thought to have been written in the 17th century B.C. as a transcript from even older Egyptian medical literature dating to the Pyramid age around 3000 B.C.(Breasted 1930). It is interesting to note that an identical remedy was used

by North American Indian tribes prior to the discovery of the new world (Major 1954). Nolagan decided to use extract of willow on account of his belief in the Doctrine of Signatures which indicates a cure of a disease from a knowledge of its cause. In view of his impression that rheumatoid arthritis was most prevalent in damp cold conditions he wrote -

> "On reflection, it seemed to me that plants whose haunts best corresponded to such a description were those belonging to the natural order Salicaceae"

The gastric irritant effect caused by salicylates was first reported in 1899 within a few years of their introduction into therapeutics. (Dresser 1899). During this century a large literature has accumulated on their action as a cause of dyspepsia (Weiss et al 1961; Smith and Smith 1966), peptic ulceration (Kern et al 1957, Krammerer et al 1958, Gedda and Moritz 1959, Bowen et al 1960, Taylor et al 1968, Chapman and Duggan 1969; and St. John et al 1973), and gastro intestinal haemorrhage (Cooke 1973, Weiss et al 1961, Salter 1968, Leonards et al 1973). Dyspepsia occurs in approximately one

third of patients taking aspirin regularly but is more common in patients with peptic ulceration (Muir and Cossar 1955). Wood and his colleagues (1962) are of the opinion that aspirin dyspepsia is at least in part psychogenic and suggest that if aspirin were more palatable its incidence would diminish. Murray and his co workers (1970) have presented evidence against this view showing a direct relationship between dyspepsia and total analgesic dose ingested with a 63 per cent incidence of severe dyspepsia when a total dose of 1 Kg. of aspirin or phenacetin is exceeded.

The evidence that salicylates are causative in peptic ulceration is also conflicting. Chapman and Duggan (1969) have presented a strong case for incriminating aspirin in the pathogenesis of gastric ulcer. Billington (1960, 1963, 1965) has documented a massive increase in incidence in gastric ulceration in the Australian community affecting young adult females. Douglas and Johnston (1961) were of the opinion that this rising incidence was due to increasing aspirin abuse and Duggan has demonstrated a close association between aspirin ingestion and the

complications of gastric ulceration such as perforation (Duggan 1965, 1967) and haemorrhage (Duggan 1968). In addition the association of gastric peptic ulceration and analgesic nephropathy has been noted (Dawborn et al 1966) although de Swiet (1970) suggests that the analgesic abuse is secondary to the chronic discomfort of the ulcer. Shay and Sun (1963) also hold the opinion that -

> "There is no conclusive evidence that aspirin per se is a cause of peptic ulcer".

It is possible that any true influence of aspirin as an agent responsible for gastric ulcer is obscured by the fact that in most series gastric and duodenal ulceration are considered together. Nicol (1941) first suggested that, while duodenal ulceration is likely to be due to endogenous factors, gastric ulceration is likely to be due to exogenous, ingested, ulcerogenic agents. Joossens (1973) has shown that the intake of common salt may well be related to the incidence of gastric neoplasm and has shown the great problems in demonstrating an aetiological role for an extremely common or universal dietary constituent in a relatively common disease.

Emmanuel and Montgomery (1971) have reviewed the evidence for aspirin

as a cause of peptic ulceration in the rheumatic diseases but concludes that the concomitant administration of corticosteroid drugs prohibits any valid conclusions from the data published to date.

Salicylate gastric irritation may be due, at least in part, to the fact that the low intra gastric pH keeps salicylic acid and acetylsalicylic acid in the unionised form allowing rapid absorption by passive diffusion (Davenport 1964). In the small intestine, the pH is higher allowing dissociation of free salicylate ion and absorption is correspondingly slower. In the stomach the intracellular pH causes a rapid change in the ionisation of the absorbed salicylic and acetylsalicylic acids occurs producing intracellular concentrations of free salicylate ion up to 15-20 times that in the gastric lumen (Levy 1960; Levy and Gagliarde 1963). Recent studies on gastric potential difference during salicylate ingestion confirm these conclusions (Murray et al 1974) and Ivey and Clifton (1974) have shown that rheumatoid arthritics taking aspirin have abnormalities in the physiological control of hydrogen ion secretion similar to those encountered in non-arthritic subjects with chronic peptic ulceration.

It is less than thirty years since corticosteroids were introduced into clinical rheumatology. In 1949 Hench et al reported the results of the first clinical trial of cortisone which had been carried out at the Mayo clinic. This was the result of many years of collaborative work between this clinic and the laboratories of Kendall and Reichstein and it was to earn these three men the conjoint award of a Nobel prize in 1950.

Addison first described the disease which still bears his name in 1855 and correctly attributed it to destruction of the adrenal glands. Brown-Séquard one year later showed by extirpation experiments in animals that these glands were essential for life. However further progress in understanding the physiology of the adrenal cortex was very slow until the third decade of the twentieth century. In 1924 Stewart wrote -

> 'the cortex is the part of the adrenal gland essential to life - how it exercises its function is utterly unknown'

By 1931 Swingle and Pfiffner were able to keep adrenalectomised animals and patients with Addison's disease alive by means of adrenal extracts. Thereafter knowledge of adrenocortical physiology mushroomed. By 1936 it was known that up to 28 different crystalline steroids could be isolated from adrenal cortex (Kendall 1934; Reichstein 1936; Wintersteiner and Pfiffner 1936).

While these laboratory efforts were progressing Hench (1940) observed that clinical remission of rheumatoid arthritis consistently occurred during pregnancy or an intercurrent attack of jaundice. He considered it likely that this was due to changes in the metabolism of adrenal steroids and so was able to utilize the availability of cortisone when sufficient became available and to record the dramatic symptomatic improvement in rheumatoid arthritis using this drug (Hench et al 1949). Confirmation of his results were soon available from a number of centres (Freyberg 1950; Copeman et al 1950; Boland 1951).

Knowledge regarding the biochemistry and physiology of steroids is now very extensive. Over 40 crystalline steroids have been isolated from the adrenal alone and it is known that to have anti-inflammatory activity corticosteroid compounds must possess certain chemical conformations such as a ketone group at carbon 3, an oxygen or hydroxyl at carbon 11; a ketone at carbon 20; hydroxyl groups at carbons 17 and 21 and a double bond at carbon 4/5 (Savage 1964). Despite this the mechanism of the anti-inflammatory action is little understood. The anti-inflammatory effect is well seen in synovial or nodular tissues in rheumatoid arthritis when systemic steroids are administered. The abolition of oedema, restoration of vascular tone and suppression of cellular and fluid exudates are all clearly evidenced.

The other metabolic actions of corticosteroids are extensive involving effects on glucose uptake and utilisation, glycogen synthesis, protein catabolism amino acid metabolism, and calcium homeostasis.

The enthusiasm with which corticosteroids were introduced into clinical rheumatology has been tempered by time and extensive studies have indicated little advantage in the long term when compared to the more traditional remedy of salicylates (Medical Research Council and Nuffield Foundation 1954; 1955; Empire Rheumatism Council 1955; 1957) and the considerable incidence of side effects encountered has led to caution and limitation in their use being exhorted by all major textbooks of clinical rheumatology (Copeman 1970; Hollander 1966 ; Boyle and Buchanan 1971).

One of the most controversial of the listed side effects of corticosteroid therapy is its effects on gastric function and gastro-intestinal mucosa. There is no doubt that adrenocortical hormones are necessary for maintaining the normal secretory function of the stomach. Gastric secretion is depressed in patients with Addison's disease and is markedly reduced after bilateral adrenalectomy in the rat, cat or dog (Crean 1963). Secretion is restored towards normal with adrenocortical replacement (Engel 1955). In therapeutic doses,

if administration is prolonged, corticosteroids cause acid hypersecretion (Clarke et al 1960). While these effects are relatively undisputed the role of therapeutically administered corticosteroids in the pathogenesis of peptic ulceration is much more problematical and the controversy has persisted for many years. Sinclair (1969) has clearly stated the evidence

> "overwhelmingly indicates that corticosteroids produce peptic ulceration"

and the diametrically opposed statement comes from Croft (1969)

> "There is no evidence that corticosteroids in the usual doses causes gastric bleeding or are otherwise harmful to the human stomach".

Nordin (1960) claimed that gastro-intestinal complications were the commonest cause of death due to steroid therapy and account for 28 per cent of such deaths. Bowen et al (1960) however produced evidence that the incidence of peptic ulceration was lower in steroid treated rheumatoid arthritis patients than in untreated subjects. In other series the incidence of peptic ulceration has varied from 7 to 32 per cent (Savage et al 1957; Howell and Regan 1956; Savage 1960). Crean(1963) in a careful review of all these studies has concluded that there is no firm evidence incriminating corticosteroid drugs in the pathogenesis of peptic ulcer. However, he concedes that a small increase in the incidence of gastric ulcer only, may be obscured in the

total data. One of the major difficulties in all these studies has been the fact that steroids are rarely given alone in rheumatoid arthritis and it is possible that any ulceration is more due to non-steroidal anti-inflammatory drugs. Certainly in those diseases where steroids are not accompanied by drugs of this type such as asthma or colitis no increased incidence of peptic ulceration has been indicated (Crean 1963). At present any verdict on this issue must remain that of the Scottish Law Courts 'Not Proven'.

Phenylbutazone owes its place in pharmacology to a whole chain of mistaken impressions and accidents. It is a derivative of antipyrin (phenazone) which was synthesised in 1884 in an effort to reproduce the antipyretic action of quinine. The successful production of a potent antipyretic, anti-inflammatory drug by these experiments is remarkable for as Alstead (1940) reports -

> "both the conception of the composition of quinine which served as a type and the conception of the composition of antipyrin first obtained were erroneous".

Although the availability of antipyrin has been restricted since 1938 its congener phenylbutazone was first introduced in 1949. Although less potent in analgesic and antipyretic actions phenylbutazone is a potent anti-inflammatory drug although as with the other drugs of this type its mode of action is not well understood (Woodbury 1965). This drug is poorly tolerated

by many patients with side effects in up to 45% of subjects receiving the drug. In up to 19% medication requires to be discontinued. The most serious side effects are aplastic anaemia and serious skin rashes but the most common are those due to gastro-intestinal irritation accounting for upwards of 60% of the adverse reactions.

Phenylbutazone has been implicated in both the production of peptic ulceration de novo and in the exacerbation of pre-existing ulcers (Beutler and Bergenstal 1954; Krainin 1953; Kirsner and Ford 1955). It seems likely that this drug is ulcerogenic as a tendency to erosive gastritis and haemorrhage is well established (Scott et al 1961; Roth 1964) although an actual increase in incidence of peptic ulceration has been difficult to establish with certainty (Sparling 1969; Croft 1969). This is probably due to the fact that excess ulcers in these series are confined to gastric ulcers which can be very large and multiple with this drug (Anon 1952; Raffensperger 1953).

Indomethacin was introduced into therapeutics in 1962. It has proved to be a potent anti-inflammatory drug both in vitro (Paulus and Whitehouse 1973) and in vivo (Michotte and Wauters 1964; Katz et al 1965).

A considerable proportion of patients cannot tolerate one or other side effect of indomethacin and this effect appears

to increase with age (Mills 1974). The most frequent side effects are those on the stomach and the central nervous system (Boardman and Hart 1967).

The mechanism of gastric intolerance induced by indomethacin is not clear but it has been shown in dogs that this drug causes changes in the gastric mucosa allowing back diffusion of hydrogen ion and that these changes persist for a long time after a single oral dose of the drug (Cohen and Silen 1971). In rats ulceration of the small intestine is well documented (Selye 1969) and the drug increases the acid response to submaximal doses of pentagastrin (Main and Whittle 1973). Reports of gastric ulceration and gastro-intestinal irritation in human disease situations have been frequent (Smyth 1965; Wright et al 1969).

Whether this drug is truly ulcerogenic or merely exacerbates the symptomatology of pre-existing ulceration is not clear. One of the most significant pieces of evidence suggesting a true ulcerogenic action is the frequency with which very large gastric ulcers in unusual anatomical sites have been reported during its use (Taylor et al 1968).

The newer anti-inflammatory drugs are too many and too recently introduced for any conclusions to be made regarding their effect on the stomach. However, there is little, from

the early clinical reports, to indicate that any of these compounds will perform substantially better in this respect than the more standard remedies reviewed above and Casadio et al(1972) have suggested that if not ulcerogenic a drug is unlikely to be anti-inflammatory.

Gastric Function - Historical Aspects

γηράσκω δ' ἀεὶ πολλὰ
διδασκόμενος

I grow old ever learning many things.

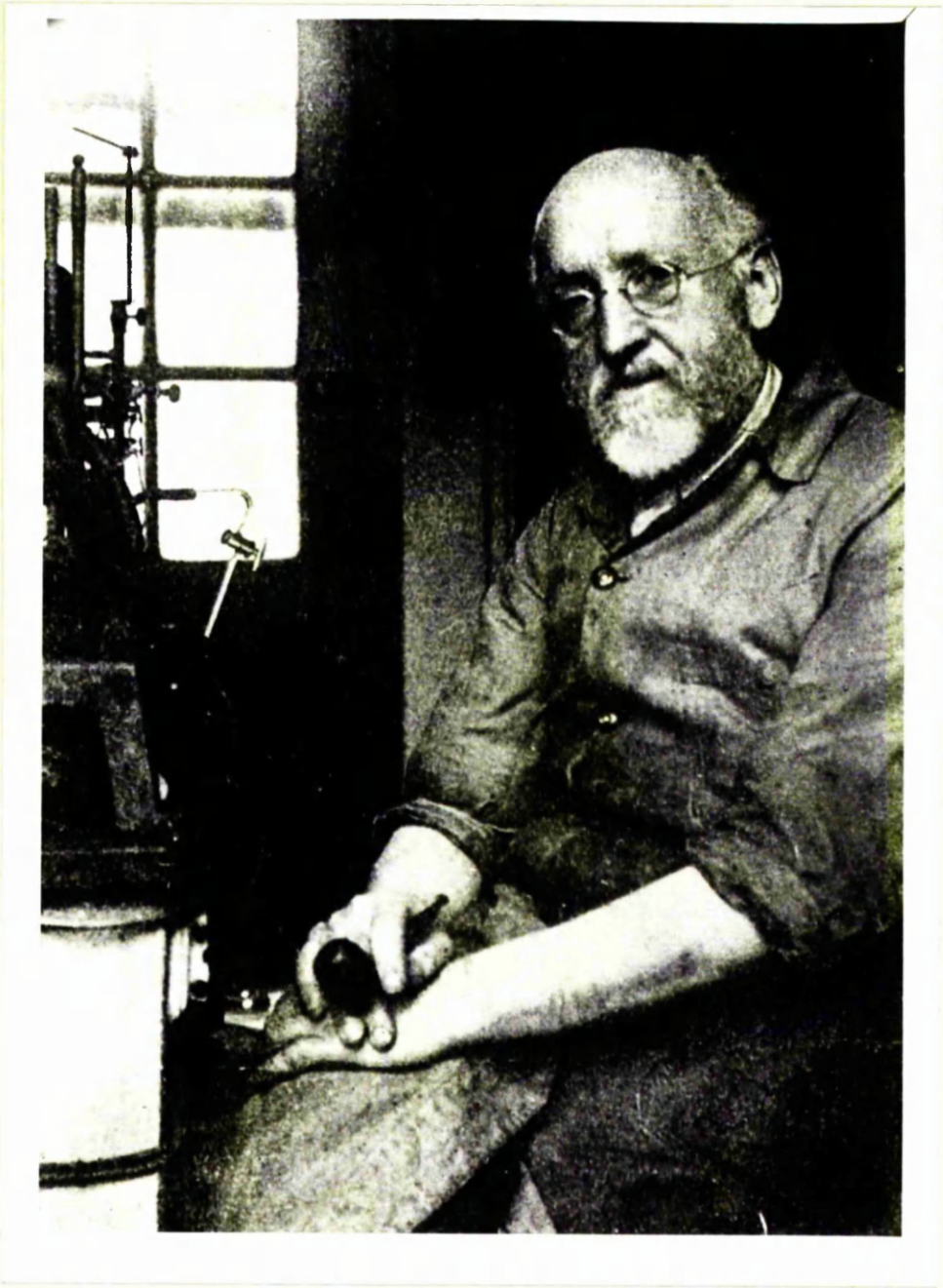
Solon C.640-C.558 BC.

Although the concept of chemical messengers arising in one organ to be carried in the blood stream for the purpose of modifying the function of another organ is confined to the twentieth century (Bayliss and Starling 1902, Edkins 1905) (Starling first coined the term hormone in 1905 from the Greek ὁρμάω - I rouse to activity,) interest in the functions and physiological control of the stomach and gastro-intestinal tract dates back into the early seventeenth century.

The stomach is represented in all vertebrate species with the exception of a few primitive fishes (Andrew 1959) and it has long been considered to be of central importance in the maintenance of normal physiology of the whole body.

> "The stomach is the great regulator
> of the whole animal machine
> which, unoffended, it nourishes,
> if outraged it disorders ".
>
> Hare 1821.

The first time that the action of gastric juice was suggested to be at least in part due to an acid ferment was as early as 1648 by Van Helmont although it was to be nearly two centuries before Prout finally put gastric hydrochloric acid secretion firmly in the tenets of physiology. Van Helmont's theory



Sir William Maddock Bayliss (1860-1924).
Portrait in laboratory. From a photograph in the Wellcome Institute for the History of Medicine.

was greeted with less than enthusiasm by his contemporaries. Silvius (1679) considered that saliva was the main component of gastric juice and the only acid secretion of the body whereas Borelli (1680) discarded all suggestion of a chemical action of the gastric juice and suggested that the whole function of the stomach was a mechanical one to churn and mill ingested food prior to assimilation. Further progress in the elucidation of the nature and function of the gastric juice was made pari-passu with the progress in the techniques of obtaining gastric juice for study. Viridet 1692 obtained gastric juice from the stomachs of animals immediately after slaughter. In addition he had observed that solutio heliotropii could detect acid by turning red in its presence. Using this observation he destroyed the theories of Silvius by showing that saliva was alkaline while gastric content was acid.

Reaumur (1752) was the first to report studies of gastric juice obtained from intact animals or rather birds. He had observed that many species of birds have the ability to

regurgitate any indigestible material. By inducing a buzzard to swallow metal cylinders filled with sponges he was able to collect the gastric juice which had saturated the sponge. He confirmed the acidity of this juice and found that meat was digested in the buzzard's stomach, but grain was not.

Reaumur's work was extended by Reuss (1786) who started a fashion for the collection of gastric juice by means of induced or voluntary emesis. He was able to show that after neutralisation of gastric juice by liberal alkaline ingestion further acid secretion could be stimulated by the ingestion of meat.

In 1772 John Hunter's post mortem observations on animals allowed him to reach these remarkably accurate conclusions regarding post mortem autolysis.

> "And observing that the half dissolved parts of the stomach were similar to the half-digested food it had immediately struck me that it was from the process of digestion going on after death that the stomach, being dead, was no longer capable of resisting the powers of that monstruum, which itself had formed from the digestion of its contents".
>
> Hunter 1772.

And he concluded from this that the action of the stomach was not mechanical.

> "but something secreted in the coat of the stomach which is thrown into its cavity and there animalifies the food or assimilates it to the nature of the blood"
>
> Hunter 1772.

He constantly found an acid present in the stomach but concluded that it was not a strong one and that the acid was the result of digestion rather than its cause (Hunter 1785).

The work of another Scot at the same time is of interest. Stevens (1777) for his thesis in Edinburgh carried out a series of experiments on a Hungarian travelling showman who earned his livelihood by swallowing stones and then regurgitating them. Stevens repeated and confirmed in the human, all Reaumur's findings in the buzzard by getting this performer to swallow perforated silver balls. These studies appear to have been carried out without any knowledge of Reaumur's experiments as he failed to use sponges to increase the yield of gastric juice. In spite of this he was the first person to carry out in vitro digestion successfully. In his experiment, Number 23, a dog was killed after an 18 hour fast and 'pure' gastric juice obtained. This was added to fresh meat

and incubated at 102°F for eight hours. Complete solution without any odour of putrefaction occurred leading him to conclude that digestion was a chemical effect and not due to trituration, putrefaction or heat.

> "In summa, haec experimenta concoctione non parum luminis offundunt. Hanc non per calorem, triturau, putredinem, vel etiam fermentationem solam, sed per humorem potentissimum, qui e tunicis ventriculi fecernitur, in cavum ejus effunditur, ibique cibum naturae sanguinis et indoli accomodat, absolvi, clare manifestoque testantur".
>
> Stevens 1777.

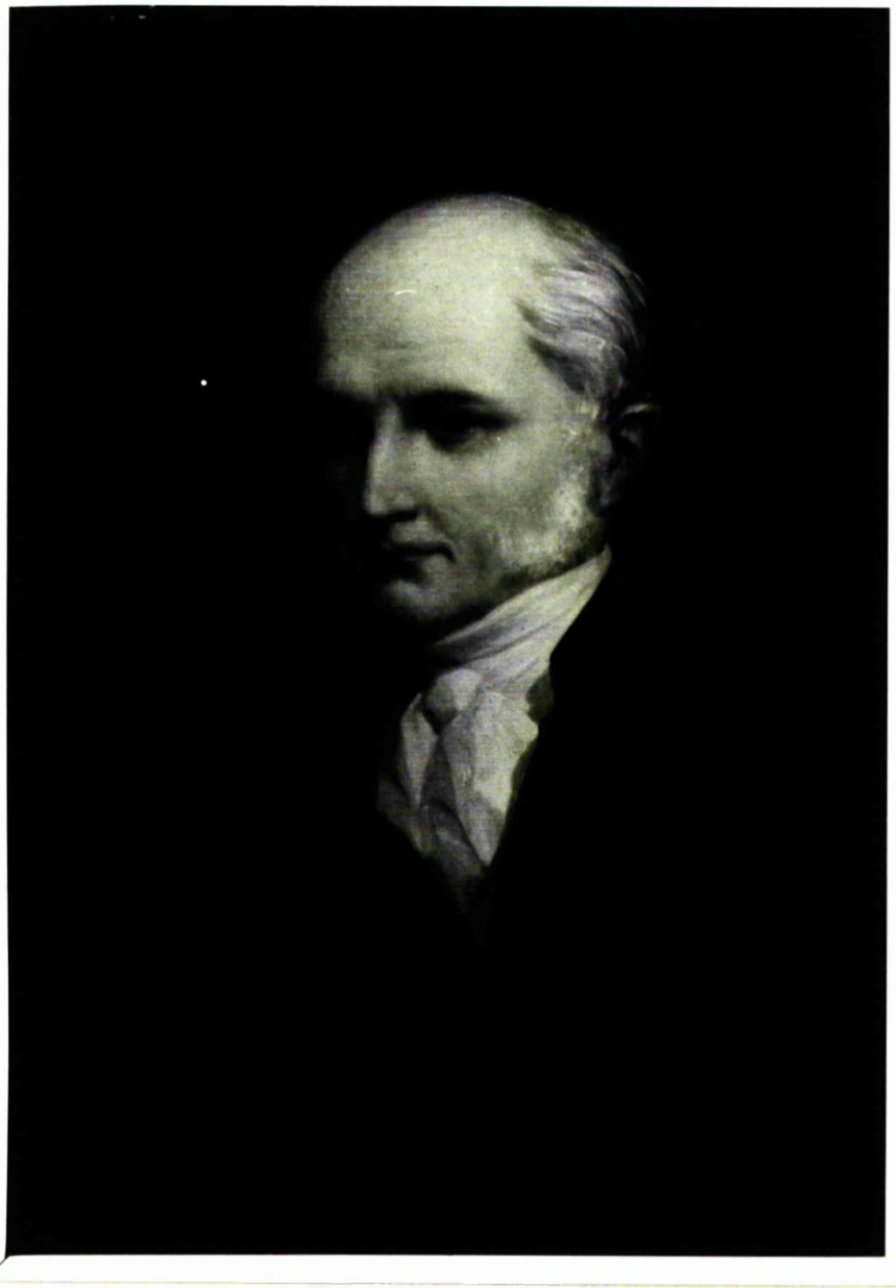
Spallanzani (1783) reported a remarkable series of experiments in hens, duck, pigeons, geese, crows, frogs, salamanders, snakes, fish, cattle, horses, owls, falcons, eagles, cats, dogs and on e human being - himself. In all these species he at first interpreted as showing digestion to be due to an acid ferment which was stimulated by certain types of food. However this publication also carries the results of the first chemical analysis of gastric juice. This was carried out on gastric juice from a crow by his friend and colleague Scopoli. In this analysis no acid was found and the constituents of gastric juice are listed as pure water, soapy and gelatinuous animal substance,

sal ammoniac, and earthy matter as found in all animal liquids.

This work was extended by Carminati (1785), the Professor of Medicine at Pavia, who showed that meat was the major dietary stimulus to the production of an acid gastric juice.

Over the next seventy years the nature of the acid in the stomach led to a prolonged and bitter controversy and it was not until thirty different investigators had expressed their varying and often totally opposing views that the matter was considered settled. Phosphoric acid, acetic acid and butyric acid had their proponents although the major controversy concerned lactic acid and hydrochloric acid. The protagonists in this debate included some of the most famous names in the history of Medicine and Physiology, including Robert Graves and Claude Bernard.

Macquart (1786) suggested initially that phosphoric acid and lactic acid were the factors responsible for gastric acidity. The work of Young (1805) and Prout (1819) also favoured phosphoric acid. It is interesting that Young's observation that production of saliva was commonly stimulated by the



William Prout. 1785-1850.

From a portrait by H.W.Phillips in the Royal College of Physicians. London.

same things that stimulated gastric juice antedated Pavlov's demonstration of the vagal coordination of these organs by nearly one hundred years. Acetic acid was suggested by Montegré (1814) and received support for its presence from Tiedmann and Gmelin (1826). Enderlin (1843) and Frerichs (1846) claimed to have shown the presence of butyric acid.

In 1813 Berzelius claimed that it was lactic acid which was present in gastric juice and moreover he claimed to have found lactic acid in all animal fluids. On 11th December 1823 Prout presented his historic communication to the Royal Society of London (Prout 1824). He showed that gastric juice contained free hydrochloric acid and that this was the only acid present. This was in spite of his claim only four years earlier that phosphoric acid was the major acid present (Prout 1819). The accuracy of Prout's work and of his conclusions are of interest. His experiments were carried out in the same way on rabbit gastric juice obtained immediately after sacrifice and on vomitus from a dyspeptic patient. The juice was divided into three equal parts. The first was evaporated to dryness and then burned in a platinum dish. The remaining mineral material was redissolved in

distilled water and the quantity of muriatic acid was determined by silver nitrate. The proportion of muriatic acid in union with fixed alkali was then determined. The second sample was supersaturated with potash and then treated exactly as the first sample to ascertain the total quantity of muriatic acid present. The third sample was exactly neutralised with a solution of potash of known strength to give the amount of free acid present. This was added to the quantity of fixed acid as previously determined and this then subtracted from the total muriate to give the amount united with ammonia. In order to give a double check this was again evaporated to dryness and the muriate of ammonia expelled by heat and collected. The quantity of muriatic acid was then determined as before. This was always the same quantity of muriate as had been determined confirming beyond doubt the general accuracy of the experiments.

Prout's conclusions were confirmed by Children (1824), Tiedmann and Gmelin (1824), Prévost and Morin (1829) and Lenderer (1851).

In the meantime Graves (1824) in Dublin had found lactic acid in the vomitus of dyspeptic patients and

from his knwoledge of the work of Berzelius he suggested that lactic acid secretion must be increased in disease. In addition Chevreul (1825) had found lactic acid in the gastric juice of Finel, a pupil of Magendie.

In 1823 the Royal Academy of Sciences had offered a prize for essays on enquiry into the digestive processes of animals. It was considered that none of the subsequent submissions were entirely satisfactory but two essays were awarded an honourable mention. The report of the Academy reads -

> "The authors have made a great number of experiments and have obtained remarkable results. For this reason, and in consideration of the expensive nature of the researches in which they engaged the Academy has adjudged to each the sum of 1500 francs".

This offer was accepted by the authors of one essay Leuret and Lassaigne (1825) but the authors of the second essay Tiedmann and Gmelin (1826) were offended by this decision and refused the award and published their work independently. Both these essays add to the controversy over the nature of gastric acid. Although the more important part of their essay relates to pancreatic function Leuret and Lassaigne studied

gastric juice by introducing sponges into the stomachs of animals prior to sacrifice. They suggested that gastric juice was 90 parts water to two parts which consisted of lactic acid, ammonium chloride, sodium chloride, organic matter, mucus and calcium phosphate. In their essay these authors were highly critical of Prout's techniques in his determination of the chloride content of the gastric juice. This stimulated Prout (1826) to reply rather bitterly.

> "I confess these remarks surprised me not a little, as I conceived the merest tyro knew how to avoid the sources of error here pointed out".
>
> Prout 1826.

Tiedmann and Gmelin on the other hand discovered hydrochloric acid independently of Prout but acknowledge his precedence in the preface of their essay.

> "Credit must be given to Prout for being the first to discover hydrochloric acid in gastric juice. We also discovered it independently of him in February 1824 on distilling several gastric liquids and it was only a month later that his treatise on this subject came to light".
>
> Tiedmann and Gmelin 1826.

William Beaumont . 1785-1853.
From J.S.Myer. Life and letters of Dr.William Beaumont 1912.
Frontispiece St. Louis, Mosby.

Apart from confirming the presence of large amounts of hydrochloric acid, Tiedmann and Gmelin also found small amounts of acetic and butyric acids which they suggested Prout had missed.

In 1833 Beaumont published his classical studies on the unfortunate Alexis St.Martin. This patient was accidentally injured by a musket in 1822 at the range of one yard.

> "The contents entered posteriorly, and in an oblique direction, forward and inward, literally blowing off the integuments and muscles of the size of a man's hand, fracturing and carrying away the anterior half of the sixth rib, fracturing the fifth, lacerating the lower portion of the lobe of the lung and diaphragm and perforating the stomach, the portion of the stomach protruding, lacerated through all its coats, and pouring out the food he had taken for his breakfast through an orifice large enough to admit the forefinger"/
>
> Beaumont 1833.

After a prolonged convalescence the patient recovered to a reasonably normal state of health although a two and a half inch diameter gastric fistula remained. Three years after the accident Beaumont began his studies. These studies led to a vogue for the production of fistulae in animals

Alexis St. Martin. 1797-1880.
From J.S.Myer. Life and Letters of Dr.William Beaumont.1912.
p.282. St. Louis, Mosby.

to enable the collection of gastric juice for study. Beaumont did not, himself, study the chemistry of gastric juice but reports Silbiman's demonstration of hydrochloric acid in juice supplied by him at Yale, and the demonstration of Dunglison and Emmett at Virginia University, of hydrochloric acid and acetic acid. He apparently also sent more than a pint of gastric juice to Berzelius in Stockholm for analysis but never received any reply.

Hunefeld (1840) was of the opinion that lactic acid was the only acid present in the stomach. Blondlot (1844) denied the presence of any free acid in the stomach because gastric juice fails to effervesce on the addition of acid calcium phosphate. This caused a stream of articles to refute this conclusion (Dumas 1846; Bernard and Barres w il 1844; Lassaigne 1844; and Melsens 1844).

The support for lactic acid as the acid of gastric juice was lent considerable prestige by the figure of Claude Bernard (1856). He at first refuted the findings of Blondlot by showing that if observed for 48 hours calcium carbonate dissolved in gastric juice with gaseous effervescence. He then drew a singular analogy between gastric juice and a mixture of lactic

Baron Jons Jacob Berzelius (1799-1848).
Line engraving by C.W.Sharpe after a painting by J. O. Soedermark (Before 1844).

acid and sodium chloride.

> "When one distilled water with lactic acid in sodium chloride, firstly only water passed over then an acid which did not precipitate silver, and finally hydrochloric acid, and gastric juice behaves in exactly the same way".
>
> Bernard 1856.

His conclusion was that lactic acid was the true acid of gastric juice and that hydrochloric acid was only formed by the action of lactic acid on the chlorides of gastric juice.

Enderlin in 1843 used yet another technique of study of gastric juice by obtaining post-mortem gastric contents immediately after the execution of a criminal in Giessen. Under these circumstances he was able to demonstrate the presence of hydrochloric acid but no acetic or lactic acid. Curiously enough in 1875 Smith had precisely the same opportunity in Philadelphia yet he found only lactic acid. He noted, however, that the criminal at Giessen could only take a little wine prior to execution whereas Smith's case had a hearty "all-American" breakfast of hard-boiled eggs, bread, butter and coffee. Further evidence for lactic acid was provided by Thompson (1845); Laborde (1874) and Lehman (1849)

The controversy between lactic acid and hydrochloric acid was such that a contemporary review of it from Germany states.

> "These two acids have days of triumph and days of defeat, staunch supporters and equally determined opponents. So far, fourteen vote for hydrochloric acid, twelve vote for lactic acid and two vote for calcium phosphate, and some reviewing the discussion somewhat philosophically say - what does it matter ? - the nature of the acid is of little importance".
>
> Anon. quoted from Robertson 1931.

However the problem was finally settled by the excellent and painstaking work of Bidder and Schmidt (1852). They demonstrated clearly that in gastric juice there was an excess of chloride over all the bases present and that this excess alone was sufficient to account for the whole acidity of gastric juice. In 1870 Bellini added independent confirmation of their work in 1870 when he showed that the addition of mercuric cyanide to gastric juice liberated hydrocyanic acid. Rabuteau (1874) and Roach (1874) interpreted the results of their experiments as adding further proof of the absence of lactic acid and the presence of hydrochloric acid in the gastric juice. Rabuteau experimented on the colour reactions of starch with iodine and with

potassium iodate and gastric juice and Reoch with the colour reaction of a mixture of ferri et quinin citrate and potassium sulphocyanide. Although Labordo (1874) attempted to revive the controversy by comparing the abilities of hydrochloric acid, acetic acid and gastric juice to split cane sugar, all subsequent publications on the subject confirm the mineral acid in its primary role in the gastric juice (Rabuteau 1875, Maly 1874, Szabo 1877 and Richet 1878). It is of interest that it was only as late as 1871 that Leube first introduced gastric intubation as a means of obtaining gastric juice for study. This was in spite of the fact that Boerhaave had pioneered its use more than one hundred years earlier in therapeutics. He used it for the nutrition of patients unable to swallow on account of corrosive poisoning or in status epilepticus and it is inconceivable that workers in the field even as early as Reaumur and Spallanzani could have failed to know of this.

> "Quando vero homines ita convulsi sunt, ut nihil deglutant, debet praesto esse canabis, metallius flexibis, qui supra linguam ad membram, quae vertebras anterior succingit, hinc in ventriculum detrudatur; per eum medicamenta injicere opportet. Quam primum vomuerunt, solent sensim ad se ipsos redire, nam malum in ventriculo est, etsi phenomena videatur capitis morbum indicare".
>
> Boerhaave 1744.

Leube's work led on directly to the clinical investigation of gastric juice by means of test meals, the earliest of which were carried out by Ewald and Boas in 1885.

Shortly before Ewald and Boas began their studies Von der Velden (1879) showed that the true reason for the difficulties in determining the nature of the acid secreted in gastric juice was due to the fact that early in digestion in the stomach lactic acid was generated from carbohydrate present in food. He also showed that the larger a meal in volume the longer it took before hydrochloric acid became detectable in the gastric content and during this period of reduced acidity carbohydrates were acted on by salivary amylase (ptyalin) with the release of lactic acid. Maly (1881) studied serial samples of gastric content both microscopically and chemically and suggested from these studies that the formation of lactic acid was a late phenomenon and always associated with bacterial overgrowth in the gastric content. This was in direct contrast to Werner (1880) who suggested that lactic acid occurred normally during the very early stages of digestion but if found during the later stages in any quantity it indicated a pathological state.

"Lactic acid is not secreted in the stomach but results from fermentation of the starch, sugar and protein substances found in the gastric contents. This formation of lactic acid is inhibited in the second stage of digestion by the normal secretion of hydrochloric acid. If there is insufficient hydrochloric secretion or if the ingest remains too long in the stomach, there is rapid development of micro-organisms introduced into the stomach by food. These giving rise to fermentation of fermentable substances of the gastric contents alcoholic as well as lactic acid fermentation can take place".

Werner 1880.

These findings were the initial sallies in what again proved to be a prolonged and bitter controversy on the cause of lactic acid production in digestion. This was considered a matter of major clinical importance in view of the stated relationship between the finding of lactic acid in test meals and gastric cancer (Boas 1892).

"Whereas much has been written about the absence of hydrochloric acid in cancer of the stomach, the question of the occurrence of lactic acid has been overlooked. Its significance is equal to that of hydrochloric acid and is a more certain criterion for the presence of cancer".

Boas 1892.

However it was eventually shown that this association was simply due to the combination of hypochlorhydria and stasis of the gastric content and was by no means specific for gastric cancer (Schmidt 1907; Robertson 1931).

> "The cause must exclusively be looked for in the combination of impaired motility and a deficiency of HCl. Only inasmuch as these two factors appear in cancer .... is lactic acid characteristic of cancer".
>
> Schmidt 1907.

Around the latter years of the nineteenth century detailed studies of gastric function were carried out in a wide variety of clinical conditions and the methods for obtaining gastric juice for analysis were becoming increasingly sophisticated. In 1904 Riegel was able to discuss in detail the available stimuli to gastric secretory function. He mentions the three earliest methods used to dismiss them. (Leube 1879). Leube had attempted to devise methods of stimulating gastric function without resort to food in order to keep the chemical analyses as simple as possible. He had tried 1) mechanical irritation of the stomach but found this inconsistent and unreliable, 2) chemical irritation with such materials as soda solution but again was dissatisfied with the results 3) thermal irritation. Leube claimed most success with the use of iced water as a stimulus to gastric secretion. Riegel, however, claims that this in his experience was unsatisfactory and goes on to list two techniques which he found more successful 4) electrical irritation of the stomach. Ziemssen 1878 and Rossi 1881 had been the first to describe the technique of gastric stimulation by Galvanic Current. However, while admitting the success of this technique in producing a flow of gastric juice Riegel 1904 dismisses it as impractical.

"There can be no doubt that percutaneous application of the galvanic current ... can stimulate the secretion of gastric juice. However, interesting all these results may be they hardly promise to be of much practical value. A good method for studying the secretion of gastric juice in pathologic cases will never be furnished in this way"

Riegel 1904.

5) digestive irritants. Riegel reaches the conclusion that this is the best form of gastric stimulation being the most natural. However, he does admit that this type of stimulation causes difficulties in standardisation and a large part of his work is devoted to the advantages of various types of meal.

Further advance in the technique of testing gastric acid function was not made until Popielski (1920) first demonstrated the potency of histamine injection in the stimulation of gastric secretion. The dangers of histamine administration inhibited the more widespread use of histamine stimulation (Bloomfield and Keefer 1927) as a gastric stimulant until 1953 when Kay introduced his augmented histamine technique covering the major toxic effects with standard anti-histamine therapy. Then

synthetic gastrin pentapeptide became available in 1966 (Wormsley et al 1966) the maximal stimulus could be applied without these precautions and to date this is the standard technique of gastric analysis.

In 1880 Fenwick first drew attention to the association between gastric atrophy and Addisonian Anaemia.

> "It will be remarked that the symptoms are those of anaemia, not of asthenia, and that in all a well-marked lesion of the glandular structure of the stomach was discovered after death capable of accounting for the deficiency of blood exhibited during the life of the patients".
>
> Fenwick 1880.

In 1904 Riegel was able to present the contrasting views as to whether the gastric atrophy which by that time had become a well established feature of pernicious anaemia was the primary defect or not. Stengel (1904) in an editorial note in the English version of Riegel's text comments -

> "It seems improper to consider achylia gastrica the cause of pernicious anaemia, and if the achylia gastrica precedes a primary anaemia it is to be regarded as coincident".
>
> Stengel 1904.

Riegel (1904) emphasises the fact that Sinhorn (1895) Ewald (1892) and himself had patients in whom normal nutrition was maintained in the face of absence of acid and pepsin secretion. By the twenties Levine and Ladd (1921) and Hurst (1923) were able to conclude that the association between total achlorhydria and pernicious anaemia was a constant one. In addition the other known physiological functions of the normal stomach were also shown to be absent in this disease; including pepsin secretion (Levine and Ladd 1921) and the active gastric secretion of neutral red dye (Davidson et al 1925).

However, it was on the lack of acid that most emphasis was laid and Cornell in a review article in 1927 states -

> "If one fact has received ample confirmation on the subject of pernicious anaemia it is this, the stomach contents do not contain free hydro-chloric acid".
>
> Cornell 1927.

Major advances in the management of pernicious anaemia which occurred in the period from 1925 to 1930 are of major significance in any historical account of the understanding of gastric function. In 1925 Robscheit-Robbins and Whipple demonstrated clearly the clinical value of liver in the management of iron deficiency anaemia. However, in the same series of articles they suggested without supporting evidence that it might be of value in other types of anaemia.

> "Even in the complex anaemias (Human pernicious anaemia, anaemia with nephritis and cancer cachexia) food factors deserve serious consideration in the clinical management of the blood condition".
>
> Whipple and Robscheit-Robbins 1925

It was this suggestion which led Minot and Murphy (1926) to try the effects of large quantities of raw or underdone liver in these complex anaemias. These workers felt that, if any response was to be obtained it would be seen mainly in the anaemia associated with cancer due to dietary deficiency caused by the anorexia encountered in such patients. Fortunately they included some patients with Addisonian anaemia and were thus able to observe and document the dramatic haemopoietic response to raw liver in this condition. Describing this response Means and Richardson (1928) write -

"The response is no less dramatic than that evoked by thyroid in myxoedema, orange juice in scurvy, pituitary in diabetes insipidus or insulin in diabetes mellitus".

Means and Richardson 1928.

The years following saw many workers involved in attempts to define the nature of the factor in liver responsible for this response and Cohn and his collaborators (1928) indicated from the results of their attempts at extraction and purification that the active principle was not any of the known vitamins but was protein or nitrogenous in character. Castle (1929) in the first of his classical series of papers reviewed what was already known about pernicious anaemia. He laid particular emphasis on the loss of gastric function, which a) had been shown to antedate the anaemia sometimes by upwards of ten years (Riley 1925); b) was resistant to subcutaneous histamine which at that time was considered to be the most powerful stimulus to acid/pepsin secretion (Bloomfield and Keefer 1927); and c) alone of the clinical abnormalities in pernicious anaemia persisted unchanged in the face of adequate liver treatment (Johansen 1929). He noted also that Shaw (1926) had reported the spontaneous remission of Addison's anaemia associated

William Bosworth Castle. Born 1897. From S.R.Kogan.
The Modern Medical World 1945. p.180. Boston.

with a return of acid secretion. All this led Castle to suggest a causal relationship between the loss of gastric function and pernicious anaemia. In 1923 Hurst had already raised such a possibility indicating that the anaemia might be due to blood destruction by an abnormal enteric bacterial flora which could be the result of the loss of the gastric acid disinfectant action. His theory had been disproved in 1928 by Davidson's demonstration of identity of flora before and after successful liver treatment. In order to examine his hypothesis Castle searched the literature for supportive evidence. He noted that in other diseases where gastric function was reported to be absent including gastric carcinoma (Brandes 1921) and gastric polyposis (Strauss et al 1928) the occurrence of an associated megaloblastic anaemia had been reported. In addition, since it had been believed since the latter part of the nineteenth century that gastric function was not essential for life or health, total gastrectomy for carcinoma or for peptic ulcer had been increasingly practised. By 1927 Miyagi was able to find ninety reported cases although in a more careful review Finney and Reinhoff (1929) only accepted nine cases where prolonged survival had occurred following operation where they could be certain all functioning gastric

tissue had been removed. Castle (1929) noted that of these nine patients two were reported as having subsequently developed Addisonian anaemia (Moynihan 1911; Hartman 1921). In addition in 1929 Dennig reported a further patient with a similar anaemia after total gastrectomy and had noted a complete response to liver therapy. In the face of all this evidence it seemed to Castle that pernicious anaemia must be a dietary deficiency state induced despite ingestion of a normally adequate diet, by the relative inability of those patients with no normal gastric function to assimilate the factor responsible.

> "An obvious possibility is that a virtual dietary deficiency might be produced in the presence of a diet, entirely adequate for a normal individual by the notable defect in the process of gastric digestion necessarily imposed by the absence of functional gastric juice".
>
> Castle 1929.

His initial thoughts about this functional deficiency centred round a protein factor in the diet as suggested by Cohn et al (1928) from their study of the active principle of liver. Castle felt that the loss of

pepsin might be the mechanism by which such a factor failed to be absorbed.

> "Since in these patients a lack of hydrochloric acid is almost invariably associated with a corresponding lack of pepsin, the environment for the peptic digestion of protein which is the chief apparent chemical function of the normal stomach would appear to be entirely unsuitable".
>
> Castle 1929.

There was certainly a good deal of analogy as Castle (1929) pointed out between the quantitative response of the liver diet (Cohn et al 1928) and that of the other known dietary deficiency states such as beri-beri.

In order to test his hypothesis Castle (1929) proposed the following experiment -

> "It was decided as a first observation to carry out the digestion of protein in the stomach of a normal man and to introduce subsequently the products of that activity into the stomach of the patient - Honi soit qui mal y pense"
>
> Castle 1929.

Normal healthy subjects were asked to ingest 300 gms. of nearly raw beef steak and after one hour to regurgitate in response to pharyngeal stimulation. This was adjusted to pH 2.5-3.5 by the addition of hydrochloric acid and then incubated for a varying period of time. After incubation the material was seived, neutralised and introduced into the stomach of patients with Addisonian anaemia by means of a stomach tube. These patients were fasting and were being maintained on a diet containing no liver, meat or kidney. Castle's rather bland understatement of the acceptability of this experiment reads -

> "On the contrary, the relief of persistent nausea or diarrhoea was occasionally surprisingly effected by a single administration of this material".
>
> Castle 1929.

The haemotological response to this treatment was equally striking and was identical to that obtained with liver extract. Control patients in whom a similar quantity of meat without predigestion was administered were not benefitted.

This experiment did not exclude the possibility that the normal stomach secretes the factor responsible for the reversal of Addisonian anaemia and that this

substance is then stored in the liver.

With the collaboration of Wilmot Townsend, Castle then proceeded to observe the effect of normal gastric juice without the addition of any nutriment. The juice was obtained by tube under the stimulus of subcutaneous histamine. Of itself the juice was entirely ineffective but when the beef was added even in vitro and incubated a highly effective preparation was obtained. Other solutions of casein or beef denatured with sodium hydroxide were ineffective.

> "These three experiments strongly suggested that some action of human gastric juice was capable of producing from 200 grm. of beef muscle, known itself to be without effect, a substance capable of causing a remission in pernicious anaemia quite comparable to the action of 150 grm. of prepared liver".
>
> Castle and Townsend 1929.

To show that the active factor was due to a chemical reaction between the gastric juice and the beef, the two constituents were incubated and administered separately. This procedure was without effect on the anaemia.

It was not until 1930 that Castle gave his concept the classical terms "extrinsic factor" now no longer used since its true identification as Vitamin B_{12} and the more lasting "intrinsic factor". It was in this paper that he showed that peptic digestion of beef was ineffective that heat inactiv ation of all digestive power of human gastric juice failed to produce the effect, and that saliva and duodenal juice contained no effective substance. The results were equally good if the incubation of gastric juice was carried out at neutrality thus excluding acid and pepsin as necessary.

Since Castle's experiments a large body of knowledge of the physiology and function of intrinsic factor has been accummulated. It has been clearly shown that the stomach is the sole source of intrinsic factor, (Sweinseid et al 1953) and that within the stomach activity is limited to the body and fundus (Meulengracht 1952). More recently it has been suggested that intrinsic factor secretion in humans is a property of the parietal cells although this is not true in other species. In the rat and mouse the chief cells appear to be involved and in the pig intrinsic factor secretion is a function of the pyloric glands and Brunner's glands (Hoedemacher 1964).

Such wide variation between hog and human is rare in comparative physiology (Meulengracht 1952). In 1929 by the time Castle had coined the name 'intrinsic factor' Sturgis and Isaacs had shown that hog stomach was an effective source of intrinsic factor for the oral treatment of pernicious anaemia. Assay of intrinsic factor was, however, hampered by the lack of a suitable animal model. In rats, hog intrinsic factor diminishes rather than enhances the absorption of B_{12} (Hildsworth and Coates 1956). Attempts have been made at developing an assay for intrinsic factor based on the binding of B_{12} by intrinsic factor concentrates but this technique has the major disadvantage that non-specific binding of B_{12} occurs with a large number of biological substances including lysozyme, saliva (Beerstecher and Altgelt 1951) and serum (Pitney et al 1954). Recent advances in radio-immunoassay have, however, proved eminently suited to the assay of intrinsic factor and the radio-labelling of Vitamin B_{12} with radiocobalt is very easy (Irvine 1965).

Two properties of intrinsic factor are essential for its biological function in relation to the absorption of Vitamin B_{12} a) its ability to combine with B_{12} and b) its ability to adhere to the surface membrane of distal small intestinal epithelium. The mechanism of

absorption of B_{12} and of its dissociation from intrinsic factor is not well defined (Wilson 1964). Intrinsic factor appears to be a mucoprotein with a molecular weight of over 100,000 but there is some evidence that smaller sub units can retain biological activity (Glass 1963). Unlike acid secretion it seems that intrinsic factor is continuously secreted in the absence of gastric secretory stimuli (Irvine 1965) and the basal intrinsic factor secretion in normal subjects greatly exceeds the minimal amount required for normal B_{12} absorption - the intrinsic factor secreted in one hour binds more than 1 pg of B_{12} (Jeffries and Sleisenger 1965) and gastrin (Irvine 1965). In almost all instances reduced secretion of intrinsic factor is due to chronic atrophic gastritis. The aetiology of this type of disorder has not been established but the presence of abnormal circulating antibodies to gastric parietal cells and to intrinsic factor as well as the association between pernicious anaemia and other diseases thought to have an immunological basis has led to the postulate that pernicious anaemia is a disease of auto-immunity (Irvine et al 1965). A small group of juvenile familial cases of pernicious anaemia has been reported with normal gastric histology but with no intrinsic factor in gastric juice (McIntyre et al 1965) and this is presumably due to a hereditary metabolic defect in the synthesis or secretion of intrinsic factor.

A historical view of the investigation of Pancreatic function and of the hormones gastrin and secretin.

"In doubtful questions 'tis the
safest way to learn what
unsuspected ancients say".

John Dryden 1631-1700.

The term pancreas is derived from the Greek all flesh. This name had been given to the organ as it had been thought to be simply a cushion of flesh on which the stomach rested. In 1671 Regnier de Graaf cannulated the duct with a goose quill and noted the flow of pancreatic juice. In his "Tractatus Anatomico" he comments on the strength of this observation that the name pancreas was inappropriate because

> "Pancreatis substantia tota glandulosa est".
>
> De Graaf 1671.

It is fair to point out, however, that the Russian name for the pancreas means "the gland under the stomach".

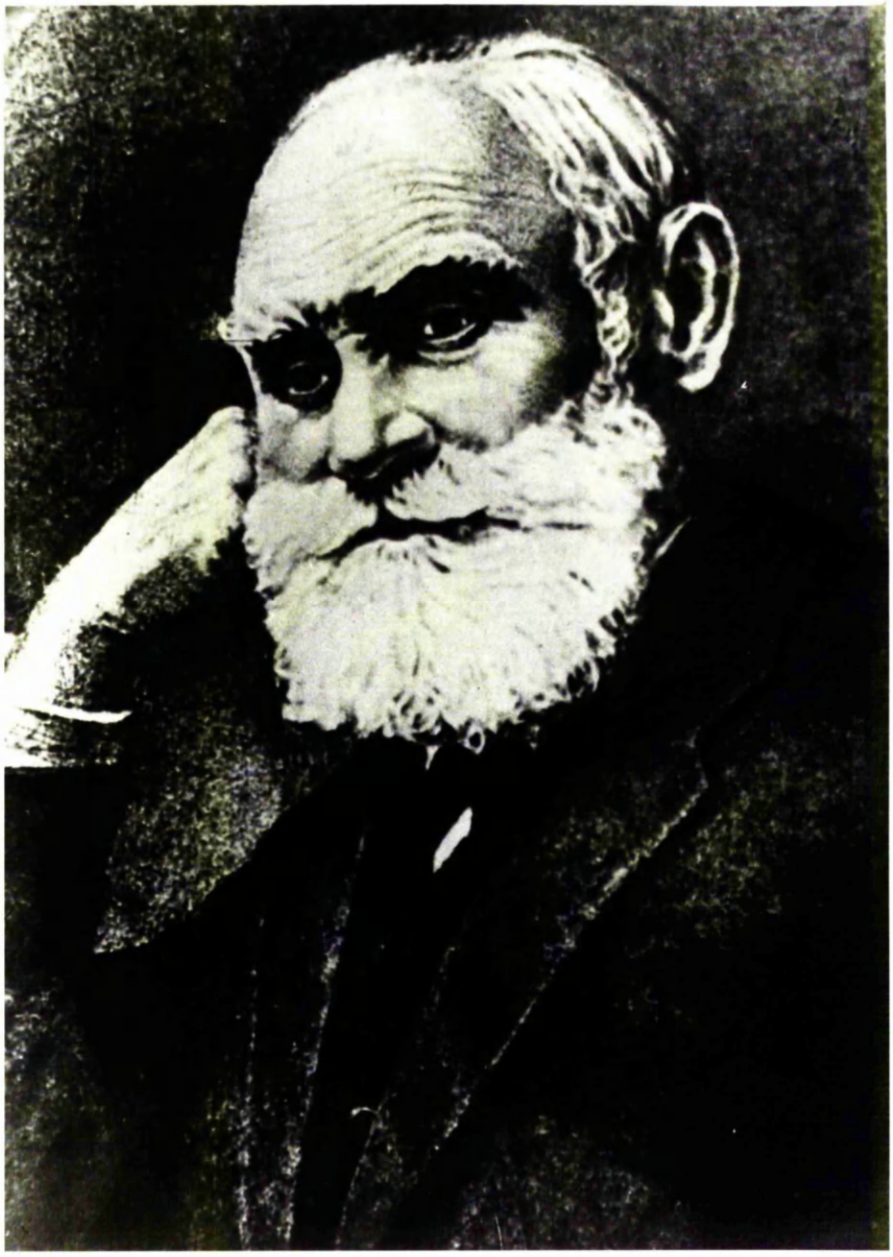
In 1825 Leuret and Lassaigne in their prize essay to the Royal Academy of Sciences reported the effect of putting acetic acid into the duodenum. This caused marked dilatation of the bile and pancreatic ducts as well as a copious flow of secretion from these ducts. They comment -

> "Since a weak acid is able to elicit secretion to the duodenum and to dilate the secretory ducts from the liver and pancreas, the acid chyme ought to have the same property. It is acidic and it is emptied into the intestine when the digestive juices are needed".
>
> Leuret and Lassaigne.

Claude Bernard (1813-1878). Performing an experiment from a photogravure of the picture in 1889 by Leon L'hermitte in the Sorbonne.

The middle of the nineteenth century was dominated by the figure of the great physiologist Claude Bernard. He was able to demonstrate the importance of the pancreas in the digestion and absorption of fats.

> "In the winter of 1846 I made a comparative study of the digestion of various substances in carnivora and herbivora. After administering fatty material orally to dogs and rabbits I followed the physical and chemical changes which this material underwent in order to be digested and absorbed by the chyle vessels. I then discovered when I opened the gut of these animals that in the dog the fat was emulsified and absorbed by the chyle vessels in the upper part of the small intestine, just beyond the pylorus, whereas in the rabbit, this phenomenon was not distinct until much further down, at a distance of 30-40 cms. from the pylorus. Surprised by this difference, I carefully investigated whether it had any anatomical cause. I then found that in the dog the two pancreatic ducts emptied very high up in the duodenum in the vicinity of the choledochus, whereas the pancreatic duct in the rabbit opened much further down than the bile duct exactly where I had seen the absorption of fat to start with great intensity. After having observed the connection between the position of the chyle-containing lymph vessels and the outflow of pancreatic juice, I

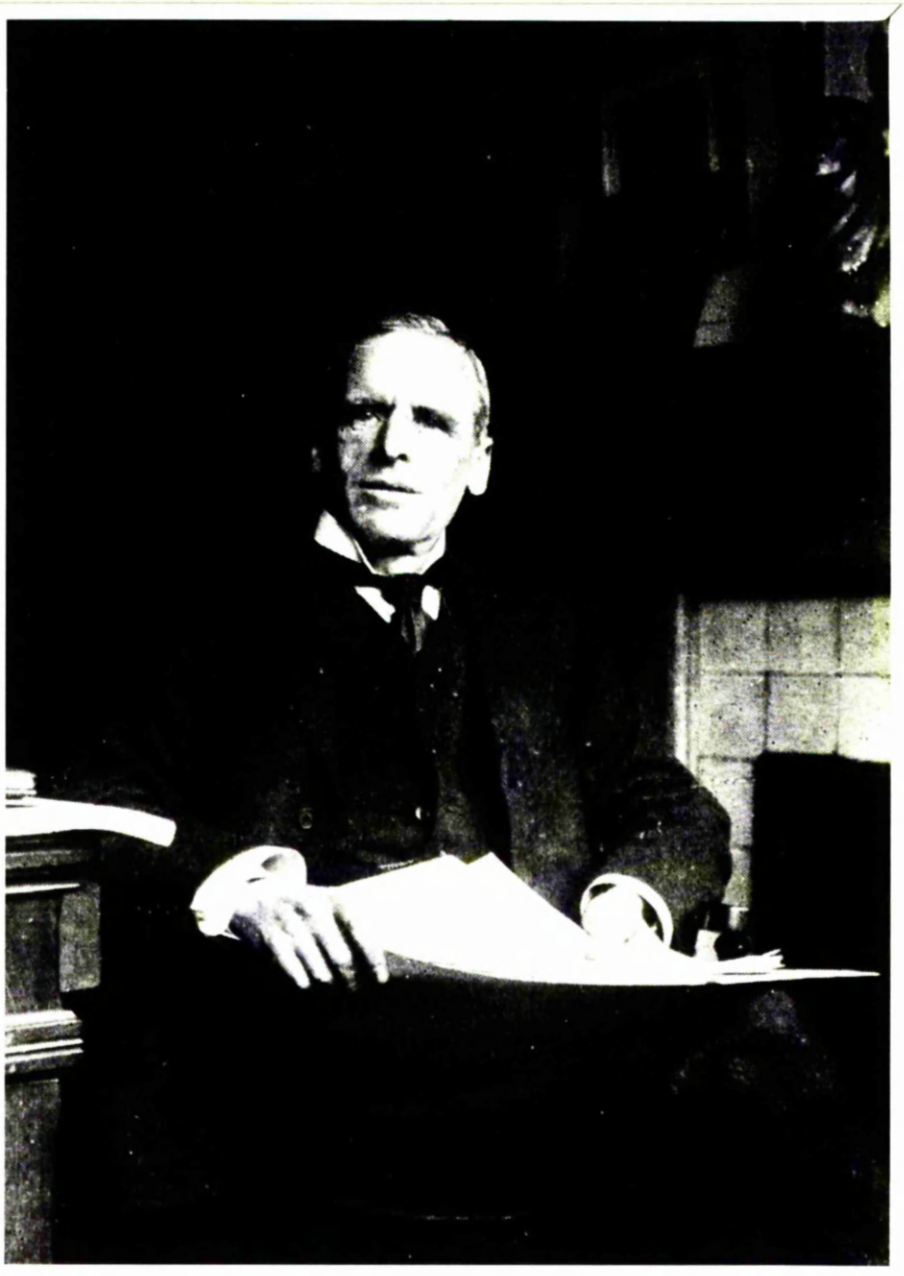


Ivan Petrovitch Pavlov (1849-1936).
From an original photograph from the Academy Pavlovsky.
Presented to the Wellcome Institute for the history of Medicine, 1956.

was naturally inclined to
believe that it is to this
fluid that we must ascribe
the ability to alter fats
so that they can be absorbed".

Claude Bernard. 1856.

In the latter part of the nineteenth century thinking in the field of gastro-intestinal physiology was profoundly influenced by the Russian school led by Pavlov. These workers demonstrated the vagal mechanism for the release of gastric acid and pancreatic juice (Pavlov 1868). Pavlov confirmed the observation of Leuret and Lassaigne that acid instilled into the duodenum would release pancreatic juice and he interpreted this as being due to a local nervous reflex. However Wertheimer and Lepage (1902) attempted to confirm this by denervating the pancreas and yet still elicited the same response. Bayliss and Starling (1902) in their classical paper were the first to interpret these observations correctly and to establish the existence of a blood-borne chemical messenger from the duodenum to the pancreas which they called secretin. In 1905 Starling himself coined the term hormone for such a messenger from the Greek $\acute{o}\rho\mu\acute{\alpha}\omega$ - I rouse to activity. Confirmation of the existence of secretin was rapidly provided in particular by Enriquez and Hallam (1903) whose evidence from their proto-type cross-circulation experiments is a further example of the excellent



Ernest H. Starling (1866-1927).
From E.H. Starling Principles of Human Physiology 1941.
Frontispiece, London. Churchill.

fundamental physiological research being performed at this time. By 1930 Ivy was able to conclude that -

> "Secretin, after going through the vicissitudes of pharmacological and physiological investigation according to the evidence at hand appears to be specific for pancreatic secretion".

Although the work of Bayliss and Starling (1902) invoked a completely new concept in physiology - the control of one organ by a chemical messenger secreted by another. Their demonstration of the first hormone was soon followed by Edkins concept of gastrin.

Edkins presented his classical paper to the Royal Society in 1905. He began as follows -

> "It has long been known that the introduction of certain substances into the stomach provokes a secretion of gastric juice and it has been thought that the nervous mechanism of the gastric glands is susceptible to certain local chemical stimuli. On the analogy of what has been held to be the mechanism at work in the secretion of pancreatic juice by Bayliss and Starling, it is probable that in the process of absorption of digested food in the stomach a substance may be separated from the cells of the mucous membrane which, passing into the

> blood or lymph, later stimulates the secretory cells of the stomach to functional activity. The following observations support this view".
>
> Edkins 1905.

Edkins then described how simple aqueous extracts of antral mucosa stimulated gastric acid secretion when injected rapidly intravenously into anaesthetised cats. He called this extract gastrin, a name derived from a contraction of "gastrin secretin" after Bayliss and Starling's work three years earlier. In this work Edkins showed that extracts of all tissues extracted and injected in the same way cause a sharp fall in blood pressure but only antral mucosa was effective in stimulating secretion. The facility of isolation of a hormone is dependent on the availability of a reliable and specific assay technique of sufficient ease and specificity of performance. The history of gastrin or as it came to be known 'the gastrin hypothesis' was bedevilled by lack of a specific assay. It was soon demonstrated by other workers that crude extracts of many organs could stimulate gastric acid secretion and that the active principle in these extracts was histamine (Barger and Dale 1910; Dale and Laidlaw 1910; Popielski 1920). In the years following this the failure to find in pyloric

mucosal extracts any stimulant of gastric secretion other than histamine (Sachs, Ivy, Burgess and Vandolah 1932; Gavin, McHenry and Wilson 1933) fostered a general belief that the activity of Edkin's extract was most probably due to this substance. It is fair to point out, however, that in 1922 Lim repeated Edkin's original experiments exactly and achieved exactly the same result. Lim showed that histamine, given rapidly intravenously in the same manner as Edkins had injected his antral extract, had no effect on gastric acid secretion and concluded that Edkins extract must contain some other stimulant. Unfortunately this work was ignored at the time. In 1938, however, Komarov was finally able to place the gastrin concept on a firm basis. He realised that Edkins' antral "hormone" might be protein in nature and that if that were so it would be removed by the extraction procedures employed by earlier workers. He was, thus, able to demonstrate that a protein extract of antrum, free from histamine, provided a potent stimulus to gastric acid secretion.

Even after Komarov's work problems in the investigation of gastrin still persisted. The best source of supply of physiologically active gastrin was hog antral mucosa but unfortunately this is a tough

organ and it contains a large amount of mucoprotein. In addition its physiological effects are confusing. In anaesthetised cats rapid intravenous injection causes acid gastric secretion (Blair et al 1961) but in conscious dogs inhibition of acid secretion occurs (Gillespie and Grossman 1963; Gregory and Tracy 1964). In 1959 Gregory and Tracy were finally able to extract gastrin in a pure form in quantities large enough for detailed studies. Their extraction was based on a technique used by Lim and his colleagues in 1934 in studies on enterogastrone . Nevertheless it was only when they adopted the extraction method described by Blair and his colleagues (1961) that large scale purification of gastrin could be achieved. It is highly salutary that the basis of Blair's technique is the same simple aqueous extract of antral mucosa described by Edkins in his original paper. Treatment of this extract with acetone was sufficient to render it free of histamine. Within one year of adopting this technique Gregory's laboratory had isolated the pure hormone (Gregory and Tracy 1961) and by 1964 had determined its full amino-acid sequence (Agarwal et al 1963) and synthesised it (Anderson et al 1964). The gastrin isolated at this time comprised 17 amino-acid units and at that time Gregory and Tracy

demonstrated the existence of two forms of the hormone gastrin 1 and gastrin 2 and these two molecules are identical except for the presence of a sulphate radicle on the tyorosine in position 12. Gastrin 1 and gastrin 2 are readily interconvertible in vitro and while it is not certain whether both exist in vivo or whether one represents an artefact of the extraction process (Gregory and Tracy 1964) both forms have been found in all species studied to date (Agarwal et al 1968).

Synthesis of gastrin had led to rapid progress in understanding the physiology of gastrin and its relationship to other foregut hormones. The main reason for this is that synthetic gastrin can be administered in the clinical situation and its physiological effects studied in detail. In addition gastrin has proven to be ideally suited to radio-immunoassay and this has permitted the detection of picogram quantities of gastrin in the circulation and in other biological fluids (McGuigan 1968). This ability to measure variations of gastrin concentrations within the normal physiological range of the hormone has enabled detailed study of factors influencing secretion, and metabolism of gastrin in both normal and diseased states.

The ease with which a hormone is isolated is dependent on the availability of a reliable and specific assay technique of sufficient ease and specificity of performance. A routine method of achieving large amounts of starting extract are also needed since only small amounts of hormone are likely to be present in any tissue, since full chemical characterisation of the hormone will require at least milligram amounts of pure material, and since in any purification technique consisting of several stages overall loss of material will be considerable.

The differing fates of the two hormones, gastrin and secretin illustrate this very well. Secretin was much easier to deal with initially since an active crude preparation from upper intestinal mucosa was easily available from the very first attempts. The specificity of this extract was never in doubt yet in spite of these advantages it was not until 1959 that complete purification was achieved (Mutt 1959) using elegant, modern techniques including gel filtration, column chromatography of proteins and electrophoresis (Jorpes et al 1962).

The early history of gastrin, as indicated above, was much more difficult. This was due mainly to the

problem of specificity of assay on account of the ubiquitous presence of histamine (Popielski 1920). Even after Komarov (1939) had fully vindicated 'the gastrin hypothesis' problems still existed in dealing with the thick tough organ supply source, hog antral mucosa, and with a hormone whose actions were rather confusing in that both gastric acid secretion (Blair et al 1961) and gastric acid inhibition (Gillespie and Grossman 1963) could occur depending on the species and method of administration.

More recently the problems have been reversed with gastrin and secretion. Although pure hormone of each type has been available for approximately the same period gastrin has proven to be remarkably well suited to radioimmunoassay (McGuigan 1968; Yalow and Berson 1970) while secretin has been much more difficult to adapt to this type of assay (Buchanan et al 1973). This has led to an explosion of knowledge regarding the function of gastrin in physiological and pathological states (Anon 1972; Anon 1973; Rooney et al 1974) while it has been difficult to establish the true role of secretin in modern gastro-intestinal physiology such that it has even been suggested that secretin may not in fact function as a hormone after all (Dormsley 1973)

A lot of work has been directed to the anatomical distribution and cells of origin of the gastro-intestinal hormones. The classical physiological type of extirpation experiments which had clearly established the other hormones such as thyroid hormone, adreno-cortical hormes and insulin with their organs of origin were not applicable in most instances to the gut .

In 1938 Freidrich Feyrter suggested that there was a "diffuse endokrine epitheliale Organe" consisting of a widely scattered system of 'clear cells' (Helle Zellen) throughout the gut and gut-assooiated organs. By 1969 it was obvious that Feyrter's cells were the cells of origin of the various gut polypeptide hormones. These hormones can now be grouped according to similarities in the sequences of their constituent amino acids (Pearse 1974) . Gastrin and cholecystokinin/ pancreozymin share the N-terminal pentapeptide (Gregory et al 1964; Mutt and Jorpes 1968). The structures of glucagan and secretin (Mutt et al 1970) and gastric inhibitory polypeptide (GIP) (Brown and Dryburgh 1971). Glucagan and secretin also share their N terminal sequence with vasoactive intestinal polypeptide (VIP) (Said and Mutt 1972). So far motilin (Brown et al 1973) appears to be entirely dissimilar from any other gastro-

intestinal polypeptide.

Attempts have been made to attribute specific hormones to specific cells (Forssman et al 1969; Pearse et al 1970; Solica et al 1973) and to date Pearse (1974) suggests that 6 of the 11 recognised types of clear cells (APUD cells) have been identified with a definite polypeptide product.

Clearly however it will be some time before the full anatomical and physiological relationships of the gut and gut associated hormones can be determined clearly.

The amino-acid structure of human heptadecapeptide

(Little) Gastrin.

GLU
↑
GLY
↑
PRO
↑
TRY
↑
LEU
↑
GLU
↑
GLU
↑
GLU
↑
GLU
↑
GLU
↑
ALA
↑
TYR - (Gastrin 1 non-sulphated Gastrin II sulphated)
↑
GLY
↑
TRY
↑
MET
↑
ASP
↑
PHE
↑
NH_2

Figure 1. Structure of Gastrin.

Radio immunoassay of Gastrin.

"measure what is measurable
and what is not measurable
make measurable".

Lord Kelvin.
1824-1907

The ideal method of assaying any hormone should be based on its specific biological action possibly even utilizing a cellular receptor site action as the specific end point. With few exceptions, however, present biological assays are insufficiently sensitive or specific to afford useful information on hormone levels in biological fluids.

In 1960 Yalow and Berson devised a method of radio-immunoassay for insulin which offered a method of hormone assay with a sensitivity, precision and, in most cases a specificity far greater than any previous method. Gastro-intestinal physiology with its plethora of polypeptide hormones, which in most instances are ideally suited to the application of radioimmunoassay, has advanced considerably on the basis of this technique.

The principles of radioimmunoassay are simple and the performance of it requires only three reagents - 1) a standard hormone, 2) an isotopically labelled hormone and 3) an antibody prepared against the hormone.

The amounts of labelled hormone and of antibody used in the assay are calculated so that the least amount of labelled hormone used is compatible with easy detectability in a radio-activity counter and the dilution of antibody is such as will bind approximately 50 per cent of the labelled hormone.

Varying concentrations of standard hormone are allowed to react with a constant amount of labelled hormone and the 50% binding dilution of antibody. The standard and labelled hormone will equilibrate such that some will be antibody-bound and some will remain free in proportion to their relative concentrations. As the concentrations of standard hormone are increased so the amount of antibody bound labelled hormone will fall. Thus increasing concentrations of standard hormone are detected by decreasing bound radioactive counts. In this way a standard curve for the hormone can be constructed. Unknown samples are treated in the identical manner to that for the standards and the resultant hormone concentration is read off from the standard curve.

The methods used to separate bound from free hormone vary widely (Greenwood 1969) and include such techniques as chromato electrophoresis, charcoal adsorption of free hormone, alcohol precipitation of antibody, solid phase adsorption of antibody and precipitation of antibody by an anti-serum to gamma globulin (double antibody technique).

The purity of the reagents in any radioimmunoassay system is relative. The antiserum may contain antibodies other than that directed against the hormone but this matters little provided the labelled hormone does not cross react with

these other antibodies. Similarly the labelled hormone preparations may contain minor impurities provided such impurities do not themselves carry the label or react with the antibody. The standard preparation should ideally be identical with the pure hormone which is to be measured but the assay can still be satisfactory if it behaves identically in an immunological sense. If it contains contaminant materials these should not react with the antibody.

In most radioimmunoassay systems the production of the antibody is the limiting factor. Supplies of many standard hormone preparations of considerable purity are available and the methodology of achieving labelling of high specific activity usually present no serious problems (Greenwood, Hunter and Glover 1963). The initial and major difficulty is the raising of a hormone antibody of high affinity and specificity. The larger the antigen in general the easier is the production of antibodies. Antibody raised against hormones with a molecular weight of 6000 or greater are usually of high affinity and result in assays of high sensitivity. However with smaller hormonal molecules antibody production becomes critical. Such hormones include gastrin, secretin, glucagon, cholecystokinin/pancreozymin, vasopressin and angiotensin. Raising antisera to these

hormones in general involves exhaustive trials of many species of laboratory animals with varying immunization schedules, and the use of adjuvant molecules such as albumin to which the hormone is bound to enhance its antigenecity. Despite all these efforts the resultant antibodies may still not possess all the qualities of those achieved with the larger molecular weight hormones.

Specificity of the antibody for the hormone in question is generally the next limiting factor, for instance, assays for glucagon detect not only the pancreatic hormone but also a cross-reacting material from the gut (Buchanan et al 1967) although this can in fact be utilised if it is possible to raise the rare antibody which is truly specific for the hormone (Heding 1971). The assay for growth hormone is known to detect human placental lactogen but is otherwise free of cross-reactivity while the immunological properties of thyrotrophin, follicle stimulating hormone, luteinising hormone, and human chorionic gonadotrophin are all similar and generally result in cross reactions im immuno-assay systems. In general it can be said that with most assays an exhaustive and tedious study of many antisera will usually reveal some with unique properties of specificity.

It is essential in any radioimmunoassay that the results

of the method be correlated with an assay of the biological effect of the hormone. This is not always possible in view of the vast difference in sensitivity between any bioassay system and that achieved by radioimmunoassay or at best the correlation can be achieved only in the higher concentrations of hormone.

It is important to remember that certain hormones such as ACTH or glucagon are partially destroyed by proteases in biological fluids so that only a fraction of the hormone present on withdrawal reaches the assay tube, although improved methods of blood collection and the use of enzyme inhibitors can partially prevent this.

Common to all assay methods performed in fluids obtained from intact organisms the assay measures concentration of hormone only at the time of sampling and so can give no indication of the continuous processes of secretion, degradation and excretion of the hormone.

Despite all these caveats radioimmunoassay has rendered possible the assay of many hormones by a technique which when established is extremely simple to perform and can easily be applied to large batches of samples. This fact is testified to by the burgeoning literature on the applications of the technique and this is perhaps suggestive that the difficulties are more apparent than real.

Methods used in the radioimmunoassay of gastrin.

Materials.

Gastrin standard.

The gastrin standard used throughout all assays referred to in this thesis was synthetic human heptadecapeptide gastrin 1 (GI 1-17) (Gastrin human type synthetic 68/4399 M.R.C.) diluted in buffer B to a stock solution of 100 ng/ml. This material was stored in 1 ml aliquots at -20°C and diluted in charcoaled human plasma directly before use to give standard gastrin dilutions in the range of 10-1000 pg/ml. The initial dilution was 1 in 10 to give a concentration of 10 ng/ml thereafter it was diluted 1 in 2 to 9. 6 pg/ml.

Buffer A

consisted of 0.04 M phosphate buffer at pH 7.4

[Na H_2 PO_4 $2H_2O$ (1.195 g) + Na H PO_4 (4.6g per litre]

Buffer B

consisted of 0.04 M phosphate buffer at pH 7.4

diluted in equal volumes of normal saline

i.e. Buffer A + 0.9 g/100 ml Na Cl.

Charcoaled human plasma -

Pooled human plasma + 1 g/100 ml decolourizing charcoal repeatedly filtered until all the charcoal was removed. This is presumed to render the plasma hormone free.

Dextran-coated charcoal -

0.5 g/100 ml charcoal + dextran in a ratio of 10 parts charcoal to 1 part dextran. This is achieved by mixing equal volumes of Norit-A neutral pharmaceutical grade decolourising carbon 1g/100 ml suspended in Buffer A and Dextran-80 0.1g/100 ml in Buffer A

Gastrin antiserum.

Gastrin antiserum R 98(2) raised in a rabbit to synthetic gastrin 1 (2-17) conjugated to chicken egg albumin by glutaraldehyde and boosted once with rabbit albumin-gastrin conjugate. Cross reactivity with cholecystokinin/pancreozymin is low being less than 1 in 10,000 on a weight basis. This antiserum is diluted 1 in 3000 to give the assay antibody.

125-Iodine labelled gastrin.

Iodination of standard gastrin utilizes the Chloramine-T method of Hunter and Greenwood (1962). The reaction is carried out at room temperature in small glass vials using -

1 ml of ^{125}I Sodium iodide solution (10 μ C_1) (Amersham)

2.5 - 10 μg of hormone in 10-50 μl solution.

Chloramine T 100 μg in 15 ul.

These reagents are mixed thoroughly and the reaction is continued for 10 seconds. It is terminated by the addition of 100 µl (240 µg) of sodium metabisulphite. Mixing is continued for a further 45 seconds before the addition of potassium iodide (6.22 mgm. in 200 µl). The reagents are maintained throughout at a pH of 7.4 Iodinated gastrin is purified by gel filtration on Sephadex G.10. The integrity of the labelled hormone was indicated by its ability to bind to antibodies raised against the hormone and to produce a sensitive standard curve for the hormone. 60-80 per cent incorporation of ^{125}Iodine into the hormone was achieved giving specific activities of 700-900 mc/µg gastrin. Approximately 80% of the labelled hormone bound to excess antibody and greater than 90 per cent adsorption to dextran charcoal was usual. The production of a good standard curve was the ultimate test of the label. The ^{125} iodine labelled gastrin, for the purposes of the assay, is diluted in buffer B so that 15000 to 20,000 counts are given by 1 µl sample in 200 seconds. This is equivalent to the addition of 8-10 pg of labelled hormone per tube.

Performance of the assay.

All controls, standards and unknowns are assayed in triplicate. Each tube contains 300 µl volume of which

100 µl is test plasma, or charcoaled human plasma. The only exception to this is the 100% tube.

The tubes include -

1. A set of 100 per cent iodine containing only -

^{125}I iodinated gastrin	100 µl

These are not subjected to the separation procedure and they give a measure of the total labelled hormone added to each tube in the assay.

2. A set of non-specific tubes containing -

^{125}I iodinated gastrin	100 µl
charcoaled human plasma	100 µl
Buffer 'A'	100 µl

As these tubes do not contain antiserum they give a measure of the total labelled gastrin available after separation.

3. A set of excess antibody tubes containing -

^{125}I Iodinated gastrin	100 µl
charcoaled human plasma	100µl
Excess antibody R 98 (2) diluted 1 in 100	100µl

4. A set of zero tubes containing -

^{125}I Iodinated gastrin	100 µl
charcoaled human plasma	100 µl
Assay antibody	100 µl.

These tubes give a measure of maximal binding of the labelled gastrin to the antibody when no 'cold' gastrin is present.

5. Sets of standard tubes containing - gastrin dilutions of 1250; 625; 312.5; 156.25; 78.12; 39.06; 19.53 and 9.76 pg/ml of standard gastrin. The amount of standard added is 1 tenth of these amounts as the volume used is 100 ul. Each standard tube contains -

^{125}I iodinated gastrin	100 µl
standard gastrin in charcoaled human plasma	100 µl
assay antibody	100 µl

6. Sets of unknown samples. These tubes contain -

^{125}I iodinated gastrin	100 µl
unknown sample fluid	100 µl
assay antibody	100 µl

The unknown sample is used undiluted unless the value obtained is greater than 500 pg/ml when the sample is assayed in doubling dilutions until the value obtained falls on the most sensitive part of the curve.

7. Sets of non-specific samples for each unknown sample. These tubes contain -

^{125}I iodinated gastrin	100 µl
unknown sample fluid	100 µl
Buffer A	100 µl

These tubes measure any non-specific interference which may occur as a result of the addition of that particular plasma to the system.

8. A further set of control samples one from a known normal serum with a concentration of gastrin around 50 pg/ml and one from a patient with pernicious anaemia whose serum has been diluted to give a gastrin concentration around 250 pg/ml.

These tubes contain -

^{125} I iodinated gastrin	100 ul
control sample	100 ul
assay antibody	100 ul

All tubes in the assay are prepared in an ice-bath and then after addition of all reagents as above each tube is vortexed for a few seconds. They are then incubated for 24 hours at $4^{\circ}C$. With the exception of the 100 per cent iodine tubes all are then separated by the addition of 1 ml of dextran-coated charcoal at $4^{\circ}C$. After further vortexing the tubes are centrifuged at 2500 r.p.m. at $4^{\circ}C$ and the supernatants are decanted. The remaining radioactivity in each tube is counted for 200 seconds in an LKB Wallac gamma counter. These represent the unbound counts. Each set of three tubes is averaged and the percentage of antibody-bound counts, using the non-specifics as the total available radioactivity are calculated for the zero sample and for each standard sample.

A calibration curve is then constructed in which the percentage of bound radioactivity is plotted against increasing

amounts of standard gastrin. From this curve the amount of gastrin in the unknown samples is then read off.

The precision of this assay has been calculated from the difference between replicate determinations of the same sample in the assay using the formula -

$$\text{standard deviation of the assay} = \sqrt{\frac{\Sigma d^2}{2N}}$$

when d = difference in replicate determinations

and N equals the number of replicate samples

(Brown, Bulbrook and Greenwood 1957).

With 54 replicates the standard deviation was found to be 0.67% bound counts suggesting that a change of 2% bound counts can be detected with 99% accuracy. The sensitivity of the assay which is the smallest amount of hormone which can be reliably detected from zero is 0.69% bound counts or 12.5 pg/ml. Reproducibility as assessed by the internal control samples did not differ by more than 15 pg/ml for the 50 pg/ml sample and by more than 25 pg/ml for the 250 pg/ml sample in ten consecutive assays.

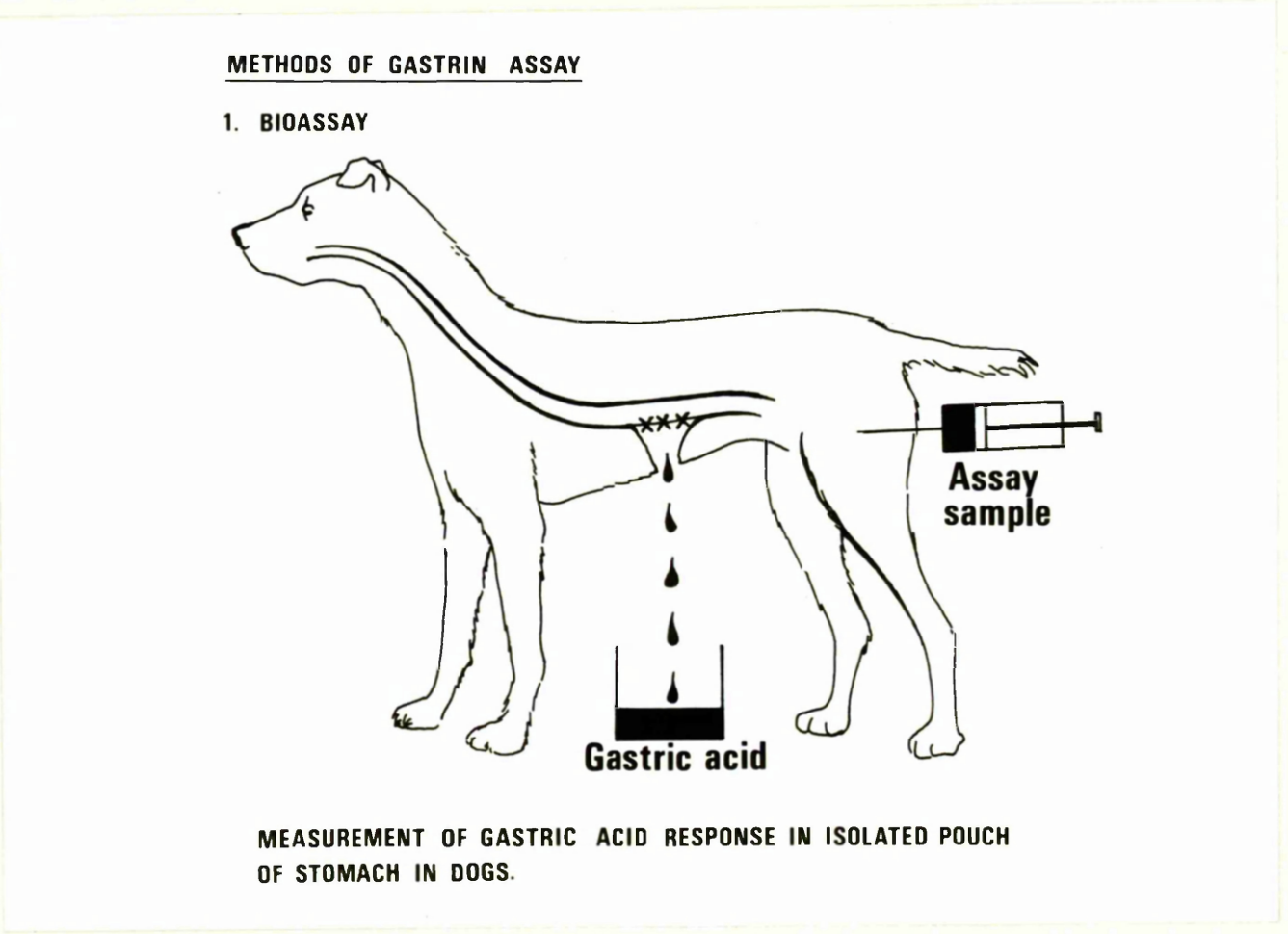


Figure 1.

Gastrin can be assayed biologically by the observation of the effect of injected material on the secretion of gastric acid. This method lacks both sensitivity and specificity.

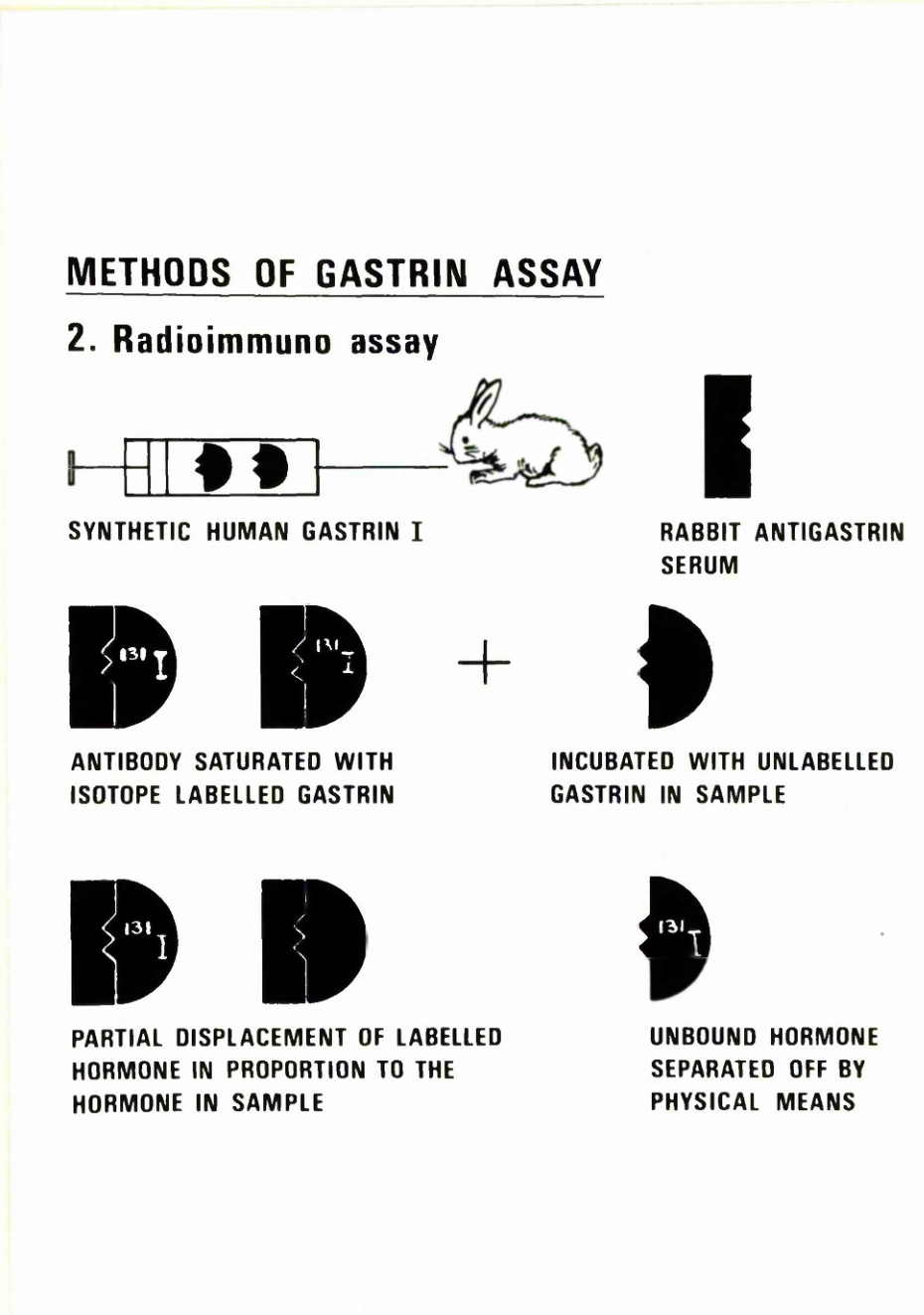


Figure 2.

Radioimmunoassay is simple, reliable, sensitive and with care, specific.

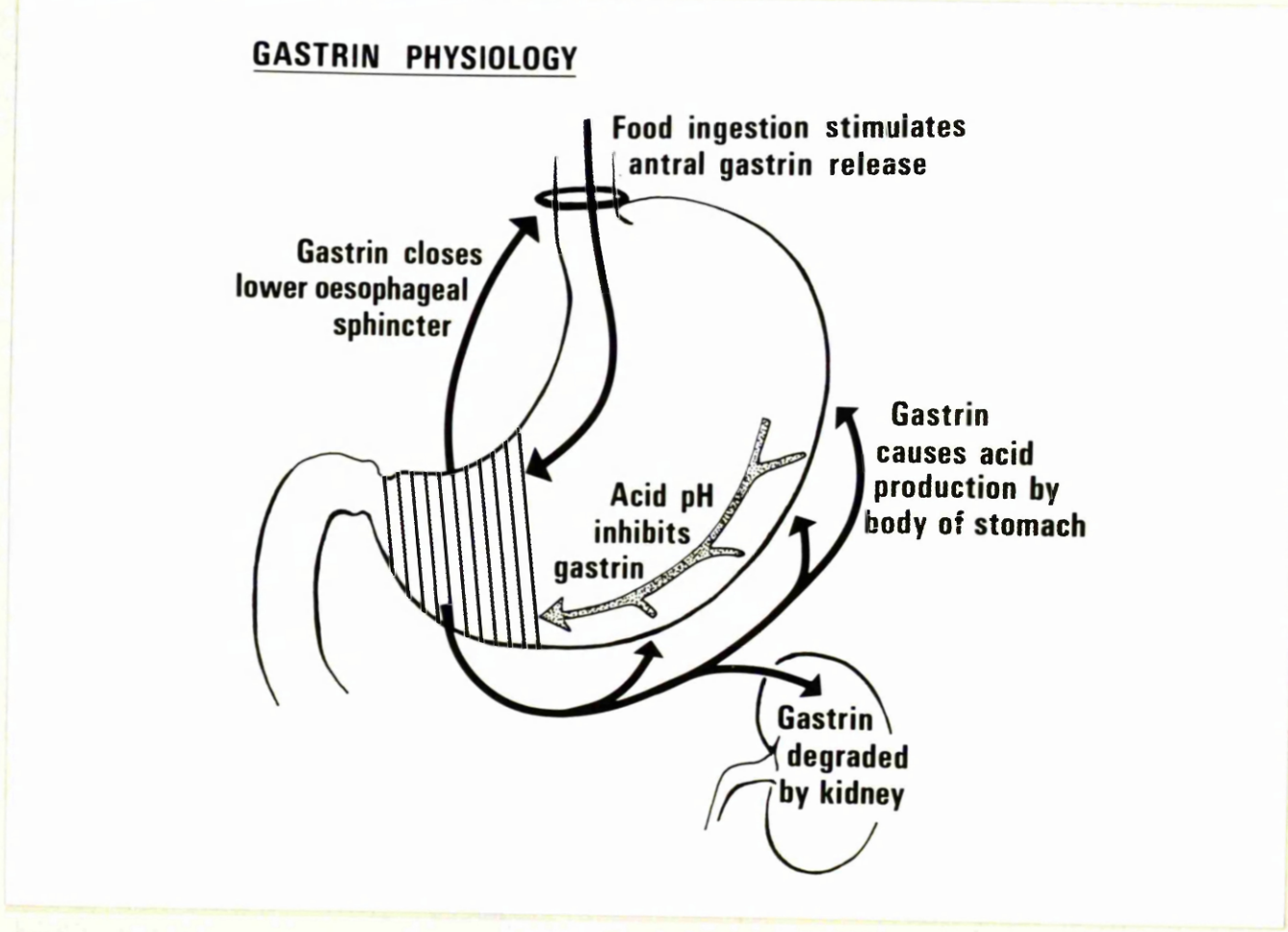


Figure 3.

A simplified view of gastrin physiology as recognised to date.

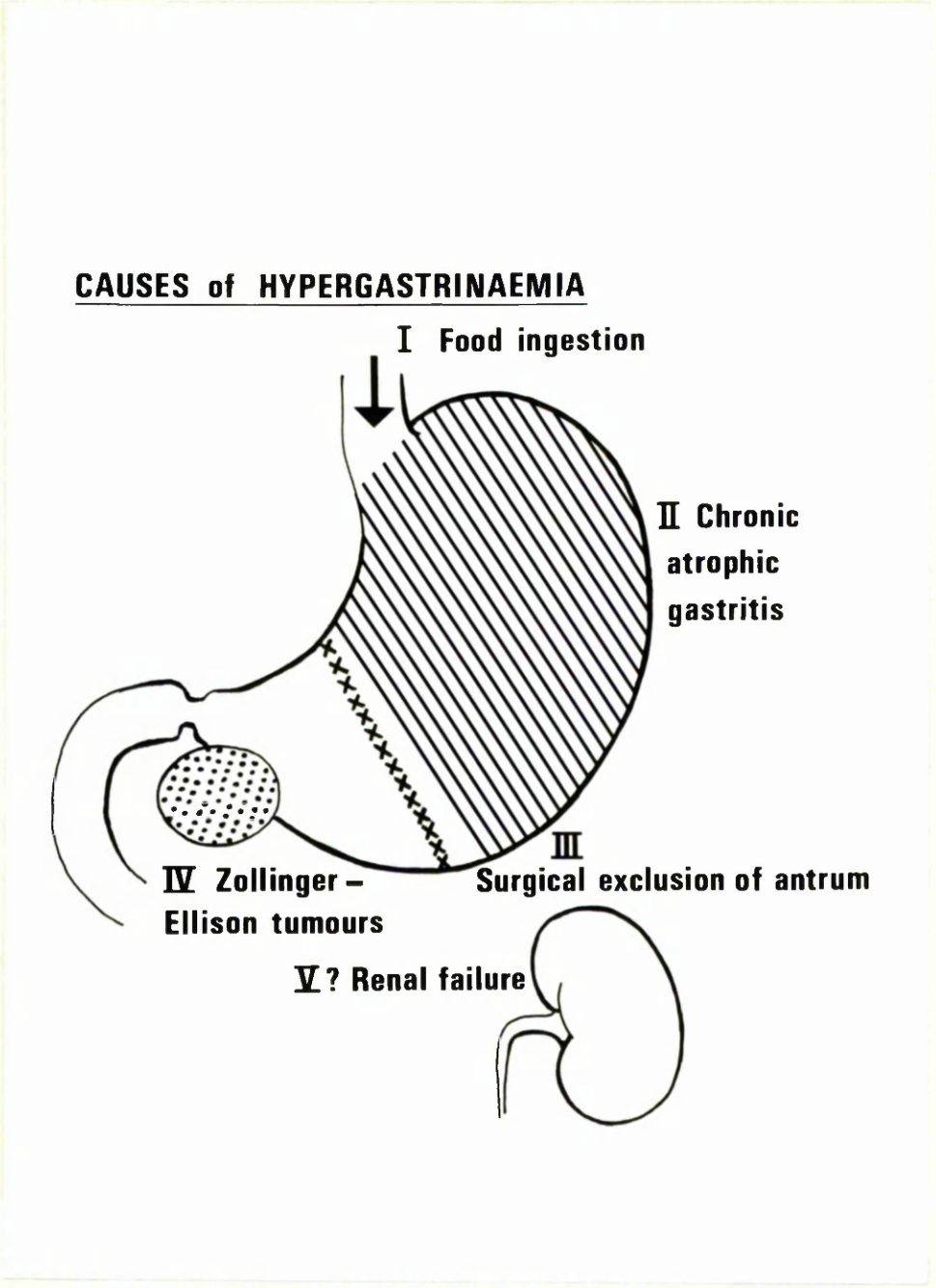


Figure 4.

Documented causes of elevation of plasma immunoreactive gastrin.

Hypergastrinaemia in rheumatoid arthritis

A new and unexplained clinical finding.

"Nought broken save this body, lost but breath
Nothing to shake the laughing heart's long peace there,
but only agony".

Rubert Brooke 1887-1915

Peace.

SUMMARY

Elevation of immunoreactive gastrin in rheumatoid arthritis is reported in this Chapter. The distribution of gastrin values in controls is shown to be log normal and in rheumatoid arthritis evidence is presented for a dual population.

The lack of effect of anti-rheumatic drugs on gastrin is reported and the normal acid output despite hypergastrinaemia discussed in detail.

The relationship of the gastro-intestinal tract, the non-steroidal anti-inflammatory drugs and inflammatory joint disease has intrigued and interested me for a considerable time. Napoleon Buonaparte is credited with the often quoted saying "an Army marches on its stomach". Since the use of most anti-rheumatic drugs is limited by their capacity to induce gastro-duodenal irritation and ulceration a similar saying could well apply to patients with rheumatoid arthritis although "marches" would more appropriately be substituted by the old Lallans Scots word 'hirples'. Despite the extensive research and vast literature on gastric function in rheumatoid arthritis that has accumulated over the years little light has been thrown on this aspect of the disorder. The new tebhnology which has given new impetus to gastro-enterological research over the past decade in the form of fibre-optic endoscopy and more importantly of radioimmunoassay of the small polypeptide foregut hormones suggested to me that the time was ripe for a fresh look at the problem.

I gratefully acknowledge my great good fortune in that Dr. Keith D.Buchanan who is eminent in the field of radioimmunoassay of this type was kind enough to

collaborate with me and to offer me the opportunity of working in his laboratory in Belfast during the course of these studies. In early pilot studies (Vince et al 1973; Rooney et al 1973) I had observed striking elevation of immunoreactive gastrin levels in about one third of patients with rheumatoid arthritis. Further studies on the prevalence, likely aetiology, and possible significance of this elevation are reported in this chapter.

MATERIALS AND METHODS

All patients who are referred to as suffering from "rheumatoid arthritis" had "definite" or "classical" rheumatoid arthritis according to the diagnostic criteria of the American Rheumatism Association (Ropes et al 1959). This means that these patients demonstrated at least five of the diagnostic criteria listed in table 1 and showed none of the excluding clinical features in table 2. All these patients were carefully characterised clinically and immunologically. A careful and detailed clinical history was obtained particular attention being paid to all past and present drug therapy and any gastro-intestinal symptomatology. A comprehensive and painstaking clinical examination was carried out on all these patients, both generally and with particular reference to the locomotor system. The severity of the inflammatory joint disease was characterised by means of grip strength (Lee, Baxter, Dick and Webb 1973) digital joint circumference (Webb, Downie, Dick and Lee 1973), duration of morning stiffness (Lee, Sturrock, Kennedy and Dick 1973), articular index of joint tenderness (Ritchie et al 1968), pain index (Lee, Webb Anderson and Buchanan 1973), and articular uptake of radioactive technetium (Dick 1972). Particular care was given to the examination of all of these patients for

any evidence of the complications of rheumatoid arthritis: vasculitis, splenomegaly, hepatomegaly, rheumatoid nodules, polyserositis, pulmonary disease or neurological impairment. A large proportion of patients with rheumatoid arthritis have Sjøgren's syndrome (Sjøgren 1943). Since this cannot always be detected by clinical examination I conducted laboratory tests to determine this. These included salivary flow rates (Mason et al 1967); sialography (Park and Mason 1966); salivary gland uptake of radioactive technetium ($99^{m}Tc$) (Hilditch et al 1967); labial gland biopsy (Whaley et al 1969); Schirmer tear-test (Williamson et al 1967); rose bengal staining of the conjunctivae (Whaley et al 1973) and biomicroscopy with the Haag-Streit or the Nikon slit-lamp (Whaley et al 1973).

Of 152 patients with rheumatoid arthritis taking part in these studies 40 were male and the mean age of the group was 56.37 years (range 25 to 82 years). The mean duration of the disease was 12.57 years (range 3 to 42 years) and all had a peripheral, symmetrical, inflammatory polyarthritis (mean articular index 19.8 score units $\pm$ S.E.M. 1.58 score units). One hundred and twenty five of these patients were sero-positive for rheumatoid factor (mean titre 1/446.2 $\pm$ 57.6 SEM),

thirty three sero-positive for anti-nuclear factor (mean titre 1/56.2 $\pm$ 11.88 SEM) and twenty one sero-positive for gastric-parietal cell auto-antibody. Thirty seven patients had documented evidence of Sjøgren's syndrome. In none of these patients was there any evidence of any coincident disease of another major system although in 23 there was evidence of a visceral complication of the rheumatoid disease (Table 3). All of these patients had received or were receiving one or more of a diverse group of non-steroidal anti-inflammatory drugs. Six patients were receiving or had received chrysotherapy and 34 were receiving long-term low-dose oral corticosteroids (< 7.5 mgm. of prednisolone or equivalent per day).

One hundred and four control volunteers, who were carefully age and sex-matched were selected for study from patients attending the Accident and Emergency Department of the Royal Victoria Hospital, Belfast with minor trauma. None of these controls had any obvious clinical evidence of rheumatic, gastro-intestinal or renal disease.

SYSTEMIC LUPUS ERYTHEMATOSIS (SLE)

15 patients with established SLE were studied. All but one were female and the mean age was 44.0 years (range 28 - 51 years). The mean duration of their disease was 11.5 years (range 2 - 25 years). All had positive tests for anti-nuclear factor in titres in excess of 1 in 64 mean ($\pm$ S.E.M.) reciprocal titre 475.2 $\pm$ 107.2. The clinical and laboratory features of these patients are shown in table 4. It will be noted that of the entire group 4 patients had clinical evidence of renal involvement. No patient in this group was shown to have elevated levels of DNA binding (in excess of 50%) at the time of study. Five patients were receiving low dose corticosteroid therapy ($<$ 7.5 mgm. of prednisolone or equivalent daily) and five were receiving high dose corticosteroid therapy(mean ($\pm$ S.E.M.) dose per day 19.4 $\pm$ 2.1 mgm. prednisolone or equivalent).

PSORIATIC ARTHRITIS

Eight patients were studied of whom 5 were male. Their mean age was 34.8 years (range 18 - 72 years) and the mean duration of their arthritis and of their psoriasis was 7.3 years (range 1 - 25 years) and 17.9

years (range 7 - 51 years) respectively. All were sero-negative for rheumatoid factor and all had an erosive, inflammatory polyarthritis of typical distribution (Duthie 1970) and evidence of sacro iliac involvement. In five patients there was evidence of characteristic nail dystrophy and in two there was evidence of periostitis on radiological examination. None of these patients was receiving corticosteroids or chrysotherapy but all were receiving a wide range of non-steroidal anti-inflammatory drugs and one patient had had a course of methotrexate which had been discontinued three months prior to the time of study.

OSTEOARTHROSIS

Twenty five patients with osteoarthrosis either of the oligo-arthritic or polyarthritic type were studied. Seven were male and their mean age was 67.4 years (range 41 to 84 years). Most (17) of these patients had severe clinical and radiological osteo-arthrosis of the hip joint. None had diabetes or evidence of disease of other major systems; all had a normal erythrocyte sedimentation rate and haemoglobin concentration and all were sero-negative for rheumatoid factor. All were receiving analgesic or non-steroidal

anti-inflammatory drugs.

ANKYLOSING SPONDYLITIS.

Seventeen male patients with ankylosing spondylitis whose mean age was 34.2 years (range 19 to 63 years) were studied. All of these patients had low back pain and stiffness, radiological evidence of bilateral sacro-iliitis and morning stiffness mean ($\pm$ S.E.M.) duration 0.6 $\pm$ 0.1 hours. All were receiving phenylbutazone, indomethacin or naproxen and none had at any time received radiotherapy. All patients had W27 tissue type antigen. Four patients had had uveitis and six had a peripheral arthritis but none had evidence of aortic incompetence.

TUBERCULOSIS

Sixteen patients with bacteriologically confirmed tuberculosis were studied. Twelve of these were male and the mean age of the group was 59.4 years (range 27 - 79 years). All had early active disease as demonstrated by systemic symptoms and chest radiology and all had been recently commenced on anti-tuberculous chemotherapy (ethambutol isoniazid, and/or rifampicin). None of these patients had clinical involvement of any other organ system.

MYOCARDIAL INFARCTION

Nine patients were studied within 3 to 5 days of an episode of prolonged typical chest pain followed by sequential cardiographic and serum enzyme changes. Seven of these patients were male and their mean age was 54.2 years (range 46 to 67 years). In every patient this was their first episode of infarction and in none were there serum lipoprotein abnormalities likely to be aetiological in the infarction (Rooney, Ballantyne and Buchanan 1975). When blood was withdrawn for serum IRG concentrations these patients had all been pain-free for at least 72 hours.

All of these patients and controls had blood withdrawn after a 10 hour overnight fast for radio-immunoassay of the foregut hormones. The effects of administration of individual anti-inflammatory drugs were studied in small sub groups of the patients with rheumatoid arthritis. Aspirin 3g daily, indomethacin 200 mgm. daily, phenylbutazone 300 mgm. daily, tetracosactrin 0.5 mgm. daily and ascorbic acid 600 mgm. daily were administered alone to at least eight volunteer patients for a period of 14 days. Fasting serum for immunoreactive gastrin was obtained before and after this time.

Twenty five outpatient volunteers from among the patients with rheumatoid arthritis were submitted to gastroscopy using an Olympus GFBK side-view, fibre-optic endoscope. Premedication was with intravenous diazepam in doses ranging from 4-47 mgms. (mean ($\pm$ SEM) 12.4 mgms. $\pm$ 4.3 mgm.). Gastric fundic mucosal biopsy was obtained through the gastroscope under direct vision in 18 subjects and submitted for histological examination.

Serum immunoreactive gastrin was assayed by the technique described in detail in the introductory chapter on radio-immunoassay.

Gastric acid output was measured by the classical technique of Kay (1953). Stimulation of acid secretion was obtained using histamine acid phosphate 40 µg/kg body weight (Kay 1953) and pentagastrin 6 µg/kg body weight (Wormsley et al 1966). Nasogastric tube positioning was obtained in the first five tests by direct X-Ray screening and thereafter by the use of the water recovery technique (Findlay et al 1972).

The analysis of distribution of immunoreactive gastrin values was by means of Pearson's second coefficient of skewness and the moment coefficient of

kurtosis (Spiegel 1972).

In 44 of the patients with rheumatoid arthritis the effect of the ingestion of 10 mls. of 0.1 molar H.Cl. on the concentration of immunoreactive gastrin was assessed over a period of 30 minutes. Similar studies were carried out on control colunteers.

During the course of my clinical studies on immunoreactive gastrin in rheumatoid arthritis I had the opportunity of studying 14 patients with classical rheumatoid arthritis in whom routine clinical management indicated the aspiration of synovial effusions of the knee. In addition in two further patients pleural aspiration was indicated for the presence of rheumatoid pleural effusions. These aspirations were all performed under fasting basal conditions and a simultaneous plasma sample was obtained. Immunoreactive gastrin measurements were carried out on all blood and serous fluid samples.

RESULTS

Striking differences are apparent between the fasting immunoreactive gastrin of the patients with rheumatoid arthritis and that of the controls. Figure 1 shows the results obtained in the first 50 patients with rheumatoid arthritis studied. The mean value of 171 pg/ml (± 38 pg/ml S.E.M.) is significantly greater than that of the controls 56 pg/ml (± 8 pg/ml S.E.M.). Even more striking however is the very great elevation of immunoreactive gastrin in some of the patients with rheumatoid arthritis. Figure 2 shows the results of the complete group of 150 patients and shows that this initial observation is borne out in the larger group. Figure 3 shows the arithmetic distribution of the results of the control subjects and it is quite obvious that the distribution of values is skewed. The curve has a coefficient of skewness of 20.0 and a coefficient of kurtosis of 50.05. For a completely normal distribution both of these figures should approach zero. Figure 4 shows that when these same results are plotted on a logarithmic scale the curve of the normal values is much closer to a normal distribution. In this instance the coefficient skewness is 3.15 and the coefficient of kurtosis 2.54. This graph also shows

a striking parallel between the control results and those obtained in rheumatoid arthritis. The curve obtained in the group of patients with rheumatoid

of incidence between 10^3 pg/ml and $10^{3.5}$ pg/ml. nd peak

If an arbitrary cut-off is made at $10^{2.5}$ pg/ml of°

If an arbitrary cut-off is made at $10^{2.5}$ pg/ml of gastrin it can be seen that of the controls only 2 lie at levels of immunoreactive gastrin greater than this whereas of the rheumatoid arthritic subjects 33 lie at greater levels. A χ^2 test of this gives a value of 20.0 which suggests a significant difference at the 0.1 per cent level although caution in this interpretation is essential in view of the small figure in the rheumatoid group. Stronger evidence for bimodality is afforded by analysis of the rheumatoid group by means of an f test using the same cut-off point. This gives a value of 17.15 ($p < 0.002$) indicating that the two sub-groups are samples of different populations.

The results of studies of maximal gastric acid output are summarised in table 5 and figure 5. These studies were carried out in 16 subjects with very markedly elevated levels of immunoreactive gastrin (Table 6). In all 16 subjects studies were carried out using pentagastrin as the secretory

stimulus while in ten a further study using histamine acid phosphate was obtained. It can be seen from table 6 that the acid outputs are normal both during the basal hour and in the post-stimulated hour regardless of the stimulus used. There was only one exception (Patient 6). This lady had histamine and pentagastrin-fast achlorhydria and was subsequently shown to have early pernicious anaemia. No relationship could be demonstrated between these acid outputs, either basal or after stimulation, and the serum concentrations of immunoreactive gastrin (Figures 6 and 7).

Attempts were made to correlate immunoreactive gastrin concentration with anti-inflammatory drug therapy. The mean ($\pm$ S.E.M.) of the immunoreactive gastrin concentrations obtained on the various drug therapies the patients were receiving at their initial admission to these studies is seen in table 7 and figure 8 whole those obtained in the more controlled situation of the studies on single drug administration are seen in table 8 and figure 9. No significant pattern is evident in these results and in particular no single drug appears to be responsible for the elevated concentrations of immuno-reactive gastrin.

In one patient at endoscopy a benign lesser curve gastric ulcer was encountered (figures 10,11). No other significant pathology was found. In particular there was no evidence of gastric mucosal atrophy either macroscopically or histologically (figure 12).

Figure 13, shows that there is no correlation between age and the concentration of immunoreactive gastrin either in the controls or in the patients with rheumatoid arthritis and this remains true even if the rheumatoid group is sub-divided into those with normal immunoreactive gastrin concentrations and those with elevated ones (figure 14 + 15). Figure 16 shows that elevation of immunoreactive gastrin cannot be correlated with the presence of either IgM rheumatoid factor or gastric parietal cell auto-antibody. Even in those patients in whom IgM rheumatoid factor is present no correlation could be found between the titre of this factor and the serum concentration of immunoreactive gastrin. This holds true whether all of the patients are included or whether only the sub-group of patients with elevated immunoreactive gastrin levels (figure 16) are considered.

No correlation between immunoreactive gastrin and any clinical or laboratory parameter of the activity of inflammatory joint disease could be demonstrated. Table 9 lists the parameters of joint disease studied in this way.

Elevated levels of immunoreactive gastrin tend to be consistent in any one patient. Table 10 indicates the repeatability of gastrin assayed in the same control subjects over a brief interval after an overnight fast. Table 11 gives the same information for subjects with rheumatoid arthritis in whom the immunoreactive gastrin was below 500 pg/ml. Table 12 is a similar study in subjects with elevated immunoreactive gastrin. The repeatability of immunoreactive gastrin on consecutive days is given in the same situations in tables 13, 14 and 15 and in the rheumatoid arthritis patients the repeatability over a period of 6 months is given in tables 16 and 17. From these last two tables it can be seen that changes in immunoreactive gastrin do occur over this period but as seen in figures 17 and 18 no correlation between these changes and the changes in clinical parameters of disease activity could be demonstrated.
Table 18 shows that of all the disorders studied apart

from rheumatoid arthritis no patient had an immunoreactive gastrin concentration greater than the highest control value of 400 pg/ml (Figure 19).

Fasting immunoreactive gastrin in eight control subjects and in 37 of the patients with rheumatoid arthritis in whom the effect of the oral ingestion of hydrochloric acid was studied showed normal initial immunoreactive gastrin values (figure 20). In these subjects no significant change was documented after acid. However, in the seven subjects with rheumatoid arthritis in whom the initial immunoreactive gastrin proved to be greater than 125 pg/ml. a paradoxical elevation was noted in response to acid ingestion although this rise did not reach levels of statistical significance ($t = 1.57$ $p < 0.10$). Table 19 indicates the values of immunoreactive gastrin in synovial fluid in patients with rheumatoid arthritis and in the pleural fluid two patients with pleural effusions attributable to rheumatoid arthritis. The table also records the corresponding plasma values from samples obtained at the same time. No significant difference exists between plasma immunoreactive gastrin levels and the levels in the serous fluids.

DISCUSSION

In the treatment of rheumatoid arthritis, the clinical value of virtually all non-steroidal anti-inflammatory drugs is limited by the incidence of gastro-duodenal irritation and ulceration. It has been suggested that these two properties of these drugs are probably interdependent and inseparable (Douglas 1965). Despite considerable research effort no convincing pharmacological explanation of this effect has been offered. It was this problem of clinical management that led to the use of the radio-immunoassay facilities offered to me in Belfast by Dr. Keith D. Buchanan in order to study the problem in greater depth.

Nonetheless the finding of striking hypergastrinaemia in some patients with rheumatoid arthritis was surprising and led to a full programme of study to try and elicit the cause of the phenomenon. My initial efforts were directed to the known causes of hypergastrinaemia. Hypergastrinaemia occurs physiologically in response to food ingestion particularly protein foods (Korman et al 1971) but the ten hour overnight fast in all patients in the present studies would exclude any

physiological food response mechanism accounting for the elevated levels encountered in those patients with rheumatoid arthritis. In the Zollinger Ellison syndrome hypergastrinaemia is the characteristic feature of the condition and is causative for the constant profuse hypersecretinn of gastric acid and pepsi n encountered in the condition (Aoyagi and Summerskill 1966) and which in turn is responsible for the severe, malignant, peptic ulceration which is the primary clinical problem in this disorder (Zollinger and Ellison 1955). This is equally true whether the condition is due, as is more common, to a tumour of the gastrin secretory cells of the stomach or pancreas (non-beta islet cell adenoma or carcinoma) or to antral cell hyperplasia where it has been suggested that the stimulus is hypophyseal in origin (Polak et al 1972). The extreme rarity of the Zollinger-Ellison syndrome, the lack of the characteristic malignant acid-peptic disease, and the normal basal and stimulated gastric acid outputs in this study exclude this as the cause of the findings.

Hypergastrinaemia has been well documented in chronic atrophic gastritis with or without concomitant pernicious anaemia (Hansky et al 1971; McGuigan et al 1970);

Ganguli et al 1971; Creutzfeldt et al 1971).

Ganguli and his colleagues (1971) suggest that the elevation of immunoreactive gastrin in this situation is inversely proportional to the amount of gastric acid that can be secreted and it is suggested that the mechanism of this hyper-gastrinaemia is loss of the antral pH physiological feedback control (Rooney, Grennan and Millar 1974). Although chronic atrophic gastritis with early pernicious anaemia was encountered in one patient (Patient No.6) such gastritis does not account for the high incidence of hypergastrinaemia in this series of patients with rheumatoid arthritis. A number of factors support this conclusion: Only 23 patients (15.3 per cent) with rheumatoid arthritis had positive tests for gastric parietal cell auto-antibody and of these only three had high concentrations of immunoreactive gastrin. While not excluding atrophic gastritis this prevalence is lower than one would anticipate from that recorded in other series (Irvine et al 1965). The normal acid outputs of those patients with elevated serum immunoreactive gastrin concentrations weighs very heavily against a diagnosis of chronic atrophic gastritis (Strickland et al 1973) and the normal histology in all

the mucosae studied by biopsy adds further weight to this conclusion. Hypergastrinaemia has been reported as a result of gastric surgery where an intact antrum has been excluded from the acid stream. This is again almost certainly due to loss of the pH inhibition feedback control mechanism (Korman et al 1972). Review of the current 150 rheumatoid patients shows that only four had had previous gastric surgery and that immunoreactive gastrin was normal in all of these. This excludes this as the aetiology of the hypergastrinaemia in the whole group. Recent reports have indicated that elevation of serum immunoreactive gastrin occurs in patients with chronic renal failure (Korman et al In this situation it appears that the elevation is accompanied by hyper-secretion of gastric acid (Wormsley 1973). In the group of patients with rheumatoid arthritis blood urea and plasma creatinine were used as indices of renal function. Only one patient had a significant elevation of these parameters (blood urea 250 mgm/100ml and plasma creatinine 2.2g/ 100ml) due to secondary amyloidosis and it is of interest that this patient's serum immunoreactive gastrin was normal. It is not possible therefore to

attribute the hypergastrinaemia in these patients with rheumatoid arthritis to renal failure.

McGuigan and Trudeau (1970) have presented evidence that serum gastrin concentration rises with age. This was confirmed by Strickland et al (1973) but detailed study of their group allowed them to conclude that this was due to an increasing incidence of chronic atrophic gastritis. In this present study no correlation could be demonstrated between serum immunoreactive gastrin and age in the control group and in the group with rheumatoid arthritis the ages of the patients with elevated immunoreactive gastrin concentrations ranged from the third to the eighth decades and statistical analysis failed to show any significant correlation with age either in the group as a whole or in the subgroup with elevated levels (Figure 15).

As noted in the general discussion on gastric physiology in the introductory chapter of this thesis gastrin is closely involved in the metabolism of calcium and probably of other divalent cation systems.

Initial studies failed to indicate any correlation between calcium and phosphate levels and the presence of hypergastrinaemia (Figure 21) but it

has recently been shown that calcium metabolism is abnormal in rheumatoid arthritis when account is taken of the protein abnormalities in the serum (Kennedy et al 1975). Further studies on the basis of these findings are reported in a later part of this work.

If the finding of elevated serum concentrations of immunoreactive gastrin in rheumatoid arthritis cannot be accounted for by any of the physiological or pathological factors known to affect gastrin homeostasis, a number of important questions are raised: Could this finding be due to any of the immunological or serological abnormalities encountered in rheumatoid disease ?; , could it be due to the chronic ingestion of analgesic or anti-inflammatory drugs which is part of the way of life of anyone suffering from rheumatoid arthritis ? ; could it be related to the activity or severity of the inflammatory process ? ; could it be due to any artefact in the assay system or to some other unsuspected mechanism ?

The sera of patients with rheumatoid arthritis contain many immunological and biochemical abnormalities. Rheumatoid factor is present in about 75 per cent of adult subjects with "classical" or "definite" disease

(Dixon 1960). This factor is a 19S macroglobulin of the IgM type with a molecular weight of about one million. It circulates combined with smaller immunoglobulins of the IgG variety as soluble complexes and it can be detected by a variety of precipitation techniques (Vaughan 1966). Of the 150 patients in this study 125 had sera positive for rheumatoid factor in a titre of 1 in 32 or greater/ Mean ($\pm$ S.E.M.) reciprocal titre 446.2 $\pm$ 57.6 /. Recent studies on the radio-immunoassay techniques for the assay of thyroid stimulating hormones have indicated that when the serum levels of immunoglobulins have been unusually high in the presence of para-proteinaemia, such immunoglobulins has interfered with the assay producing unexpectedly high levels of hormone (Chapman, Hunter, and Hatter 1974). It seemed important to see if the hypergastrinaemia in our series could be due to similar interference by IgM rheumatoid factor. As figure 16 demonstrates no correlation is present between the presence or titre of rheumatoid factor and immuno-reactive hypergastrinaemia. Nor could any correlation be found between raised concentration of immunoreactive gastrin and the presence of anti-nuclear factor in

the sera of patients with rheumatoid arthritis (Fig.22). This study does not exclude the possibility of this effect being due to the smaller more soluble IgG rheumatoid factors.

As has been indicated all subjects with rheumatoid arthritis in the present study were taking anti-inflammatory drugs for their joint disease. Particular attention has been paid to this in the anticipation that such drugs may well interfere with secretion or metabolism of gastrin and the possibility that the capacity of these agents to induce gastro-duodenal irritation and/or ulceration is mediated through this type of endocrine change. The possibility of these agents or their metabolites in the serum interfering in the radio-immunoassay must also be borne in mind. Initially the drug therapy of the first 50 patients with rheumatoid arthritis was studied in detail. No pattern was evident in this group which would have suggested that any particular anti-inflammatory compound was responsible for the elevation of serum immuno-reactive gastrin. The mean immunoreactive gastrin ($\pm$ S.E.M.) of the patients on each individual anti-inflammatory agent are shown in Table 7. Although the

mean immunoreactive gastrin of each group varies widely it can be seen that the standard errors of these means are very large and statistical analysis confirms that these groups are not significantly different. The variation is almost certainly due to the fact that each group contains a few patients with very high concentrations of immunoreactive gastrin. However even when these groups are subdivided into patients with high and those with low levels of immunoreactive gastrin there is still no significant pattern to the results (Table 20). The studies carried out on the synovial fluid of patients with rheumatoid arthritis would appear to indicate little or no difference between such fluid and plasma in respect of immunoreactive gastrin (Table 19). However no patient with very high level of immunoreactive gastrin has yet been encountered in whom I have had the opportunity of obtaining a sample of joint fluid during therapeutic or diagnostic aspiration especially as such subjects would also require to be fasting. It must therefore remain a possibility, however remote, that the gastrin in those patients with elevated levels derives from the joint fluid.

Despite there appearing to be no correlation between anti-inflammatory drug therapy and immuno-reactive gastrin it seemed important to reaffirm this in a less complicated clinical situation and to ascertain, if possible, whether elevation of immuno-reactive gastrin in some patients was a property common to a wide group of anti-inflammatory compounds. A series of anti-inflammatory drugs were administered alone to small groups of volunteer patients. The drugs used included ascorbic acid as a placebo to attempt to assess the immunoreactive gastrin status of patients who were not taking any anti-inflammatory drugs. As the results clearly show no anti-inflammatory drug studied caused any significant change in immunoreactive gastrin. These studies clearly indicate that the hypergastrinaemia is not related to interference in the assay system by any of the anti-inflammatory drugs and they strongly suggest that elevation of immunoreactive gastrin in rheumatoid arthritis is not due to anti-inflammatory drug exhibition. This latter conclusion however must remain guarded as the time scale of drug therapy in rheumatoid arthritis is measured in years rather than days so it must remain, at present, an untested possibility that

long-term therapy with any or all of the anti-rheumatic drugs can in some instances cause immuno-reactive hypergastrinaemia.

These studies of immunoreactive gastrin have been carried out over a period of two years. Throughout this time intensive efforts have been made to find a clinical parameter which would correlate with immuno-reactive hypergastrinaemia. Initial efforts were directed to the manifestations of the inflammatory reaction in the joints of the patients with rheumatoid arthritis in the expectation that gastrin would prove to be yet one more in the long list of acute phase reactants (Freeman 1970). Detailed study was made in the group of 50 patients first studied and reported (Rooney et al 1973) to correlate immunoreactive gastrin with disease activity. No correlation could be found between immunoreactive gastrin and the following clinical and laboratory parameters : age, sex, duration of disease, articular index of joint tenderness, digital joint circumference, grip strength, duration of morning stiffness, pain index, articular uptake of radio-active technetium, haemoglobin concentration, erythrocyte sedimentation rate, total haemolytic complement and immunoglobulin concentration.

In addition to these initial studies an attempt has been made to correlate changes in immunoreactive gastrin concentrations with changes in activity of inflammatory joint disease. While it can be seen that immunoreactive gastrin does change in some patients with time there was no evidence that such changes correlated with disease activity.

All of these studies have been carried out in the knowledge that immunoreactive hypergastrinaemia in rheumatoid arthritis might turn out to be an assay artefact. My earliest worry regarding this related to rheumatoid factor. As has been demonstrated earlier my studies have excluded IgM rheumatoid factor as the cause of such an artefact. IgG rheumatoid factor is much more difficult to eliminate from such a possibility. However if the hypergastrinaemia were due to such low molecular weight rheumatoid factor binding anti-gastrin antibody this interference would apply in the non-specific samples as well and render this interference obvious. This same argument could be used for the other abnormal serum factors encountered in rheumatoid arthritis such as circulating soluble complexes although these are less likely interfering factors as similar complexes have been demonstrated in

other inflammatory joint diseases in which we have to date been unable to demonstrate immunoreactive hypergastrinaemia.

It is possible that other abnormal proteins in the sera of patients with rheumatoid arthritis could cross react with the anti-gastrin antibody but this is unlikely in view of the identical dilutional curves obtained in the radioimmunoassay system especially when similar small peptide molecules such as pancreozymin/ cholecystokinin and secretin fail to show identity in this way even although in the case of cholecystokinin the C terminal tetrapeptide chain is identical to that of gastrin (Mutt and Jorpes 1967).

A bioassay system of gastrin would afford the opportunity of checking whether the immunoreactive material in rheumatoid arthritis is also biologically active. However since the advent of radioimmunoassay the availability of such a system has declined and the sensitivity of most bioassays is orders of magnitude different from that of the radioimmunoassay. To date I have been unable to set up a satisfactory bioassay system to study this particular problem. Lack of availability of appropriate surgical assistance and lack of animal house space have been the main factors in this.

TABLE 1

Diagnostic Criteria for Rheumatoid Arthritis
Diagnostic Criteria for Rheumatoid Arthritis
of the American Rheumatism Association

1. Morning Stiffness.

2. Pain on movement or tenderness in at least one joint (observed by a physician)

3. Swelling due to soft tissue or fluid in at least one joint (observed by a physician).

4. Swelling of at least one other joint within three months.

5. Symmetrical joint swelling with the same joints affected on both sides of the body at the same time.

6. Subcutaneous nodules over bony prominences on extensor surfaces.

7. X-Ray changes typical of rheumatoid arthritis.

8. Positive agglutination test (i.e. demonstration of rheumatoid factor by any method which in two laboratories has been positive in not more than 5 per cent of normal controls.

9. A poor mucin precipitate from synovial fluid with shreds and a cloudy solution.

10. Characteristic histological changes in synovial membrane: villous hypertrophy; proliferation of synovial cells; chronic inflammatory infiltrate with a tendency to form lymphoid nodules; deposition of compact fibrin and foci of cell necrosis.

11. Characteristic histological changes in nodules; i.e. granulomatous foci with central zones of necrosis and peripheral fibrosis and chronic inflammatory cell infiltrate.

TABLE 2

Exclusions from classification of rheumatoid arthritis

1. A typical rash of systemic lupus erythematosis.
2. High concentrations of L.E. cells.
3. Histological evidence of periarteritis nodosa.
4. Dermatomyositis or muscle weakness.
5. Definite scleroderma.
6. A characteristic clinical picture of acute rheumatic fever.
7. A characteristic clinical picture of gouty arthritis.
8. Tophi.
9. A characteristic clinical picture of acute infective arthritis.
10. Histological or bacteriological evidence of joint tuberculosis.
11. A characteristic clinical picture of Reiter's syndrome.
12. A characteristic clinical picture of the shoulder hand syndrome.
13. A characteristic clinical picture of hypertrophic pulmonary osteoarthropathy.
14. A clinical picture characteristic of neuroarthropathy.
15. Homogentisic acid in the urine.
16. Histological evidence of sarcoid or a positive Kveim test.
17. Multiple myeloma.
18. Characteristic skin lesions of erythema nodosum.
19. Leukaemia or lymphoma.
20. Agammaglobulinaemia.

TABLE 3

Visceral complications of rheumatoid encountered in 23 of 150 patients studied.

Complication	Number of patients
Scleritis	5
Cutaneous vasculitis	4
Neuropathy	4
Lymphadenopathy	3
Atlanto-axial subluxation	3
Felty's syndrome	2
Pericarditis	2
Pulmonary fibrosis	1
Amyloidosis	1
Digital arteritis and gangrene	1
Brown-Kelly Patterson oesophageal web	1

TABLE 4

Clinical features of 15 patients with S.L.E.

Total Number		15
	Female	14
	Male	1
Renal Lupus		4
	Proteinuria	4
	Azotaemia	2
	D.N.A. Binding	0
	Positive renal biopsy	1
Lung Involvement		4
Joint disease		14
Skin Involvement		8
C.N.S. Lupus		1
Sjøgrens syndrome		3
Liver Disease		4
Positive Coomb's test		2

TABLE 5

Acid Output in 16 patients with elevated immunoreactive gastrin

Patient	Basal Acid Output		Pentagastrin Stimulated Output		Histamine Stimulated Output	
	Vol. (mls)	Outp. (meq)	Vol. (mls)	Outp. (meq)	Vol. (mls)	Outp. (meq)
1	20	0.72	181.5	19.8		
2	85	1.71	187	20.9	160	19.2
3	55	0	48	7.05		
4	30	0.44	139	10.95		
5	14	0.97	151.5	13.3	200	12.0
6 *	4	0	15	0	20	0
7	35	1.73	138	12.69	150	13.95
8	8.4	0.08	279	23.4	206	16.5
9	69	918	230	50.6		
10	15	1.5	56	4.20		
11	17.5	3.06	245	39.4	193	33.4
12	88	5.28	181	17.6	130	12.1
13	72	1.65	118	10.15		
14	23	3.17	129	17.0	150	13.9
15	29	4.43	179	27.6	110	17.8
16	18	0.94	203	31.2	150	24.5
Mean		2.22		19.1		16.3
S.D.		2.55		13.2		8.7
S.E.M.		0.64		3.3		2.7

Standard error of difference between histamine and pentagastrin stimulated acid output 1.1 meq.

* Patient 6 confirmed to have pernicious anaemia.

Table 6

Immunoreactive gastrin concentrations in subjects with rheumatoid arthritis submitted to gastric analysis

patient	Immunoreactive gastrin	Basal Acid Output (meq)	Maximal acid output (pentagastrin stimulation) (meq)
1	800	0.72	19.8
2	400	1.71	26.9
3	500	0	7.05
4	700	0.44	10.95
5	1000	0.97	13.3
* 6	500	0	0
7	800	1.73	12.69
8	700	0.08	23.4
9	1300	9.8	50.6
10	1550	1.5	4.2
11	400	3.06	39.4
12	1000	5.28	17.6
13	1500	1.65	10.15
14	900	3.17	17.0
15	1000	4.43	27.56
16	1300	0.94	31.2
Mean	896.9	2.22	19.1
S.D.	369.9	2.55	13.2
S.E.M.	92 .5	0.64	3.31

* Patient 6 subsequently shown to have pernicious anaemia.

Table 7

Drugs being exhibited in 150 patients studied and the results of immunoreactive gastrin assay.

Drug	Number of Patients	Mean Gastrin	± S.E.M.
Aspirin	63	276.8	69.2
Indomethacin	80	224.2	46.8
Phenylbutazone	11	416.8	167.9
Corticosteroids and corticotrophin	51	227.3	55.2
Ibuprofen	6	346.6	165.3
Alclofenac	1	500	

TABLE 8

Drug	Dose	Immunoreactive gastrin pg/ml Mean ± S.E.M.		Articular Index Mean ± S.E.M.		ESR. Mean ± S.E.M.	
		Pre	Post	Pre	Post	Pre	Post
Aspirin	4g/day	102 ± 11	111 ± 9	15.6 ± 3.2	12.8 ± 2.4	11.4 ± 3.2	11.2 ± 3.7
Indomethacin	200 mg/day	152 ± 27	143 ± 22	17.4 ± 3.4	16.4 ± 2.8	8.2 ± 3.2	11.2 ± 2.4
Phenylbutezone	300 mg/day	127 ± 9	138 ± 11	5.3 ± 4.0	7.2 ± 3.0	15.1 ± 3.9	16.4 ± 5.2
Tetracosactrin	2.5 mg/day	111 ± 9	118 ± 7	17.3 ± 3.2	12.1 ± 3.2	15.2 ± 1.8	12.1 ± 3.4
Ascorbic Acid	600 mg/day	172 ± 33	162 ± 24	12.1 ± 1.1	12.2 ± 1.7	15.8 ± 3.2	14.9 ± 2.4

Table 9

Assessments of disease activity of rheumatoid arthritis studied in relation to elevation of immunoreactive gastrin.

1. Pain Index
   (Lee, Webb, Anderson and Buchanan 1973)

2. Articular index of joint tenderness
   (Ritchie et al 1968)

3. Duration of morning stiffness
   (Lee, Sturrock, Kennedy and Dick 1973)

4. Grade of morning stiffness
   (Bäckland and Tiselius 1967)

5. Grip Strength
   (Lee, Baxter, Dick and Webb 1973)

6. Proximal interphalangeal joint circumference
   (Webb, Downie, Dick and Lee 1973)

7. Erythrocyte sedimentation rate
   (Lansbury 1965)

8. Patients assessment of change
   (Lee, Webb, Anderson and Buchanan 1973)

Table 10

Immunoreactive gastrin concentrations (pg/ml) in the same control subject in two samples withdrawn within 30 minutes in the fasting state.

20	25
30	30
10	15
60	50
15	10
25	35
0	0
10	10
25	20
15	10
Mean 21.0	20.5
S.D. 16.3	14.8
SEM. 5.2	4.7

Standard error of difference

$\pm$ 1.9 pg/ml.

Table 11

Immunoreactive Gastrin Concentrations (pg/ml) in the same patient with rheumatoid arthritis in two samples withdrawn within 30 minutes in the fasting state (No patient with initial immunoreactive gastrin greater than 500 pg/ml included).

20	10
60	50
70	15
265	300
55	50
155	130
60	45
55	70
90	105
70	50
200	190
10	10
50	70
Mean 89.2	84.2
S.D.73.5	82.4
SEM. 20.4	22.9

Standard Error of Difference $\pm$ 6.4 pg/ml.

Table 12

Repeatability of immunoreactive gastrin concentrations (pg/ml) in six patients with rheumatoid arthritis with initial values greater than 500 pg/ml. The two samples withdrawn within 30 minutes in the fasting state.

900	1065
1125	1145
1250	1000
750	700
1550	1500
1270	1200
Mean 1140	1102
S.D. 285.3	262.0
SEM. 116.4	106.9

Standard error of difference

$\pm$ 55.0 pg/ml.

Table 13

Fasting immunoreactive gastrin concentrations (pg/ml) in plasma samples taken on consecutive days in the same control subject.

	Day 1	Day 2	Day 3
	10	10	15
	55	70	60
	20	20	20
	25	40	40
	30	40	35
	15	10	10
Mean	25.8	31.6	30.0
SD	15.9	23.1	18.7
SEM	6.5	9.5	7.6

Standard Error of difference -

Day 1 to Day 2 $\pm$ 3.5 pg/ml
Day 1 to Day 3 $\pm$ 2.7 pg/ml
Day 2 to Day 3 $\pm$ 2.1 pg/ml

Table 14

Fasting immunoreactive gastrin concentrations (pg/ml) in plasma samples taken on consecutive days in the same patients with rheumatoid arthritis (no patient included whose initial value exceeded 500 pg/ml)

Day 1	Day 2	Day 3
70	65	70
125	140	140
200	225	210
10	10	10
65	70	65
80	80	85
175	185	190
105	125	110
90	90	85
Mean 102.2	110.0	107.2
SD 58.0	66.0	63.3
SEM 19.3	22.0	21.1

Standard error of difference -

Day 1 to 2 $\pm$ 3.5 pg/ml

Day 1 to 3 $\pm$ 2.3 pg/ml

Day 2 to 3 $\pm$ 2.6 pg/ml

Table 15

Fasting immunoreactive gastrin (pg/ml) on consecutive days in six patients with initial values greater than 500 pg/ml.

Day 1	Day 2	Day 3
1250	1000	1100
900	500	650
1550	1500	1500
1000	750	750
1250	1500	1250
800	875	850
Mean 1125	1021	1016
SD 277.0	406.3	325.0
SEM 113.1	165.9	132.7

Standard error of difference -

Day 1 to 2 ± 98.4 pg/ml
Day 1 to 3 ± 52.3 pg/ml
Day 2 to 3 ± 56.4 pg/ml

Table 16

Immunoreactive gastrin in the same patients with rheumatoid arthritis over a period of six months (no patients with an initial value greater than 500 pg/ml included). Articular index on same days also recorded.

Initial Immunoreactive gastrin (pg/ml)	Initial Articular Index (Units)	Immunoreactive gastrin at least 6 mths. later (pg/ml)	Articular Index (Units)
20	23	80	20
80	3	85	11
450	11	400	12
80	19	100	20
10	11	10	11
110	0	30	2
150	5	200	8
155	8	100	12
30	21	70	14
70	14	75	6
85	9	20	7
60	7	300	9
70	6	100	5
85	5	25	8
90	9	110	7
200	6	20	5
10	7	10	12
Mean 103.2	9.7	97.8	9.9
SD 103.1	6.3	108.9	4.9
SEM 28.0	1.5	26.4	1.2

Standard error of difference of immunoreactive gastrin $\pm$ 20.9 pg/ml.

Table 17

Repeatability over a period of 6 months of immunoreactive gastrin in patients with rheumatoid arthritis in whom the initial value exceeded 500 pg/ml. Articular index on same day also recorded.

Initial immunoreactive gastrin	Initial Articular Index	Immunoreactive gastrin at least 6 mths. later.	Articular Index.
1200	17	1100	8
900	35	1100	17
1100	4	75	4
700	2	750	13
1560	5	1500	7
1750	0	2050	2
Mean 1200	10.5	1096	8.5
SD 393.7	13.4	668.7	5.6
SEM 160.7	5.5	272.9	2.3

Standard error of difference of immunoreactive gastrin $\pm$ -

Table 18

Immunoreactive gastrin in non-rheumatoid inflammatory disease

	Controls	Rheumatoid Arthritis	Psoriasis	S.L.E.	Ankylosing Spondylitis	Osteo-Arthrosis	T.B.	Myocardial Infarction
Mean I.R.G	56	312	68	67	25	59	86	90
S.D.	53	108	95	38	54	58	43	75
S. E.M.	8	57	16	14	6	11	16	16

Table 19

Joint Fluid Studies

Name	Serum Gastrin	Joint Fluid
Gibson	255	80
Kennedy	180	120
Ferguson	170	110
Bates	95	95
Hendry	40	160
McLarmon	110	215
Carrigan	75	45
Craig	125	135
Anderson	75	60
Wilson	90	115
Cullen	80	60
Johnstone	10	25
McLeod	65	45
Callaghan	85	10
Mean	103.92	91.07
Variance	3904.53	3158.3
S.D.	62.48	56.19
S.E.	16.70	15.01

$t = 0.658$ $p > 0.5$ N.S.

	Serum	Pleural Fluid
Callaghan	85	70
Bradley	110	100

Table 20

			Pre	Post
Aspirin	Low initial LRG Samples	(mean)	43.8	55.4
	High initial LRG Samples	(1 sample)	550	500
Indomethacin	Low initial LRG Samples	(mean)	45.1	49.2
	High initial LRG Samples	(1 sample)	900	800
Phenylbutazone	Low initial LRG Samples	(mean)	28.8	30.0
	High initial LRG Samples	(mean)	575	600
Tetracosactrin	Low initial LRG Samples	(mean)	36.5	52.4
	High initial LRG Samples	(mean)	67.5	800
Ascorbic acid	Low initial LRG Samples	(mean)	50.0	47.5
	High initial LRG Samples	(mean)	600	620

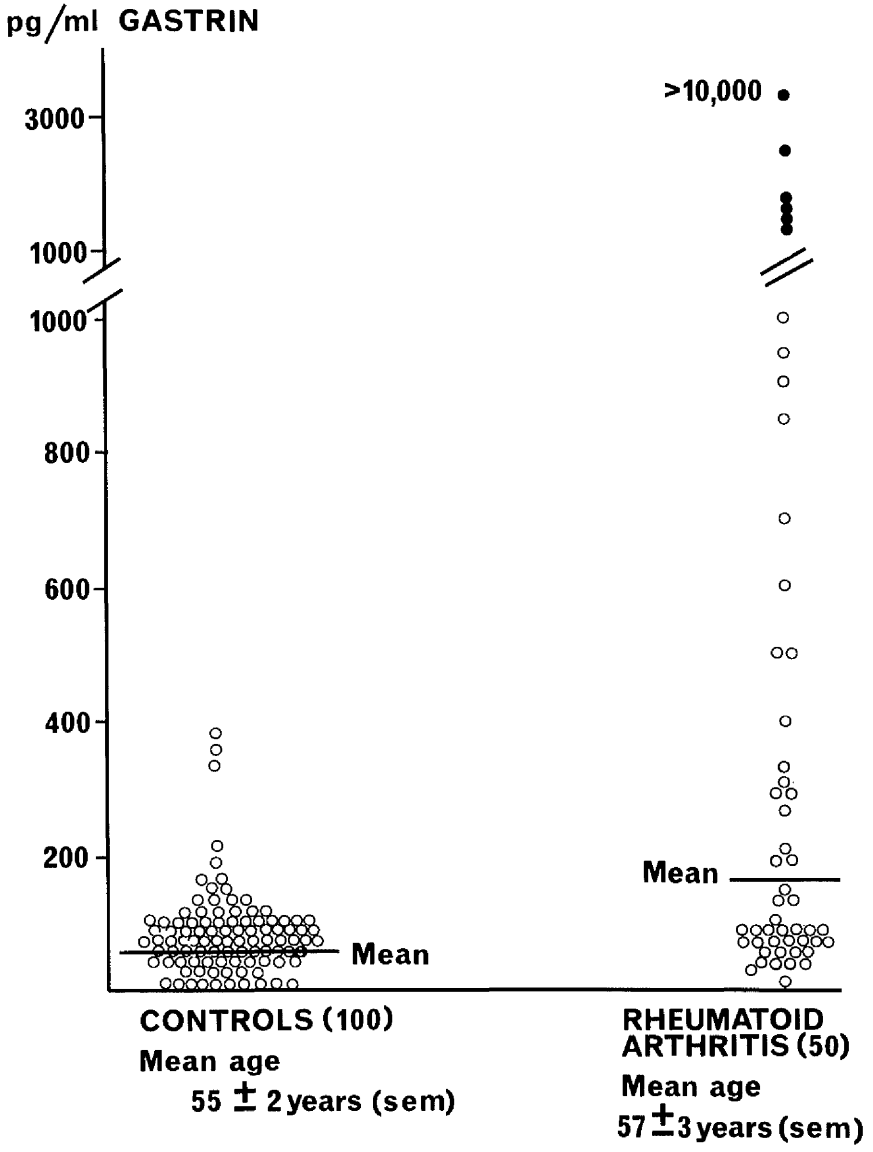


Figure 1.

Fasting immunoreactive gastrin concentration in 50 patients with rheumatoid arthritis and 100 control normal subjects.

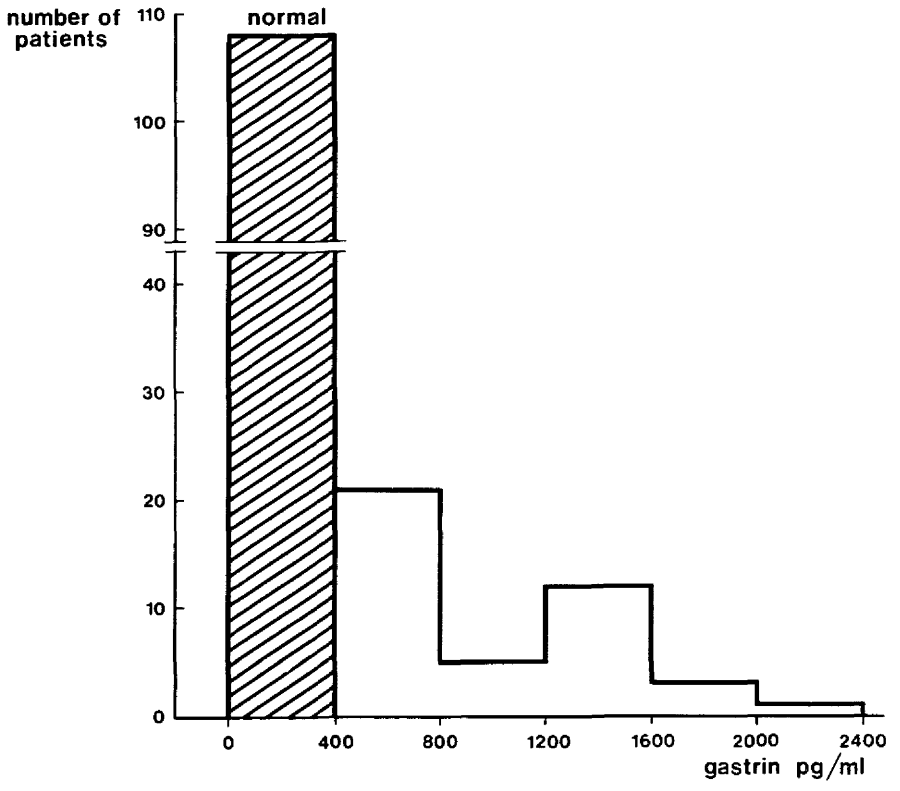


Figure 2.

Fasting immunoreactive gastrin concentrations in 150 patients with rheumatoid arthritis.

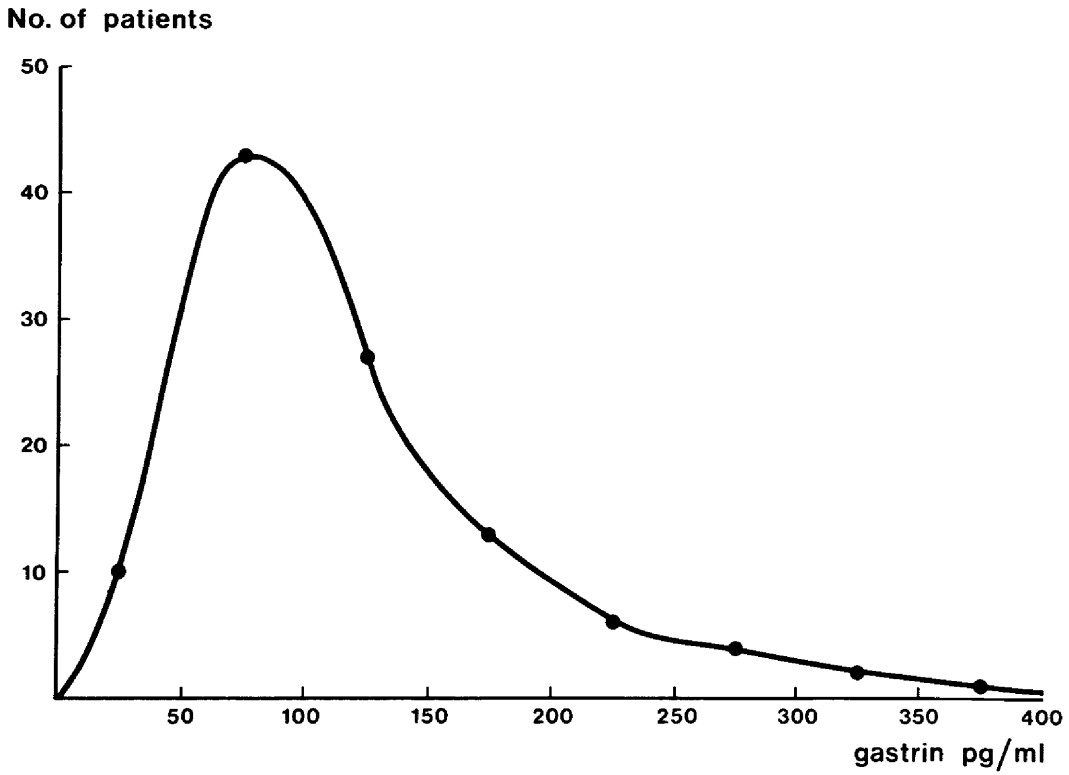


Figure 3.

Arithmetic distribution of fasting immunoreactive gastrin concentrations in 100 control subjects.

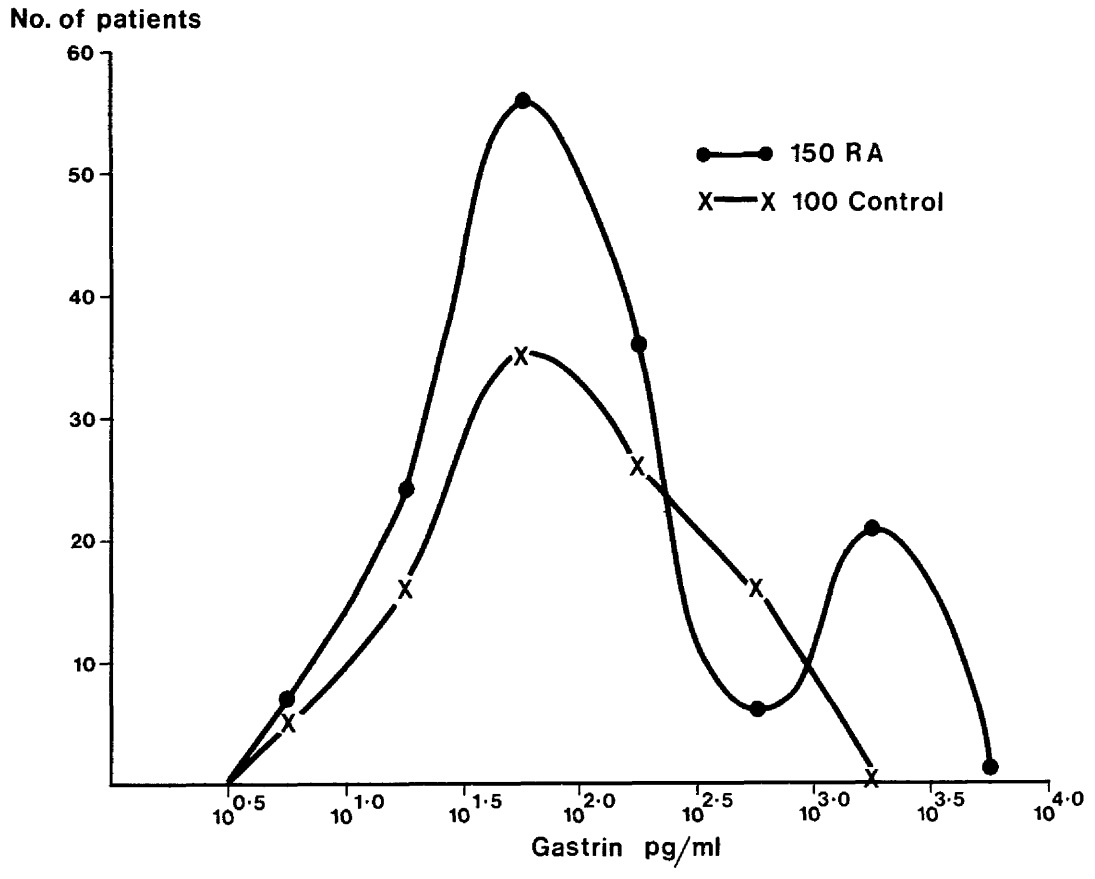


Figure 4.

Logarithmic distribution of fasting immunoreactive gastrin concentrations in 100 control subjects and 150 patients with rheumatoid arthritis. Note bimodal distribution in rheumatoid group.

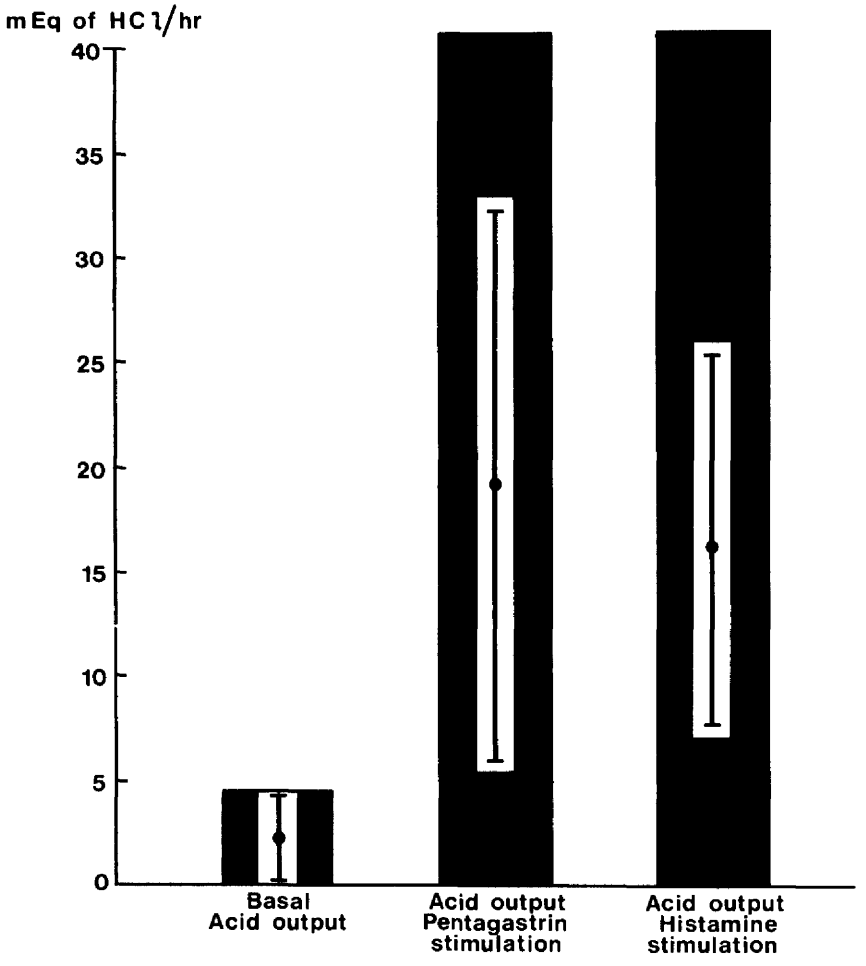


Figure 5.

Basal and maximal acid outputs in 16 patients with rheumatoid arthritis and elevated plasma immunoreactive gastrin.

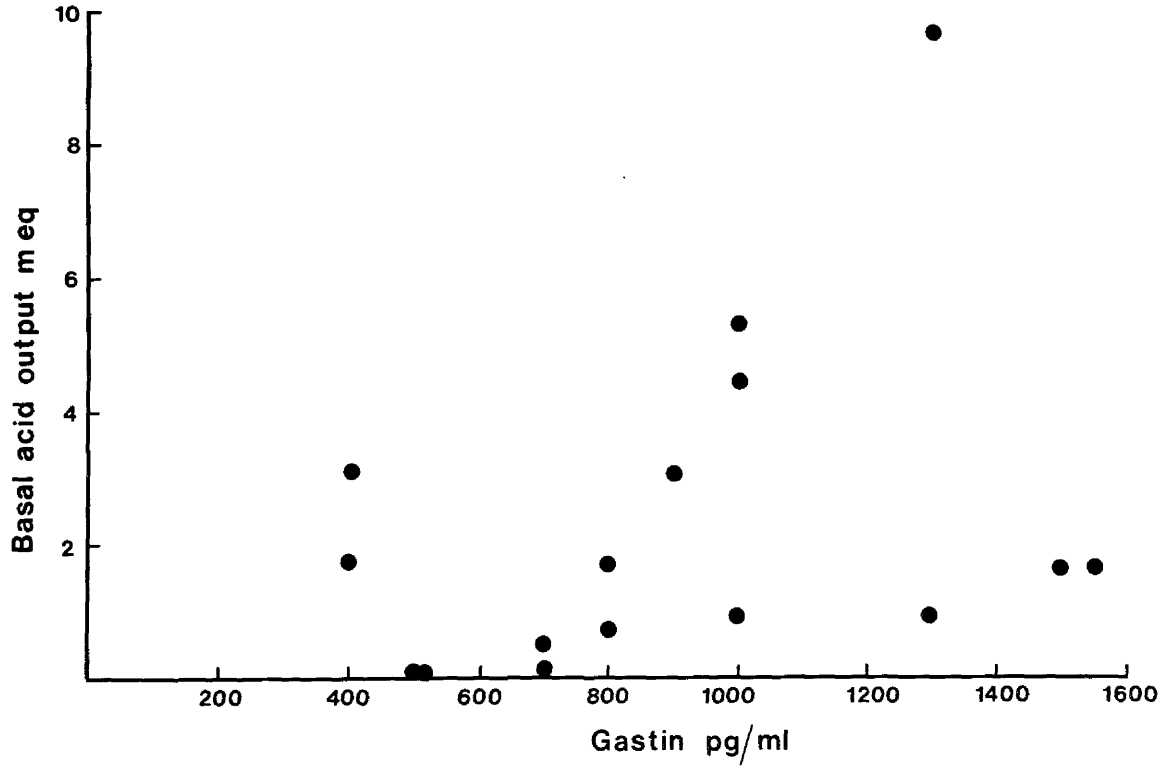


Figure 6.

Correlation between fasting immuno reactive gastrin concentration and basal acid output ($r = 0.32$; NS) in 16 patients with rheumatoid arthritis.

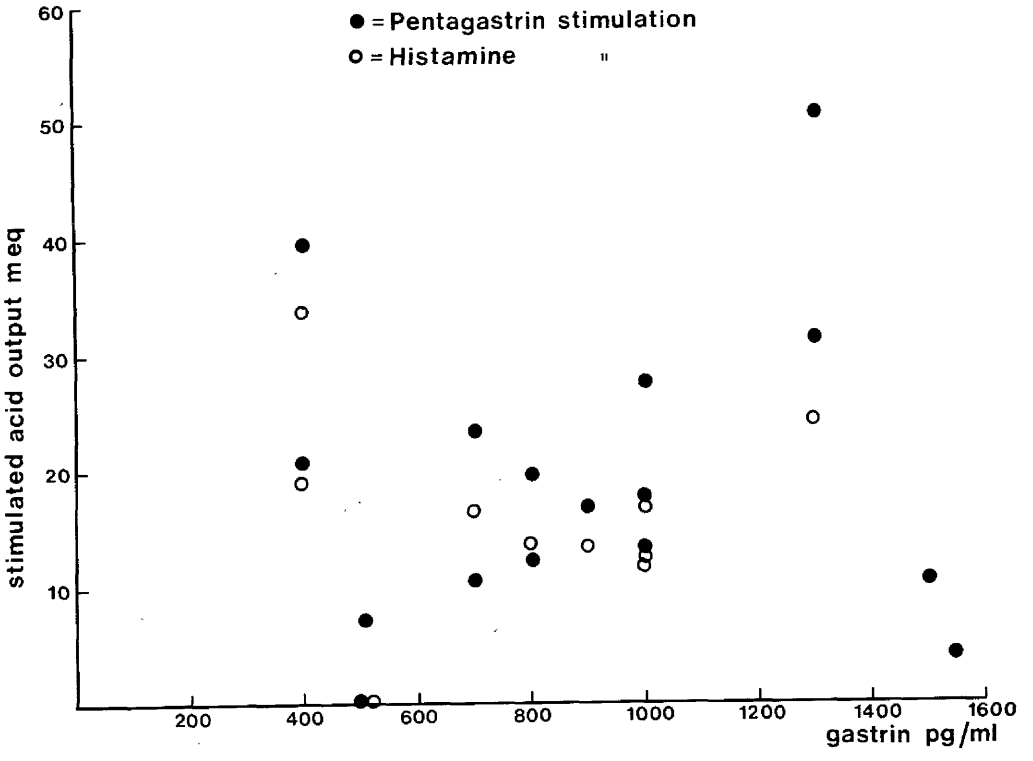


Figure 7.

Correlation between fasting immunoreactive gastrin concentration and stimulated gastric acid output in 16 patients with rheumatoid arthritis.

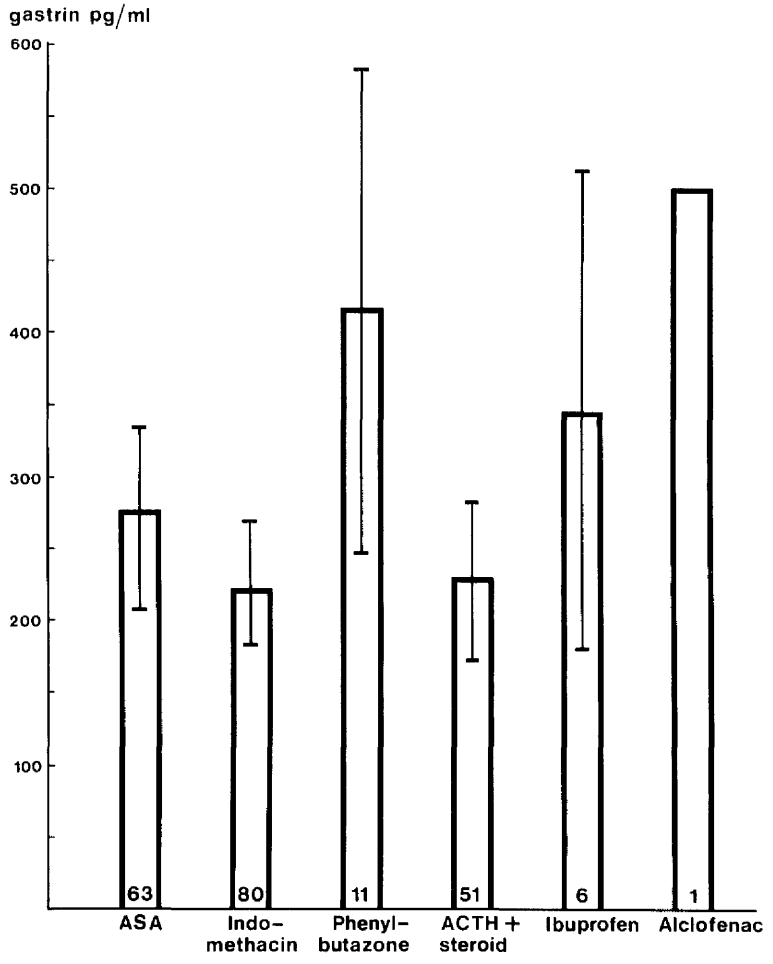


Figure 8.

Mean and Standard error of immunoreactive gastrin concentrations in 150 patients with rheumatoid arthritis divided according to the drugs being prescribed at the time of study. Numbers in boxes refer to number of patients taking each drug.

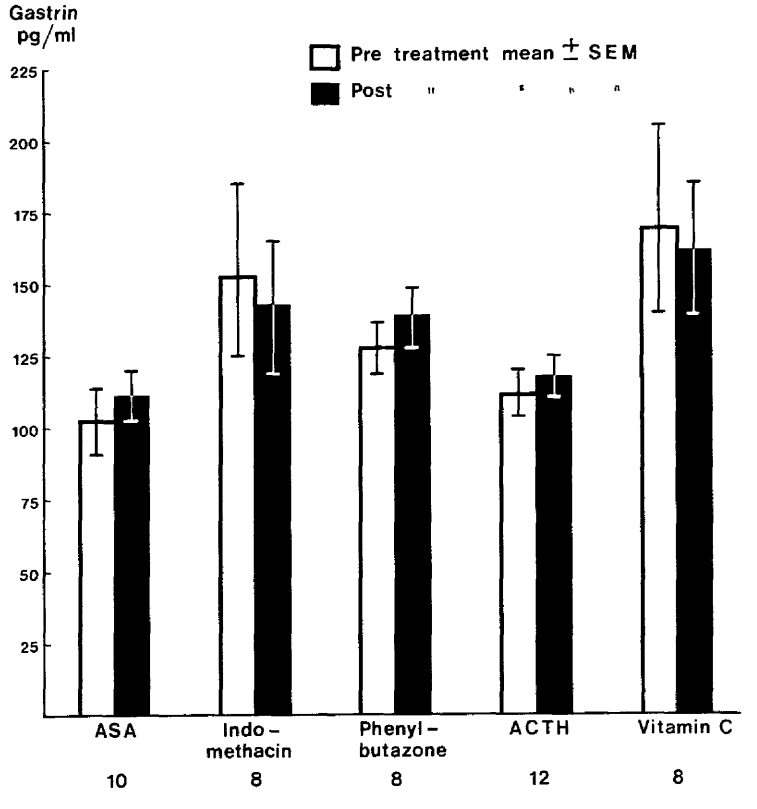


Figure 9.

Mean and standard error of immunoreactive gastrin in patients with rheumatoid arthritis taking single anti-inflammatory drugs for periods of 14 days. Numbers below each drug refer to number of patients taking each compound.

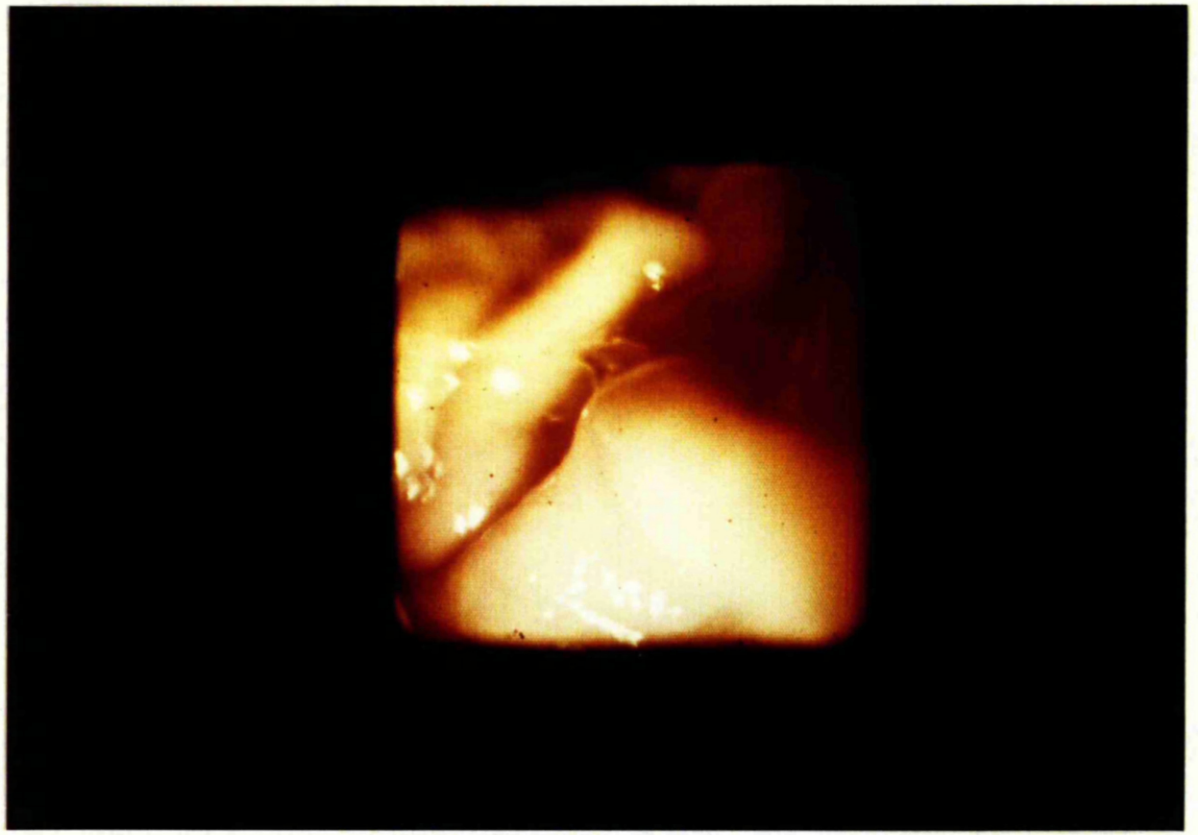


Figure 10.

Swollen mucosal folds at edge of chronic benign gastric ulcer.

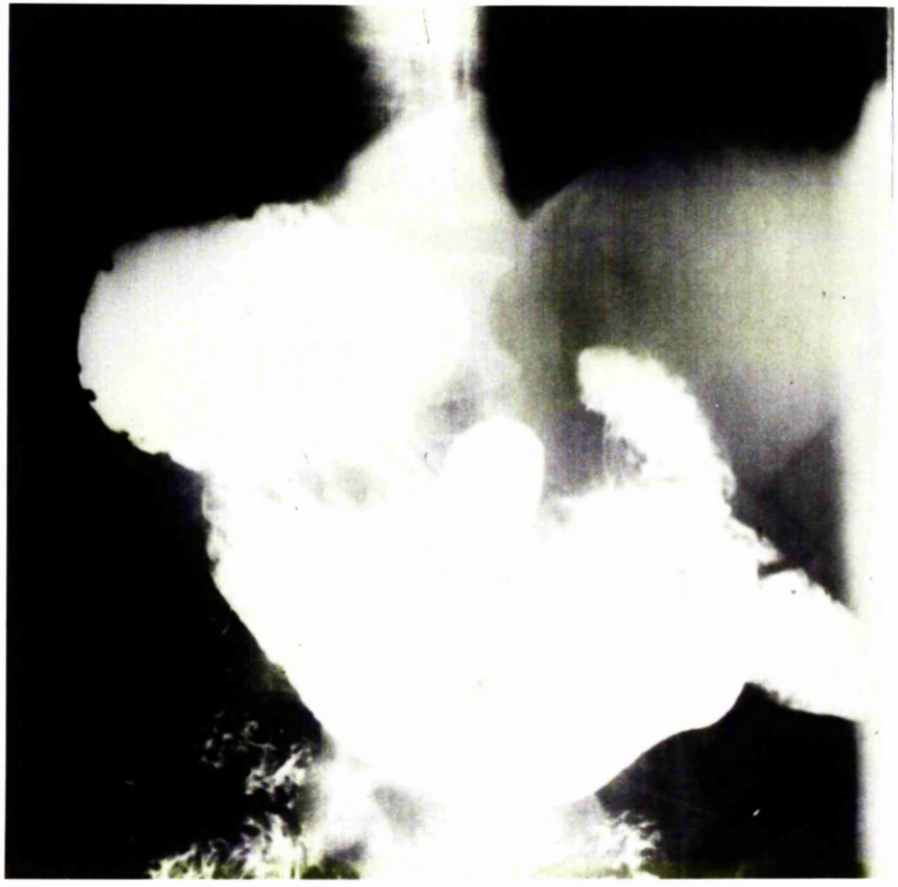


Figure 11.

Barium meal showing chronic gastric ulcer. (Same patient as in Figure 10).

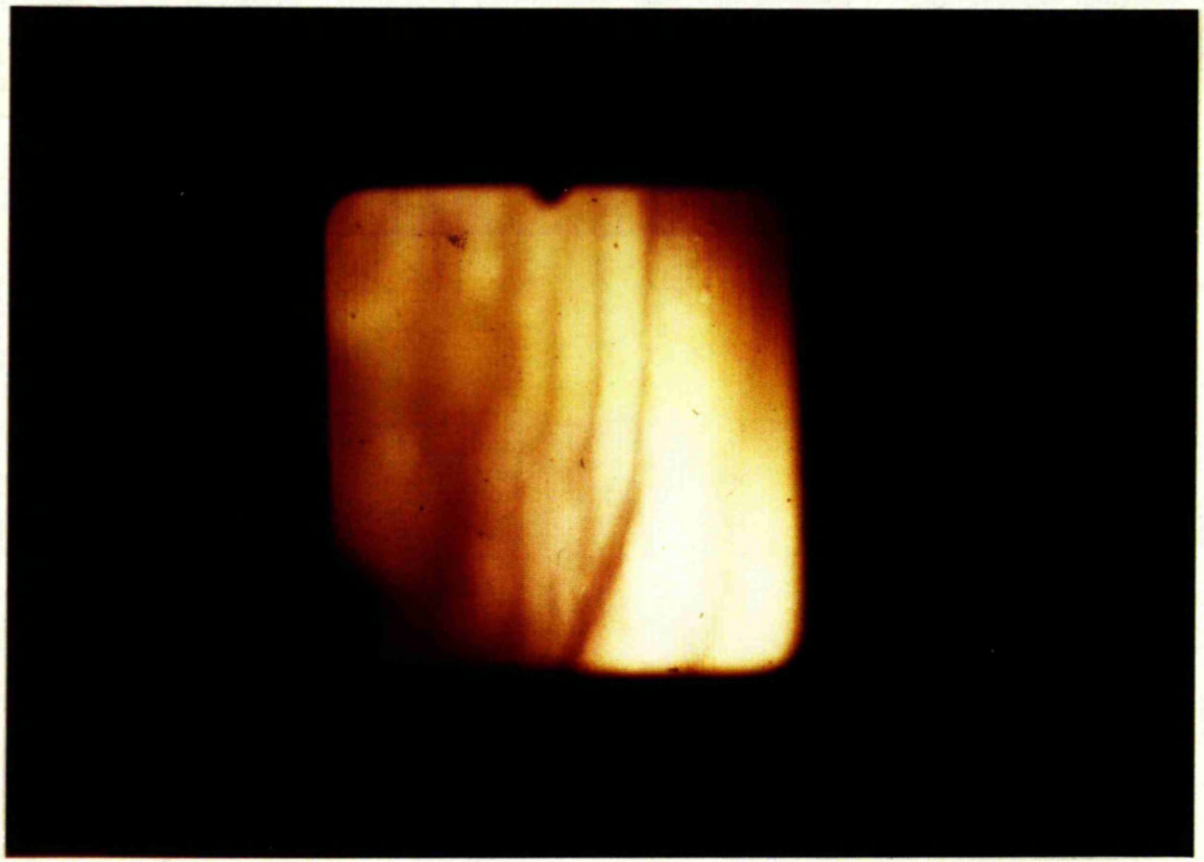


Figure 12.

Normal gastric mucosa seen at endoscopy. Pylorus visible at lower left corner of photograph.

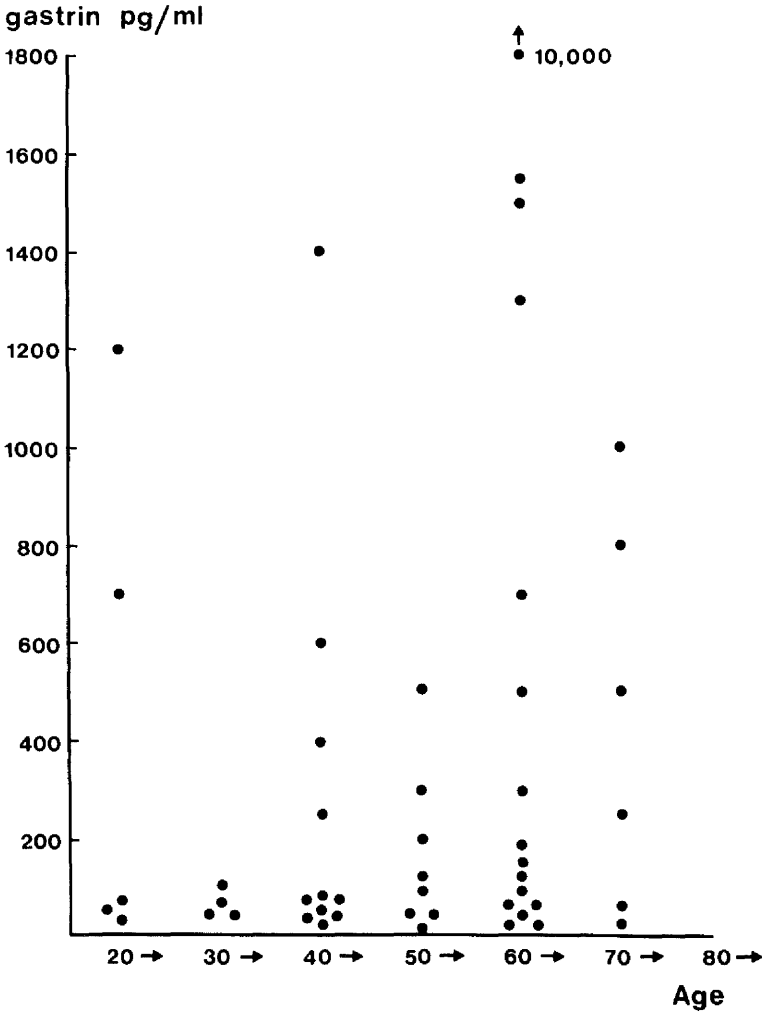


Figure 13.

Age distribution of 50 patients with rheumatoid arthritis and fasting immunoreactive gastrin concentrations.

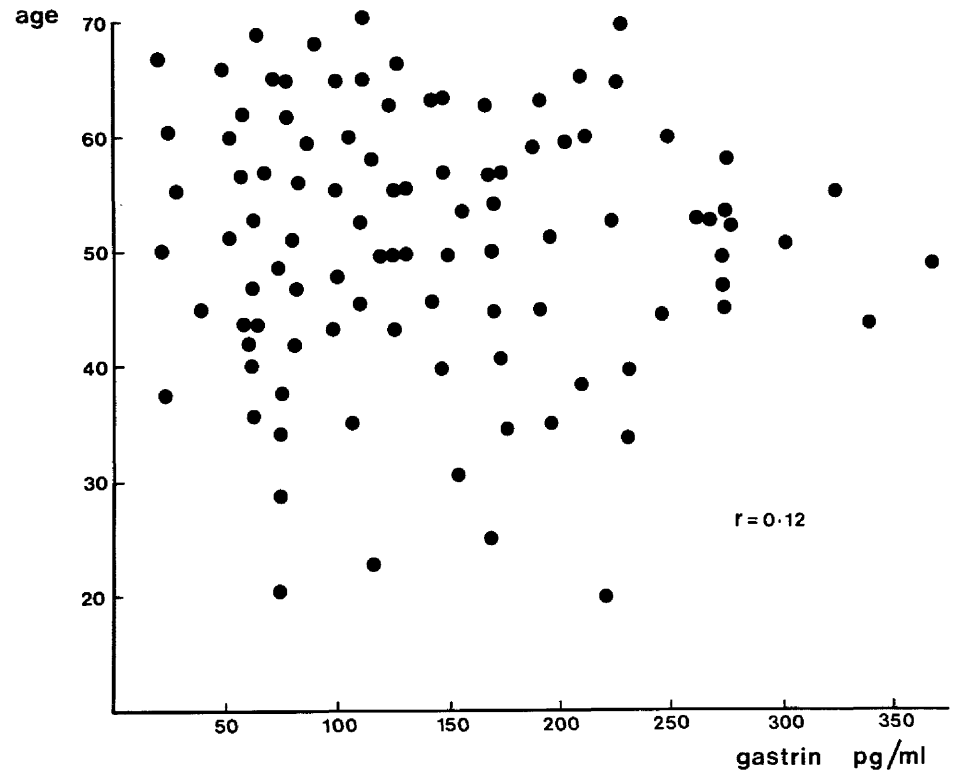


Figure 14.

Correlation between age and fasting immunoreactive gastrin in patients with rheumatoid arthritis and normal immunoreactive gastrin.

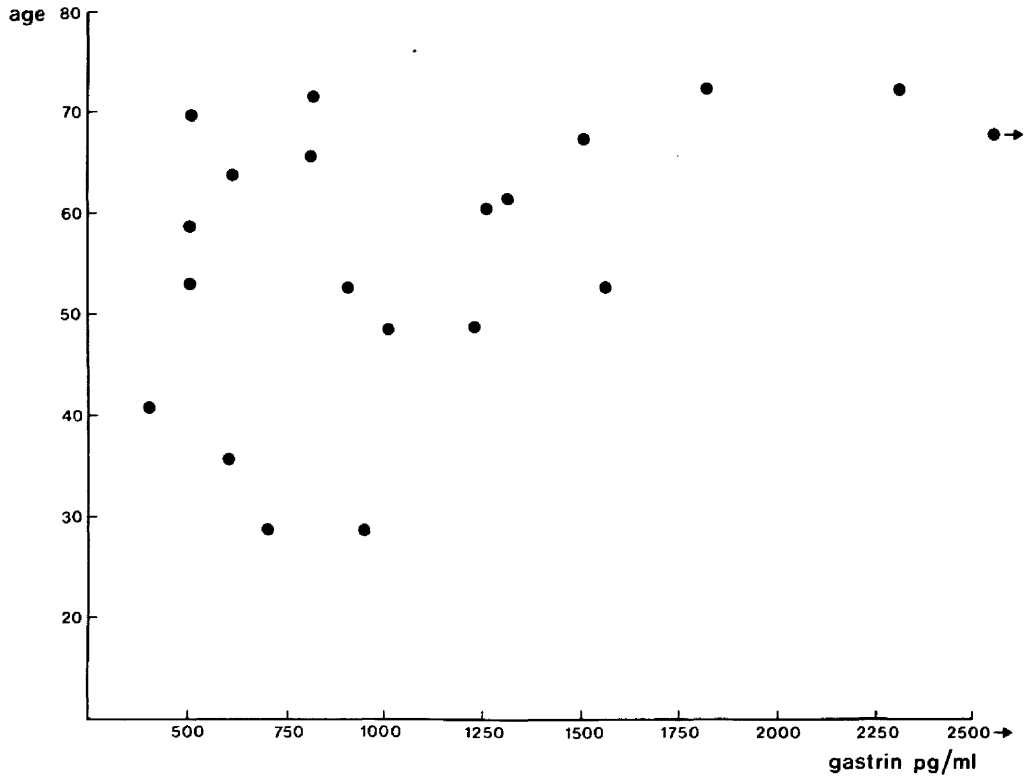


Figure 15.

Correlation between age and fasting immunoreactive gastrin in patients with rheumatoid arthritis and elevated immunoreactive gastrin.

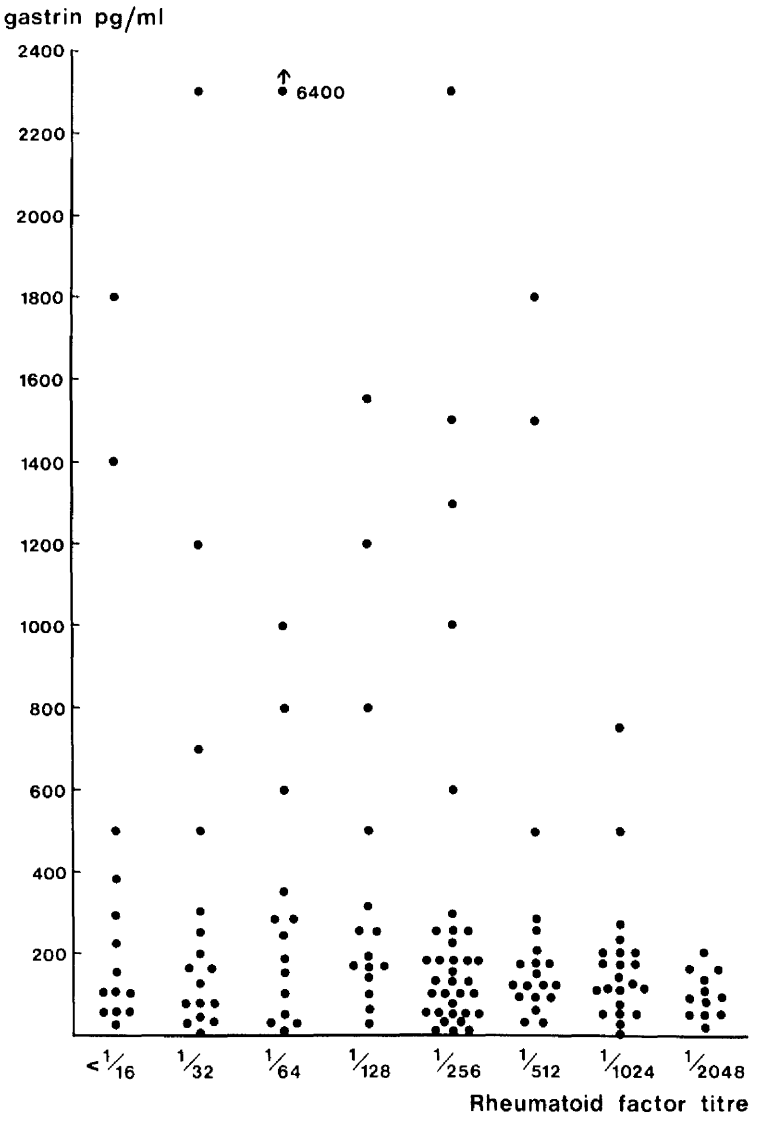


Figure 16.

Distribution of immunoreactive gastrin levels in 150 patients with rheumatoid arthritis according to rheumatoid factor titres.

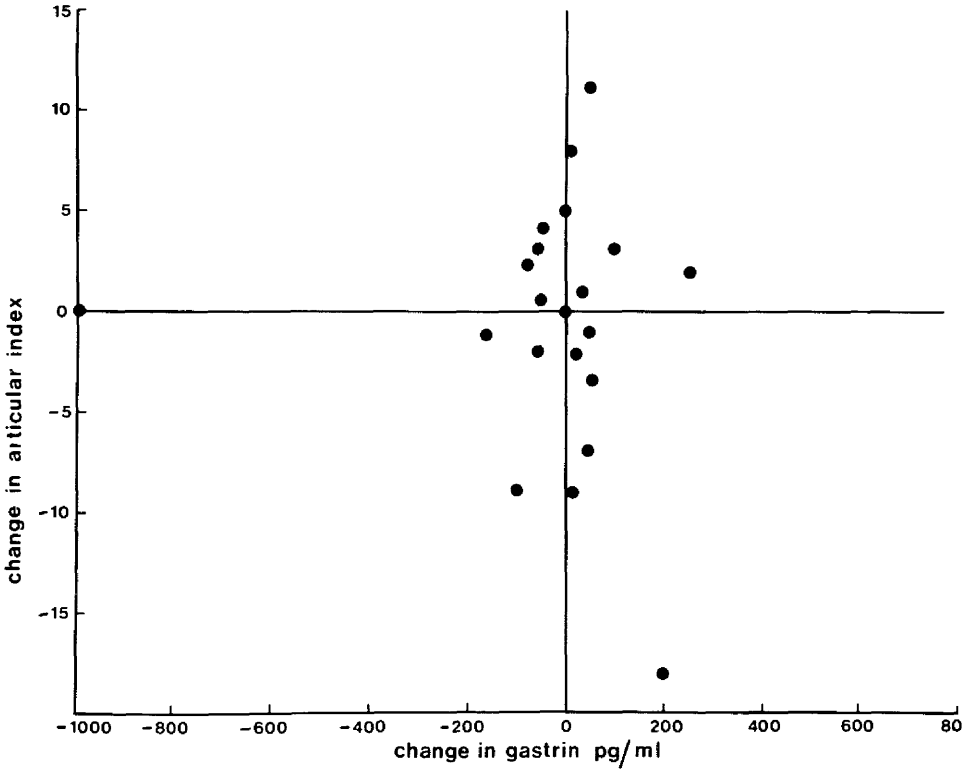


Figure 17.

Change in immunoreactive gastrin and change in articular index over 6 months in 20 patients with rheumatoid arthritis and normal immunoreactive gastrin.

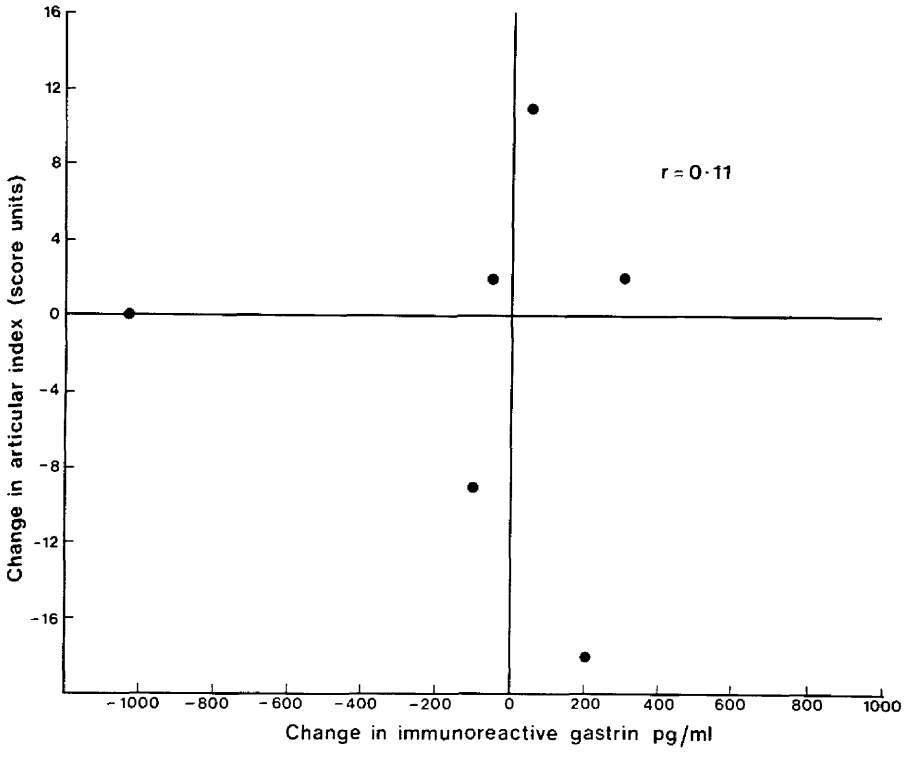


Figure 18.

Change in immunoreactive gastrin and change in articular index over 6 months in 6 patients with rheumatoid arthritis and initially elevated immunoreactive gastrin.

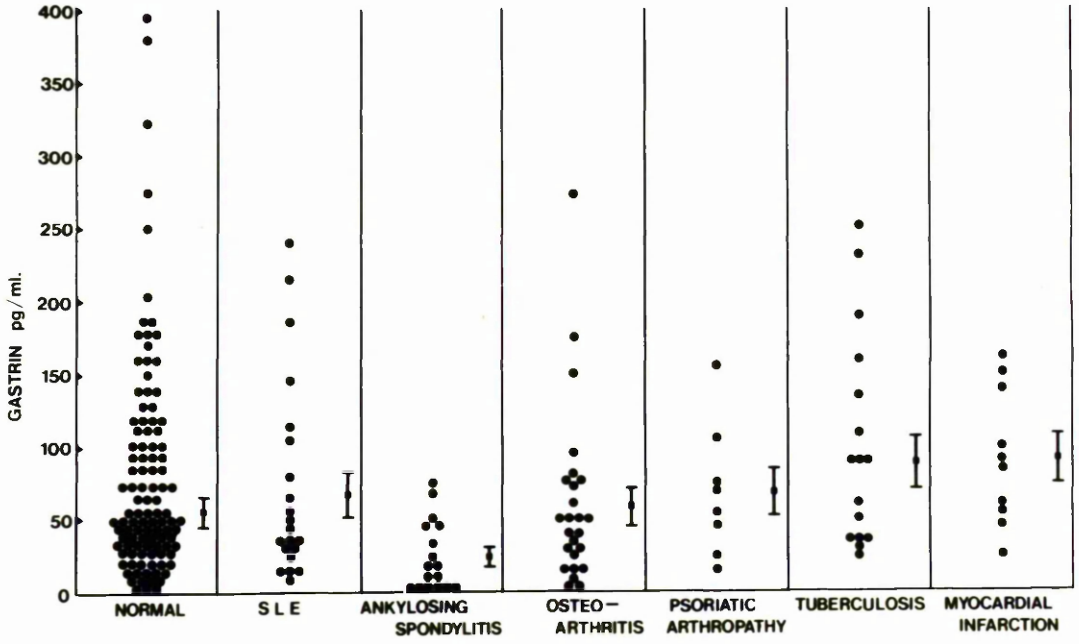


Figure 19.

Immunoreactive gastrin in chronic inflammatory and degenerative diseases other than rheumatoid arthritis.

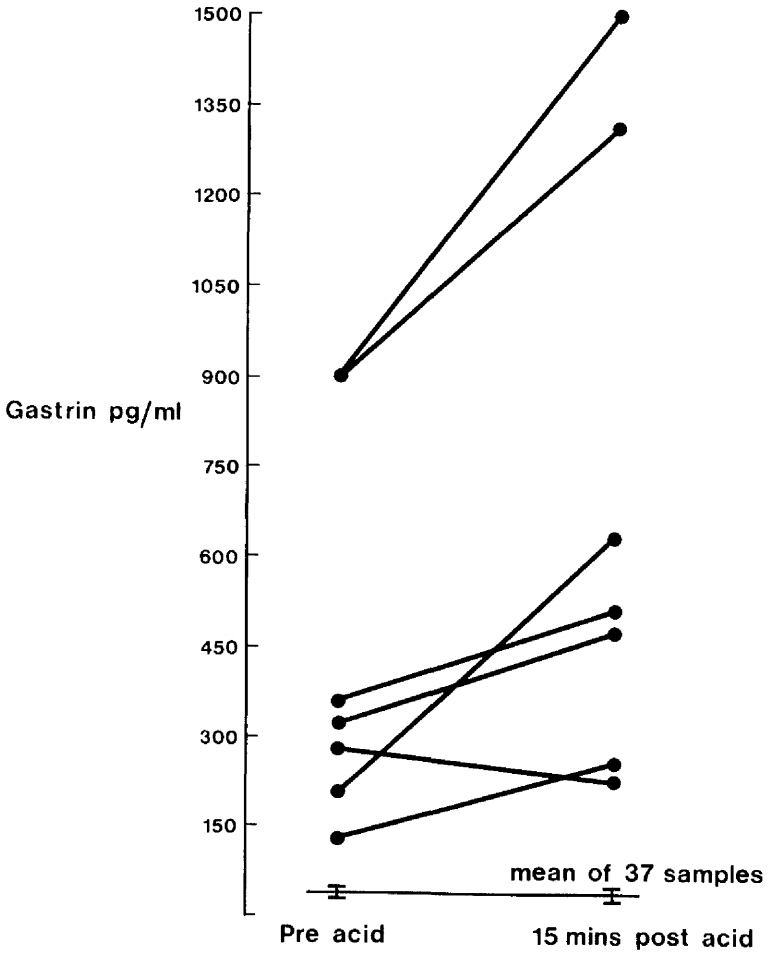


Figure 20.

Change in immunoreactive gastrin in patients with rheumatoid arthritis induced by ingestion of hydrochloric acid.

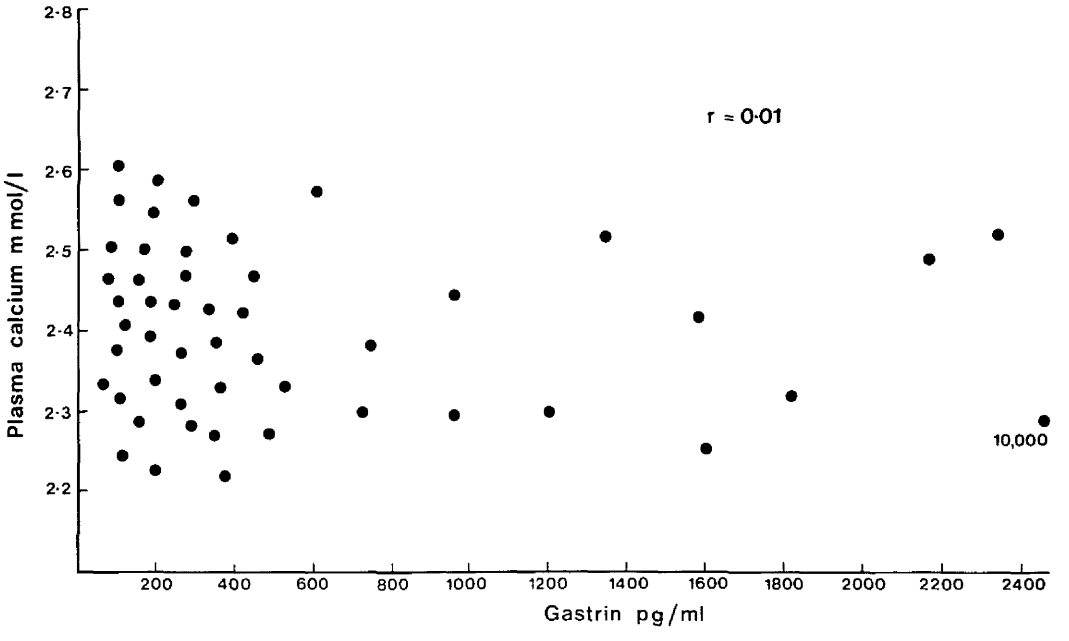


Figure 21.

Correlation between immunoreactive gastrin and total plasma calcium in 50 patients with rheumatoid arthritis.

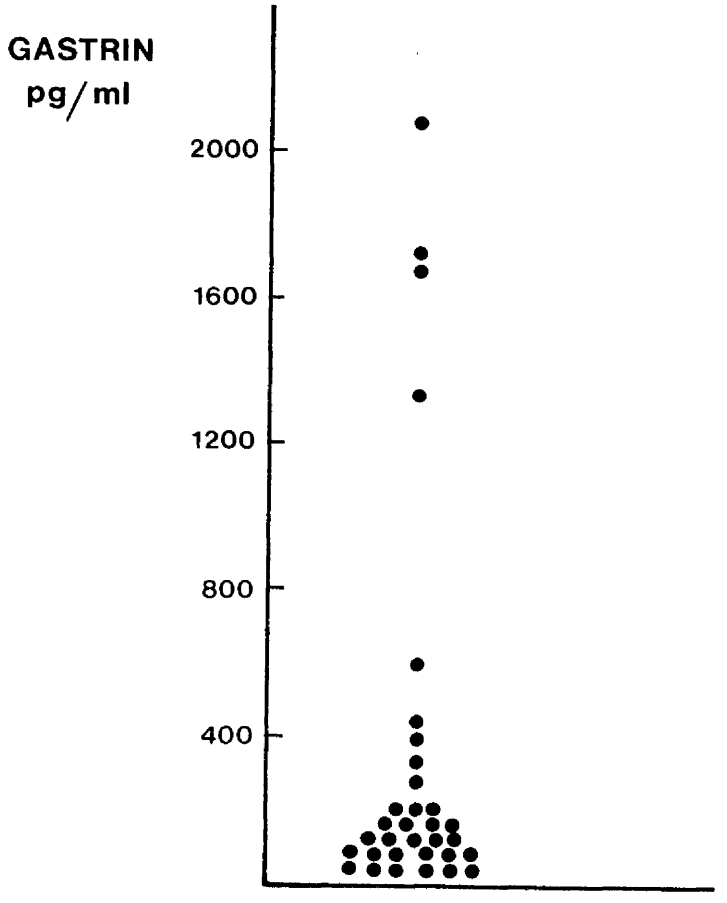


Figure 22.

Distribution of immunoreactive gastrin values in 33 patients with rheumatoid arthritis who were sero-positive for anti-nuclear factor.

Studies on carbenoxolone sodium

in rheumatoid arthritis.

"Sugarally watter - black as the lum,
A' bring yer penny an' ye'll a' get some"

Glasgow childrens' street song.

SUMMARY

In this chapter the development and pharmacology of carbenoxolone sodium is considered. It is shown to be an unique drug in possessing both anti-inflammatory and ulcer healing properties. The extensive effects it demonstrates on the endocrine system are reviewed.

Studies of carbenoxolone sodium in patients with rheumatoid arthritis in control subjects are reported. No effect on plasma immunoreactive gastrin could be shown but the original observation that this drug elevates immunoreactive secretin levels is reported and the possible significance of this in terms of the ulcer healing properties of the drug discussed in detail. The lack of beneficial effect of this drug on rheumatoid inflammatory joint disease is reported and this is shown to be likely to be due to the high incidence of side effects encountered by patients during the use of the drug.

Carbenoxolone sodium. Historical aspects.

"How I can make a patient vomit and how I can purge or sweat him are matters which a druggists shop-boy can tell me off hand. When, however, I must use one sort of medicine in preference to another, requires an informant of a different kind - a man who has no little practice in the arena of his profession".

T.Sydenham 1624-1687.

As described in Chapter 3 of this thesis I have investigated the possibility of anti-inflammatory drug administration being responsible for elevation of immunoreactive gastrin in rheumatoid arthritis. At the same time I took the opportunity of studying the drug carbenoxolone sodium. The unique actions and effects of this compound have held a particular fascination for me during my post graduate career both when I was involved in the clinical care of patients in the field of gastro-enterology and more recently when dealing with the care of patients with inflammatory joint disease.

The development of carbenoxolone was based on the old folk remedy, liquorice or, as it is known colloquially in the West of Scotland; sugarally. The liquorice plant, Glycyrrhiza glabra, grows wild in sub-tropical regions of China, Asia and the Middle East. It is a shrub which grows to a height of around five feet and it is the root rhizomes which are harvested for their unique flavour. In 'folk medicine' liquorice has long held a prominent place as a therapy for digestive complaints (Historia Botanica Practica 1744) and as a

'Protection against the
acrimony of food'.

quoted by Avery Jones 1967.

In 1946 Revers claimed therapeutic value in peptic ulceration for liquorice. He had observed that many patients with peptic ulcers improved after taking a proprietory preparation purchased from a local pharmacist in a small Netherlands town. This preparation contained a mixture of succus liquiritae, fructus anisi and ferrum reductum. Revers made the assumption that the beneficial effect he had noted was due to the succus liquiritae (liquorice extract; 'sugarally watter'). When he tested this clinically he noted complete radiological healing of some gastric ulcers in ambulant patients within three weeks. In 1948 he again reported this effect but on this occasion noted frequent side-effects of the treatment consisting of headache, dyspnoea of effort and oedema.

In 1949, in his thesis to the University of Utrecht, Nelemans-Stamperius showed that liquorice had a powerful spasmolytic effect and that it prevented experimental gastric ulceration in rodents. He could not, however, reproduce the oedema in any of his experimental animal models. Molhuysen and his colleagues (1950) in an interesting series of clinical experiments suggested that the actions of liquorice mimic but are not identical to the actions of ACTH or deoxycortone and suggested the use of the drug in the therapy of Addison's disease. Over

the next few years a number of reports of the value of liquorice and its extractable constituents in the control of Addison's disease appeared (Borst 1950; Borst et al 1953; Groen et al 1952; Card et al 1953; Pelser et al 1953; Calvert 1954). This observation has recently been reconfirmed by a patient, reported by Cotterill and Cunliffe, (1973) who established that self- medication with liquorice sweets controlled all the symptoms of her, as then undiagnosed, Addison's disease.

These investigations into the corticosteroid effects of liquorice were paralleled by the equally fascinating studies of its healing effects on peptic ulceration. In this regard the active principle of succus liquiritae was concluded to be enoxolone (β-glycyrrhetic acid) (Johnston et al 1974) and from this the semi synthetic compound carbenoxolone (β glycerrhetinic acid) was developed and presented commercially as its disodium salt (Biogastrone ^{R}). It is fortunate that at the time this compound was under trial as an ulcer-healing agent Richard Doll was pursuing his very careful enquiry into factors influencing peptic ulcer healing and so was able to demonstrate in a very clear way the effect of this drug on the healing of gastric ulcer in ambulant patients (Doll et al 1962). Since this early study a large series of trials have confirmed the effectiveness of this drug (Table 1).

Carbenoxolone is the succinic acid ester of enoxolone and is a triterpenoid compound (fig.1) with a relatively low acute toxicity (Khan and Sullivan 1968). It is a weak acid with a pK_a of 7.1 (Parke 1968) and is stable in neutral and acid solutions. In alkaline solution it is hydrolysed to the parent compounds enoxolone and succinic acid.

Carbenoxolone is unsuitable for administration by injection as high local concentrations cause tissue necrosis. However, despite its fairly large molecular size it is rapidly and virtually completely absorbed from the stomach (Parke 1968). Recently Parke (1972) has suggested that there may be proteins in gastric mucosa with a special affinity for the drug.

It is of interest that early studies of the metabolic fate of carbenoxolone (Carlat et al 1959) were interpreted as showing that virtually no absorption occurred since virtually all of an oral dose of tritiated (^3H) carbenoxolone can be recovered from the faeces in an unchanged form. Parke (1972) has been able to demonstrate absorption of over 80 per cent of an oral dose and has been able to explain these discrepant results by demonstrating that the drug is almost all excreted back into the bowel via the bile

usually as sulphate or glucuronate compounds which are subsequently broken down in the bowel under bacterial action to its original state (Parke et al 1963).

Mayer and Guttman (1968) have emphasised the influence that protein-binding has on the distribution, pharmacological actions and excretion of drugs. Carbenoxolone is remarkable in the extent of its protein binding being more than 99.9 per cent bound in therapeutic plasma concentrations (10-100 µg/ml) (Sullivan 1970). It is probably this factor which causes carbenoxolone to be excreted so exclusively in bile (Downer et al 1970). It also accounts for its lack of distribution to other tissues such as kidney, body fat, brain, muscle etc. which show extremely low concentrations of drug relative to the plasma or bile concentrations (Parke 1972). Protein-binding may also influence the metabolic fate of carbenoxolone. Oxidative degradation of the succinate part of the molecule can occur but more usually the only change in the drug is its conjugation in the liver with glucuronate or sulphate. The terpenoid structure is not metabolised at all and it has been suggested that this is because its affinity for the plasma protein prevents it from entering the microsomal membrane and hence encountering the cytochrome p 450 drug metabolising enzymes (Parke 1972).

In vitro, carbenoxolone has been shown to be a potent uncoupler of oxidative phosphorylation but this effect is completely inhibited in the presence of bovine albumen (Whitehouse et al 1967). Uncoupling has been confirmed by Parke (1972) but only in vitro and he feels that in vivo the plasma protein binding prevents the drug gaining access to the mitochondrial proteins which control the oxidative phosphorylation process (Weinboch and Carbus 1969).

Carbenoxolone is an anti-inflammatory drug. This action was first reported by Finney and Somers (1958) and in view of its ulcer-healing action (vide infra) it is virtually unique among such drugs. Its anti-inflammatory action has been confirmed by other authors and the effect fairly extensively investigated (Finney and Tornoky 1960; Cygielman 1963; Robinson 1965). In animal studies the anti-inflammatory potency of carbenoxolone is about one third of that of hydrocortisone and it can be demonstrated that the effect is dependent on the presence of intact adrenal glands. The pituitary on the other hand is not essential (Khan and Sullivan 1968). It is possible that these findings are due either to displacement of glucocorticoids from binding by plasma proteins (Khan and Sullivan 1968) or to the inhibition of the metabolism of

corticosteroids in the liver (Atherden 1958). Cygielman (1963) has presented evidence that the anti-inflammatory action of carbenoxolone is mediated principally through the cellular elements of the inflammatory process.

Carbenoxolone sodium has a major influence on water and electrolyte metabolism. This usually consists of a dose-related anti-diuretic effect causing retention of sodium and water. The effect the drug has on potassium is dependent on the sodium load to the kidney. A high sodium load causes a significant kaliuresis (Sullivan 1972). This effect is of major clinical significance since sodium and water retention and hypokalaemia are the principal side-effects of therapy with carbenoxolone in man and it has been shown that if salt intake is kept to a minimum and there is an adequate intake of potassium these side effects are rarely seen (Bank and Marks 1970).

The hypertension and hypokalaemia seen during therapy with carbenoxolone sodium are very suggestive of primary hyperaldosteronism (Conn 1955) but the fluid retention and oedema are more typical of the secondary type of aldosterone excess . Doll et al (1968) were able to show that the aldosterone antagonist, spironolactone was effective in blocking these side effects of carbenoxolone but were disappointed to note that it also blocked the beneficial effect

on ulcer healing. Hausman and Tarnoky (1968) have suggested that these actions of carbenoxolone could arise in three ways .

1. Carbenoxolone has aldosterone activity per se.

2. It can stimulate the secretion of the hormone.

or 3. It could displace aldosterone from its protein binding sites to shift the equilibrium towards the free and active forms of the hormone.

Hausman and Tarnoky (1968) considered the second of these possibilities unlikely since considerable evidence existed which made such an effect of the parent compound, liquorice extract improbable (Drosdowski et al 1961; Carcin et al 1961; Salassa et al 1962). Baron and his colleagues (1969) have also presented direct evidence against the drug causing secretion of aldosterone.

Porter (1970) has studied the effects of carbenoxolone on the metabolism of aldosterone using an elegant, in vitro bioassay system (Crabbé 1961; Crabbé and de Weer (1964). This system is dependent on the stimulation by aldosterone of active sodium transport in the bladder and skin of the toad (Bufo marinus (Porter 1970). In these experiments Porter (1970) was able to show that carbenoxolone had no intrinsic mineralocorticoid activity but it showed marked

synergism with aldosterone and as with aldosterone itself this effect could be blocked by spironolactone. Porter (1970) has adduced evidence to suggest that this effect is not due to an action on the sodium conductance of the membrane. He suggests it is due, either to an allosteric interaction by carbenoxolone at the receptor site to increase the receptor occupancy by aldosterone, or to an effect on aldosterone induced protein (Edelman and Fimognari 1967; Fanestil et al 1968) to improve the efficiency of the sodium pump.

As well as potentiating the effect of aldosterone, Sullivan (1972) has presented some evidence of a direct effect of carbenoxolone on the kidney by showing that some sodium retention still occurs after adrenalectomy.

The most significant effect of carbenoxolone sodium is its healing action on gastric peptic ulceration. As Avery Jones stated in his introduction to an international symposium on the drug in 1967 -

> 'It is not uncommon to have a new treatment put forward for peptic ulcer, but it is uncommon to have a treatment which seems to be effective ... Carbenoxolone sodium is the first drug which convincingly has been shown to accelerate the healing of chronic gastric ulcer'

It was in 1962 that Doll and his colleagues reported the

results of their very carefully controlled study of the effects of carbenoxolone in outpatients with gastric ulcers. The drug was effective in doubling the rate of healing as assessed radiologically. Since this first study more than twenty similar studies have confirmed this original report (Table 1).

The mechanism of this intriguing and now undisputed effect has never been adequately explained. The initial misconception by Carlat and his colleagues (1959) that no absorption of carbenoxolone occurred suggested that the drug had a direct, local action on the ulcer. Despite the more recent and detailed knowledge of the metabolism and pharmacology of carbenoxolone this concept of a local action persists (Hunt 1972). This unlikely idea is offered as the 'rational' basis for the failure of carbenoxolone in duodenal ulceration and as the justification for the, admittedly ingenious, but expensive, technology of the imploding "duogastrone" [R] capsule. In the field of gastric ulceration it has now been adequately demonstrated that the systemic effects are intimately related to the healing action. This is most apparent when one considers the efforts made to reduce the side-effects of the treatment. Doll, Langman and Shawdon in 1968 clearly demonstrated that spironolactone could prevent the side-effects of fluid retention, hypokalaemia and hyper-

tension. However, they also showed clearly that the healing action was also inhibited at the same time.

An animal model to study the mechanism of ulcer healing has not been easy to produce (Sullivan 1972) but since 1968 rats and guinea pigs models have been available in which enhanced healing has been shown in ulcers produced by, electro-cautery, of the gastric mucosa (Khan and Sullivan 1968), by the histamine liberator 48/80 (Dean 1968), by prednisolone (Hoffmeister and Hoffmeister 1971), and by restraint (Lipkin and Ludwig (1968).

From these experiments Lipkin and Ludwig (1968) showed that carbenoxolone increased the amount of periodic-acid Schiff staining material (presumably mucus) in histological sections of gastric mucosa. Dean (1968) also showed an increase in adherent mucus in the stomach. Parke (1972) has suggested that carbenoxolone may act directly on the mucus cells to regulate the synthesis of glycoproteins.

The protective effects of mucus in all aspects of animal physiology is very interesting. It is present very early in the evolutionary scale and its main function appears to be protection against sudden osmotic changes.

Its possible importance in the stomach as part of the protection of the mucosa against acid/peptic digestion has been emphasised (Jones 1972) and the possibility considered that the healing action of carbenoxolone is due to its effect on mucus (Jones 1972).

The importance of cell turnover kinetics in the maintenance of an intact gastric mucosa has been recognised in recent years (Max and Monguy 1970) and it has been suggested that if excess loss of cells occurs, RNA loss which occurs with the cells may cause inhibition of mucoprotein synthesis (Kim, Kerr and Lipkin 1967; Imondi, Balis and Lipkin 1968). Lipkin (1970) has demonstrated that under the influence of carbenoxolone the life span of the gastric epithelial cell is prolonged and the rate of extrusion of cells is decreased. As it seems to be the mature gastric epithelial cells which are most efficient in the secretion of mucosubstance this effect of cell turnover may be related to the effect observed on mucus (Lipkin 1970),.

The effect of carbenoxolone on steroid metabolism is thought to be due, at least in part, to the displacement of hydrocortisone from protein-binding (Khan and Sullivan 1968). Bojonowicz (1966) has presented some rather tenuous evidence that another corticosteroid deoxy-

corticosterone acetate can contribute to ulcer healing and it is possible that carbenoxolone acts by displacing similar steroids from competitive protein binding.

Many workers have studied the influence of carbenoxolone on the digestive ferments of gastric juice. No consistent effect on gastric acid secretion has been observed (Cocking and McCaig 1969; Berstad, Peterson and Myren 1970) but following the suggestion of Taylor (1959, 1970a) that the pepsins of gastric juice are abnormal in patients with peptic ulceration and that pepsin should be considered as one of the aetiological factors in gastric ulceration (Taylor 1970b) several studies of the effect of carbenoxolone on the peptic activity have been reported. Henman (1970) has observed that carbenoxolone decreases total peptic activity of gastric juice in rats and Berstad (1972) has confirmed this finding in the human situation. Roberts and Taylor (1973) have shown that carbenoxolone is inhibitory to pepsinogen and pepsin in vitro and in vivo in human gastric juice and that this effect varies with the individual pepsin involved in a pattern consistent with Taylor's original hypothesis (Taylor 1970b).

Studies on the effect of carbenoxolone sodium on the inflammatory arthritis and foregut hormones in patients with rheumatoid arthritis.

Patients may recover in spite of drugs or
because of them.

J.H.Gaddum. 1900 - 1965.

Carbenoxolone exerts a marked effect on several hormones (vide supra), including aldosterone (Porter 1970), hydrocortisone (Khan and Sullivan 1968) and insulin (Baron et al 1970). Its effects on a number of aspects of gastric physiology have also been detailed above. It seemdd important to consider whether these effects on the gastro-intestinal tract were also mediated by an endocrine mechanism particularly through an influence on the hormones gastrin and secretin. At the same time, in view of the anti-inflammatory properties of the drug the opportunity was taken to assess any therapeutic benefit afforded by carbenoxolone on the inflammatory joint disease.

MATERIALS AND METHODS

A series of 16 in-patient volunteers with classical or definite rheumatoid arthritis (Ropes et al 1959) were included in the initial study. In addition six control volunteers from the staff of the Centre for Rheumatic Diseases were also studied. The clinical data on these subjects is summarised in table 2.

These subjects were given orally 200 mgm. carbenoxolone sodium in 4 divided doses each day for eight days. All other medications were withdrawn for the period of the study. On day 1 and day 8 of the study blood was withdrawn after a ten hour overnight fast.

On each blood sample the plasma sodium, potassium, chloride and carbon dioxide concentrations were measured by the routine biochemical auto-analyser methods. In addition plasma immuno-reactive gastrin and secretin were assayed. Plasma immunoreactive gastrin was measured by the method detailed in chapter 2 of this work. Plasma immunoreactive secretin (Buchanan et al 1973) was measured using the anti-serum (BB33) raised against synthetic porcine secretin (S.G.18773, batch XXX1-14A) kindly donated by Dr. Miguel Ondetti, Squibb Pharmaceuticals. The synthetic secretin was labelled

with ^{125}iodine (Amersham) using the enzyme lactoperoxidase (Holohan et al 1973) and this material was used as standards. Serum was extracted by an alcohol precipitation method (Heding 1971) in order to rid the serum of proteolytic activity and non-specific interference in the assay. The assay to date has been specific for secretin, in that no cross-reaction has been noted with pork pancreatic glucagon (Medical Research Council), pork gut glucagon-like immunoreactivity (Murphy et al 1973) human insulin, (Medical Research Council) human synthetic gastrin (Imperial Chemical Industries) 99 per cent pure pancreozymin/cholecystokinin (kindly donated by Dr.V.Mutt) motolin, or gastric inhibitory polypeptide (both kindly donated by Dr.J.C.Brown). Extracts of human jejunum can be shown to have the same dilution slopes as the porcine secretin standard. The sensitivity of the assay, that is the lowest concentration of hormone that can reliably be distinguished from zero, was 30 pg/ml (p 0.05).

In a further series of 13 in-patient volunteers with definite or classical rheumatoid arthritis (Ropes et al 1959) the effects of the same oral dose (200 mgm. daily) of carbenoxolone on the activity of the inflammatory joint disease were assessed over a period of

14 days. The clinical details of these patients are summarised in table 3. This second study was designed as a double-blind cross-over trial. On entry to the study all other medication was withdrawn and each patient randomly allocated to the initial treatment period with either four 50 mg. tablets of carbenoxolone daily or four placebo tablets daily. Blood for electrolyte estimation was withdrawn on days 1, 7 and 14 of each treatment sequence. On completion of the first treatment sequence immediate cross-over to the second occurred and, as with all such drug studies carried out at the Centre for Rheumatic Diseases, a trap-door provision in the design permitted any patient who had intolerable symptoms at any time during the trial to proceed immediately to the next treatment stage.

The activity of joint inflammation was assessed on entry to the study and at the end of each treatment period. The assessments used were a pain index (Lee et al 1973), articular index of joint tenderness (Ritchie et al 1968), digital joint circumference (Webb et al 1973), grip strength (Lee et al 1974) and radio-technetium uptake over joints (Dick 1972). Any side-effects of the treatments were elicited by the question " Have the tablets upset you in any way ?". The possible dangers of electrolyte imbalance during the

period of treatment with carbenoxolone were avoided by the review of the electrolyte status by an independent physician. Any patient showing dangerous hypokalaemia (less than 3.0 mmol/l) was to be withdrawn from the trial immediately. An independent physician was used as it was felt that my knowledge of the electrolytes might permit identification of the active treatment and influence the outcome of the trial.

RESULTS

Significant changes in the plasma electrolytes were noted in the patients with rheumatoid arthritis during the initial study. These are summarised in table 4. Similar changes were observed in the control subjects although the degree of change was much smaller (table 5).

No consistent change in immunoreactive gastrin was observed in either the patients or controls. However, immunoreactive secretin rose significantly in the patients with rheumatoid arthritis and a similar trend is observed in the control group although the rise in this group does not reach levels of statistical significance. These results are summarised in table 6. The extent of the change in immunoreactive secretin in the patients with rheumatoid arthritis can be shown to be related to the degree of change in plasma potassium ($r = 0.83; p < 0.001$) and in plasma carbon dioxide ($r = 0.63; p < 0.01$). The patients with rheumatoid arthritis who underwent the double-blind cross-over study showed very similar electrolyte changes during the period when they were receiving carbenoxolone sodium although the extent of these changes was much less and did not require any patient to be withdrawn from the study. No such change

was observed during the placebo period (tables 7-10).

The effects of the two treatment periods on the assessments of disease activity are summarised in table 11. As can be seen by no assessment index was carbenoxolone significantly better than placebo and by some it performed worse.

During this study four patients dropped out, two on each treatment, with side effects sufficiently distressing to prevent their continuation with the study. It is of interest that both the patients dropping out during the carbenoxolone period did so on account of intolerable dyspepsia. Neither of these subjects had suffered any dyspeptic symptoms on any other non-steroidal anti-inflammatory drug. Of the nine patients completing the study five stated a preference for the placebo period and only one for the carbenoxolone treatment period ($\chi^2 = 3.99$; $0.05 > p > 0.01$). This may be due at least in part to the considerable excess of reported side effects on carbenoxolone (Table 12).

DISCUSSION

The known actions of carbenoxolone sodium; the promotion of healing in gastric ulceration (Doll et al 1962) and anti-inflammatory actions in experimental animals (Finney and Somers 1958) suggested that it should be ideal in the management of rheumatoid arthritis where the use of all other non-steroidal anti-inflammatory agents is associated with a considerable incidence of dyspepsia and probably of gastric ulceration (Casadio et al 1967; Emmanuel and Montgomery 1971; Ivey and Clifton 1974). In addition, the observation that immunoreactive gastrin is elevated in some patients with rheumatoid arthritis increased the relevance of a drug known to affect gastric physiology. It was rather disappointing, therefore, to find that carbenoxolone sodium, in common with the other anti-inflammatory drugs had no effect on immunoreactive gastrin, in the doses and time course of this study.

Nonetheless, all the other reported actions of carbenoxolone sodium have been attributed, at least in part, to changes in associated hormone levels: Porter (1970) has clearly demonstrated the effects on aldosterone metabolism; Khan and Sullivan (1968) have implicated an effect on endogenous glucocorticoid metabolism in the anti-

inflammatory action of the drug; Baron and his colleagues (1970) have indicated that carbenoxolone influences insulin metabolism and Bank and Marks (1970) reported a patient with persistent galactorrhoea during the use of this drug.

It was against this background, that despite having observed no effect on immunoreactive gastrin, I decided to assay the immunoreactive secretin content of the same plasma samples. I was fortunate in having available to me the radio-immunoassay recently developed in Dr. Keith Buchanan's laboratory. This assay uses the less vigorous iodination technique using lactoperoxidase (Holohan et al 1973) and the secretin is labelled on the histidyl residue. This study has clearly demonstrated that, in patients with rheumatoid arthritis, carbenoxolone causes a significant rise in immunoreactive secretin. A similar trend was noted in the control subjects but the changes failed to reach levels of statistical significance. It is interesting to speculate on the possible relationship of this finding to the ulcer healing properties of the drug.

Although secretin was the first hormone to be discovered in 1902 by Bayliss and Starling and was the corner stone of the hormone hypothesis for many years, its true functional and pathogenetic role has been very scantily

investigated, so much so that in 1973, Wormsley, in an extensive review, found little or no evidence that secretin was in fact secreted. There is no doubt about the effects of injected secretin. From very early studies its apparently unique action in stimulating the secretion of bicarbonate-rich pancreatic juice encouraged its use as a functional test for the pancreas (Chiray et al 1930; Agren et al 1936). Despite Wormsley's (1973) scepticism, Farooq et al (1974) have presented evidence that small amounts of secretin stimulate the pancreas to flood the duodenum with alkaline pancreatic juice and Winship and Robinson (1974) have shown that there is no other physiologically important mechanism controlling this function.

Increasing evidence implicating secretin in the mechanism or effects of peptic ulceration is being presented. In patients with duodenal the mean gastric acid production is increased (Baron 1963) and duodenal acid neutralisation is impaired (Wormsley 1967). Although in these subjects pancreatic sensitivity appears to be normal (Banks et al 1967; Wormsley and Mahoney 1967) the duodenal mucosal release of secretin in this circumstance appears to be impaired (Bloom and Ward 1975). Grossman (1972) has been attempting to use secretin as a

therapy for duodenal ulceration and cites as his rationale the effects of secretin in a) neutralising the contents of the duodenum (Anderson and Grossman 1966) b) inhibition of gastrin stimulated acid secretion (Berstad and Petersen 1970) and of c) gastrin induced parietal cell hyperplasia (Stanley et al 1972). It is also known that secretin injection relieves the pain of duodenal ulceration (Holst et al 1971).

This suggestion that the ulcer healing action of carbenoxolone is mediated via its effect on plasma immunoreactive secretin is not at variance with other proposed mechanisms for its mode of action (vide supra). As I have discussed in the introduction to this chapter carbenoxolone has been shown to have an effect on mucus (Lipkin and Ludwig 1968). More recently Vagne and Farger (1973) have shown that secretin influences the amount and chemical composition of gastrin mucus. The slowing of gastric epithelial cell extrusion (Lipkin 1970) during carbenoxolone therapy may also be hormonally mediated. Gastrin is well recognised to be trophic to the gastric epithelium (Willems et al 1972) and the competitive inhibition which exists between gastrin and secretin in terms of gastric secretory functions (Johnson and Grossman 1971) is likely to exist also in this closely allied physiological action.

This study is also consistent with the known pharmacology of carbenoxolone sodium in one other important respect. The lack of effect on plasma immunoreactive gastrin levels correlates well with the known lack of effect of carbenoxolone on gastric acid secretion (Geismar and Mosbech 1970). If the effects on plasma secretion reported here can be confirmed in patients with peptic ulceration and can be shown to correlate with ulcer healing, the physiological role of the foregut endocrine system in this type of disease will be re-emphasised and important avenues of study opened up in relation to the pathogenesis of peptic ulcer. An interesting observation made during these studies with this drug was the very high incidence of heartburn and dyspepsia induced by carbenoxolone sodium both in the patients, two of whom discontinued the controlled study of anti-inflammatory effect for this reason, and in the control subjects all of whom reported this side effect. It is possible that this symptom is due to acid reflux into the oesophagus as it is known that secretin is inhibitory to the lower oesophageal sphincter (Johnston and Grossman 1971).

Despite its action as an anti-inflammatory drug in experimental animals (Finney and Somers 1958) no

beneficial effects were noted in the short term.as regards the activity of rheumatoid arthritis during these studies. Indeed carbenoxolone is almost pro-inflammatory in comparison to the placebo preparation. This may be in part due to the fluid retention associated with the use of this drug as well as to the very increased incidence of side effects which were noted during the carbenoxolone treatment period. It is a little surprising that the use of carbenoxolone in rheumatoid arthritis has not been reported previously. However it is interesting to note that in 1950 Molhuyson and his colleagues noted the lack of effect of liquorice extract in a single patient with rheumatoid arthritis, despite its obvious adrenocorticol effects on fluid and electrolyte metabolism.

Table 1

Number of patients	Type of Patient Out or In patient	Result	Reference	
50	Outpatient	Benefit	Doll et al	1952
46	Outpatient	Benefit	Doll et al	1965
53	Outpatient	Benefit	McCaig	1970
20	Outpatient	Benefit	Bank et al	1967
36	Outpatient	Benefit	Cooking & McCaig	1969
33	Outpatient	Benefit	Horwich & Galloway	1965
8	Outpatient	No Benefit	Geismar & Mosbech	1970
27	Outpatient	Benefit	de Marcos Perez	1967
56	Outpatient	Benefit	Doll et al	1968
31	Inpatient	No Benefit	Middleton et al	1965
12	Inpatient	Benefit	Turpie & Thomson	1965
70	Outpatient	Benefit	Gosch & Ottenjohn	1971
9	Inpatient	Benefit	Kunz	1971
7	Outpatient	Benefit	Lenz et al	1971
27	Inpatient	Benefit	Doyle et al	1968
70	Outpatient	Benefit	Montgomery	1967
30	Outpatient	Benefit	MacCaig	1967
36	Outpatient	Benefit	Ottenjohn & Gosch	1970
19	Outpatient	Benefit	Pulvertaft	1968
15	Outpatient	Benefit	Geismar et al	1973
33	Outpatient	Benefit	Longman et al	1973

TABLE 2

	Rheumatoid Arthritis	Controls
Total number	16	6
Mean age (years)	56 (range 21-76)	32 (range 28-43)
Mean duration of disease (years)	10.5 (range 2-33)	-
Mean articular index (score units)	22.6 (range 1-57)	-
Sero positive	14 (mean titre 112^{-1})	-
X-Ray positive	16	-
Low-dose corticosteroid therapy (10 mg/day)	3	-
Gold therapy	0	-
Drugs used prior to study -		-
Aspirin	8	
Indomethacin	6	
Naproxen	2	
Alclofenac	2	

Table 3

Clinical features of 13 volunteer patients with rheumatoid arthritis who participated in double blind study of carbenoxolone sodium.

Number of patients with rheumatoid arthritis	13
Mean age (years)	45.5 (range 29-63)
Mean duration of disease (years)	5.0 (range 2-8)
Mean articular index on admission to study	17.7
Sero positive	13
X-Ray positive	13

TABLE 4

Electrolyte changes in 16 patients with rheumatoid arthritis during eight days treatment with carbenoxolone sodium.

	(mmol/l) Sodium		(mmol/l) Potassium		(mmol/l) Chloride		(mmol/l) Carbon Dioxide	
Day	1	8	1	8	1	8	1	8
Patient No.1	139	141	4.1	3.2	105	103	24	27
2	144	146	4.5	3.7	102	108	28	26
3	140	141	4.3	4.3	104	104	21	25
4	143	144	3.5	2.8	105	101	20	26
5	142	145	4.0	2.9	104	107	22	26
6	143	144	3.7	2.6	105	103	20	28
7	140	147	3.9	2.4	103	107	22	24
8	146	147	3.7	2.7	105	107	26	26
9	143	144	3.1	2.4	101	99	26	33
10	143	144	3.5	2.8	105	101	20	26
11	141	140	3.9	3.5	105	107	22	21
12	144	147	4.0	3.4	105	106	28	30
13	142	141	4.0	3.5	106	102	25	29
14	143	145	3.8	2.5	105	103	26	30
15	138	144	3.7	2.5	103	101	24	26
16	142	145	4.1	3.2	106	107	25	28
Mean	142.0	144.0	3.86	3.02	104.3	104.1	23.19	26.9
S.D.	2.04	2.26	0.33	0.54	1.4	2.8	2.75	2.76
S.E.M.	0.51	0.56	0.08	0.13	0.35	0.71	0.68	0.69
t	3.75		9.0		0.48		4.69	
p	<0.005		<0.0005		>0.1 N.S.		<0.005	

Table 5

Electrolyte changes in six control subjects during eight days treatment with carbenoxolone sodium.

	Sodium (mmol/l)		Potassium (mmol/l)		Chloride (mmol/l)		Carbon Dioxide (mmol/l)	
Day	1	8	1	8	1	8	1	8
Subject 1	141	142	3.6	3.9	106	106	26	27
2	141	143	4.0	4.3	100	103	31	30
3	142	143	3.9	3.7	102	104	28	30
4	142	141	4.0	3.6	102	103	26	29
5	139	141	4.0	3.7	101	105	27	26
6	139	140	3.7	3.4	98	99	29	29
Mean	140.6	141.6	3.8	3.7	101.5	103.3	27.8	28.5
S.D.	1.37	1.2	0.17	0.31	2.7	2.4	1.9	1.64
S.E.M.	0.56	0.49	0.07	0.12	1.1	0.99	0.79	0.67
t	2.24		0.77		3.05		1.0	
p	0.10 > p > 0.05		> 0.1 N.S.		0.05 > p > 0.01		> 0.1 N.S.	

TABLE 6

Change in immunoreactive gastrin and secretin in sixteen patients with rheumatoid arthritis and in six controls during eight days treatment with carbenoxolone sodium.

	Gastrin				Secretin			
	R.A.		Controls		R.A.		Controls	
Day	1	8	1	8	1	8	1	8
Subject 1	50	100	70	70	100	150	65	70
2	120	65	65	65	110	55	70	70
3	45	50	35	55	50	125	45	100
4	25	5	80	80	-	-	110	65
5	45	45	80	50	55	75	50	65
6	145	110	65	35	0	100	95	140
7	120	110			210	110		
8	270	105			35	0		
9	185	610			0	70		
10	205	270			10	40		
11	950	820			25	165		
12	50	100			70	85		
13	30	70			70	100		
14	110	90			70	105		
15	125	70			90	120		
16	90	70			55	70		
Mean	160.3	168.1	65.8	59.2	63.3	91.3	72.5	85.0
S.D.	221.6	223.9	16.6	15.9	53.0	42.3	25.4	30.0
S.E.M.	55.4	55.9	6.8	6.5	13.7	10.9	10.3	12.2
t	0.28		0.83		1.82		0.86	
p	> 0.1 N.S.		N.S.		< 0.05		N.S.	

TABLE 7

Plasma Sodium (m mol/l) in thirteen patients with rheumatoid arthritis.

	Carbenoxolone			Placebo		
Day	1	7	14	1	7	14
1	142	148	146	146	143	142
2	142	145	147	143	141	142
3	142	142	143	143	141	142
4	140	141	141	141	141	140
5	142	144	144	144	141	143
6	143	144	145	145	141	140
7	143	149	150	150	145	146
8	139	139	143	143	142	142
9	139	142	142	142	140	140
10	142	--	--	--	--	--
11	139	--	--	137	136	139
12	142	141	--	--	--	--
13	--	--	--	143	--	--
Mean (Nos.1-9 only)	141.3	143.8	144.5	144.1	141.7	141.9
S.D.	1.58	3.2	2.8	2.7	1.5	1.9
S.E.M	0.53	1.1	0.93	0.9	0.5	0.6

-- No result due to patients drop-out from trial

t test on day 14 of each treatment $t = 4.44$ $0.005 > p > 0.001$.

TABLE 8

Plasma potassium (m mol/l) in thirteen patients with rheumatoid arthritis.

	Carbenoxolone			Placebo		
Day	1	7	14	1	7	14
1	3.4	3.1	3.0	3.0	3.3	3.2
2	4.2	3.6	3.4	4.1	4.0	4.2
3	4.4	4.0	3.7	4.2	4.4	4.4
4	3.6	3.2	3.0	3.9	3.7	3.6
5	3.5	3.7	3.6	3.6	3.7	3.6
6	4.4	3.9	3.7	3.7	3.9	3.9
7	3.8	3.1	2.8	3.7	3.6	3.8
8	3.4	3.2	3.2	3.2	3.6	3.6
9	4.6	4.0	4.1	4.1	4.3	4.3
10	4.0	-	-	-	-	-
11	4.2	-	-	4.3	4.2	4.2
12	4.0	4.1	-	-	-	-
13	-	-	-	4.2	-	-
Mean (No.1-9 only)	3.92	3.53	3.39	3.72	3.83	3.84
S.D.	0.48	0.39	0.42	0.41	0.35	0.39
S.E.M.	0.16	0.13	0.14	0.14	0.12	0.13

- No result due to patient drop-out from trial

t test between day 14 on each treatment $t = 4.07$ $0.005 > p > 0.001$

TABLE 9

Plasma chloride (m mol/l) in thirteen patients with rheumatoid arthritis

	Carbenoxolone			Placebo		
Day	1	7	14	1	7	14
1	106	104	104	104	107	107
2	106	106	106	107	107	106
3	105	106	106	104	103	105
4	104	107	107	103	102	104
5	101	103	105	105	104	104
6	106	108	106	106	106	106
7	105	107	107	103	103	105
8	105	106	108	108	107	106
9	104	105	107	107	106	105
10	103	--	--	--	--	--
11	104	--	--	105	103	104
12	105	107	--	--	--	--
13	--	--	--	104	--	--
Mean (Nos.1-9 only)	104.7	105.8	106.2	105.2	105.0	105.3
S.D.	1.6	1.6	1.2	1.9	2.0	1.0
S.E.M.	0.5	0.5	0.4	0.6	0.7	0.3

-- No result due to patient drop-out from trial

t test between day 14 on each treatment t = 1.51 p N.S.

TABLE 10

Plasma Carbon Dioxide (m mol/l) in thirteen patients with rheumatoid arthritis.

	Carbenoxolone			Placebo		
Day	1	7	14	1	7	14
1	25	27	28	28	25	25
2	23	26	26	22	23	23
3	25	24	24	25	24	25
4	22	23	23	23	23	22
5	20	21	23	23	23	21
6	22	24	24	24	24	24
7	26	27	30	25	25	26
8	23	23	24	24	21	21
9	22	22	23	23	20	20
10	23	-	-	-	-	-
11	24	-	-	23	24	24
12	23	25	-	-	-	-
13	-	-	-	23	-	-
Mean (Nos.1-9 only)	23.1	24.1	25.0	24.1	23.1	23.0
S.D.	1.9	2.1	2.5	1.8	1.7	2.1
S.E.M.	0.6	0.7	0.8	0.6	0.6	0.7

- No result due to patient drop-out from trial.

t test between Day 14 of each treatment $t = 3.62$ $0.01 > p > 0.005$

TABLE 11

Disease activity during therapy with carbenoxolone sodium and placebo.

	Initial	Placebo	Carbenoxolone
Pain index (Mean ± SEM)	2.0 ± 0.24	1.9 ± 0.35	2.33 ± 0.24
Articular index (Mean ± SEM)	17.7 ± 4.37	12.7 ± 3.4	19.5 ± 4.0
Grip Strength (R) (Mean ± SEM)	161 ± 24.6	147 ± 20.8	141 ± 17.3
Grip Strength (L) Mean ± SEM)	161 ± 21.8	137 ± 21.4	137 ± 18.5
Ring Size (R) (Mean ± SEM)	281.7 ± 4.5	281 ± 4.2	281 ± 5.1
Ring Size (L) (Mean ± SEM)	272.7 ± 5.2	272.8 ± 4.1	274.5 ± 4.4
$T^{C}99$ uptakes			
R. Knee (Mean ± SEM)	18.61 ± 1.82	17.94 ± 1.80	17.89 ± 1.96
L. Knee (Mean ± SEM)	17 .27 ± 1.73	15.99 ± 1.16	15.63 ± 1.04
R. Wrist (Mean ± SEM)	15. 28 ± 1.68	16.11 ± 1.77	15.21 ± 1.27
L. Wrist (Mean ± SEM)	14. 48 ± 1.49	13.04 ± 0.81	14.05 ± 1.06

TABLE 12

Side effects encountered during therapy with carbenoxolone sodium and placebo.

	Carbenoxolone	Placebo
Nausea	5 *	-
Vomiting	2	-
Abdominal Pain	4 *	-
Heartburn	4 *	-
Rash	-	1
Itch	-	1
Headache	3	-
Ankle Oedema	1	-
Dyspnoea	1	-

* Two patients dropped out on account of abdominal side effects.

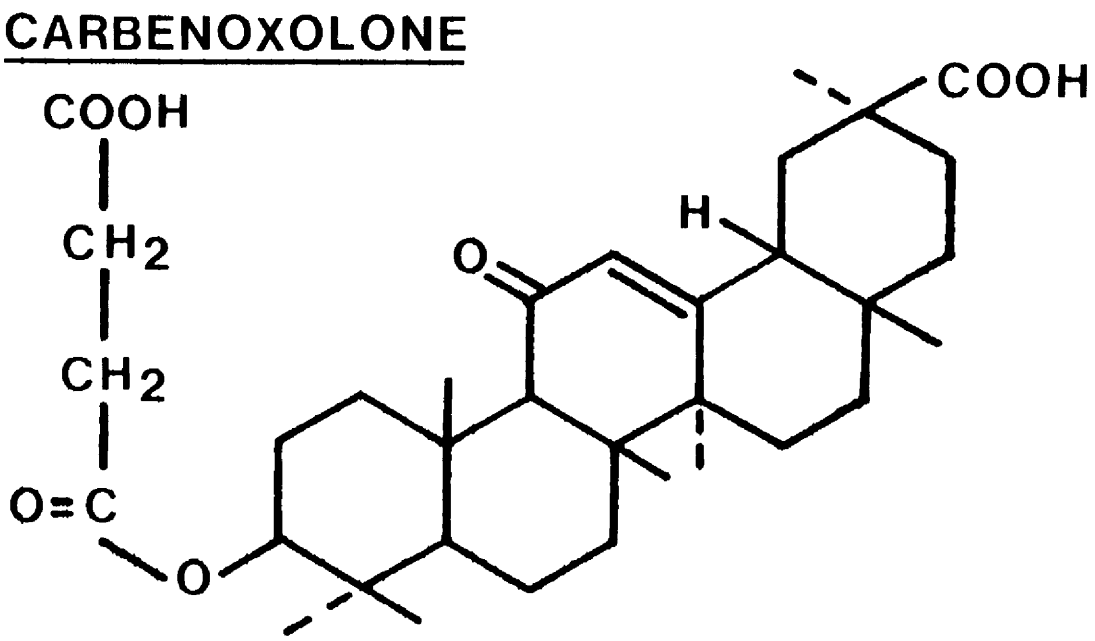


Figure 1.

Chemical structure of carbenoxolone.

Studies on immunoreactive gastrin

in adjuvant induced arthritis in rats.

Rats !
They fought the dogs and killed the cats
And bit the babies in their cradles
And ate the cheeses out of the vats
And licked the soup from the cooks' own ladles
Split open kegs of salted sprats
Made nests inside men's Sunday hats
And even spoiled the women's chats
    By drowning their speaking
    With shrieking and squeaking
In fifty different sharps and flats.

Robert Browning.

The Pied Piper of Hamelin.

SUMMARY

Elevation of plasma immunoreactive gastrin during the induction of adjuvant induced arthritis in rats is reported. Its time course in relation to the onset of the inflammatory arthritis is considered in some detail as is the effect of varying the strain of rat used in these experiments. The concomitant reduction of gastrin acid output when adjuvant arthritis is induced is reported from studies in rats with gastric fistulae. The lack of effect of non-steroidal anti-inflammatory drugs on this elevation of immunoreactive gastrin is compared with the similar phenomenon in patients with rheumatoid arthritis reported elsewhere in this thesis. Some evidence is presented for a pro inflammatory effect of exogenous synthetic gastrin.

INTRODUCTION

In an effort to explain the mechanism of the elevation of immunoreactive gastrin in rheumatoid arthritis, the study of laboratory animal models of inflammatory arthritis was undertaken.

In view of its known similarities to human rheumatoid arthritis, its ease and consistency of induction and the fact that laboratory facilities and skilled assistance and advice were available it was decided initially to restrict these studies to the model of adjuvant induced arthritis in the rat (Pearson 1964). I am grateful to Dr. Robert Imrie and to Mr. David Turner for their help and advice and to Beecham's research laboratories whose animals and animal house facilities were used in these studies.

MATERIALS AND METHODS

The studies reported here were carried out on adult male rats weighing around 300 g. In some of these studies the animals used were Sprague-Dawley rats and in some Wistar rats (Southern Biological).

In all instances induction of adjuvant arthritis was by a standard procedure involving the sub-plantar injection of 0.5 mgm killed Mycobacterium butyricum in 0.1 ml heavy mineral oil into the left hind paw. The progress and severity of the adjuvant disease was assessed by means of weight change and paw scores (Currey and Ziff 1966) immediately prior to withdrawal of blood.

Plasma immunoreactive gastrin was measured by the method described in detail in Chapter 2 of this thesis using the same antiserum R98(2) raised in rabbits to human synthetic gastrin . Blood was obtained either by venepuncture of the rat tail vein or by direct cardiac puncture. All blood samples with the exception of those obtained in experiment 1 were obtained after a 15 hour overnight fast during which the rats were kept in wire-bottomed cages to prevent coprophagia.

Gastric fistula rats were prepared surgically at least two weeks prior to test. The surgery comprised the formation of a simple gastric fistula at the junction of the main stomach

and the rumen. Patency was maintained by means of a titanium steel cannula.

Experiment 1.

In the first study 13 Sprague Dawley rats were studied before and 15 days after the induction of adjuvant arthritis. Assessment of weight, paw scores and immunoreactive gastrin were measured on both days. No attempt was made to fast the rats during this study.

Experiment 2.

In this study 22 Sprague Dawley rats were used. Assessments of weight, paw scores, and immunoreactive gastrin were carried out on the day prior to the injection of adjuvant and on the fourteenth day after injection. In 8 rats assessments were carried out on both days while in 9 only the pre adjuvant assessments were made and in 5 only the post induction assessments.

Experiment 3.

In six Sprague Dawley rats an identical experiment was performed except that from day 10 to day 14 each rat was administered indomethacin in an oral dose of 0.5 mg per day.

Experiment 4.

In a group of 10 Sprague Dawley rats using the same experimental protocol time course studies on immunoreactive gastrin were carried out. Blood in these animals was withdrawn on days 0, 1, 7, 14 and 21 after induction of adjuvant induction.

Experiment 5

A similar time course experiment was performed using Wistar rats. In this experiment the days on which blood was withdrawn were 0, 2, 4, 6 and 8 after adjuvant injection.

Experiment 6.

In this study the influence of exogenous synthetic pentagastrin on the course and severity of adjuvant arthritis was assessed.

Each group of 16 rats in this study were injected with different doses of adjuvant. In one group the dose used was 1 mg. of Mycobacterium butyricum which would normally be expected to induce arthritis in 90 per cent of animals. In the second group 0.3 mg. was used and in the third group 0.1 mg. 8 rats in each of these groups was also treated with 5 μg synthetic pentagastrin (ICI) subcutaneously twice daily for three weeks from the day of adjuvant injection. Paw scores in all animals were assessed on days 10, 12 and 17 after injection of adjuvant.

Experiment 7

The effect of induction of adjuvant arthritis on gastric acid output was assessed in this study. 16 rats with gastric fistulae were studied. 8 of these were submitted to the

injection of adjuvant and 8 acted as controls. On days 0, 7, 14 and 21 after adjuvant injection assessment was made of weight, paw scores, immunoreactive gastrin and basal acid output in terms of both volume and concentration over a period of 30 minutes. Acid titration was carried out against sodium hydroxide using a pH meter.

RESULTS

Experiment 1.

The results of this study are demonstrated in table 1. It can be seen that the induction of significant adjuvant disease is confirmed by weight reduction and the change in paw scores. No significant difference, however, is apparent in the concentration of immunoreactive gastrin.

Experiment 2.

When the rats are fasted it can be seen that quite a different result is obtained. In this study the mean weight of the seventeen rats studied prior to the injection of adjuvant was 306.6 g ($\pm$ 5.1g SEM) whereas in the 13 rats studied after induction of adjuvant disease the mean weight was 293.8 g ($\pm$ 7.2g SEM). This reduction however is not statistically significant ($t = 1.50$ NS). The mean paw score of these rats was 2.7 ($\pm$ 0.6 SEM) indicating a moderately severe arthritis. The immunoreactive gastrin was significantly higher in the post-adjuvant group having a mean level of 401.4 pg/ml ($\pm$ 41.7 pg/ml SEM) in comparison to a mean of 234.6 pg/ml ($\pm$ 35.9 pg/ml SEM) ($t = 2.91$; $0.01 > p > 0.001$). The results in those eight rats in which assessments were available both before and after induction of adjuvant arthritis are summarised in table 2. Of this group of animals all but one lost weight and the mean initial weight 311.8 g ($\pm$ 7.0 g SEM) fell to a mean of 283.0g ($\pm$ 10.5g SEM).

This difference is statistically significant ($t = 3.415$; $0.02 > p > 0.01$). Similarly in all of these rats a rise in immunoreactive gastrin concentration was recorded. The initial mean level of 211.8 pg/ml ($\pm$ 41.1 pg/ml SEM) rose to a mean of 478.7 pg/ml ($\pm$ 72.7 pg/ml SEM). This is again statistically significant ($t = 3.182$; $0.05 > p > 0.02$).

Experiment 3.

The results in the six rats in which indomethacin was exhibited are demonstrated in table 3. It can be seen that in this group of rats the mean weight rose from 302.5 g ($\pm$ 12.5 g SEM) to 312.5 g ($\pm$ 13.5 g SEM) although this rise is not statistically significant ($t = 1.10$; NS). In addition the paw scores in this group of rats are considerably lower indicating that these rats were being, at least in part, protected from the induced arthritis by the anti-inflammatory effects of indomethacin. Despite this these rats again showed a statistically significant rise in immunoreactive gastrin from a mean of 243.3 pg/ml ($\pm$ 44.7 pg/ml SEM) to a mean of 375.8 pg/ml ($\pm$ 40.3 pg/ml SEM) ($t = 4.42$; $0.001 > p > 0.0005$).

Experiment 4

The time course of these effects on immunoreactive gastrin in response to the induction of adjuvant arthritis is indicated by table 4 and figure 1. It can be seen that the peak level of

the rise in immunoreactive gastrin in this study was reached on day 7 coinciding with the appearance of the secondary inflammatory response in the injected paw. Thereafter as the generalised arthropathy ensues the immunoreactive gastrin level tends to fall. The mean level of immunoreactive gastrin on days 7 and 14 can be shown to be statistically significantly higher than on the day prior to injection (table 4).

Experiment 5.

The time course studies carried out on Wistar rats are summarised in table 5. In these rats the level of immunoreactive gastrin recorded is considerably lower than in the Sprague Dawley strain. A small rise in immunoreactive gastrin is noted between day 4 and day 6 and this rise is just significant. However the level reached on day 6 is not significantly different from the initial immunoreactive gastrin level. The severity of the secondary arthritis is also considerably less in this group of rats.

Experiment 6.

The studies on the effect of immunoreactive gastrin on the course of adjuvant induced arthritis are summarised in tables 6 and 7 and in figures 2 & 3. It can be seen that treatment with pentagastrin appears to cause earlier onset of generalised adjuvant induced arthritis and at a lower dose of adjuvant than

in the controls. The difference in mean paw scores at 12 days in both upper doses of adjuvant between those rats receiving gastrin and those not is highly significant. A similar effect is seen at the middle adjuvant dose (300 ug) at day 17 but in view of the severity of disease in both groups at the full dose this effect is no longer apparent.

Experiment 7.

The results of this experiment are summarised in table 8. It is clearly seen that at 7 days after induction of adjuvant arthritis a significant fall in gastric acid output occurs. However the results of immunoreactive gastrin assay in this experiment (table 8.) show that while this rises at 7 days the rise is not statistically significant and a similar rise occurs in the control rats although again no statistical significance is reached.

The arthritis in the rats with fistulae appears to be less severe than in non-fistula rats as the mean paw score was only 1.37 ($\pm$ 1.2 SEM). However significant weight differences were noted between these rats and the controls (table 8).

DISCUSSION

Although Bywaters (1958) has reported a naturally occurring joint disease of primates which bears some similarities to rheumatoid arthritis there is no true animal counterpart to the human disorder. Gardner (1960) in an extensive review has also indicated that the laboratory experimental models of arthritis are all rather unsatisfactory with none of them bearing more than a superficial resemblance to human rheumatoid disease. In spite of this the use of such animal models is helpful in elucidating the mechanisms of the inflammatory reaction and its associated phenomena, which are common to all types of inflammatory disease regardless of the cause.

One of the most commonly used animal models of inflammatory arthritis is that of adjuvant arthritis in the rat. Pearson (1964) concluded that this disorder represents one of the best laboratory models of rheumatoid disease, a view which is reflected in its continuing extensive use (Trnavska et al 1972; Mowat and Garner 1972; Liyanage et al 1975).

Adjuvant arthritis is produced by the single injection of classical Freund's adjuvant (killed tubercle bacilli, heavy mineral oil and an emulsifying agent) into one of several depot sites; intra cutaneously; into the paw pad; or into the base of the tail. Usually over the first 24 hours after injection a local irritative erythema develops which rapidly subsides only to recur much more

severely and often with associated ulceration around the fifth to the eighth day after injection. These local phenomena remain the only obvious sign of disease until about the twelfth day when an extensive arthritis and periarthritis ensues (Pearson 1964). Many joints are affected including both the peripheral joints as well as the joints of the tail and spine. The arthritis is often migratory with remissions and relapses but it can also be very severe and persistent. The severity is often influenced by the particular strain of rat used (Swingle et al 1969), and it is of considerable interest that the response of immunoreactive gastrin to the induction of this disease reported here also varied with the strain of rat used. This may reflect the difference in the induced disease itself or it is conceivable that the immunoreactive site of the gastrin molecule varies between these two strains of rat thereby reducing its capability of being 'recognised' by the rabbit antiserum which is raised against the human synthetic gastrin molecule.

Histopathologically adjuvant arthritis consists of an acute and sub acute synovitis with proliferation of the synovial lining cells. Infiltration with inflammatory cells which are predominantly mononuclear occurs in the articular and peri-articular tissues and is accompanied by invasion of bone and cartilage by connective tissue pannus. Extensive periosteal new bone

formation is common especially adjacent to the affected joints (Pearson 1963) and in severe or chronic cases joint destruction is extensive often with fibrous or bony ankylosis.

Careful studies of adjuvant arthritis have failed to demonstrate any cultivable microbiological agent (Sharp et al 1961; Jones and Ward 1963) and transfer of the disease is possible only by the transfer of large numbers of viable lymph node or spleen cells from animals with very early generalised adjuvant disease (Pearson 1964). Many of the studies on adjuvant arthritis testify to an immune mechanism for the polyarthritis. Adjuvant disease can be inhibited by whole body irradiation, by heterologous anti lymphocyte globulin (Currey and Ziff 1968) by lymphadenectomy or by immuno-suppressive drugs (Quagliata et al 1968). The induction of tolerance to the tubercle bacillus by exposure in the early neonatal period will suppress or prevent the subsequent induction of adjuvant disease (Pearson 1964) as will interference in the immune reaction by the exhibition of gram negative bacterial extra cellular products (Wood and Pearson 1962; Quagliata and Taranta 1972).

Significant elevation of fasting immunoreactive gastrin occurs during the induction of adjuvant arthritis. It is of note that feeding obscured this effect which was only evident when strict fasting of the rats was achieved. Fasting the rats

for the puposes of these studies proved to be a considerable problem as these animals which are generally night feeders managed to eat their wooden cages, plastic water bottles and even their own faeces. It was not until they were secured in wire mesh cages for the period of fasting that suitable experimental conditions were attained.

The mechanism of the elevation of immunoreactive gastrin in adjuvant induced arthritis is not clear. If it reflects the same phenomenon as reported in this thesis in patients with rheumatoid arthritis then it is clear that drug therapy and specific rheumatoid factors are not implicated in its pathogenesis and it is likely that it is part of the cascade of phenomena occurring in the inflammatory response (Ward 1974; Rooney et al 1973; McQueen 1973).

The action of gastrin in adjuvant arthritis appears to be a phlogistic one as evidenced by the effect of exogenous gastrin on the onset and severity of the joint disease. Despite this the potent anti-inflammatory action of indomethacin had no influence on the rise in immunoreactive gastrin. This parallels again the human disease situation reported in Chapter 3 of this work as no evidence could be obtained in those patients with rheumatoid arthritis of any effect of anti-inflammatory drugs on the concentrations of immunoreactive gastrin

The peak concentration of immunoreactive gastrin was reached in these rats at 7 days after adjuvent injection. This was prior to the peak of the inflammatory response and taken in conjunction with the evidence that exogenous gastrin enhances the inflammatory effect this would suggest that gastrin is involved as an active mediator in the initiation or maintenance of the inflammatory reaction.

However, the reduction in the usual biological effect of gastrin, the secretion of gastric acid, in the face of increasing concentrations of the hormone suggest that gastrin itself may not be the primary agent of this effect. Prostaglandins are well recognised to be involved in the inflammatory reaction (Greaves et al 1971). and prostaglandin E_2 and its methylated analogues have been shown to inhibit gastric acid secretion whether it be mediated via gastrin, histamine or cholinergic mechanisms (Karim et al 1973). It is conceivable that involvement of prostaglandins or other related mediators in inflammation results in gastric acid inhibition with a resultant feed-back effect causing hypergastrinaemia. The possible relationship of the elevation of immunoreactive gastrin to the stress of the induced arthritis has been considered as it has been demonstrated that catecholomines stimulate the release of gastrin (Stadil and Rehfeld 1973). This seems unlikely on two counts.

The major stress in these experiments to the rats appeared to be the 15 hour fast and this affected the control rats as well as those injected with adjuvant. In addition the peak immunoreactive gastrin level was reached at least one week prior to the maximal inflammatory reaction in the joints.

Kalliomaki and his co workers (1965) demonstrated neuroendocrine changes in rats following the induction of adjuvant disease particularly affecting the neurohypophysis. With the recent suggestion by Polak et al (1972) that gastrin may be, at least partly, under pituitary control a mechanism for hypergastrinaemia linking these two observations has to be considered. However what the nature of such a mechanism may be could be no more than fairly wild speculation at the present time.

As Freund's adjuvant is used in the production of the anti-gastrin antiserum in rabbits which is used in the radio-immunoassay it is conceivable that the hypergastrinaemia reflects a cross reaction with the antiserum by the adjuvant used to induce the arthritis. However why this should vary with time as has been shown is difficult to explain and there is clear evidence of immunological identity in the assay with human gastrin as demonstrated by identical dilution curves for rat serum with synthetic human gastrin.

Further studies on this interesting animal model of inflammatory arthritis are indicated in view of the importance of establishing whether the hypergastrinaemia in these rats has the same basic cause as that encountered in rheumatoid arthritis.

Table 1

Weight (g) paw scores (0-5) and immunoreactive gastrin concentrations (pg/ml) in non-fasting rats on the day prior to and 14 days after injection of adjuvant.

	Pre-Adjuvant			Post-Adjuvant		
Rat	Weight	Paw-Score	Gastrin	Weight	Paw-Score	Gastrin
1	320	0	315	330	2	315
2	375	0	250	360	4	185
3	350	0	250	345	1	185
4	425	0	270	350	1	400
5	280	0	400	280	2	285
6	325	0	240	350	1	205
7	400	0	355	355	3	400
8	380	0	175	380	4	175
9	340	0	265	300	1	285
10	310	0	335	320	2	295
11	350	0	550	300	1	485
12	325	0	355	310	4	315
13	400	0	655	370	5	295
Mean	352.3		339.7	334.6		294.2
SEM	11.55		± 36.9	8.46		± 26.0

Paired t test comparing weight before and after induction of adjuvant

$t = 2.22$; $0.05 > p > 0.01$

Paired t test comparing immunoreactive gastrin before and after induction of adjuvant.

$t = 1.46$ N.S.

Table 2

Weight (g) paw scores (0-5) and fasting immunoreactive gastrin concentrations (pg/ml) in 8 rats before and after injection of adjuvant.

	Pre Adjuvant			Post Adjuvant (Day 14)		
Rat	Weight	Paw-score	Gastrin	Weight	Paw-Score	Gastrin
6	300	0	120	275	3	505
8	300	0	405	250	2	455
10	355	0	130	325	3	790
13	325	0	355	300	5	470
16	295	0	245	250	2	610
17	310	0	135	275	1	610
19	310	0	215	270	2	230
20	300	0	90	325	1	160
Mean	311.8		211.8	283.7		478.7
SEM	± 7.0		± 41.1	± 10.5		± 72.7

Table 3

Weight (g) Paw scores (0-5) and immunoreactive gastrin concentrations (pg/ml) in 6 rats before and after induction of adjuvant in whom indomethacin 0.5 mgm.daily had been exhibited from day 10 to day 14.

	Pre-Adjuvant			Post-Adjuvant (day 14)		
Rat	Weight	Paw-score	Gastrin	Weight	Paw-Score	Gastrin
1	305	0	300	280	1	490
2	290	0	325	320	2	325
3	275	0	130	275	1	260
4	325	0	390	350	1	505
5	350	0	180	350	1	335
6	270	0	135	300	1	340
Mean	302.5		243.3	312.5		375.8
SEM	12.56		44.7	13.5		40.3

t tests weight $t = 1.10$ N.S.

gastrin $t = 4.42$; $P < 0.00005$

Table 4

Immunoreactive gastrin (pg/ml) in 8 rats on the day prior to and days 1, 7, 14 and 21 after induction of adjuvant arthritis.

	DAY				
Rat	0	1	7	14	21
1	60	85	105	250	105
2	115	155	-	165	180
3	165	125	155	165	50
4	145	135	230	135	135
5	210	125	495	105	260
6	75	65	240	180	80
7	135	85	230	250	145
8	125	-	210	260	230
Mean	128.7	110.7	237.8	188.7	157.8
SEM	± 16.9	± 12.3	± 46.7	± 20.3	± 27.3

t test between days 0 and day 7 $t = 2.31$ $0.01 > p > 0.025$

0 and day 14 $t = 2.30$ $0.01 > p > 0.025$

Table 5

Immunoreactive gastrin (pg/ml) in 10 Wistar rats studied 0, 2, 4, 6 and 8 days after adjuvant injection.

	Gastrin					Paw Score	
Days after adjuvant injection	0	2	4	6	8	0	6
Rats 1	55	30	10	20	30	0	1
2	20	-	35	75	20	0	1
3	25	0	0	20	20	0	1
4	5	0	0	25	0	0	2
5	15	30	15	15	30	0	1
6	15	15	35	25	15	0	2
7	10	0	25	15	15	0	1
8	15	40	0	15	0	0	3
9	5	20	0	45	20	0	1
10	-	0	25	5	5	0	1
Mean	18.33	15.0	14.5	26.0	15.5	0	1.41
S.E .M.	± 5.1	± 5.3	± 4.6	± 6.4	± 3.5	0	

paired t test comparing days 0 and 6

$t = 1.14$ NS.

paired t test comparing days 4 and 6

$t = 2.27$ $0.025 > p > 0.001$

Table 6

Mean paw scores (± SEM) in the injected paw in rats with varying doses of adjuvant and with concomitant treatment with synthetic gastrin pentapeptide (ICI) in a dose of 5 ug per rat twice daily.

Days after adjuvant		Dose of Adjuvant 100 ug Without gastrin	100 ug with gastrin	300 ug Without gastrin	300 ug with gastrin	1000 ug without gastrin	1000 ug with gastrin
10	Mean	0	0	0	0.5	0.62	1.0
	SEM	-	-	-	± 0.19	± 0.18	± 0.19
	t	-		-		1.43	
	p	-		-		NS	
12	Mean	0	0	0.87	2.12	2.13	3.63
	SEM	-	-	± 0.39	± 0.44	± 0.44	± 0.32
	t	-		2.10		2.74	
	p	-		0.005 > p > 0.025		0.01 > p > 0.005	
13	Mean	0.75	0.87	2.62	4.0	4.12	4.5
	SEM	± 0.37	± 0.23	± 0.53	± 0.46	± 0.35	± 0.19
	t	0.29		1.95		0.94	
	p	NS.		0.05 > p > 0.025		NS.	

Table 7

Total paw scores (± SEM) in rats with varying doses of adjuvant and with concomitant treatment with synthetic gastrin pentapeptide (ICI) in a dose of 5 ug per rat twice daily.

Days after adjuvant		Dose of Adjuvant: 100 ug without gastrin	100 ug with gastrin	300 ug without gastrin	300 ug with gastrin	1000 ug without gastrin	1000 ug with gastrin
10	Mean	1.12	1.25	3.62	4.75	4.87	5.75
	SEM	± 0.12	± 0.25	± 0.32	± 0.53	± 0.29	± 0.31
	t	0.45		4.20		2.03	
	p	NS		0.0001 > p > 0.00005		0.05 > p > 0.025	
12	Mean	0.87	1.37	4.62	7.37	7.0	8.62
	SEM	± 0.26	± 0.26	± 0.37	± 0.88	± 0.49	± 0.32
	t	1.44		2.68		2.62	
	p	NS		0.01 > p > 0.005		0.01 > p > 0.005	

Table 8

Mean values (± SEM) for weight (g), volume of gastric juice (mls), total titratable acidity (meq) and plasma immunoreactive gastrin concentration (pg/ml) in rats with gastric fistulae (8 control rats and 8 after induction of adjuvant arthritis).

Day		Weight	Volume	T.T.A.	Gastrin
0	Control	286.3 ± 3.8	0.57 ± 0.09	90.03 ± 24.98	37.9 ± 10.2
	Arthritis	290.5 ± 4.2	0.56 ± 0.13	94.36 ± 28.04	56.4 ± 7.8
7	Control	322.8 ± 5.1	0.48 ± 0.05*	89.60 ± 15.01*	74.3 ± 12.6
	Arthritis	305.1 ± 4.3	0.25 ± 0.04	37.43 ± 6.34	82.9 ± 8.7
14	Control	341.5 ± 4.9*	0.58 ± 0.06	93.12 ± 10.62	45.8 ± 3.0
	Arthritis	302.7 ± 5.1	0.59 ± 0.11	74.19 ± 27.47	62.1 ± 10.3
21	Control	368.4 ± 8.3*	0.56 ± 0.06	96.86 ± 17.50	62.9 ± 15.5
	Arthritis	298.5 ± 8.2	0.59 ± 0.05	66.98 ± 15.57	61.4 ± 8.1
28	Control	403.1 ± 9.4*	0.73 ± 0.10	90.96 ± 14.95	-
	Arthritis	310.4 ± 8.2	0.83 ± 0.08	68.96 ± 11.31	-

T.T.A. - total titratable acidity

* t test shows significant difference $0.01 > p > 0.001$.

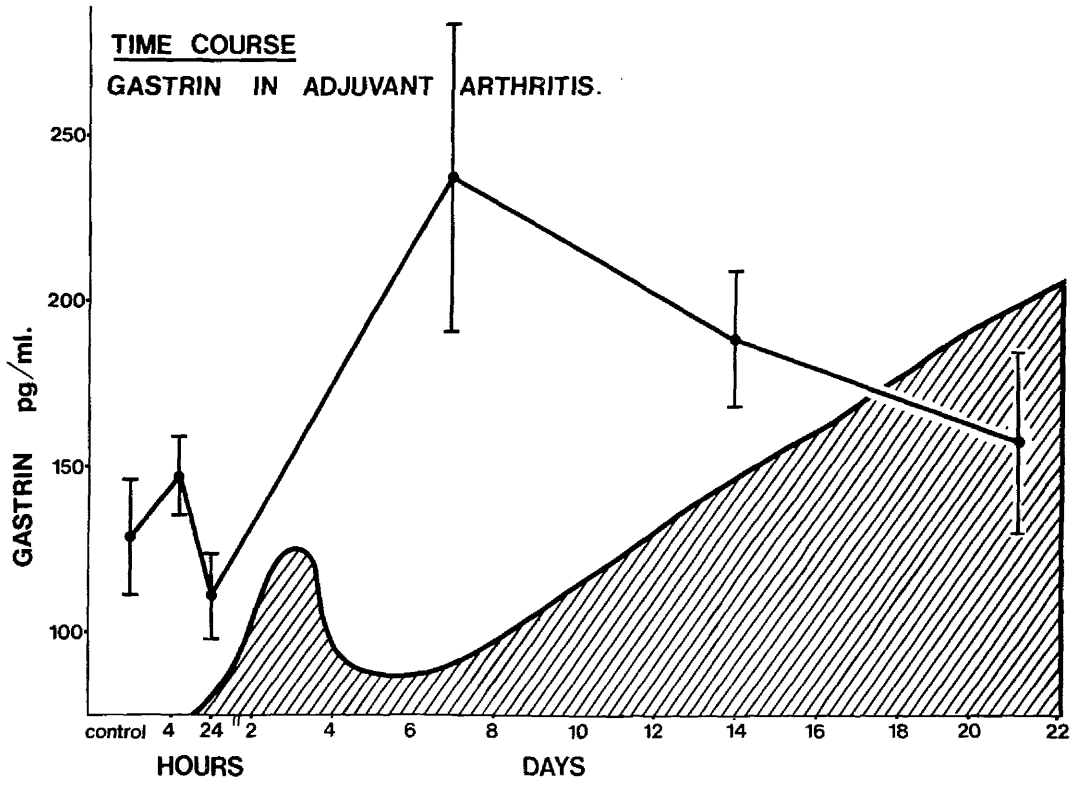


Figure 1. Time course of the elevation of immunoreactive gastrin in rats with adjuvant arthritis. (Shaded area represents time course of the inflammatory joint disease.)

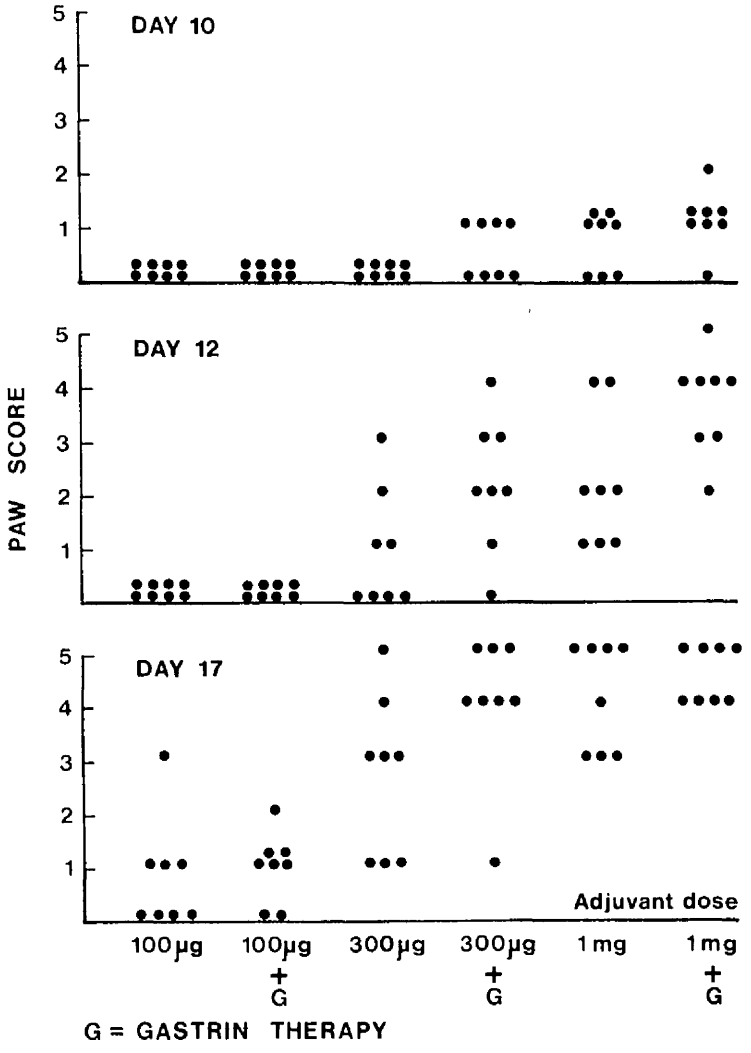


Figure 2. Effect of therapy with gastrin on single paw scores of rats given varying doses of adjuvant.

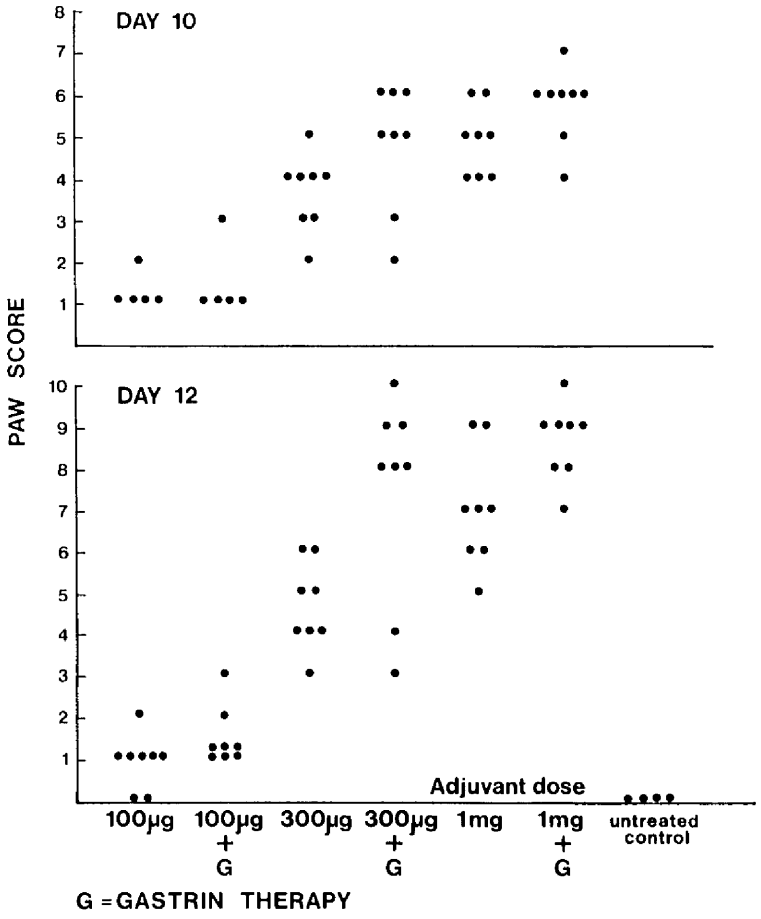


Figure 3. Effect of therapy with gastrin on total paw scores of rats given varying doses of adjuvant.

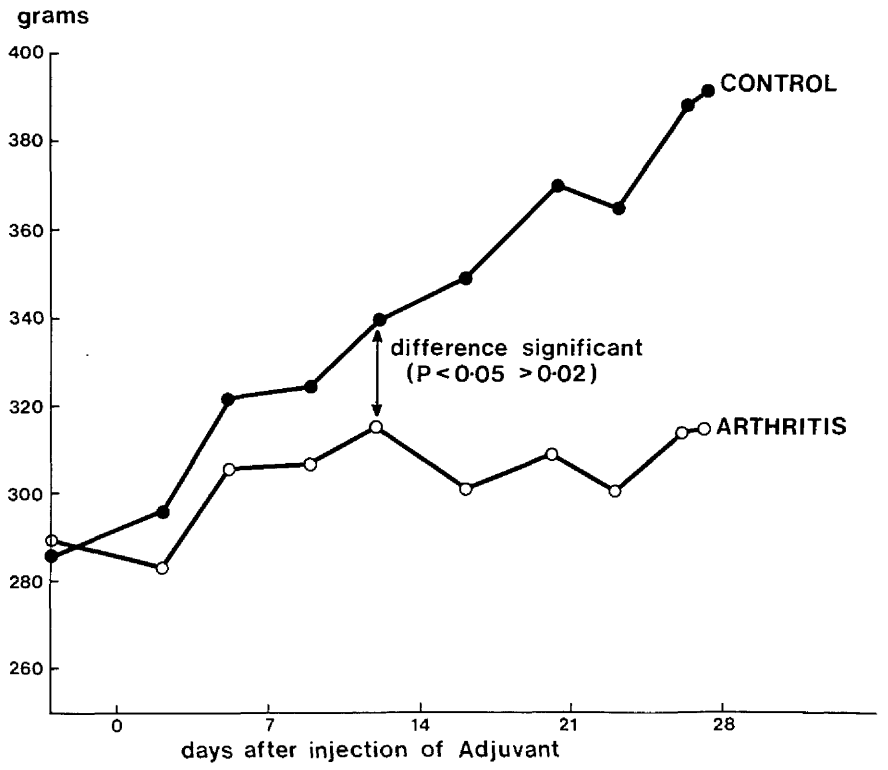


Figure 4. Time course of weight of rats during induction of adjuvant arthritis (fistula experiment).

Histamine and gastrin :

They both good servants, both ill masters be.

Fulke Greville 1554-1628.

SUMMARY

In this chapter the literature is reviewed regarding a possible physiological role for histamine. Its function in inflammation is reviewed with particular emphasis on its probable role as a vascular mediator. The interrelationship between gastrin and histamine in the control of gastric function is extensively discussed and the evidence for the presence of two types of receptor for histamine analogous to the α and β adrenergic receptors is presented. The possible interaction of gastrin and histamine H_2 receptors in parietal cells is considered. With this background studies are reported on the effect of histamine and gastrin on the microcirculatory control of synovial joints in dogs. These studies were conducted using the technique of monitoring the clearance of injected radioactive ^{133}Xenon. The evidence that this technique reflects changes in the venous drainage of synovial tissue is reviewed. Experiments wherein clearance of radioactivity from the joints is directly compared with the radioactivity in the femoral vein of the same limb are reported and their possible significance considered.

Evidence from the effects of administration of H_1 and H_2 receptor antagonists on this system is presented to support the contention that the major histamine effect on subsynovial blood vessels is mediated via receptors of the H_2 type. The failure of gastrin to influence small blood vessel control is demonstrated and the lack of any interaction between gastrin and histamine on the cells responding to H_2 receptor stimulation in this system is considered.

Histamine - current thoughts on its physiology and relationship to the stomach and to the inflammatory response.

'In medias res'

At the heart of the matter

Horace 65-8 BC.

In 1907 Windaus and Vogt succeeded in synthesising histamine from imidazole-propionic acid and in 1910 Barger and Dale isolated this same compound from the alkaloids of ergot. In the same year Dale and Laidlaw published the first study on its pharmacological effect. However, in spite of extensive studies over more than sixty years the physiological role of histamine remains in doubt. It occurs widely in nature in both plant species, such as the tomato or nettle, and in animals. Its presence in virtually all the tissues of man bespeaks a physiological function especially as the tissue content of many organs represents toxic or even lethal doses of the amine. In such organs it is maintained perforce in an inactive state sequestered in storage granules of tissue mast cells (Kelly et al 1971). In plasma there is under normal circumstances less than 1 ng/ml (Adam et al 1957) and although Emmelin et al (1941) demonstrated a threshold dosage of histamine to induce specific effects, the plasma concentrations which elicit gastric secretion are so low as to allow Obrink (1948) to conclude that at least in this aspect no threshold existed.

Injected histamine is rapidly destroyed but when the amine is formed in vivo it is stable for many days with an average tissue half life of upwards of fifty days (Grollman 1962).

Histamine is thought to be a local hormone especially in the skin where it initiates vasodilator axon reflexes (Tolia et al 1970). It is of interest that the greatest concentrations of the amine are in tissues in contact with the external environment, skin, lung and bowel. It is formed in tissues by decarboxylation of histidine (See fig.1.). This in most tissues is achieved by the action of the specific enzyme histidine decarboxylase. In the gastric mucosa (vide infra) the enzyme involved in the formation of histamine appears to vary in different species. Free histamine is rapidly removed from the circulation and the larger part of it is metabolised or excreted (Cross 1973). However it does cause a fall in the histamine forming capacity of the tissues (Kahlson et al 1965). Histamine manifests its pharmacological effects through three main groups of actions. It is a measure of the original studies of Dale and Laidlaw (1910) that with one major exception the pharmacological effects described by them are very similar to those of any modern textbook of pharmacology; the exception being the stimulatory effects of histamine in gastric secretion which was not described until 1920 by Popielski.

Histamine exerts profound and complex effects on the vascular system. The effect on capillaries varies with their initial tone (Rocha e Silva 1966) although its usual effect is to produce marked vasodilatation with subsequent increase in capillary permeability and exudation of plasma into the tissues. These actions are quite independent of any neuronal reflex (Naranjo and Naranjo 1958). Clinically this can be seen as urticaria or as the triple response of Lewis (Lewis 1927). When extensive this response is associated with a profound fall in blood pressure (Dale and Laidlaw 1910, Folkow et al 1948). Until recently it had been believed that histamine had no direct effect on the myocardium (Alstead 1960). However, Kuye and Levi (1972) have demonstrated direct effects in isolated guinea pig heart with resultant sinus tachycardia, atrio-ventricular block and a reduction in coronary blood flow. Smooth muscle in almost all sites is affected by the action of histamine, particularly uterus (Dale and Laidlaw 1910; Dews and Graham 1946), gut (Dale and Laidlaw 1910; Ash and Schild 1966) and bronchi (Dale and Laidlaw 1910; Ash and Schild 1966).

Histamine is a powerful secretory stimulus to many glands, the most important of which are the parietal cells

of the stomach (Popielski 1920; Kay 1953) (vide infra) but other glands such as the salivary glands, pancreas and adrenal medulla also respond to histamine (Werle and Lorenz 1966; Lorenz et al 1968).

The pioneer work of Dale and his colleagues (Barger and Dale 1910; Dale and Laidlaw 1910; Dale 1950) and of Lewis and his colleagues (Lewis and Grant 1924; Lewis 1927) established clearly that histamine could reproduce the acute vascular changes of injury as seen in the triple response. Such changes include vasodilatation and increased vascular permeability.

Clear evidence exists that histamine is released from the tissues following a wide variety of injurious stimuli including thermal (Spector and Willoughby 1963) chemical (Spector and Willoughby 1959) and anaphylactic (Lichtenstein and Osler 1964) damage. Although unable to show a major effect on the whole inflammatory response, using only conventional H_1 antagonists, Spector and Willoughby (1959), and Wilhelm and Mason (1960) clearly demonstrated an influence of histamine on the early vascular responses.

Spector and Willoughby (1964) in their excellent review

of this subject conclude that the

> 'role of histamine in many types of inflammation is to initiate vascular change and to sustain them for perhaps the first hour or so after injury, later being superseded by other mechanisms'.

Despite the large volume of work on inflammation and on its mediators (Rooney et al 1973; Velo, Willoughby & Giroud 1974) since this time there has been no reason as yet to change this interpretation of the role of histamine in inflammation.

Throughout the years during which histamine has been studied its intimate relationship to gastric acid secretion is striking. Histamine was first shown to have a gastric secretagoguic effect in 1920 by Popielski. This demonstration combined with the almost universal presence of histamine in tissue extracts (Abel et al 1919) threw considerable doubt on the 'gastrin concept' until the clear separation of the hormonal effect from that of the amine by Komarov in 1938.

Despite this clear establishment of the separate identity of gastrin and histamine there was no good evidence of separate function and it remained possible that gastrin was

was a general trigger for the release of histamine. It was not until 1966 that this was conclusively disproved. In that year Blair was able to demonstrate that gastrin had no effect on plasma histamine concentration and that urinary histamine output could not be correlated with gastric acid secretion. Although Code in 1965 showed that the urinary excretion of histamine increases after a meal Blair (1966) was able to show that this was due to intestinal absorption of ingested amine and it could not be correlated with the gastric phase of digestion.

As early as 1938 McIntosh presented an alternative theory linking gastrin and histamine. He suggested that gastrin acts by the local gastric mucosal release of histamine. This concept has been succintly stated by Codo (1956).

> 'Histamine is the final common local chemostimulator of the parietal cells of the gastric mucosa".

Histamine has been found in the mucosa of the body of the stomach in all species in which it has been sought (Blair 1966; Code 1965). The mucosal tissue histamine concentration is highest in the region of the neck of the oxyntic glands (Feldberg and Harris 1953; Smith 1959) but it is not clear

whether this is due to this being the area of highest concentration of parietal cells or whether it is due to the high rate of turnover of non-parietal cells in this area (Grossman 1967).

In rats it has been clearly demonstrated that all stimuli for the gastric secretion of hydrochloric acid are associated with a reduction in the mucosal histamine content (Kahlson et al 1965, Shore 1965)and when porta-caval shunts are established, a rise in tissue histamine content occurs which is associated with a rise in gastric acid secretion (Fischer and Snyder 1965). It has not proved possible, however, to reproduce these results in other species such as the dog, cat or guinea pig (Blair 1966; Code 1965) and their true significance is as yet unknown especially in relation to human physiology. It may be that they reflect the presence in rat gastric mucosa of the specific histidine decarboxylase as this enzyme is not present in the mucosae of other species (Code 1965). Histamine is largely metabolised by deamination by histaminase or diamine oxidase, or by ring methylation by imidazole-N-methyl transferase. The rat is unique among the species studied to date in that the gastric mucosa of all other species contain the ring methylating enzyme (Brown et al 1959) but no histaminase or diamine oxidase

(Code 1965). In the rat the possible presence of the ring methylating enzyme as well as the deaminating ones is disputed (Brown et al 1959; Fischer and Snyder 1965).

Recently Code (1973) has delineated a possible role for methylation as a means of local control of gastric acid secretion. Navert and his colleagues (1969) have shown that when ^{14}C histamine is administered, its ring N methylated derivative (tele methylhistamine) is the major radio-active compound accummulating in the gastric mucosa (Ragins et al 1964). It is now felt that methylation is the major if not the only pathway of metabolism of histamine in gastric mucosa (Code et al 1972).

However, in addition to the ring methylated compounds Code and his colleagues (1972) have identified radio-active histamine derivatives which were methylated on the side chain, N-methylhistamine and N-N- dimethyl histamine. While ring methylated histamine does not stimulate gastric secretion (Grossman et al 1952) the side chain methylation produces compounds which are more potent than histamine itself (Lin et al 1962) as well as acting more rapidly (Chilvers and Code 1971). These compounds appear to have a very transient half life in the mucosa whereas the ring methylated compound is held in the mucosa (Navert et al 1967). From all this evidence Code (1973) has postulated that

methylation of histamine is the local control for gastric secretion. He has suggested that it must be side-chain methylated to act and that control of secretion is by switching from ring to side-chain methylation. Amino-guanidine inhibits the deaminating enzymes histaminase and diamine oxidase. It has been shown that it augments both basal and stimulated acid secretion (Code 1965; Haverback et al 1965) whether such stimulation be induced by histamine, by side-chain methylated histamine or by gastrin (Code and Maslinski 1971). Code (1973) has now changed his hypothesis to stating that :

> "histamine and its side-chain-methylated derivatives, N methyl histamine and N N dimethyl histamine provide a common mechanism for the activation of the parietal cells"

The role of histamine in allergic and inflammatory responses has led to many attempts to find drugs which could inhibit the actions of the amine. It was more than a quarter of a century after Dale and Laidlaw's description of its action that Bovet and Staub (1939) managed to find a compound with histamine-blocking activity. Although too toxic for clinical use the compound they described, thymoxyethyldiethylamine protected guinea pigs from a lethal dose of exogenous histamine, and lessened the symptoms of anaphylactic shock (Staub and Bovet 1937).

These observations and investigations were extended by Halpern (1942), by Bovet and his colleagues (1944) and by Loew et al (1946) to produce the early clinically useful and effective anti-histamine drugs.

Bovet (1950) has reviewed the main structure activity relationships of the conventional anti-histamines and it is fairly clear that the major part of the activity in these compounds lies in the substituted ethylamine core which probably competes with histamine for cell receptors (Douglas 1965) (Fig.2). The conventional anti-histamines antagonise most but not all of the pharmacological actions of histamine by means of competitive antagonism (Marshall 1955). These include the effects on smooth muscle, in the gut, uterus and respiratory tract. They also antagonise the vasoconstrictor effects of histamine on the large blood vessels and have some effect on the histamine response in the smaller blood vessels although this is incomplete.(Folkow et al 1948). These conventional anti-histamine drugs have no effect on histamine induced gastric acid secretion (Payne et al 1961) and it is this lack of effect on some actions that led Ash and Schild (1966) to postulate the existence of two types of histamine receptor. This was adequately confirmed when Black et al (1972) introduced a new group of compounds by means of which they were able to identify and

separate both types of receptor. In their search for active H_2 agonist and inhibitor compounds these workers synthesised and tested about 700 compounds in a period of eight years (Black et al 1972). This culminated in the production of the highly effective H_2 receptor antagonists, burimamide and metiamide. There are marked chemical distinctions between these H_2 receptor antagonists and the conventional H_1 receptor antagonists. The H_1 antagonists possess aryl or heteroaryl rings which need not have any structural relationships to the imidazole ring of histamine itself (Van den Brink 1967). These make the molecule strongly lipophilic and probably act as binding groups while the H_1 antagonism rests in the side chain which is positively changed at physiological pH (Fig.2). H_2 receptor antagonists on the other hand are dependent for their activity on the presence of an imidazole ring identical to that of histamine so that it is likely that this ring is the part of the molecule of histamine which activates the H_2 receptor (Durant et al 1973). (Fig.3). The identification of these H_2 receptors antagonists has stimulated a large amount of research into their possible therapeutic value especially as possible anti-ulcer agents. Black et al (1972) had clearly demonstrated inhibition of histamine stimulated acid secretion and that this inhibition was of competitive type. These workers also

presented evidence that H_2 receptor antagonists also inhibited the gastric acid secretion stimulated by pentagastrin and feeding but had little or no effect on vagally induced secretion. These observations were largely confirmed and extended by Parsons (1973) although Black (1973) has suggested that the relationship between gastrin and histamine may be less clear cut.

Some personal observations on the role of histamine and gastrin in the microcirculation of synovial joints.

Desire of knowledge, like the
thirst of riches, increases ever
with the acquisition of it.

Laurence Stern 1713-1768.

Tristram Shandy.

The fact that histamine has long been known to have both pressor and depressor effects on the general circulation under different circumstances and the fact that H_1 receptor antagonists only partly antagonise these effects (Rocha e Silva 1966) can now be explained by the demonstration that large and medium sized blood vessels contain both H_1 and H_2 receptors which interact in the final control of vascular tone (Persons and Owens 1973; Powell and Brody 1973). The relevance of histamine receptors to the control of the microcirculation, however, remains unclear. Studies were designed to examine the respective roles of histamine H_1 and H_2 receptors in the control of the peripheral circulation using the clearance of ^{133} Xenon from a diarthrodial joint as a monitor of histamine induced changes in the synovial microcirculation as well as to study the influence of exogenous gastrin on this system both of itself and during the studies with histamine

Materials and Methods.

These experiments were carried out on adult mongrel dogs weighing between 18 and 25 Kg. at the Wellcome Surgical Research Institute in Glasgow. In every experiment

anaesthetic induction and maintenance, and monitoring of blood pressure and blood gases were standardised. Anaesthetic agents employed were thiopentone, nitrous oxide and oxygen with up to one per cent halothane. In all experiments in which gastrin was used the dogs were totally fasted for 10 hours prior to the start of the study. Radioactive ^{133}Xenon (0.1 ml. of a solution of 10ml. of ^{133}Xenon dissolved in 3 mls of 0.9 per cent sterile saline, Amersham, England) was injected intra-articularly and the count rate monitored by a thallium activated, sodium iodide scintillation crystal (1 inch x 1 inch, P1062), photomultiplier and pulse height analyser (7050) and rate meter (7070) and recorded upon a potentiometer recorder (Smiths RE.520). Particular attention was paid to injection technique, injected volume and other methodological details known to have a potential influence on isotope clearance (Dick 1972). The half life ($T\frac{1}{2}$) (minutes) was obtained from a semi-logarithmic plot of the count rate against time and was derived from sections of the graph 15 to 20 minutes duration before and following injections of active compounds. Changes in clearance rate reflect changes in tissue perfusion although expression of results in units of blood flow is less securely based (Dick 1972). Accordingly the results of the studies on Xenon clearance are expressed in terms of the relevant $T\frac{1}{2}$

values. These $T\frac{1}{2}$ values show considerable variation in the individual base-line values but this is in accord with previous studies (Dick et al 1970)and the technique has been shown to have acceptable reproducibility during repeat studies on the same joint.

Drug Studies

Each agonist and antagonist was injected into each joint in increasing doses following a control monitoring period and the effects of each drug on the clearance rate of ^{133}Xe were recorded. The effect of pre-treatment with each antagonist upon the histamine response was determined at increasing dose ratios (weight to weight) of antagonist to agonist and similarly the effect of pre-treatment with H_2 receptor blocking drugs upon the response to isoprenaline and noradrenaline was studied at different dose ratios. For each experiment control monitoring periods were inserted between injections of different drugs to allow the clearance rates to return to base-line values. In addition a series of experiments were undertaken to study the duration of the effect of the H_2 receptor antagonist metiamide. In these experiments histamine was injected at increasing intervals following an injection of metiamide. A further series of experiments were carried out to

establish if gastrin (synthetic human pentapeptide) had any influence on the clearance of ^{133}Xenon and also to see if gastrin exerted any influence on the histamine responses or on the blockade of such responses by specific H_1 and H_2 receptor antagonists.

Histamine was supplied as histamine acid phosphate (Evans Medical), isoprenaline as isoprenaline sulphate (Boots), noradrenaline as noradrenaline acid tartrate stabilised in sodium metabisulphite (Winthrop), mepyramine as mepyramine maleate (May and Baker). Metiamide was kindly donated by Smith, Kline and French Laboratories Ltd. Gastrin was synthetic human gastrin pentapeptide (CIBA Geigy). All drug dilutions were accomplished with sterile 0.9 per cent saline.

Isotope Recovery

The dog femoral vein was exposed in the groin and femoral venous blood was diverted through a siliconised brass coil placed round a thallium activated sodium iodide crystal connected to a pulse height analyser, a rate meter and potentiometric recorder. Two vertical side arms were machined into the coil at the inflow and outflow points and the difference in height of the blood columns in each side arm was read in millimeters and plotted linearly against

time. Resistance to flow through the coil was minimised by machining the coil inflow and outflow tracts smoothly at their insertions into the venous system. The coil was constructed of brass to reduce absorption of ^{133}Xenon into the metal. To allow comparison between the count rate obtained from the coil and from the joint it was necessary to convert both into percentages of total count obtained from the standard amount of ^{133}Xenon injected and measured under the different conditions obtained in the knee joint and femoral vein coil.

Prior to the start of the experiment the coil was filled with a solution containing the same amount of radioactive ^{133}Xenon as to that to be injected into the joint. The count rates of this same dose of ^{133}Xenon injected into the joint and into the coil were measured at the start of the experiment (T 0) and designated 100 per cent. The count rates over the joint and in the coil were then monitored continuously and the percentage of the base-line count leaving the knee was compared with the percentage of the base-line count detected in the coil. The latter figure was derived from the count of the coil and the femoral venous flow rate at that time calculated from the difference in height of the columns of blood in the two vertical side arms with reference to a calibration

curve. At the conclusion of each experiment the coil outflow tract was disconnected, femoral venous flow was measured by free flow and compared with the femoral venous flow rate derived from the vertical side arms. Additional in vitro standardisation was achieved by plotting the difference in heigth of the two columns of blood in the side arms against known flow rates measured by free flow. A calibration curve was constructed by conducting warmed heparinised dogs' blood through the coil from an elevated reservoir and varying the flow rate by partially clamping the inflow cannulae.

RESULTS

Histamine produced a consistent vasodilator effect as shown by shortening of the $T\frac{1}{2}$ values (minutes) of ^{133}Xenon clearance in doses from 0.1 ug to 1.0 ug (Table 1).

Metiamide alone produced no consistent change in ^{133}Xenon clearance (Table 2) but a dose ratio of metiamide to histamine of 500:1 and above consistently abolished the action of histamine. At a dose ratio of 200:1 (metiamide: histamine) the action of histamine was abolished on three of the seven occasions on which it was observed, while in all cases at a dose ratio of less than 200:1 (metiamide: histamine) the effect of histamine was clearly evident (Table 3).

Mepyramine alone in doses of 125 ug and above produced a change in ^{133}Xenon clearance rate (Table 5). It did not abolish the effect of histamine given after a return of the clearance rate to a base-line value in any dose ratio tested (up to 1000:1) (Table 4). When metiamide was given before mepyramine in a dose ratio of 1:1 (metiamide: mepyramine) this effect of mepyramine was diminished (Table 5). In four experiments histamine and ^{133}Xenon were injected one hour after a blocking dose of metiamide had been given and on three of these four

occasions a histamine response was seen (Table 6). Previous experiments had shown that the blocking action of metiamide was still present thirty minutes after injection.

Isoprenaline was injected after 500 ug and 1000 ug of metiamide and on all occasions it produced an increase in ^{133}Xenon clearance rate in a dose of 1.0 ug (Table Similarly a decrease in ^{133}Xenon clearance rate was subsequently obtained when noradrenaline (1.0 ug) was injected (Table 6). The changes in ^{133}Xenon clearance rate produced by isoprenaline and noradrenaline when given after pre-treatment of the joint with metiamide were of similar magnitude to those produced by these agents given without prior administration of metiamide.

The administration of pentagastrin in all doses tested 2.5×10^{-5}, 2.5×10^{-7}, 2.5×10^{-9} gm. produced no change in the clearance of ^{133}Xenon (Table 8). Similarly no difference in the threshold or magnitude of the response to histamine could be produced by the concomitant administration of gastrin in the same doses (Table 9).

The results of the recovery experiments are shown in Table 10 . It can be seen that at 10, 20, 30 and 40

minutes after injection 92, 91, 98 and 93 per cent of radio-activity leaving the joint could be recovered in the femoral vein. Femoral venous flow rates obtained were of the order of 140 ml per minute which is a realistic order of magnitude. When the derived femoral flow rates (110 ml/min; 160 ml/min; and 140 ml/min) were compared with the respective values obtained by free flow (100 mls/min., 155 ml/min., and 145 ml/min.) at the conclusion of the experiments, close agreement was obtained.

DISCUSSION

In these studies the clearance rate of ^{133}Xenon from the synovial cavity has been used to provide an indirect measure of synovial perfusion (Dick 1972; Dick et al 1970). Following intra-articular injection ^{133}Xenon diffuses into the synovial membrane and is cleared by the superficial subsynovial blood vessels to the femoral vein. This contention is supported by the fact that clearance is halted by occlusion of the femoral vein with a tourniquet and recommences when the tourniquet is released (Dick 1972). The work of Stone and Miller (1949) who could detect no radio-activity in the lymphatic system following the clearance of intra-articularly administered isotopes is supported by our own experience that only a maximum of 10 per cent of radio-activity is available to be cleared by all other channels and we could detect no ^{133}Xenon in lymphatic channels during any of our experiments. These factors render significant clearance via a lymphatic pathway unlikely.

The slow diffusion rate of ^{133}Xenon in tissues (Unsworth et al 1969) makes clearance through avascular cartilage unlikely at least in the early stages after injection at a time when our studies were conducted.

Furthermore had any significant clearance been occurring through cartilage and thence through blood vessels in bone it is unlikely that an inflated tourniquet placed proximal to the joint but distal to the site of emergence of nutrient bone blood vessels would stop clearance.

Finally the results reported in the isotope recovery experiment described here show that a very large proportion of the radio-activity leaving the joint at any point in time following intra-articular injection can be accounted for in the femoral vein adding further experimental support for a vascular clearance route of intra-articularly injected ^{133}Xenon. The clearance method for the determination of capillary flow has been used by many workers (Kety 1951; Lassen et al 1964; Sjerson 1967; Dick et al 1970). On theoretical grounds the rate of clearance of an inert gas from the synovial cavity could be considered to be dependent on tissue perfusion, partial pressure gradiant between the synovial cavity and subsynovial vessels, the partition coefficient of the tracer substance between synovial tissues and blood, membrane permeability and the diffusion rate of the isotope across the synovial membrane. ^{133}Xenon is an inert lipid-soluble gas and hence membrane permeability can be discounted. Previously measurement has been made of the partition coefficient of ^{133}Xenon for synovial

tissues from various sites with respect to blood and shown this to be approximately constant with a maximum range from 0.87 to 1.1 and a mean of 1.0. The diffusion rate of ^{133}Xenon in synovial tissue has been shown to be slow and constant (1.0×10^{-5} cm^2, sec^{-1} at 37°C) (Unsworth and Gillespie 1969). Gillespie (1968) has studied the effects of partial pressure gradients on tissue clearance of an inert radioactive gas and has concluded that in a homogeneous tissue the partial pressure at any stage during clearance could be assumed to be approximately constant. Large errors may occur if tissue adjacent to the region under study possessed markedly different flow rates but there is no reason to suppose that an analogous situation obtains in the present context. A small tissue volume which possesses uniform diffusion and partition coefficient and from which gas clearance is mono exponential can be assumed to be functionally homogeneous (Perl et al 1965). ^{133}Xenon clearance from the synovial cavity is mono exponential and hence in the presence of constant diffusion rate, partition coefficient and partial pressure of the gas synovial perfusion can reasonably be considered to be proportional to the clearance rate.

In this study the clearance rate of ^{133}Xenon has been used to investigate the effect of gastrin and histamine and

and the antagonists of histamine on synovial perfusion. This is supported by previous studies in which substances known to be vasodilator or vasoconstrictor to vascular beds elsewhere have been shown to produce the anticipated changes in ^{133}Xenon clearance following intra-articular injection (Dick et al 1971). A similar method has been used to investigate the effects of drugs on the microcirculation of muscle (Gosselin 1966). It has been suggested that if one uses a control monitoring period prior to the administration of a drug it has to be proposed that all the injected drug reaches the isotope containing tissue quickly and uniformly, (Gosselin 1966). In these studies we have compared the effects of a vasoactive drug when given simultaneously with isotope with its effects when given after a control monitoring period and found that results in the same range were obtained using both techniques.

It could be argued that these studies do not distinguish a direct vasodilator effect of histamine on the microcirculation from an effect on the distribution in blood flow within the joint after histamine injection. Thus as ^{133}Xenon is highly fat soluble diversion of venous blood to or from subsynovial fat would produce longer or shorter $T\frac{1}{2}$ values of ^{133}Xenon clearance respectively. If redistribution of blood to lipid rich tissues were

occurring it might be expected that ^{133}Xenon clearance would no longer appear mono exponential as a mono exponential clearance is dependent on tissue homogeneity. This is not our experience. Further, the clearance rate of ^{133}Xenon from adipose tissue has been measured and differs by an order of magnitude from any of the results shown in this study (Larsen et al 1966). Moreover to our knowledge there is no evidence for the existence of a "counter current" change in the distribution of blood flow between synovium and subsynovial adipose tissue as this contention would suggest . Even if this were the mechanism of histamine induced changes in ^{133}Xenon clearance the large dose related changes which have been shown to occur predictably with administration of the amine can reasonably be supposed to denote agonist vascular effect.

Accepting the difficulties in interpretation, the bulk of the evidence supports the conclusion that ^{133}Xenon leaving the counting area is being cleared from a reasonably homogeneous localised, subsynovial area via its effluent venous sytem and thence to the femoral vein and that changes in ^{133}Xenon clearance rate may be interpreted as "vasodilator" or vasoconstrictor" responses.

In this study metiamide produced a dose related

abolition of the histamine vasodilator response in the synovial microcirculation suggesting that there are H_2 receptors in this vascular bed. The pharmacological antagonism of metiamide has been shown by other workers to be specific to the H_2 system (Black and Spencer 1973). In these experiments metiamide produced no abolition of the microcirculatory response to α and β sympathetic agents further supporting the likelihood that its abolition of the histamine vaso dilator response was due to H_2 receptor antagonism. These results also su gest that H_2 receptor blockade from metiamide under these experimental conditions lasts less than one hour.

Histamine H_1 receptors have been shown to play a part in the histamine vasodilator response in large vessels in dogs and cats (Parsons and Owens 1973; Powell and Brady 1973) and to mediate vasoconstriction in rabbit ear arteries (Parsons and Owens 1973). In our experiments, however, we could find no significant effect of mepyramine on the antagonism of the histamine vasodilator response in the synovial microcirculation, nor was any significant vaso-constrictor effect apparent when histamine was administered after H_2 receptor blockade as might be expected if H_1 receptors were present in this situation mediating vaso-constriction. However these findings in a relatively crude, in vivo, experimental situation with local administration of

of antagonists and agonists do not completely exclude H_1 receptors in the synovial microcirculation. Mepyramine alone produced a vasodilator response in high doses. It has previously been shown that mepyramine and other H_1 receptor antagonists (Mota and Dias da Silva 1960; Lorenz et al 1968) may themselves cause histamine release and this might explain the effect of mepyramine on the microcirculation demonstrated in this study. This is supported by the decrease in this effect when mepyramine was given after metiamide in certain dose ratios. On the other hand these results could be interpreted to suggest that mepyramine has an H_2 agonist action. This seems less likely since no evidence of such an action is known from previous work with antihistamines. It is interesting that gastrin had no effect in this experimental model. Gregory and Tracy in 1964 showed that high doses of the hormone caused a drop in blood pressure. There is no evidence, however, of any direct effect of gastrin on blood vessels (Rooney et al 1974) and there is no evidence that gastrin is a general releaser of histamine (Blair 1966). These studies would also militate against the possibility of gastrin producing a local histamine release in synovium. Grossman (1972) has put forward the suggestion that in the case of the parietal cell there are separate but interacting receptors for gastrin, histamine and acetylcholine.

These studies provide no evidence for the existence of similar interacting gastrin receptors in another system despite good evidence that the histamine receptors are also of the H_2 type. It would seem unlikely, under these circumstances, that the hypergastrinaemia associated with synovial inflammation in rheumatoid arthritis (Chapter 3) or adjuvant arthritis (Chapter 5) is due primarily to the changes in the microcirculation of the joints or of its mediator control mechanisms.

Table 1

^{133}Xenon clearance T½ values (minutes) following histamine alone in various doses

Dose (g)	Pre	Post	Dose (g)	Pre	Post
0.1	90	51	1.0	135	22
0.1	45	35	1.0	42	16
0.1	58	35	1.0	35	16
0.1	54	49	1.0	75	26
0.1	138	87	1.0	25	16
0.1	89	53	1.0	68	30
Mean per cent change		32% ± 5.6			54% ± 4.4

Table 2

^{133}Xenon clearance T½ values (minutes) with Metiamide alone in increasing doses

Dose g	Pre	Post
25	115	115
50	20	20
50	45	55
200	49	51
360	50	50
500	47	50
500	40	34
500	24	25
500	35	38
1000	60	58
1000	150	150
Mean	57	59
± SEM	12	12

Mean per cent change 1%

Table 3

^{133}Xenon clearance T½ values (minutes) with g histamine following metiamide given in increasing Metiamide:histamine dose ratios

200:1		200:1		500:1		1000:1	
Pre	Post	Pre	Post	Pre	Post	Pre	Post
115	29	20	12	31	31 †	39	49 †
22	11	38	24	47	47 †	28	33 †
28	13	25	25 †	26	26 †	150	150 †
60	21	32	29	60	58 †	58	58 †
150	59	50	25	37	33 †		
36	15	34	24	35	35 †		
59	29	48	48 †				
Mean 66	24	35	28	39	38	69	73
± SEM 18	6	4	4	5	5	28	26
Mean per cent change	59%		19%		0%		0%

† denotes instances of complete H_2 receptor blockade.

Table 4

^{133}Xenon clearance $T_{\frac{1}{2}}$ values (minutes) with histamine following increasing dose ratios of mepyramine:histamine

	100:1		500:1		1000:1	
	Pre	Post	Pre	Post	Pre	Post
	60	22	71	39	48	15
	38	18	40	23	73	24
	55	31	73	25	36	23
	150	37	39	13	32	18
			32	21	43	23
			96	51	66	18
					47	36
Mean	76	27	59	29	49	22
± SEM	25	4	10	8	6	3
Mean per cent change	60%		50%		51%	

Table 5

^{133}Xenon clearance T½ values (minutes) with mepyramine given alone and with mepyramine following metiamide in two dose ratios

mepyramine : metiamide

	Mepyramine		Metiamide 1.5 : 2.5		Mepyramine 1 : 1	
Dose ug	Pre	Post	Metiamide	Mepyramine	Metiamide	Mepyramine
125	71	31	95	37	80	61
250	40	17	80	33	23	17
250	113	63	145	72	62	52
500	54	31			25	19
500	62	9			60	64
600	32	8			35	31
500	125	18			29	31
500	65	9			69	69
Mean	70	23	107	47	48	43
± SEM	11.6	6.6	19.6	12.3	7.8	7.3
Mean per cent change	65%		56%		13%	

Table 6

^{133}Xenon clearance $T\frac{1}{2}$ values (minutes) with histamine 1 ug given 1 hour after a blocking dose of metiamide.

Pre	Post
63	34
42	22
80	80 +
68	17

+ denotes continuing H_2 blockade.

Table 7

^{133}Xenon clearance $T\frac{1}{2}$ values (minutes) before and after isoprenaline (10 ug) alone and before and after isoprenaline (1.0 ug) after metiamide

Isoprenaline		Metiamide	Isoprenaline	
Pre	Post	Dose ug	Pre	Post
25	8	500	28	12
46	15	500	28	13
43	36	500	28	19
45	13	1000	60	24
26	22			

Table 8

^{133}Xenon clearance T½ values (minutes) following administration of various doses of gastrin.

Gastrin dose pg	Pre	Post
2.5	76.3	79.1
2.5	30.1	31.3
250	47.6	51.5
250	115.2	101.5
25,000	28.5	27.7
25,000	28.8	27.0
25,000	23.6	27.2
25,000	27.1	21.9
Mean per cent change		2.1%

Table 9

^{133}Xenon clearance $T_{\frac{1}{2}}$ values (minutes) following varying doses of gastrin given with 0.1 ug and 1.0 ug histamine.

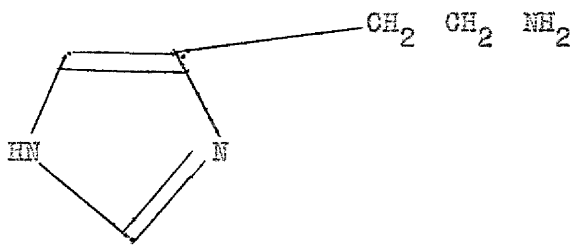
Gastrin dose (pg)	Histamine dose (ug)	Pre	Post
2.5	0.1	76	68
2.5	1.0	51	25
250	0.1	23	12
250	1.0	29	13
25000	0.1	22	12
25000	1.0	89	53

Table 10

Cumulative percentages of 100% dose cleared from stifle joint and cumulative percentage of 100% dose accounted for in femoral vein 10, 20, 30 and 40 minutes after injection.

	10 min.		20 min.		30 min.		40 min.	
Dog No.	Joint	Femoral Vein	Joint	Femoral Vein	Joint	Femoral Vein	Joint	Femoral Vein
2	11.9	11.3	19.4	16.7	25.0	19.2	30.6	20.4
6	12.5	12.6	20.3	20.7	28.6	29.5	32.2	35.3
13	1.9	1.1	17.3	12.0	23.0	18.3	30.8	24.9
16	-	-	33.7	30.1	55.9	52.8	70.6	72.9
18	5.4	7.6	10.7	14.3	18.2	22.3	24.6	30.6
19	4.7	2.4	5.9	5.1	35.1	28.6	61.4	51.7
21	9 .0	7.2	15.5	13.9	23.0	20.2	25.0	25.8
22 L	5.1	7.1	16.0	16.5	33.1	25.9		-
22 R	12.5	8.7	27.0	22.2	35.4	28.4	48.8	38.8
Mean	7.9	7.2	18.4	16.8	30.8	27.2	40.5	37.5
	92		91		88		93	

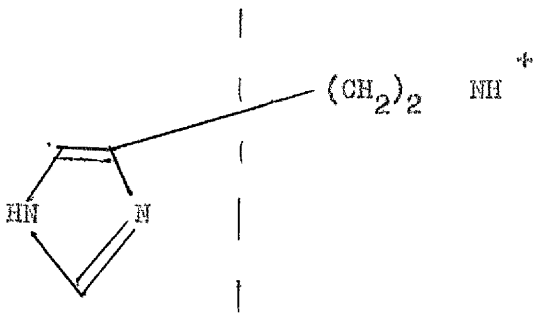
Percentage of counts cleared from joint which can be detected in femoral vein.



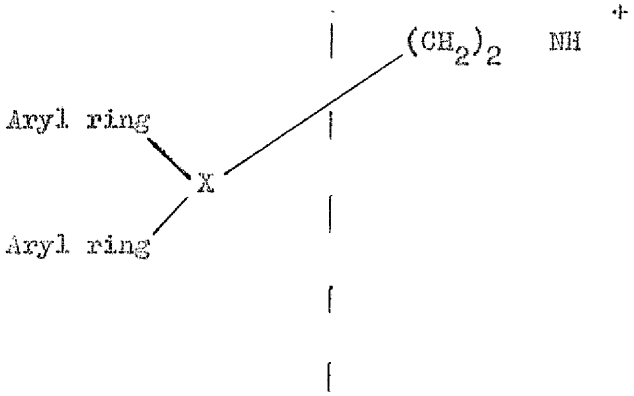
Histamine

Figure 1.

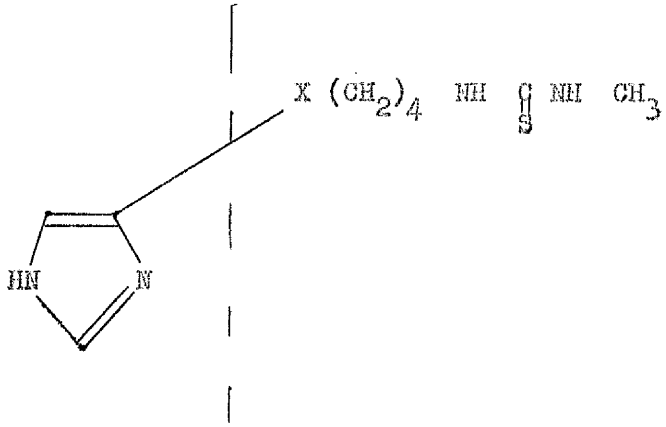
Structure of Histamine.



Histamine



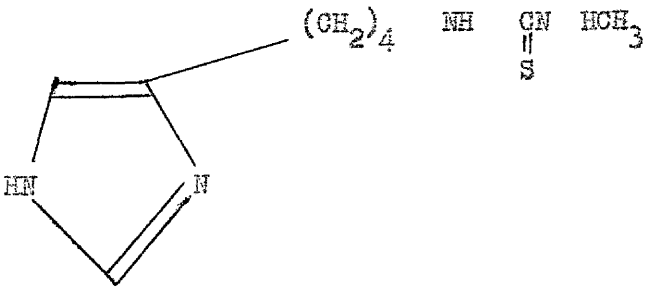
H_1 Antagonist



H_2 Antagonist

Figure 2

Structure of Histamine and its antagonists.



Metiamide

Figure 3.

Structure of Metiamide.

Gastrin in pregnancy, puerperal

and early post-natal physiology.

There all the learn'd shall at the labour stand,
and Douglas lend his soft, obstetric hand.

A. Pope (1688-1744).

Dunciad.

SUMMARY

In this chapter the change in immunoreactive gastrin occurring during pregnancy and parturition are reported. The relationship of these observations to the known physiology of gastric function in pregnancy is discussed. The occurrence of elevation of gastrin in early post natal life is noted and its clinical significance discussed. Some studies of the effects of pregnancy on gastrin and on rheumatoid arthritis are reported.

The effects of pregnancy in relieving the symptoms of rheumatoid arthritis are striking and it was as a result of observing these effects (Hench 1938) that Hench (1949) pioneered the clinical use of corticosteroids in this disease. It is now considered unlikely that these effects of pregnancy are due solely to changes in corticosteroid metabolism (Hill and Holley 1960) but the true mechanism remains unknown.

Similarly significant changes in the physiology of the gastro-intestinal tract occur during pregnancy (Hunt and Murray 1958; Crean 1963) and this is reflected in striking changes in the symptomatology of pathological conditions. The symptoms of duodenal ulceration are almost universally alleviated (Clark 1953) yet during pregnancy many women complain of heartburn de novo, and the precise mechanism for this alteration in symptoms is unknown (Atlay et al 1973).

In view of the noted elevation of immunoreactive gastrin in rheumatoid arthritis it seemed important to establish if the gestational changes in both gastro-intestinal physiology as well as in the symptomatology of rheumatoid arthritis were humorally mediated via the gastrin mechanism.

During these studies the opportunity was taken to study

in greater detail the influence of adrenocortical function on immunordactive gastrin as well as to study the role of immunoreactive gastrin in neonatal physiology.

I would like to acknowledge the help and collaborative efforts of Dr. Thomas Dow of the Royal Maternity Hospital Glasgow in these studies. My thanks are due to Professor MacNaughton for permission to study his patients.

PATIENTS AND METHODS

A series of 62 normal, nulliparous, non-gravid female control subjects who were all within the child-bearing age range (mean age 19.75 years $\pm$ 0.27 years S.E.M.) and who were not using any oral contraceptive preparation were selected from volunteers among the staff of the Centre for Rheumatic Diseases, the Glasgow Royal Maternity Hospital, and the Royal Infirmary, Glasgow. A group of 129 normal, healthy pregnant patients attending the Glasgow Royal Maternity Hospital were also studied (mean age 27.18 years $\pm$ 0.64 years S.E.M.). Details of parity, gestation and the occurrence of any gastro-intestinal symptoms were noted. For the purposes of this study these subjects were arbitrarily divided into groups according to gestation : 0-12 weeks (first trimester), 12-28 weeks (second trimester), 28 weeks to delivery (third trimester) and 0-2 weeks post partum. A further ten patients were studied at the time of vaginal haemorrhage during the course of otherwise normal pregnancies. These patients comprised four primigravid subjects and six multigravidae. The mean age of this group was 26.1 years (range 19 - 39 years). At the time of study most (seven)were at less than 16 weeks gestation, although three were studied during ante-partum haemorrhage during the third trimester.

All of these patients and controls had blood withdrawn for assay of immunoreactive gastrin after a ten hour overnight fast. 14 of the normal, pregnant subjects were also studied immediately after parturition. These patients had all undergone artificial induction of labour by rupture of the membranes and all had been fasting for at least six hours prior to delivery. Parallel blood samples were obtained from these patients and from the umbilical cords of their children immediately on completion of the second stage of labour. In all 14 infants cord vein blood was obtained and in six cord artery blood was also sampled.

A further group of six patients with classical rheumatoid arthritis were studied during the course of a pregnancy. Four of these patients were primigravidae and the mean age of the group was 27.1 years ($\pm$ 3.8 years S.E.M.). At monthly intervals assessment was made of the activity of their joint disease by means of an articular index of joint tenderness, grip strength digital joint circumference and pain index. In addition assay of fasting immunoreactive gastrin was carried out.

In a group of eleven patients with classical rheumatoid arthritis who had been receiving long term corticosteroid therapy and in whom studies of the integrity of the

hypothalamo-pituitary-adrenocortical axis were being carried out, the opportunity was taken of carrying out concomitant studies of immunoreactive gastrin. In six of these patients (5 female and 1 male) studies were carried out during the performance of insulin hypoglycaemia (Conn 1963). The mean age of this group was 54.2 years (± 3.1 years S.E.M.) and the mean duration of arthritis was 7.8 years (± 2.9 years S.E.M.). Corticosteroid therapy in low dose (7.5 mgm. prednisolone per day or equivalent) had been administered for a mean of 3.1 years (± 1.1 years S.E.M.).

Insulin hypoglycaemia was achieved in all subjects using an insulin dose of 0.1 units per kilogram body weight and all of the tests were carried out with an indwelling intravenous cannula inserted in case of adverse reaction to the hypoglycaemia. Blood was withdrawn for plasma glucose, plasma cortisol and plasma immunoreactive gastrin assay immediately prior to and at 15, 30, 60, 90 and 120 minutes after injection of insulin.

In eight of the 11 patients (5 female and 3 male) similar studies of immunoreactive gastrin were carried out following tetracosactrin injection (Nelson et al 1966). The mean age of this group was 59.1 years (± 5.4 years S.E.M.). and the mean duration of arthritis 11.7 years (± 4.3 years S.E.M.).

In these subjects low dose corticosteroids had been administered for a mean of 7.2 years (± 1.1 years S.E.M.) with the exception of one patient a man who had been receiving 15 mg. prednisolone daily for two years prior to this study. (The reason for this elevated dose which had been prescribed in another city was never ascertained.).

In this group blood was withdrawn immediately prior to and 30 and 60 minutes after the injection of 0.25 mgm. tetracosactrin, for assay of plasma cortisol and plasma immunoreactive gastrin.

Plasma glucose was measured by a glucose analyser in the routine biochemical laboratory. Plasma cortisol was measured by the fluorimetric method of Mattingly (1962). Immunoreactive gastrin was assayed using the technique described in Chapter 2 of this thesis.

RESULTS

The results of this study in the normal pregnant subjects are summarised in tables 1-4 and in figure 1. As can be seen there is little change in plasma immunoreactive gastrin concentration until the third trimester of pregnancy, parturition and the early puerperium, when immunoreactive gastrin concentrations rise sharply. Although the difference between primigravidae and multigravidae is not statistically significant these changes appear to be more pronounced in primigravid subjects.

In the ten patients who were studied during episodes of vaginal bleeding (table 5) the mean immunoreactive gastrin is considerably higher than in any of the groups of normal subjects.

In the patients with rheumatoid arthritis (table 6) considerable alleviation of the joint symptoms during pregnancy was noted so that by the tenth week of gestation all but one were completely off anti-rheumatic drug therapy and the remaining one was being maintained on a considerably reduced dosage of indomethacin. In this small group of subjects no elevation of immunoreactive gastrin was found during pregnancy and indeed it can be seen that the immuno-

reactive gastrin paralleled closely that in the normal group (table 6).

The results of the studies on immunoreactive gastrin in response to tests of adrenocortical function are summarised in tables 7 and 8. It can be seen that in neither group of subjects do adequate adrenal responses significantly alter plasma immunoreactive gastrin.

In the infants there was considerably higher immunoreactive gastrin concentrations than in the mothers (table 4) and there was no significant difference between the concentrations in the umbilical artery and vein.

DISCUSSION

Little change occurs in immunoreactive gastrin in early pregnancy. However in the third trimester the concentration of the immunoreactive hormone rises significantly and appears to reach a peak at parturition. The high level at this time could be due at least in part to the shorter period of fasting in these subjects prior to study or it could be due to catecholamine response to the stress of labour (Stadil and Rehfeld 1973). In addition the contribution to these levels from the foetus in whom the levels have been shown to be considerably higher is problematical.

These studies have not determined the functional significance of the elevation of immunoreactive gastrin but it is interesting that previous studies of gastric secretion in pregnancy show a pattern of results which bear a striking similarity to that shown here. Hunt and Murray (1958) in human subjects and McCarthy et al (1954) and Clark (1957) in bitches have demonstrated that gastric acid secretion is normal or slightly diminished during early pregnancy but during the later stages of gestation and particularly at parturition gastric acid secretion rises markedly.

In this study serum immunoreactive gastrin falls

during the early puerperium from its peak at parturition. When it returns to pre gestational levels has not been determined. Nevertheless, it is of note that in all patients in this study lactation was suppressed. Although in both human and animal studies acid output continues at a high level during lactation, it is also recognised that when lactation is suppressed gastric acid output falls (Hunt and Murray 1958; McCarthy et al 1954; Clark 1957). Klein (1933) using a totally denervated pouch preparation in dogs interpreted these changes during lactation as indicating a humorally-mediated acid secretory mechanism.

The high levels of immunoreactive gastrin encountered in the patients studied during episodes of vaginal haemorrhage are of interest. At first sight these would suggest that the stimulus to gastrin hypersecretion is placental in origin especially associated with placental separation. It is a little surprising , however, that this response appears to be much greater in those patients whose haemorrhage occurred early in pregnancy. It may be that the area of placental separation is crucial as five of the seven patients with early bleeding went on to complete spontaneous abortion whereas the three patients with third trimester bleeding came to term and were safely delivered of normal infants.

The known action of prostaglandins in inducing abortion (Corlett and Ballard 1973) and their implication in labour and spontaneous abortion (Karim and Devlin 1967; Karim 1968) as well as their influence on gastric secretory function (Karim et al 1973) suggest that this group of compounds may, in some way be implicated in the changes in gastrin and gastric acid secretion noted in pregnancy.

The significant remission of arthritis in the six patients with rheumatoid disease is of note especially in view of the 'normality' of the immunoreactive gastrin levels in these subjects. The relationship of these two factors is as yet quite unknown.

The striking difference in immunoreactive gastrin between the mothers and their offspring noted in this study confirms some work by Rogers and his colleagues (1974). These authors indicated that immunoreactive gastrin levels were high in neonates and that this elevation persists throughout the first week of post-partum life. This hypergastrinaemia correlates well with the high acid outputs known to occur in neonates and with the known increased prevalence of peptic ulceration at this period of life (Bird et al 1941). Gastrin in adult patients with pernicious anaemia has a half life of around 12 minutes

(Ganguli et al 1971). Unless in neonates metabolism of the hormone is virtually non existent it seems unlikely that an effect of this duration can be due to gastrin acquired from the mother before birth. It is conceivable that the elevation of immunoreactive gastrin in the maternal circulation in late pregnancy and at parturition could be of foetal origin but the gastric hypersecretion known to occur during lactation makes this an unlikely possibility. Further studies in this respect are clearly indicated.

This study has done little to elucidate the problems of dyspeptic symptoms in pregnancy. It has been shown that the lower oesophageal sphincter is yet another important structure under foregut endocrine control (Castell and Harris 1970) with varying constrictor and relaxant effects being attributed to the foregut polypeptide hormones such as gastrin (Castell and Harris 1970) and secretin (Lipshutz and Cohen 1972).

There appears to be little or no relationship between gastrin and the hypothalamo-pituitary-adreno cortical axis despite some evidence for a pituitary role in gastrin secretion (Polak et al 1972) and evidence that the adrenal medulla also influences gastrin levels (Stadil and Rehfeld 1973). It seems unlikely therefore that these effects of

pregnancy on immunoreactive gastrin simply reflect the known changes in the binding and metabolism of corticosteroids (Mills 1960; Wallace and Carter 1960; Oka 1958). There is no doubt however that the complex inter relationships of all of these varying parts of the endocrine system in diseases such as rheumatoid arthritis will be of increasing importance as the technology of hormone assay becomes increasingly sophisticated.

Table 1

Immunoreactive gastrin (p g/ml) in normal, pregnant subjects and in controls.

		Number of subjects studied	Immunoreactive gastrin Mean	± SEM.
Controls		62	30.08	± 3.45
All patients	First Trimester	22	28.41	± 6.85
	2nd Trimester	33	30.0	± 5.3
	3rd Trimester	55	41.3	± 5.5
Primigravidae	First Trimester	14	21.1	± 6.9
	2nd Trimester	21	26.9	± 5.1
	3rd Trimester	16	42.5	± 9.1
Parturition		14	58.2	± 13.3
Post partum patients		19	45.0	± 7.8

Table 2

Immunoreactive gastrin in normal pregnancy.

Statistical Evaluation of the results by

Student t test.

GROUPS COMPARED		First Trimester	Second Trimester	Third Trimester
Control Subjects and All Gravid Subjects	Degrees of Freedom	82	93	115
	t	0.34	0.502	1.73
	p	NS	NS	$0.05 > p > 0.01$
Control subjects and Primigravidae	Degrees of Freedom	74	81	76
	t	0.73	0.40	1.37
	p	NS	NS	$0.05 > p > 0.01$
Post Partum Subjects and all Gravid Subjects	Degrees of Freedom	39	50	72
	t	1.46	1.65	0.12
	p	NS	NS	NS
Post Partum Subjects and Primigravidae	Degrees of Freedom	31	38	33
	t	2.2	2.06	0.34
	p	$0.05 > p > 0.01$	$0.05 > p > 0.01$	NS
Control Subjects and Post Partum Subjects	Degrees of Freedom	79		
	t	2.04		
	p	$0.05 > p > 0.01$		

Table 3.

Immunoreactive gastrin in normal pregnancy.
Comparison by student t test between patients at different stages of gestation.

	First Trimester V Second	2nd Trimester V Third	3rd Trimester V First
All gravid subjects			
Degrees of freedom	53	86	75
t	0.18	1.24	1.37
p	NS	NS	NS
Primigravidae			
Degrees of freedom	33	35	28
t	0.61	1.50	1.83
p	NS	NS	$0.05 > p > 0.01$

Table 4

Immunoreactive gastrin (pg/ml) in mothers and children taken at the same time immediately after completion of the second stage of labour.

		Number of subjects studied	Immunoreactive gastrin Mean	± S.E.M.
Maternal		14	58.2	± 13.3
Foetal	Cord Vein	14	169.3	± 34.5
	Cord Artery	6	134.1	± 25.9

Statistical comparison (by student t test)

	Maternal V Cord Vein	Maternal V Cord Artery	Maternal V Cord Vein
Degrees of freedom	13	5	5
t	3.24	2.59	0.35
p	$0.005 > p > 0.001$	$0.05 > p > 0.01$	NS

Table 5

Fasting immunoreactive gastrin in 10 patients during episodes of vaginal bleeding in pregnancy.

Patient	Age (years)	Primigravid	Gestation (weeks)	Immunoreactive gastrin (pg/ml)
1	26	+	8	265
2	22	+	12	200
3	32		14	135
4	27		16	45
5	39		11	75
6	19	+	12	200
7	25	+	14	25
8	22		32	50
9	20		34	75
10	25		36	30
Mean	25.7			110.0
SEM	± 1.89			± 26.8

Mean (± SEM) immunoreactive gastrin of the seven first trimester = 135.0 pg/ml (± 34.2 SEM)

Comparison with 20 selected samples from patients in the normal pregnant group matched for age, parity and gestation

$t = 2.84$; $0.003 > 5 > 0.001$

Table 6

Mean (± SEM) assessment scores in 6 pregnant subjects with classical rheumatoid arthritis.

Gestation	Articular Index (wrists)	Grip Strength R.hand (mm Hg)	Digital Joint Circumference R.hand (mm)	Immunoreactive gastrin (pg/ml)
12 weeks (app.)	6.4 ± 2.3	85 ± 3	292.3 ± 6.7	31.2 ± 8.1
16 weeks	5.6 ± 1.8	95 ± 7	291.2 ± 7.1	39.2 ± 8.2
20	5.1 ± 2.1	98 ± 8	295.1 ± 7.3	35.1 ± 6.4
24	4.6 ± 1.8	91 ± 7	294.9 ± 6.9	38.3 ± 8.1
28	5.1 ± 1.9	99 ± 5	295.1 ± 8.5	36.5 ± 6.3
32	3.1 ± 1.7	108 ± 6	298.1 ± 8.1	45.4 ± 6.1
36	4.5 ± 1.9	103 ± 8	298.1 ± 9.4	49.8 ± 7.3
40	4.1 ± 1.9	101 ± 10	295.1 ± 9.2	48.7 ± 8.2
4 weeks Post partum	12.2 ± 4.1	78 ± 11	291.2 ± 6.8	28.5 ± 3.1

Table 7

Response of plasma immunoreactive gastrin (pg/ml) to insulin hypoglycaemia in 6 patients with classical rheumatoid arthritis who were receiving long term steroid therapy.

		Minutes after insulin injection				
	Pre insulin	15	30	60	90	120
Gastrin						
Mean	217.5	235.4	215.0	210.7	225.3	215.7
SEM	38.5	39.4	50.3	38.8	39.5	35.8
Cortisol (n mol/l)						
Mean	283.4	290.8	350.5	412.7	400.8	308.8
SEM	± 17.1	± 18.4	25.6	20.3	20.5	25.8
Blood Sugar (m mol/l)						
Mean	4.84	4.28	2.05	2.12	3.86	4.95
SEM	± 0.86	± 1.06	1.08	1.13	1.06	0.92

Table 8

Response of plasma immunoreactive gastrin (pg/ml) to the injection of 0.25 mg. tetracosactrin intramuscularly in 8 patients with classical rheumatoid arthritis who were receiving long term corticosteroid therapy.

	Pre-ACTH	Minutes after ACTH 30	60
Gastrin (pg/ml)			
Mean	247.2	250.1	242.1
SEM	± 58.7	± 49.3	± 54.1
Cortisol (n mol/l)			
Mean	283.1	485.6	512.1
SEM	± 27.1	± 31.1	± 42.3

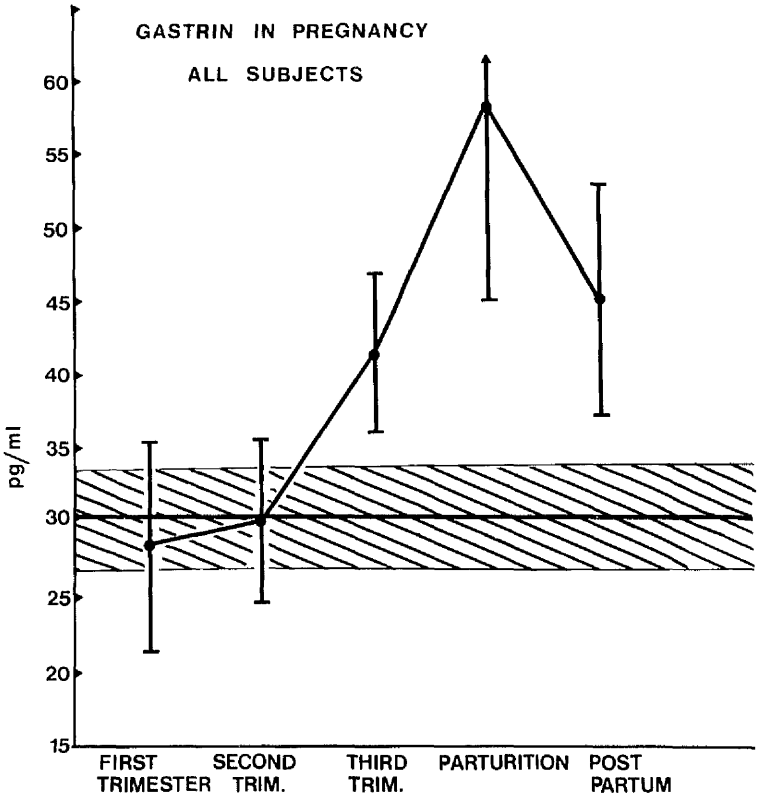


Figure 1.

Immunoreactive gastrin in pregnancy (normal female control range represented by shaded area).

Some studies on Calcium Metabolism in

Relationship to Gastrin in Rheumatoid Arthritis.

They are ill discoverers that think there is
no land,
When they can see nothing but sea.

Francis Bacon 1561-1626
Advancement of Learning.

SUMMARY

Recent studies are reported wherein it has been shown that plasma calcium is elevated in a considerable proportion of patients with rheumatoid arthritis. Some evidence is presented to suggest that this is due to parathyroid overactivity.

The possible relationship between this observation and the elevation of gastrin in rheumatoid arthritis is considered.

Calcium has been shown to be closely associated with release of gastrin and with the control of basal and stimulated gastric acid secretion (Gray and Adkinson 1941; Kaplan and Peskin 1969). When hypercalcaemia of the order of 3.0 - 4.0 m mol/l is induced by intravenous infusion of calcium gluconate in man, a significant increase in serum gastrin and gastric acid secretion occurs (Reeder et al 1970). Atropine diminishes the acid response but does not decrease the gastrin concentration. Hypergastrinaemia has also been associated with hyperparathyroidism (Turbey and Passaro 1972; Barreras 1973).

Recently a close association between calcitonin and gastrin levels has been demonstrated in medullary carcinoma of the thyroid, and in the Zollinger-Ellison syndrome, and it has been suggested that calcitonin may be inhibitory to gastrin release while gastrin in turn, stimulates calcitonin secretion (Sizemore et al 1973).

Abnormalities in bone, which is the major store of calcium in the body, are very common in rheumatoid arthritis. These consist mainly of juxta-articular and generalised osteo-porosis (Kennedy et al 1974; Kennedy et al 1975) as well as periarticular erosions and geodes (Castillo et al 1965). Despite this, no consistent abnormalities of serum

calcium metabolism in rheumatoid arthritis have been reported (Cockel et al 1971; Maddison and Bacon 1974). Recently a number of authors have emphasised the importance of relating serum calcium levels to serum protein concentrations especially serum albumin, and have published various procedures to correct calcium levels taking this into account (Orrell 1971; Berry et al 1973; Payne et al 1973). It seemed important to reconsider the calcium status of patients with rheumatoid arthritis in the light of these findings.

I should like to acknowledge my colleagues in this work, Dr. A.C.Kennedy of the Centre for Rheumatic Diseases and Dr. B. Allam of the Department of Biochemistry, Royal Infirmary, Glasgow.

MATERIALS AND METHODS

The studies reported in this chapter encompassed a total of three hundred and ninety patients with "classical" or "definite" rheumatoid arthritis (Ropes et al 1959). Of these the larger part (364) were included in a retrospective study of their previous biochemical results. These patients comprised 229 female patients with a mean range of 51.6 years ($\pm$ 12.4 years S.D.) and 138 male subjects with a similar mean age of 56.9 years ($\pm$ 10.2 years S.D.). Of the female subjects 73 were receiving low dose corticosteroid therapy ($<$ 7.5 mgm. prednisolone daily or equivalent) and of the males 24 were receiving this type of therapy.

A further group of twenty female patient volunteers with "classical" or "definite" rheumatoid arthritis and in whom there was radiological evidence of generalised osteoporosis as measured by the metacarpal index of Barnett and Nordin (1960) were admitted to a biochemical study of mineral metabolism. These patients included 12 who had never received treatment with corticosteroids and who had a mean age of 58 years ($\pm$ 8.6 years S.D.) as well as 8 subjects who, at the time of study, were receiving low dose corticosteroid therapy and whose mean age was similar (54.5 years $\pm$ 7.8 years S.D.). In this

study a biochemical, mineral metabolism screen was performed on each subject. This involved the collection of an accurately timed four hour urine specimen and, during the period of urine collection the withdrawal of 20 ml of blood without venous occlusion. The blood was divided into two 10 ml aliquots one of which was permitted to clot and the other was collected into lithium heparin. All these studies were carried out with the patients in the fasting state after a ten hour overnight fast.

The plasma and serum were separated within half an hour and the following estimations performed :- on plasma - urea and electrolytes; on serum - calcium, phosphorus, creatinine, total proteins, albumin and alkaline phosphatase; on urine - calcium, phosphorus, creatinine and urea.

In 24 female patient volunteers, all with "classical" rheumatoid arthritis a radioisotopic estimate of calcium absorption was carried out. These subjects included 18 of the patients in the study of mineral metabolism. Of these 24 patients 14 were on non-steroidal anti-inflammatory drug regimens at the time of study and 10 were on low dose corticosteroid therapy. Eight normal, healthy, control volunteers also participated in these studies. These

subjects were recruited from the staff of the Centre for Rheumatic Diseases and the Royal Infirmary, Glasgow, and were matched for age and the date of the menopause with the rheumatoid arthritis group. Each of these patients and controls was administered orally a 5 μC dose of Ca^{47} in 50 mgm. calcium chloride carrier. Venous blood samples were obtained at thirty minute intervals for two and a half hours thereafter. Plasma Calcium ^{47} radioactivity was measured in a gamma scintillation counter (Gamma-Guard). The value obtained expressed as plasma activity per litre was multiplied by 15 per cent of the total body weight in kilograms to assess the total amount of radio-calcium in the blood at the time of withdrawal (Nordin 1967).

The final group of twenty three patient volunteers with "definite" or "classical" rheumatoid arthritis were selected from the initial large group of patients on the basis of having had elevation of the 'corrected' serum calcium within the six months prior to this part of the study. Three of these subjects were receiving corticosteroid therapy and the mean age of the whole group was 51.7 years (± 15.6 years S.D.). For this group of patients nine age-matched, normal, female, control volunteers were also obtained from the hospital staff as in the previous study. These controls had a mean age of 49.5 years

(± 10.2 years S.D.). From each patient and control a sample of blood was withdrawn without venous occlusion after a ten hour overnight fast. On this sample total serum calcium, serum ionised calcium, serum proteins, serum albumin and plasma immuno-reactive gastrin were assayed.

Total calcium was measured by atomic absorption spectro-photometry using initially Unicam S.P.90 and subsequently Perkin-Elmer 403. Serum was diluted in E.D.T.A. and urine in lanthanum chloride. Serum total proteins were estimated by the biuret reaction as automated for auto analyser (Technicon Auto Analyser Method N.14a) and serum albumin by the bromocresol green binding method as modified for auto-analyser by Northam and Biddowson (1965). Inorganic phosphorus was measured in serum and urine by the molybdate reaction as modified for auto-analyser by Yee (1968). Serum alkaline phosphatase was estimated by the method of Kind and King (1954) as modified for auto-analyser by Axelson et al (1965). Plasma electrolytes and urea were measured on a 5-channel Technicon auto-analyser; electrolytes by Technicon auto-analyser methods N20b, N5b, and N8b, and urea by the diacetyl monoxime reaction as modified for auto-analyser by Haslam (1966). Creatinine was estimated

by the alkaline picrate reaction (Technicon auto analyser method N11b).

Serum calcium levels were corrected for albumin using the following formula.

Ca (corrected) = [(47 - alb.)X0.0194] + Ca.

where Ca is total serum calcium in m mol/l, alb. is serum albumin in g/l and Ca (corrected) is serum calcium in m mol/l corrected for albumin. 47 represents the mean albumin in g/l or the normal healthy population and the figure 0.0194 represents the slope or regression coefficient of calcium on albumin. It indicates that calcium changes by 0.0194 m mol/l for every 1g/l change in albumin. It was obtained from the regression equation. (derived from the results of one hundred and fifty consecutive laboratory serum specimens) $y = 0.0194x + C$ where x = serum albumin in g/l, y = serum calcium in m mol/l and C is a constant which equals 1.434 denoting the position of the regression line or the value of y when $x = 0$. All normal biochemical values in these estimations were obtained from two hundred and eighty normal healthy subjects of both sexes (age range 16-67 years).

Serum ionised calcium was estimated by a flow-through electrode system (Orion 99-20) at 37°C and corrections were made for pH and for direct interference of sodium on

electrode readings (Watson 1975).

Plasma immunoreactive gastrin was measured by the radioimmunoassay technique described in detail in Chapter 2 of this work.

"Osteoporosis" was measured by the metacarpal index (Barnett and Nordin 1960); femoral index (Barnett and Nordin 1960); clavicular cortical width (Anton 1969) and standard aluminium equivalents (Anderson et al 1966).

RESULTS.

The mean plasma albumin of the patients included in the large retrospective study of rheumatoid arthritis is lower in both the males (35.2 g/l ± 5.2 g/l S.D.) and the females (33.0 g/l ± 4.7 g/l S.D.) than in the normal (47 g/l ± 0.5 g/l S.D.). The total serum calcium of the patients with rheumatoid arthritis is normal with a mean of 2.38 m mol/l (± 0.16 m mol/l S.D.) in the males and 2.38 m mol/l (± 0.15 m mol/l S.D.) in the females. Of the whole group 13 patients were hypocalcaemic and 12 had serum calcium levels above the normal range (2.20 - 2.60 m mol/l). However when the calcium is corrected for albumin the mean serum calcium is elevated having a value of 2.61 m mol/l (± 0.16 m mol/l S.D.) in the males and 2.66 m mol/l (± 0.16 m mol/l S.D.) in the females. It is then apparent that only three subjects remain hypocalcaemic whereas 168 patients have serum calcium levels in the hypercalcaemic range.and of these 52 had other biochemical abnormalities of the phosphorus and alkaline phosphatase highly suggestive of hyperparathyroidism namely reduction in phosphorus, elevation of alkaline phosphatase or a combination of both these changes.

No significant relationship could be shown to exist between corrected serum calcium and any of the measured

indices of osteoporosis; metacarpal index ($r = 0.026$); femoral index ($r = 0.045$); clavicular cortical thickness ($r = 0.015$); or standard aluminium equivalent ($r = 0.066$). Nor was there any relationship between corrected calcium and duration of disease ($r = 0.067$).

The results of the smaller study using biochemical, mineral metabolic assay are largely similar. In this group the mean total serum calcium was within the normal range 2.27 m mol/l ($\pm$ 3.1 m mol/l S.D.) and 5 patients were in the hypocalcaemic range with none of the patients above the upper limit of normal. However the albumin was again significantly lower than normal with a mean of 36.9 g/l ($\pm$ 4.3 g/l S.D.) in comparison to the normal group mean of 4.7 g/l ($\pm$ 4 g/l S.D.) ($t = 6.42$ $p < 0.001$). When the calcium was corrected for albumin the mean level rose to 2.47 m mol/l ($\pm$ 0.18 m mol/l S.D.) and four of the patients could be shown to be hypercalcaemic with only two remaining below the normal range.

In addition to these changes in calcium a number of other metabolic abnormalities are apparent in this group of subjects. 18 (76%) had elevation of the plasma chloride the mean of the whole group being 107.8 m mol/l ($\pm$ 2.4 m mol/l S.D.) in comparison to the normal group where the mean was 100 m mol/l ($\pm$ 2.5 m mol/l S.D.). In addition 2 patients (10%) had serum phosphate levels below the normal range (< 0.8 m mol/l)

and 3 (15%) had a reduced renal tubular reabsorption of phosphate (<0.7 m mol/l) in spite of normal renal function. The studies on radiocalcium absorption demonstrate that at the peak of absorption (two hours after ingestion of the calcium) significant differences exist between the patients with rheumatoid arthritis and the control subjects. The mean peak absorption of Ca^{47} in the control group was 18.1% $\pm$ 6.1%. whereas in the rheumatoid arthritis subjects the mean was 29.5 $\pm$ 8.3 ($t = 8.4$ $0.001 > p > 0.0005$).

In the final group of patients in these studies a very similar pattern of results emerged (Table 1)with one patient hypocalcaemic, and one patient hypercalcaemic. The albumin is again low (mean 38.2 g/l $\pm$ 3.9 g/l S.D.) so that when calcium is corrected for serum albumin 7 patients were hypercalcaemic and none were below the normal range. This table also shows the ionised calcium values for the control group and these subjects have a mean ionised calcium of 1.062 m mol/l $\pm$ 0.03 m mol/l S.D. Among the patients with rheumatoid arthritis there are 10 subjects whose ionised calcium value was greater than 1.1. which is the calculated upper limit of the normal group. The normal range of the control group was assessed as $\pm$ 2 standard deviations from the mean, the validity of this being dependent on a Gaussian distribution of serum ionised calcium concentrations in the normal population. The mean ionised calcium

concentration of the patients with rheumatoid arthritis was much higher than that of the controls being 1.13 m mol/l and this difference is statistically significant ($t = 3.32$ $0.01 > p > 0.005$). In this group of patients a correlation can be demonstrated between uncorrected serum calcium and ionised calcium ($r = 0.73$ $0.05 > p > 0.01$). However when serum calcium is corrected for albumin this correlation can be shown to be much closer ($r = 0.71$ $0.005 > p > 0.001$). If the immunoreactive gastrin results obtained during this study are considered (Table 2) it can be seen that only one of these subjects has a significantly elevated immunoreactive gastrin. It is interesting however that this patient is the patient with the highest corrected calcium and the highest ionised calcium. A correlation exists between corrected calcium and immunoreactive gastrin ($r = 0.58$; $0.001 > p > 0.0005$) and this holds even when this single patient is discounted as being only one member of a different population (Chapter 2) although the correlation is not quite so close ($r = 0.4$, $0.1 > p > 0.05$). This relationship is maintained when ionised calcium is considered ($r = 0.55$; $0.001 > p > 0.0005$)

DISCUSSION

Few authors have reported abnormalities of calcium in rheumatoid arthritis. Cockel et al (1971) demonstrated a number of abnormalities in 100 patients with rheumatoid arthritis. Twenty per cent of these patients had hypocalcaemia and 26 per cent elevated serum alkaline phosphatase levels, although only four had demonstrable hepatic dysfunction. Although Cockel and his colleagues (1971) interpreted the hypocalcaemia they encountered as being due to low albumin concentrations they made no effort to correct for this factor. Throughout these studies total serum calcium concentrations in rheumatoid arthritis have been normal. However, when, as has been encouraged by various authorities recently (Orrell 1971; Berry et al 1973; Payne et al 1973) correction is made for serum albumin concentrations a high percentage of patients with rheumatoid arthritis can be shown to be hypercalcaemic. Indeed in some patients the pattern of results is highly suggestive of hyperparathyroidism. It is of interest that Cockel et al (1971) reported one case of hypercalcaemia among their patients but the extent of this abnormality and details of other associated biochemical abnormalities were not provided. None of the patients in these studies had a positive test for mitochondrial auto antibody which can

be associated with focal biliary cirrhosis and may account for some of the elevated serum alkaline phosphatase levels encountered in patients with rheumatoid arthritis (Whaley et al 1970; Webb et al 1975).

The evidence suggesting parathyroid overactivity in these patients with rheumatoid arthritis is significantly greater when the results of the calcium absorption studies are considered. These results show unequivocally that calcium absorption is increased in rheumatoid arthritis and that the results obtained are very similar to those reported in primary hyperparathyroidism (Nordin 1967).

Maddison and Bacon (1974) have reported 5 cases of overt vitamin D deficiency osteomalacia in rheumatoid arthritis. Osteomalacia could account for the occasional patient with rheumatoid arthritis and a low serum calcium (Cockel et al 1971). However, vitamin D deficiency could also account for the findings in this study if secondary hyperparathyroidism had ensued. In the cases reported by Maddison and Bacon (1974) they were able to conclude that the hypovitaminosis D was due to dietary causes. It is also conceivable that hypovitaminosis D could be due to long term anti-inflammatory drug therapy in the same way that long term anti-convulsant therapy can cause it via the mechanism of hepatic microsomal enzyme induction.

Oral corticosteroid therapy reduces the serum calcium concentration in hypercalcaemia of various aetiologies with the notable exception of hyperparathyroidism. In this study there was no significant difference between the calcium concentrations of those patients receiving oral corticosteroids and those who had not had any treatment of this type.

It is of interest that articular erosions similar to those seen in rheumatoid arthritis have been reported in hyperparathyroidism (Bywaters 1959).

Additional evidence of the relevance of these findings to the metabolism of bone and calcium is adduced from the studies on ionised calcium. Ionised calcium is generally considered to be the physiological part of the serum calcium and so it is likely that the hypercalcaemia which has been found in rheumatoid arthritis is of physiological significance. It is interesting also that it appears from these studies to be episodic. Although all the subjects had been selected as being hypercalcaemic within the previous six months, 12 of them had normal calcium status at the time of this study. No information is available as yet as to whether this fluctuation relates to changes on the activity of the inflammatory joint disease.

As can be seen from table 2 there is a relationship between corrected, or ionised, calcium and immunoreactive gastrin. It is not apparent from this table, however, whether calcium abnormalities can account for all elevated levels of immunoreactive gastrin in rheumatoid arthritis as only one of these subjects had a significantly elevated level. In view of this a retrospective study was made of the data available on the first 50 patients described in detail in Chapter of this work. Thirty one of these patients had had serum calcium and plasma albumin estimated within one week of examination of their immunoreactive gastrin status. It is to be noted that in none of these patients was the calcium estimated in the fasting state. However, the results of this study are noted in table 3. It can be seen that the relationship between corrected calcium and immunoreactive gastrin is maintained ($r = 0.41$, $0.05 > p > 0.01$). If however this group is separated into those with elevated immunoreactive gastrin (> 500 pg/ml) and those with normal levels it can be seen that the relationship between calcium and immunoreactive gastrin levels is maintained only for those with normal levels ($r = 0.56$, $0.01 > p > 0.001$).

While this study has not clarified the reason for

elevated immunoreactive gastrin it has demonstrated that serum calcium is abnormal in a large proportion of patients with rheumatoid arthritis and that this ion has a significant effect on immunoreactive gastrin at least within the physiological range of the hormone. This finding is in agreement with the known influence of serum calcium on immunoreactive gastrin in other situations (Kaplan and Peskin 1969; Reeder et al 1970). Taken in conjunction with the known abnormalities in other divalent cations: zinc (Kennedy et al 1975), copper (Plantin and Strandberg 1965), iron (Roberts et al 1963) which occur in rheumatoid arthritis this suggests that the metabolism of such cations is considerably disturbed in rheumatoid arthritis and that the endocrine system which is inter-related in their normal control is also abnormal.

It is not unlikely that it is a combination of factors involving many or all of the divalent cations and the foregut polypeptide hormones which is ultimately responsible for elevation of immunoreactive gastrin in rheumatoid arthritis.

Table 1

	RHEUMATOID ARTHRITIS PATIENTS				CONTROL
	Serum Calcium (Uncorrected) m mols/l	Serum Albumin g/l	Serum Calcium (corrected) m mols/l	Serum Ionized Calcium m mols/l	Serum Ionized Calcium m mols/l
	2.21	31	2.50	1.11	1.08
	2.54	43	2.60	1.12	1.10
	2.60	57	2.60	1.11	1.09
	2.44	37	2.65	1.19	1.02
	2.33	39	2.50	1.19	1.07
	2.64	39	2.80	1.19	1.06
	2.50	38	2.65	1.12	1.03
	2.34	36	2.55	1.09	1.04
	2.32	32	2.60	1.13	1.03
	2.60	40	2.75	1.25	
	2.36	43	2.45	1.10	
	2.40	40	2.55	1.13	
	* 2.49	39	2.65	1.16	
	* 2.41	35	2.65	1.16	
	2.34	36	2.55	1.17	
	2.15	33	2.40	1.09	
	2.30	42	2.40	1.09	
	* 2.38	37	2.60	1.09	
	2.40	38	2.55	1.04	
	2.40	44	2.45	1.08	
	2.35	35	2.60	1.05	
	2.30	40	2.45	1.05	
	2.58	34	2.85	1.27	
Mean	2.41	38.2	2.58	1.13	1.06
S.D.	0.13	3.9	0.12	± 0.06	0.03

* On corticosteroid therapy .

Table 2

Plasma immunoreactive gastrin (pg/ml) and serum corrected and ionised calcium (m mol/l) in 22 patients with rheumatoid arthritis.

	Immunoreactive gastrin pg/ml	Serum Corrected calcium m mol/l	Serum Ionised Calcium m mol/l
	55	2.50	1.11
	70	2.60	1.12
	105	2.60	1.11
	75	2.65	1.19
	40	2.50	1.19
	145	2.80	1.19
	125	2.65	1.12
	95	2.55	1.09
	120	2.60	1.13
	125	2.75	1.25
	105	2.45	1.10
	50	2.55	1.13
	325	2.65	1.16
	60	2.65	1.16
	75	2.55	1.17
	110	2.40	1.09
	30	2.60	1.09
	45	2.55	1.04
	30	2.45	1.08
	95	2.60	1.05
	50	2.45	1.05
	1250	2.85	1.27
Mean	144.5	2.59	1.13
S.D.	254.5	0.11	0.06
S.E.M.	54.3	0.02	0.01

Table 3

Albumin (g/l), corrected calcium (mmol/l) and immunoreactive gastrin (pg/ml) in 31 patients with rheumatoid arthritis.

Patient	Albumin	Calcium	Corrected Calcium	Immunoreactive gastrin
1	42	2.42	2.52	90
2	41	2.32	2.44	90
3	39	2.2	2.36	55
4	33	2.17	2.43	160
5	47	2.60	2.60	50
6	36	2.25	2.47	75
7	40	2.35	2.49	200
8	32	2.10	2.40	50
9	36	2.15	2.37	30
10	40	2.27	2.41	75
11	42	2.37	2.47	75
12	40	2.27	2.41	200
13	40	2.27	2.41	100
14	40	2.32	2.46	75
15	35	2.55	2.79	300
16	39	2.15	2.31	50
17	32	2.22	2.52	50
18	37	2.25	2.45	75
19	21	2.02	2.54	75
20	40	2.22	2.36	950
21	37	2.47	2.67	700
22	42	2.65	2.75	600
23	34	2.45	2.71	1000
24	34	2.35	2.61	900
25	35	2.37	2.61	1550
26	28	2.20	2.58	1300
27	34	2.47	2.73	10,000
28	39	2.45	2.61	1500
29	41	2.37	2.49	500
30	38	2.25	2.43	2300
31	38	2.45	2.63	1800
Mean	37.16	2.32	2.52	805.6
S.D.	4.9	0.15	0.13	1815.1
S.E.M.	0.9	0.03	0.02	325.9

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