The Influence of Endocrine and Metabolic Dysfunction on the type of Chronic Atrophic Gastritis which is accompanied by Humoral Antibody to Gastric Mucosa.

Thesis submitted to the University of Glasgow
for the Degree of Doctor of Medicine

by

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To

J. L. MARKSON

for stimulating my interest
in the problem of gastritis.

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INTRODUCTION

These studies have their origin in the work on thyroid auto-antibodies and auto-allergic thyroiditis of Rose and Witebsky (1956) in the U.S.A. and of Roitt, Doniach, Campbell and Hudson (1956) in England. A more immediate stimulus was provided by the demonstration of antibody to intrinsic factor in some cases of pernicious anaemia (Taylor, 1959) and of an association between pernicious anaemia and myxoedema (Tudhope and Wilson, 1960). At the time my investigations were commenced it was already a commonplace to suspect that the chronic gastritis or gastric atrophy present in pernicious anaemia might be analogous to the chronic thyroiditis of Hashimoto's disease. The first investigation undertaken showed that there was an antibody to a cytoplasmic component of human gastric body mucosa in the serum of many patients with pernicious anaemia and this was thought to be good supportive evidence for the hypothesis of an auto-allergic pathogenesis. The same antibody was present, though less frequently, in cases of iron-deficiency anaemia. The other studies were undertaken to elucidate known or suspected associations - pernicious anaemia and diabetes mellitus, pernicious anaemia and renal tubular acidosis, myxoedema and malignant disease or reticulosis.

I.

INVESTIGATIONS

1. Gastric Auto-antibody in Pernicious Anaemia and Iron-deficiency Anaemia.

This investigation was made in collaboration with J. L. Markson. A communication on the subject was given to the Scottish Society for Experimental Medicine (Markson and Moore, 1962a) and a paper was published in the same year (Markson and Moore, 1962b).

(a) Evidence for auto-allergy in pernicious anaemia.

Taylor (1959) demonstrated antibody to hog intrinsic factor in the serum of some patients with pernicious anaemia and this was confirmed by Schwarz (1960). All of the patients in Taylor's series and a few of those in that of Schwarz had never been treated with preparations of hog stomach. The method consisted of incubating a large volume of serum with intrinsic factor and then assaying this mixture for intrinsic factor in a patient with pernicious anaemia.

On the analogy of auto-allergic thyroiditis it seemed that there might be present in addition an antibody to a cytoplasmic constituent of gastric mucosa and, if so, it would of course be a great convenience if it could be demonstrated by a simple method using small quantities of serum and without an assay procedure. Accordingly, I looked for such an antibody by the two methods readily available to me at the time, namely precipitin and complement-

fixation tests using crude cytoplasmic extracts of human gastric mucosa in buffered saline. The complement-fixation test gave positive results with serum from some patients with pernicious anaemia but the precipitin test did not. Extracts of autopsy material were tested and although the material was mostly unusable because of its excessively mucoid nature an occasional potent and non-mucoid extract was obtained, e.g., from a child 11 years old dying of acute leukaemia. An extract from the autopsy mucosa of one patient with pernicious anaemia was also not unusually mucoid but it showed no activity in complement-fixation tests. Consequently all complement-fixation tests recorded were performed using extracts from the gastric mucosa of stomachs obtained in the operation of partial gastrectomy for duodenal or stomal ulcer. Such extracts have been less variable in potency than those obtained from thyrotoxic thyroid glands for the detection of thyroid microsomal antibody.

(b) Comparison of extracts from body mucosa and from pyloric antrum mucosa.

Since the gastritic or atrophic lesion of pernicious anaemia affects the body and fundus of stomach only (Magnus and Ungley, 1938) it seemed logical to see if the active principle in the extracts was present in those derived

from body mucosa only. Such a correlation would link the findings more definitely with pernicious anaemia. This proved to be so. Extracts from body mucosa were effective in complement-fixation tests whereas those derived from antral mucosa were not.

(c) <u>The results of gastric mucosa complement-fixation tests in Pernicious anaemia, iron-deficiency anaemia, other anaemias, Hashimoto's disease, and controls.</u>

In the published results of Markson and Moore, already mentioned, rather weak extracts were used. They were prepared from the whole mucosa of a gastrectomy specimen by freezing, slicing thinly, and shaking a saline suspension for 5 minutes at room temperature. With such extracts diluted 1 in 3 and using a 1 in 5 dilution of serum, 41.7% of 83 patients with pernicious anaemia, 4.2% of matched controls, and 4.5% of 134 random controls gave positive results in the gastric mucosa complement-fixation (G.M.C.F.) test. This was a statistically-significant difference ($P<0.01$). Extracts used subsequently were made from that portion of stomach which was unequivocally body mucosa and the shaking was carried out for 1 hour at $4^{\circ}C$. With such extracts diluted 1 in 15 and using a serum dilution of 1 in 4, 70% of sera from pernicious anaemia cases gave a positive result and the proportion was higher if neat serum or serum diluted 1 in 2 was used. This compares with a figure of 8 - 10% in control hospital patients. The

details of the extraction and complement-fixation techniques are described in the technical appendix.

The proportion of positive G.M.C.F. tests in other types of patient using the original technique was as follows:-

	Number tested	G.M.C.F. test positive	
Iron-deficiency anaemia	34	17.6%	($P<0.05$)
Hashimoto's disease	41	12.2%	(no significant difference)
Other anaemias	40	5.0%	(no significant difference)

The results in pernicious anaemia are supported by the independent work of Irvine et al., (1962) and by Taylor et al., (1962) and those in iron-deficiency anaemia by Dagg et al.,(1964).

(d) Antibody nature of the serum component giving a positive G.M.C.F. reaction and its relation to intrinsic factor antibody.

It was established by testing the gamma and non-gamma fractions of a high-titre serum that the serum component giving a positive G.M.C.F. reaction was probably an antibody. Dr. J. R. Anderson very kindly fractionated the serum on a DEAE column. The activity was found to be present in the gamma fraction and absent from the non-gamma fraction.

To determine if this antibody was distinct from the antibody to intrinsic factor described by Taylor and by Schwary, three experiments were made:-

(1) A gastric mucosal extract known to be suitable for use in complement-fixation tests was tested for intrinsic factor activity by Dr. M. D. Smith. This was done by determining its effect on the absorption of ^{58}Co-labelled vitamin B12 in two patients with pernicious anaemia, as measured by the urinary excretion method of Schilling (1953). A dose of 0.5 ug. of labelled vitamin B12 was administered. The values for urinary radioactivity were 3.2% and 1.1% respectively. When the same dose was administered in 20 ml. of gastric mucosal extract (about one fifth of the volume obtained from one entire gastric mucosa) the values for urinary radioactivity were 4.8% and 4.6%. These differences were not significant and it was concluded that there was little or no intrinsic factor in active form present in the extract.

(2) Separate extracts of body mucosa and antral mucosa were made from the stomachs of freshly-killed hogs. Again antigen similar to that obtained from human stomach was present in extracts of body mucosa but not in antral extracts. Since intrinsic factor is present in the hog in the antral mucosa but not in body mucosa (Meulengracht, 1935), this provides a neat if indirect proof that the antibody described above is not antibody to intrinsic factor.

(3) Saline solutions of differing strength were prepared from dried hog stomach of known intrinsic factor potency and tested by complement-fixation for the presence of antigen comparable to that obtained in extracts of body mucosa from human stomach. No complement-fixation was detected.

It was concluded that the antibody described was distinct from antibody to intrinsic factor.

(e) Some properties of the G.M.C.F. antigen.

A) Lability. The antigen is readily destroyed by heat. When extracts are heated at 45°C. for 30 minutes most of the antigen is destroyed and none of it survives heating at 50°C. for the same length of time.

B) Tissue specificity. Activity similar to that obtained with extracts of body mucosa was not present in extracts of the mucosa of pyloric antrum, first part of duodenum, or colon. By means of testing extracts of kidney and liver with appropriate sera it was shown that the G.M.C.F. reaction was distinct from the A.I.C.F. (auto-immune complement-fixation) reaction described by Gajdusek (1958). The kidney and liver used in the first instance were obtained from autopsy material, but in later tests freshly-killed guinea pigs were used as being a more standard source of material. Using extracts of human thyrotoxic thyroid gland it was also shown that the G.M.C.F. reaction was distinct from the thyrotoxic complement-fixation reaction frequently detected in patients with Hashimoto's disease. A comparison of

results obtained in Hashimoto's disease and pernicious anaemia makes this clear:-

	Pernicious anaemia	Hashimoto's disease
Number of patients tested	72	41
G.M.C.F. test positive/ T.C.F. test negative	23 (32%)	1 (2.5%)
G.M.C.F. test negative/ T.C.F. test positive	5 (7%)	30 (74%)
Both tests positive	7 (10%)	4 (10%)

C) Species non-specificity. As already mentioned on p.6 antigen similar to that present in the gastric body mucosa in human subjects is present in the body mucosa of the hog. Saline extract of the body mucosa of a hog gave positive complement-fixation tests with 7 of 8 sera positive with human extract and negative results with 6 sera negative with human extract and negative results with 6 sera negative with human extracts.

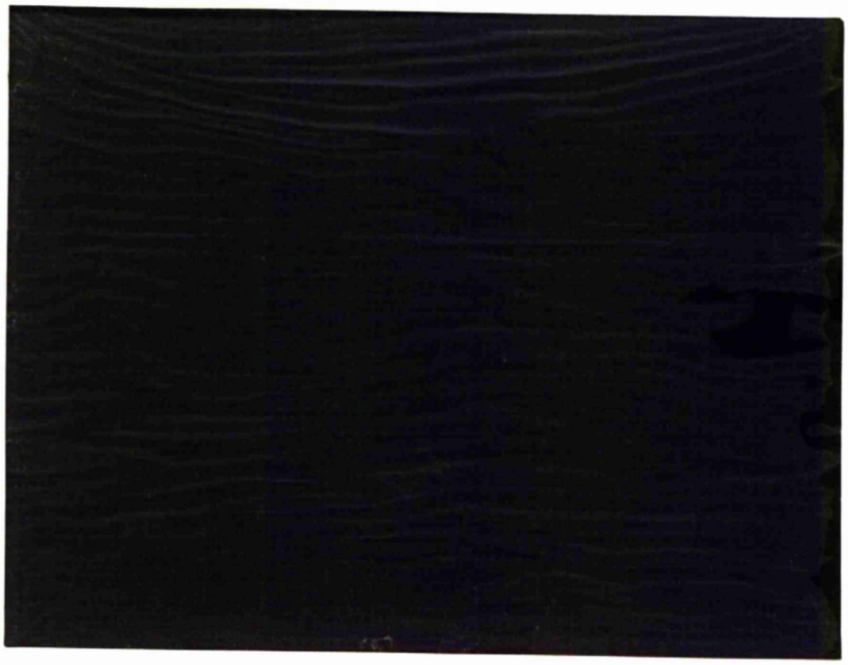
The difficulties inherent in characterising chemically this gastric mucosal antigen are likely to be considerable if, as seems likely, it resembles thyroid microsomal antigen. The latter has been investigated by Roitt et al., (1964) who showed that it was an insoluble lipoprotein intimately bound to small smooth-surfaced vesicles in the microsomes of the cytoplasm of thyroid epithelial cells.

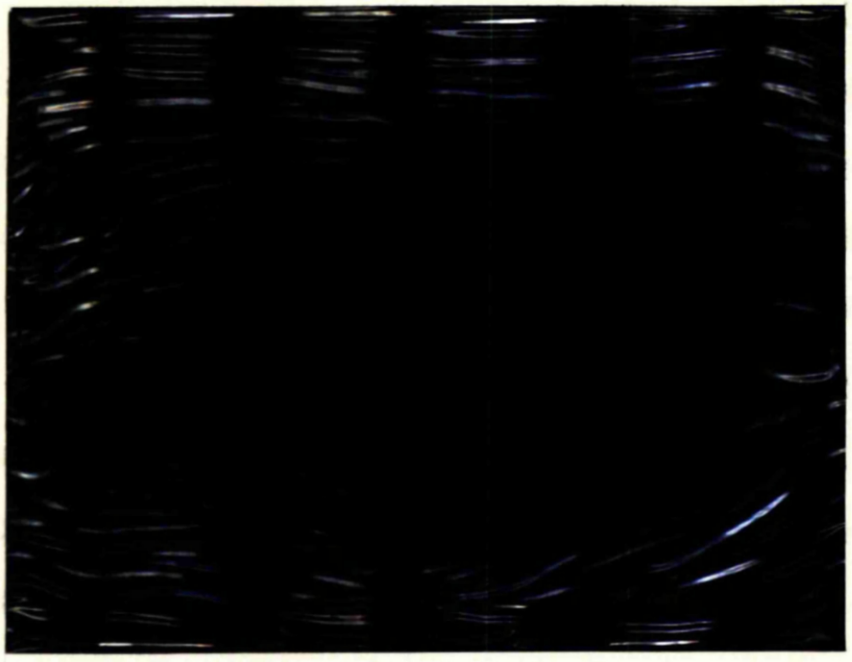
An interesting digression during the investigation was provided by the occurrence of an A.I.C.F. reaction in

the serum of a youth aged 18 years who had been under observation for 12 years on account of recurring iron-deficiency anaemia associated with idiopathic pulmonary haemosiderosis. The reaction was obtained with extracts of kidney, liver, thyroid, and gastric mucosa and it gradually declined in strength and disappeared in the year and a half following the first test. A positive A.I.C.F. reaction was also obtained in the only other case of idiopathic pulmonary haemosiderosis subsequently encountered. The individual concerned was a 19-year-old male with anaemia at the time tested, a two year history of bouts of haemoptysis and epistaxis, and evidence of haemosiderosis in a biopsy of lung. The aetiology of this uncommon condition is unknown. One would be inclined to speculate that haemosiderin in the tissues provoked the A.I.C.F. reaction if it were not for the fact that one of the reported features in some cases is a glomerulonephritis. This suggests that some kind of hyper-reactivity is present. In neither of the cases mentioned here were there overt signs of nephritis.

(f) Specific reaction of G.M.C.F. antibody with gastric parietal cells in an immunofluorescent test.

When the investigation had been carried to this stage it was realised that use of the immunofluorescent technique might be helpful. Unfortunately, such facilities were not available at the time. Taylor et al., (1962) reported





Immunofluorescence of the cytoplasm of human gastric parietal cells treated with the serum of a young diabetic patient known to have atrophic gastritis.

X 125

that the gastric antibody detected by complement-fixation tests in the serum of patients with pernicious anaemia gave specific immunofluorescence with the cytoplasm of gastric parietal cells. This is illustrated opposite.

In the three succeeding investigations it has been my practice to use the technique as an adjunct to complement-fixation tests in order to verify specificity. In most instances the use of extracts from other tissues would give this information without resort to the more tedious immunofluorescent method but there are exceptions, e.g., the young female with pernicious anaemia and diabetes (Moore & Neilson, 1963a), in whom strong non-specific serum reactions were obtained and yet the serum showed strong parietal-cell activity in immunofluorescent tests.

2. Gastric and Thyroid Auto-antibodies in Diabetes Mellitus

(a) The association of pernicious anaemia and diabetes mellitus.

This investigation was made in collaboration with Dr. J. McE. Neilson and the results have been published (Lancet, 1963a). It was prompted by a fact long known to physicians dealing with diabetes, namely, that pernicious anaemia is relatively common in any diabetic population (Joslin, 1959). Varying figures have been given but the average figure is around 10 per 1000 and this was the

proportion found by Dr. Neilson in just over 600 patients attending the diabetic clinic at Stobhill General Hospital. A general practitioner survey (Scott, 1960) found that the rate of occurrence of pernicious anaemia in the general population in the Glasgow area was in the neighbourhood of 1.5 per 1000.

Pernicious anaemia only becomes manifest when the body's stores of vitamin B 12 have been depleted over a period of years consequent on a probably very slow process of atrophic change in the gastric mucosa. It was thought therefore that the existence of an atrophic gastritis not causing or not yet causing pernicious anaemia might be demonstrable in diabetic patients by the presence in their serum of gastric parietal cell antibody. It was also thought that thyroid auto-antibodies should be looked for in view of the association between pernicious anaemia and myxoedema (Tudhope and Wilson, 1960).

(b) Materials and Methods.

The serological methods are described in detail in the Technical Appendix. 83 diabetic patients were tested, 29 of these being male and 54 female. In 60% of cases the disease was of "maturity onset" type. Those with pernicious anaemia, iron-deficiency anaemia, or thyroid disease were excluded. Of the total, 63 patients were making a routine

visit to the hospital diabetic clinic and 20 were in hospital for stabilisation of their diabetic state or, in a few instances, on account of complications. Each diabetic serum was controlled by serum from two hospital patients who did not have diabetes, pernicious anaemia, iron-deficiency anaemia, or thyroid disease, matched with the diabetic for age and sex. All sera were tested for parietal cell antibody and for thyroid microsomal antibody by the complement-fixation technique. Only the first 65 diabetic sera and 65 matched control sera were tested for antithyroglobulin by the tanned red cell agglutination (T.R.C.A.) test.

(c) Results.

(1) In all patients tested.

Both gastric parietal cell antibody and thyroid microsomal antibody were detected with significantly greater frequency in diabetic patients than in controls ($P<0.01$ in each case) but there was no such increase in antibody to thyroglobulin. The details of the results were as follows:-

Antibody present	Diabetes mellitus	Controls
Antithyroglobulin	6/65 (9%)	5/65 (8%)
Thyroid microsomal antibody	14/83 (17%)	7/166 (4%)
Gastric parietal cell antibody	18/83 (22%)	13/166 (8%)

(2) In "early onset" and "late onset" groups.

It seemed desirable that the results should be analysed in terms of "early onset" and "maturity onset" categories in view of the accepted differences between such groups of diabetic subjects. Since it would have been difficult to classify certain of the patients investigated, it was decided to make an arbitrary allotment of diabetic patients to an "early onset" group if clinical onset of the disease occurred before the age of 40 years and to a "late onset" group if it occurred at the age of 40 years or later. The number of males and females in each group was as follows:-

	Total	Males	Females	Male:Female Ratio.
"Early onset" group	33	14	19	1:1.35
"Late onset" group	50	15	35	1:2.33
Total	83	29	54	1:1.86

In the "early onset" group the youngest patient was aged 15 years and 8 were over 40 years of age at the time of the investigation. The duration of clinical diabetes in this group ranged from 1 year to 35 years and in 8 cases diabetes commenced in the early teens. The results in the two groups were as follows:-

Onset group	Patients tested	Antibody present: Anti-thyroglobulin.		Thyroid microsomal		Gastric parietal cell.	
Early	Diabetics	3/25	(12%)	6/33	(18%)	8/33	(24%)
	Controls	1/25	(4%)	2/66	(3%)	1/66	(1.5%)
Late	Diabetics	3/40	(7.5%)	8/50	(16%)	10/50	(20%)
	Controls	4/40	(10%)	5/100	(5%)	12/100	(12%)

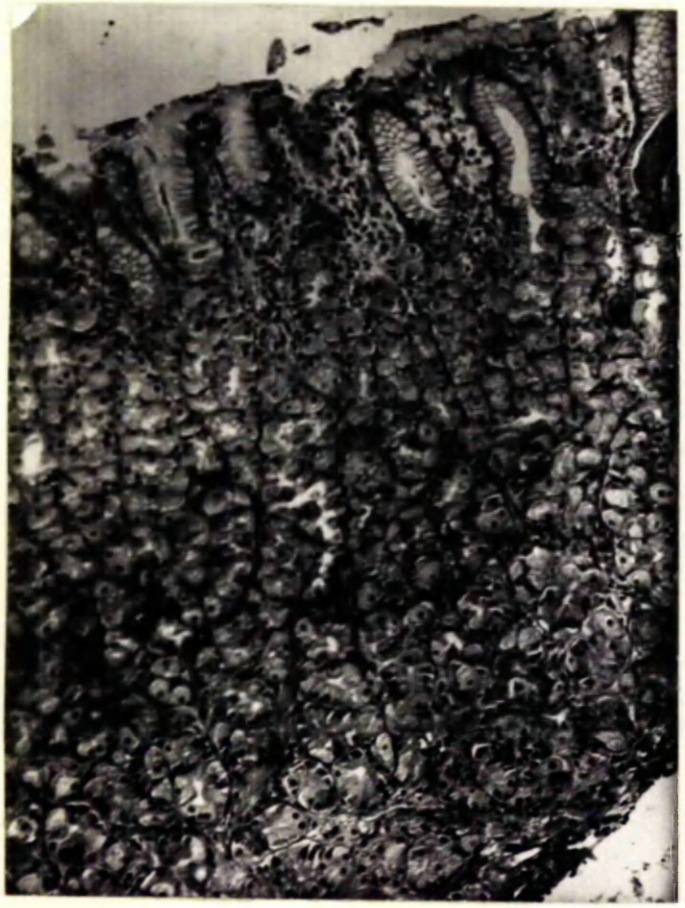
These published figures have been challenged by Irvine and Davies (1963). They reported that they did not find an increased frequency of parietal cell antibody in comparison with controls in "early onset" diabetes who were aged less than 40 years when tested. They did find a higher incidence of both gastric and thyroid antibodies in a group of "early onset" diabetics aged 40 - 60 years than in blood donors of the same age group, but the difference was not pronounced. Both complement-fixation and immunofluorescent techniques were used in their investigation.

Because of this report more intensive consideration was given to parietal cell antibody in our "early onset" group and the results of this have been published (Moore and Neilson, Lancet, 1963b). All of our results had been obtained with one technique, namely complement-fixation, and while it seems reasonable to suppose that a technique able to detect this antibody originally would have been capable

of detecting it in this investigation, nevertheless it was desirable to use the immunofluorescent test as a check on specificity. Furthermore all of the results reported in the G.M.C.F. test had been obtained with one particularly good gastric extract and, while it might have been expected that any increased sensitivity of the test in diabetics would be offset by a higher proportion of positive results in controls, this may not have happened. Accordingly the G.M.C.F. test was repeated on all seropositive "early onset" patients for whom serum was still available employing two other gastric extracts in current use in addition to the original extract, which was known to have deteriorated somewhat because of age. 7 of the 8 sera originally reported as parietal cell positive were available and in good condition. The results obtained are shown below:-

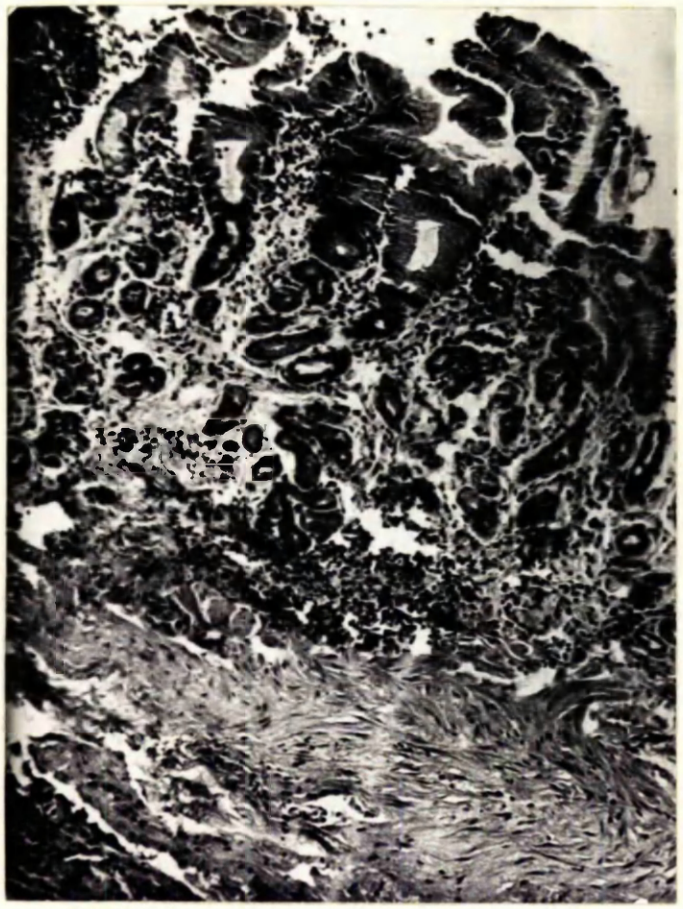
G.M.C.F. test - serum dilution 1/4
(titre in parenthesis)

Case no.	Age of onset of diabetes (yr.)	Present age (yr.)	Sex	Original Antigen: original test	Original antigen: retested	Current antigen A	Current antigen B
1.	14	19	F	+ (1/30)	+	+	+
2.	34	35	M	+ (1/60)	+	+	+
3.	19	27	F	+ (1/7)	-	-	-
4.	28	29	F	+ (1/10)	+	+	+
5.	34	39	F	+ 1/80)	+	+	+
6.	21	27	M	+ (1/7)	No serum available		
7.	25	31	F	+ (1/7)	-	-	-
8.	21	28	F	+ (1/30)	+	+	+



Normal gastric mucosa.

H. & E. x 80



Marked atrophic gastritis in a young diabetic patient.

H. & E. x 80.

An aliquot of each serum was sent to Dr. J. R. Anderson who kindly tested them for parietal cell immunofluorescence at a serum dilution of 1 in 4. He found this to be absent or doubtful in cases 3 and 7 and present in the other 5. It can be seen from the table that the serum from cases 3 and 7 which originally gave a titre of 1/7 in the G.M.C.F. test now gave negative results with 3 extracts when re-tested. This indicates that 3 of the 8 G.M.C.F. positive results reported were obtainable only with the original antigen in its pristine condition. The performance of this antigen is further shown by the results of routine tests on pernicious anaemia sera carried out during the period of the diabetic investigation. 9 were tested and of these 6 were positive at a 1/4 screening dilution (titres 1/7, 1/7, 1/15, 1/30, 1/30, 1/30) 2 were positive at a titre of less than 1/4, and 1 was negative.

Thus by generally acceptable criteria 15% of "early onset" diabetics had gastric parietal cell antibody in their serum. This is an abnormally high proportion for such an age group. The serological findings in 8 unselected "early onset" diabetic patients tested subsequently confirm that the group is abnormal in the respect claimed. Details of the 3 seropositive cases are shown in the table below. The gastric biopsy in Case No.9 showed marked atrophic changes (seen opposite, with a normal mucosa for comparison).

Case no.	Age of onset of diabetes (yr.)	Present age (yr.)	Sex	G.M.C.F. test (titre in parentheses) Original antigen: original test	Current antigen A	Current antigen B	Parietal cell immuno-fluorescence
9	33	41	M	(1/120)	+	+	+
10	22	22	M	(1/40)	+	+	+
11	16	19	F	not tested	+(1/15)	+	+

Therefore in this expanded group 19.5% of 41 "early onset" diabetics had gastric parietal antibody in their serum. The patients tested have been carefully assessed by Dr. Neilson and found to be a true sample of the diabetics in their age group attending the hospital's diabetic clinic, in respect of treatment, complications and all other recorded features. Unless the patients of Irvine and Davies were radically different from ours in some unsuspected way it is difficult to explain the discrepant results. That the above results are the more likely to be correct is indicated by the gastric investigations of Angervall and his associates (Angervall et al., 1961 and 1962). They found that achlorhydria or hypochlorhydria was common in diabetic patients, particularly in younger diabetic patients, and that this was associated with the presence of chronic atrophic gastritis.

Pettit, Landing, and Guest (1963)demonstrated that thyroid auto-antibody was present in the serum of juvenile diabetics significantly more often than in control sera.

Further evidence bearing on such an association was obtained by the team of workers at the Middlesex Hospital, London. In a study of juvenile thyroiditis patients they encountered a high incidence of diabetes mellitus in the blood relations of such cases (Dr. D. Doniach, personal communication, 1963).

3. Auto-antibody Studies in Three Cases of Renal Tubular Acidosis of Adult Type

This investigation was undertaken in collaboration with Dr. J. Wilson Chambers, whose vigilance led to a correct initial diagnosis in the 3 cases investigated. The more florid, disabling form of renal tubular acidosis with which this investigation is concerned affects adults, females more commonly than males in the ratio of 2 or 3 to 1, and has a familial incidence in some instances. The condition is uncommon. I have been informed of 12 cases attending Glasgow hospitals in 1964 apart from the 3 cases studied here. The majority of these were under the care of a medical unit with a particular interest in calcium metabolism.

The kidneys of an affected individual are unable to produce urine of pH less than about 6.5 even when ammonium chloride is administered. It has been thought that this defect results from an enzyme deficiency in the renal tubule, but this has not been proved. Whatever its nature, the defect eventually produces a systemic acidosis with an alkaline urine and without retention of nitrogen. There is frequently

also a urinary loss of calcium mobilised from the bones and of phosphate and potassium. The patient may come under medical care because of any of the following:-

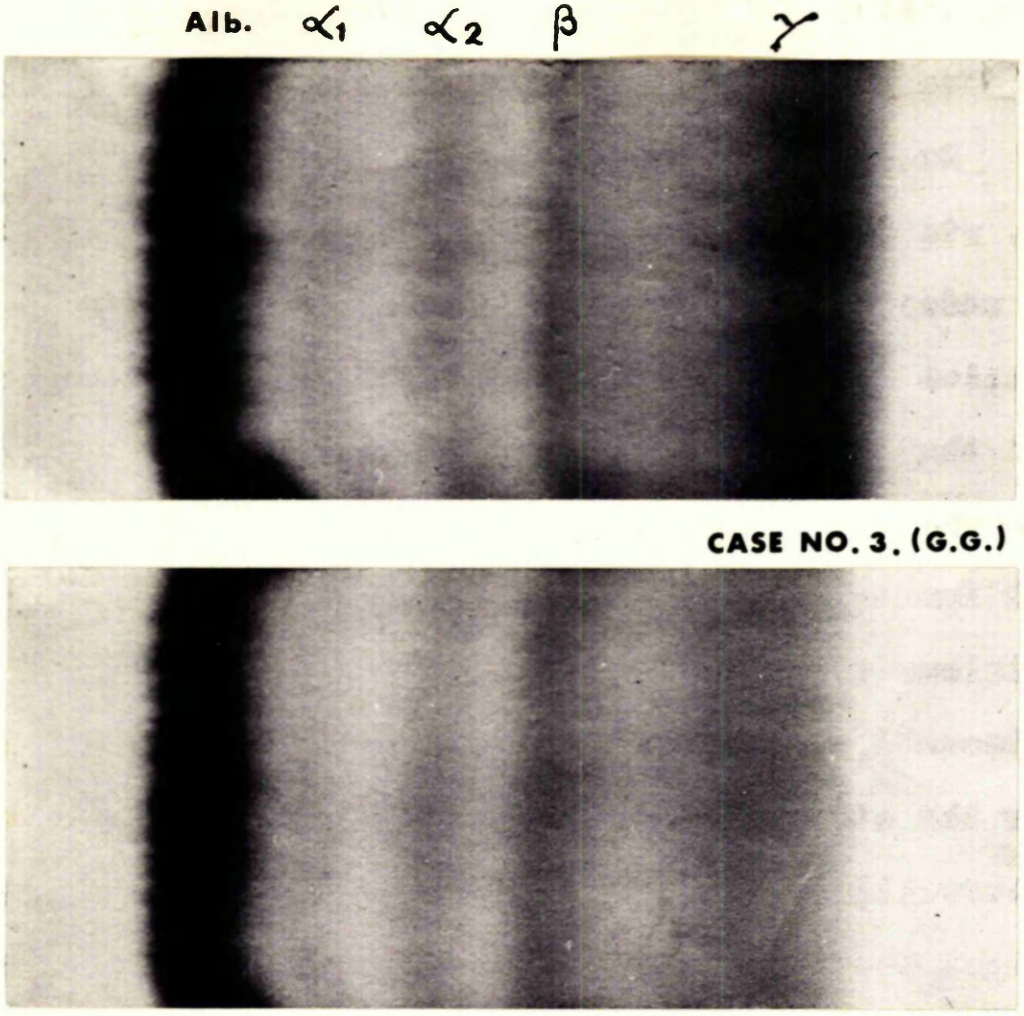
(1) renal calculi

(2) osteomalacia

(3) potassium deficiency

(4) pyelonephritis

Case 1 (Section VI) presented clinically with locomotor disability due to osteomalacia and cases 2 and 3 with weakness due to potassium deficiency. All were women in their forties when diagnosed. My interest was aroused when case 1 developed pernicious anaemia 8 years after she came under observation for renal tubular acidosis. Was this a merely random association? Pernicious anaemia is not a rare disease. As already stated it occurs in the Glasgow area at the rate of about 1.5 per thousand of population (Scott, 1960) although the rate may be somewhat higher than this in the fifth decade since it is a disease affecting mainly the middle-aged and elderly. The adult form of renal tubular acidosis is very much rarer than this. The 3 cases studied were detected in hospitals of the Glasgow northern group over a period of 8 years. Something like 100 cases of pernicious anaemia would have been detected in this group of hospitals during that time. I concluded that this association of the two diseases was unlikely to be coincidental. This belief was strengthened



Normal (Case 1) and increased (Case 3) gamma globulin concentration in serum demonstrated by paper electrophoresis.

when a survey of iron deficiency in cases of pernicious anaemia attending the Victoria Infirmary, Glasgow, indicated that one case showed the same association (Gibson et al., 1963). Dr. Ian Anderson, Clinical Biochemist to that hospital, stated that this was the only case of renal tubular acidosis under surveillance there at the time (personal communication to J. W. Chambers). It remains only to say of case 1 that there was gastric parietal cell antibody in her serum when this was tested by complement-fixation (titre 1/40) and immunofluorescent methods and that her mother also had pernicious anaemia.

Because of this curious association it seemed worthwhile to review the other 2 cases of renal tubular acidosis under regular surveillance at the hospital. The type of abnormality being sought was something related to pernicious anaemia, such as a family history of the disease, latent pernicious anaemia, or the presence of gastric parietal cell antibody in the serum. There was in fact no such abnormality but something equally interesting was apparent. One patient (case 2) had unequivocal rheumatoid arthritis and in the other (case 3) unexplained hypergammaglobulinaemia had been present for at least 5 years (see opposite). The results of laboratory investigations in these two cases are summarised in the table below (clinical and biochemical details in Section VI). They indicate that the cases have much in common.

Case No.	2 (C.W.)	3 (G.G.)
Clinical disease other than RTA	rheumatoid arthritis	none
Clinical presentation of RTA	potassium deficiency	potassium deficiency
Serum proteins (GN./100 ml.)		
Albumin	3.3	3.4
Globulin	4.9	5.0
Rose-Waaler test	+	-
Latex globulin test (Stayne)	+	+
Nuclear immunofluorescence		
serum dil. 1/4	+	+
serum dil. 1/10	+	-
A.I.C.F. test*	+	+

* A.I.C.F. = Auto-immune complement-fixation.

The only other features worth mentioning in these 2 cases were that case 2 was deaf and that case 3 had a brother with diabetes mellitus. The deafness has not been investigated but there is a known association of another form of hereditary renal disease and deafness (Perkoff et al., 1958).

In the literature on renal tubular acidosis there is no mention of pernicious anaemia apart from the case of Gibson et al., (1963). Rheumatoid arthritis is mentioned in passing by Partridge and Duthie (1963) in a 42-year-old male, case 7 of their series of rheumatic patients. Unexplained hypergammaglobulinaemia is mentioned on 3 occasions. The cases were as follows:-

(a) Subject No.34 in the very large series of cases of Wrong and Davies (1959). Kveim and Mantoux tests were negative. The patient was a 52-year-old female.

(b) A single case in a 42-year-old female reported by Fourman and McCance (1955). Liver function tests were normal and sarcoidosis was excluded.

(c) One of 2 cases described by Carroll and Davis (1964). This was in a female aged 42 years and their only additional information was that Bence-Jones proteinuria was absent.

One group of conditions of possible or probable auto-allergic nature not encountered in our patients but mentioned twice in American reports was a history of thyroid disorder. Details are as follows:-

1. Owen and Verner (1960) found that 3 out of 9 cases of renal tubular acidosis had thyroid disease or a history of past thyroid disease. All 3 cases were in females. One aged 45 years had a partial thyroidectomy for thyro-toxicosis 12 years previously. The second aged 23 years had a partial thyroidectomy for thyrotoxicosis 6 years previously. This patient also had a histamine-fast achlorhydria and, although in some thyrotoxic subjects achlorhydria is associated with atrophic gastritis, it may be present occasionally in the absence of gastritis or

gastric atrophy (Bock and Witts, 1963). The third was aged 36 years and suffered from myxoedema.

2. Huth et al., (1959) described a case of thyrotoxicosis and renal tubular acidosis in a 44-year-old female. They suggested that the renal disorder was an acquired condition secondary to hypercalcaemia associated with thyrotoxicosis. They did so mainly because they thought that there was no evidence of a familial incidence. However they did say that the patient's mother died of pyelonephritis and uraemia and this could have been a complication of unrecognised renal tubular acidosis. The same could be said of our case 2 whose mother died in her forties during an operation for uterine fibroids, possibly because of potassium deficiency.

4. Gastric and Thyroid Auto-antibodies in Malignant Disease and Reticulosis

Increasing interest has been shown in the possibility that immunological processes play a part in the development of cancer (Green, 1958; Hieger, 1961; Goudie, 1963; Grace, 1964) and in regulation of normal growth (Burwell, 1963). The work reported here was undertaken in an attempt to clarify the observation by colleagues J. L. Marksen and G. E. Flatman that myxoedema developed in 5 patients following irradiation

for, respectively, carcinoma of the breast, anaplastic carcinoma or reticulum cell sarcoma of the laryngeal portion of pharynx, giant follicular lymphoma affecting cervical and axillary lymph nodes, carcinoma of the upper end of oesophagus, and carcinoma of the nasopharynx. In the course of radiotherapy for these conditions, the thyroid gland of all these patients was exposed to radiation. The amount of this radiation ranged from 2800 - 5000 r which is considerably below the minimum reported to be necessary to ablate a non-thyrotoxic gland (Felix et al., 1961). The interval between irradiation and the detection of myxoedema was 4 to 12 months in three cases and 3 years in one case. The patient with follicular lymphoma was locally irradiated for an abdominal recurrence 3 years after irradiation of the neck and axilla, and she developed myxoedema 1 year after the second irradiation. Four of the patients were in the age group 57 to 66 years, and one, the patient with follicular lymphoma, was aged 39 years. All were females and all responded well to treatment with thyroxine. The explanation of this observation made by Markson and Flatman may be that the association was a random one but, if it was not, alternative explanations might be listed as follows:-

(1) myxoedema and cancer or reticulosis were preceded by a state of hypothyroidism which facilitated the development of cancer;

(2) either radiation or cancer/reticulosis or both induced a pathological state in the thyroid gland leading to myxoedema;

(3) a common cause produced both cancer/reticulosis and a pathological state in the thyroid gland leading to myxoedema.

The kind of pathological state in the thyroid which is envisaged is either an auto-allergic thyroiditis or some kind of induced biochemical defect. Marksom and Flatman took the view that the association was probably not coincidental and in order to investigate the matter further I collaborated with them by testing serum from a number of patients with cancer or reticulosis for the presence of gastric and thyroid auto-antibody. The results of a pilot investigation suggested that cancers of breast, ovary, and endometrium were of most interest in this respect. Attention was therefore concentrated on these tumours which are stated in the reports of the Cancer Registration Scheme to constitute 14% of all cancers occurring in the West of Scotland and 29% of cancers occurring in females.

Results.

The tests used (details in Technical Appendix) were the T.R.C.A. test for anti-thyroglobulin and the thyroid and gastric complement-fixation (T.C.F. and G.M.C.F.) tests for thyroid microsomal and gastric parietal cell antibodies.

Sera giving a positive G.M.C.F. test were subjected to the gastric immunofluorescent test to confirm that the reaction was specific to parietal cells. The results were controlled by testing similarly the serum from a large number of non-cancerous patients from medical and psychiatric wards who were of the same sex and age group as the cancer patients. No patient known to have thyroid disease, pernicious anaemia, iron-deficiency anaemia, or diabetes was included in the control group.

1. Cancer of Breast.

A significantly greater proportion of positive results was obtained in the 57 patients with cancer of the breast in tests for both anti-thyroglobulin (22.8%) and thyroid microsomal antibody (15.7%) than in controls (8.5% and 4.9% respectively). Titres in the seropositive cancer patients ranged from 1/5 - 1/250 in the former and from 1/4 - 1/80 in the latter, i.e., they were moderate or low. In 3 patients both thyroid antibodies were present and in the remainder there was one only. The proportion of positive results in the G.M.C.F. tests (12.3%) was not significantly increased in the breast cancer group. However, a comparison of the proportion of positive gastric tests in breast cancer patients who also had thyroid antibody in the serum with the proportion in breast cancer patients who had no thyroid antibody, namely 21.0% and 7.8% respectively, suggests although it does not prove that a

woman who is suffering from breast cancer and who has thyroid antibody in her serum is substantially more likely to have chronic gastritis than a non-cancerous woman of the same age.

2. Pelvic Cancer.

21 cases of endometrial cancer, 33 of ovarian cancer, and 52 of cancer of uterine cervix were tested as above. No increased proportion of thyroid antibodies was detected in these patients but the proportion positive in G.M.C.F. tests was 33.3% in endometrial cancer, 21.2% in ovarian cancer, 11.5% in cervical cancer, and 9.2% in controls. The increase is significant if the first two groups are combined, so giving 25.9% of positive tests in 54 patients.

It should be noted that these figures do not discriminate between patients who had been irradiated and those who had not. Radiation had been administered therapeutically to 75% of breast cancers, 14% of endometrial cancer 21% of ovarian cancers, and 15% of cervical cancers. It proved impossible with the type of study employed to assess with confidence the effect of irradiation on the production of auto-antibodies but in the 300 or so patients with cancer or reticulosis investigated there was a tendency for antibodies to be present more commonly in those who had been irradiated than in those who had not. Thus in the combined group of ovarian and endometrial cancers a positive G.M.C.F. test was obtained in 5/10 (50%) irradiated patients and in 9/44 (20.4%) non-irradiated patients.

Conclusion

From these results I conclude that thyroiditis of some degree occurs with increased frequency in breast cancer and that the same is true of chronic gastritis in the combined group of ovarian and endometrial cancers. In relation to the occurrence of myxoedema in cancer patients following irradiation which was the starting point of the investigation the results are not directly helpful, except perhaps to suggest that myxoedema occurring in the patient with breast cancer may not have been present fortuitously. The results do support certain lines of thought which will be developed in Section III.

II

Clinical and Serological features of Chronic Atrophic Gastritis

1. Definition.

By the term chronic atrophic gastritis is meant that type of diffuse, chronic inflammation of part-thickness or full-thickness of human gastric mucosa which is confined to the area of mucosa containing the gastric glands, namely body and fundus, which tends to produce destruction of the specialised peptic and parietal cells, and in which the predominating inflammatory cells are lymphocytes and plasma cells. Strictly speaking this definition refers only to a proportion of cases of pernicious anaemia, but it seems certain that very many other examples of chronic gastritis in patients not suffering from pernicious anaemia are of the same type. It is assumed without proof that most examples of gastric atrophy are the end result of chronic atrophic gastritis so defined.

2. Relation to Gastric Parietal Cell Antibody.

Gastric biopsy was carried out on 9 patients in whom I had detected parietal cell antibody. The biopsy was made in 8 instances by Dr. J. L. Markson and in 1 by Dr. J.McE.Neilson. Chronic gastritis of some degree was present in all. None of the patients had pernicious anaemia. Adams et al., (1964) also found biopsy evidence of chronic gastritis in every one of 20 patients with parietal cell antibody who did not have

pernicious anaemia. Thirteen had severe atrophic gastritis with complete or nearly complete loss of parietal cells, 5 had atrophic gastritis with less severe atrophy, and 2 had superficial gastritis with little or no atrophy. The authors thought that in iron-deficiency anaemia the contrast between the common occurrence of gastritis and the relatively infrequent occurrence of parietal cell antibody indicated that the chronic gastritis found in that condition may comprise a heterogeneous group of conditions and that only in a minority may the lesion be of the same nature as that found in pernicious anaemia. This contention could well be correct but in my opinion it would be desirable to compare the antibody incidence in histologically similar stages of gastritis from clinically diverse conditions. te Velde et al., (1964) made an interesting study of the relation between parietal cell antibody and chronic gastritis in the relatives of patients with pernicious anaemia. They tested sera with gastric mucosa of the rat by the immuno-fluorescent technique. Positive results were obtained in 87% of cases of pernicious anaemia, 20% of relatives of patients with pernicious anaemia and parietal cell antibody, and 7% of controls. Gastric biopsy was performed on 20 of these relatives who had parietal cell antibody. A normal mucosa was found in 4 and these were aged 20, 28, 39, and 54 years respectively. Chronic gastritis was present in the remainder.

te Velde et al., commented that:-

> "It is possible that the early involvement of the gastric fundus is patchy, therefore we cannot state with certainty that the presence of the parietal cell antibody precedes the development of atrophic gastritis, but this auto-antibody clearly may be present in an early stage."

Joske, Finckh and Wood (1955) made observations which suggested that gastritis could be patchy. They took a biopsy sample from two different sites in 726 cases. In 73.8% the histological appearances were similar and in the remainder there were considerable differences. Likewise when they carried out serial biopsies in 123 patients the appearances were in agreement in 76.4% and not in agreement in the remainder. Parietal cell antibody is present in the serum of a high proportion of cases of pernicious anaemia but in my experience of that condition and in that of Doniach and Roitt (1964) the titre in complement-fixation tests is seldom above and usually below 1/40. On the other hand high titres have been obtained in other types of patient, some of whom had free acid in the gastric juice and therefore a gastric lesion less advanced than that found in pernicious anaemia.

3. Possible Auto-allergic Nature.

Much of the ensuing discussion proceeds from the hypothesis that an auto-allergic state is present in the chronic gastritis which is accompanied by parietal cell antibody. The discussion will be concluded by an examination of this hypothesis.

III

Endocrine and Metabolic Dysfunction as Factors in the Pathogenesis of Chronic Atrophic Gastritis

It is considered that the serological investigations described in Section I point to the importance of two factors in the pathogenesis of some examples of chronic atrophic gastritis. These are endocrine dysfunction, of which thymo-lymphatic dysfunction may be a part, and metabolic upset.

1. Endocrine Dysfunction

On the striking association between pernicious anaemia and thyroid dysfunction (Tudhope and Wilson, 1960; McNicol, 1961) current views give prominence to the common developmental origin of thyroid and stomach, the implication being that they will react in the same way to harmful influences. However, there is no bond of common development between the stomach and the adrenal cortex and yet they are closely allied in the immunological sense (Irvine, 1963). It is therefore at least

a possibility that the endocrine dysfunction associated with myxoedema and thyrotoxicosis has an influence on the development of gastritis in pernicious anaemia. For this reason chronic gastritis is a better condition in which to study factors in pathogenesis than is chronic thyroiditis since it is difficult to imagine chronic gastritis having much effect on endocrine function, even if the stomach is, as it seems to be, a target organ for hormones.

The particular kinds of endocrine dysfunction other than thyroid which have a known relation to chronic gastritis are idiopathic hypoparathyroidism and Addison's disease. To these I would add tentatively the endocrine upset frequently found in carcinoma of ovary and endometrium.

(a) <u>The association of idiopathic hypoparathyroidism and pernicious anaemia</u>.

Ikkala et al., (1964) have reviewed the literature on the association of these two rarities. I say two rarities because the pernicious anaemia seen in this connection occurs in children or young people. Less than 100 cases of idiopathic hypoparathyroidism have been reported and 7 of these were associated with pernicious anaemia. Ikkala et al. added to this total 1 very thoroughly investigated case in which the gastric biopsy showed total atrophy. These workers also investigated 4 patients with badly-controlled post-operative hypoparathyroidism. The operation in question was presumably

that of partial thyroidectomy for thyrotoxicosis but this was not stated. Gastric biopsy showed that severe superficial gastritis was present in all 4 patients and that this persisted in the 2 cases who had a further biopsy taken after an effective course of treatment with parathyroid hormone. The gastritis in these 4 patients might have been associated with preceding thyrotoxicosis but this is difficult to assess. It is possible but not in my opinion probable that in idiopathic hypoparathyroidism there occurs a disuse atrophy of stomach since Keynes et al., (1962) have shown in animal experiments that parathyroid deficiency is commonly associated with reduced secretion of hydrochloric acid and pepsin and that this can be reversed by raising directly the level of blood calcium.

There is some rather more tenuous evidence of another link between parathyroid dysfunction and chronic gastritis. Doig and Wood (1958) remarked in a review article that 1./13 cases of chronic pancreatitis investigated by Mackay showed chronic gastritis. The connection is provided by the also unexplained association of chronic pancreatitis with primary hyperparathyroidism (Keynes, 1962).

(b) Addison's disease and chronic gastritis.

Idiopathic hypoparathyroidism is associated with pernicious anaemia and also with Addison's disease (Irvine et al., 1963). Rarely the 3 conditions are found in combination (Morse et al., 1961).

The literature on the relationship between adrenal glands and the stomach was reviewed by Gray, Ramsay and Thorn (1956). From their survey it can be concluded that adrenalectomy in animals produced a different lesion in the stomach from that found in Addison's disease. In the former there developed a purely atrophic lesion within a short period of time, whereas chronic atrophic gastritis was found in those cases of Addison's disease, the majority, with achylia. This effect of adrenalectomy in animals and the well known ulcerogenic effect of adrenal steroids in human subjects was thought to indicate that under certain circumstances the adrenal cortex exerted a trophic effect on the gastric mucosa. Pernicious anaemia is very much less commonly associated with Addison's disease but it does occur and, in the case reported by Ura and Barile (1963) in a middle-aged male, thyrotoxicosis developed in a daughter. Irvine (1963) tested 15 cases of idiopathic Addison's disease for gastric parietal cell antibody by the complement-fixation method and obtained a positive result in 6. From the results of this and other tests for auto-antibody he suggested that the overlap of Addison's disease of idiopathic type, chronic thyroiditis, and pernicious anaemia pointed to a basically similar pathogenesis.

(c) <u>Parietal cell antibody in carcinoma of endometrium and ovary.</u>

As already described parietal cell antibody was detected with greater frequency in a group of patients who had carcinoma

of the endometrium or of the ovary than in comparable controls. This was a very odd and unexpected finding but carcinoma of the endometrium at least is a very odd tumour (Lancet, 1961).

Way (1956) listed what he considered to be important associated features. These were:-

(1) later menopause.

(2) diabetes mellitus common.

(3) frequent occurrence of endometrial hyperplasis and polyps.

(4) high incidence of associated feminising ovarian tumours.

(5) probable inherited predisposition.

Benjamin (1960) supported Way's contention about diabetes and endometrial cancer. He also drew attention to some pertinent epidemiological data, summarised in the following table:-

Race	Carcinoma of cervix	Carcinoma of endometrium	Diabetes mellitus
Japanese	common	practically unknown	rare
Jewish	rare	common	common

Way considered that excessive anterior pituitary function accounted for the features enumerated. A rather more explicit pathogenesis has been postulated by Sherman and Woolf (1959). They suggested that, under the influence of luteinising hormone (L.H.), cells of the ovarian hilum elaborate a hormone

carcinogenic for the endometrium and, as a corollary, endometrial cancer does not occur if the ovaries have been removed. These workers claimed that an assay procedure showed an increased output of L.H. in all cases of endometrial cancer tested. Their work was confirmed in part only by Varga and Henriksen (1963) who showed that raised L.H. levels were present in 30% of endometrial cancers tested, irrespective of the presence or absence of ovaries, whereas normal levels were present in controls.

Berggren (1963), in a review of thyroid disorders in pelvic cancer, summarised the findings of Kimbach which were that 7.8% of 204 cases of endometrial cancer occurring in women over 50 years of age had previously undergone thyroidectomy and in one third of these the operation had been performed in the 3 years prior to the operation for cancer. In a control group of 408 patients with benign gynaecological disorders 0.74% had undergone thyroidectomy. Kimbach's interpretation of this was that thyroidectomy produced hypothyroidism and that this favoured the development of endometrial cancer in 2 ways - (1) by diminishing cell respiration generally, and (2) by stimulating the pituitary to produce more of both gonadotrophic and thyrotropic hormones, in turn leading to an increased output of ovarian follicular hormone.

Carcinoma of the ovary also has some peculiar features. Unlike other malignancies the prognosis is worse in older subjects than in younger. Nulliparity predisposes to the

development of ovarian cancer as it does to breast cancer. The florid masculinising or feminising tumours, which constitute 10% of all ovarian malignancies, carry a relatively good prognosis.

Cancer of the ovary is occasionally associated with Cushing's syndrome as are cancers of thymus, bronchus, and pancreatic islets. Parsons and Rigby (1958) reviewed the literature and described such a case in a woman aged 64 who developed the disease soon after excision of an ovarian carcinoma with localised spread. One feature of the Cushing's disease was unstable diabetes and this resulted in a fatal urinary infection soon afterwards. At autopsy the cortex of both adrenal glands was hyperplastic and the pituitary gland showed no abnormality. Parsons and Rigby concluded that in their patient the stress of severe and long-standing malignant disease might have provoked hyperactivity of the adrenal cortex. They may however be wrong. There are good grounds for supposing that the neoplastic cells in some examples of bronchial, oat-celled carcinoma produce hormones resembling those of the adrenal cortex or having a corticotrophic action on that organ (Lancet, 1964a). The same may have been true of the ovarian tumour in question and, if so, lesser degrees of the same phenomenon may occur in other ovarian cancers.

Wren and Frampton (1963) have drawn attention to the frequently reported presence of endometrial hyperplasia and increased output of oestrogens in non-feminising malignant

ovarian tumours occurring in post-menopausal women. This contrasts with the low levels of oestrogen found in normal post-menopausal women. Using a crude vaginal cornification index, these workers found that oestrogenic activity was increased in post-menopausal women with ovarian tumour compared with those who had no tumour, in the ratio of 2 to 1. Where endometrial hyperplasia, established histologically, was used as the index, evidence of oestrogenic activity was found in 40% of post-menopausal women with ovarian tumour and in 1% of those with no tumour. It is not known where the oestrogenic substances giving such effects originate. The cells of the ovarian hilum and those of the adrenal cortex have both been suggested as possible sources.

It has been mentioned above that Way described as one feature of endometrial cancer a high incidence of associated feminising ovarian tumours. Miller et al., (1962) made one observation on the incidence of associated primary endometrial and ovarian cancers. In their series of 214 cases of primary ovarian carcinoma 13 had a second, primary, malignant tumour in another site. In 4 (1.9%) the second primary was in the endometrium.

Berggren (1963) analysed the records of 187 cases of primary ovarian cancer in Sweden and compared these with those of 4935 non-cancerous, gynaecological controls taking age into account. He found that there was thyroid disease

or a history of it in 21 of the ovarian cancer group whereas the expected number calculated from the controls was 6.9.

Thymo-lymphatic dysfunction

I have postulated that endocrine function may give rise to disturbed function in the thymus and lymphoid system. There can be no dispute that this system is responsive in a quantitative way to changes in hormone secretion elsewhere. It hypertrophies markedly in thyrotoxicosis and Addison's disease and atrophies as markedly in adrenal cortical hyperfunction and myxoedema (Yoffey and Courtice, 1956; Williams, 1962). No doubt also lesser changes occur in other types of endocrine function, but do the sort of changes mentioned cause a disturbance of thymo-lymphatic functions? A very striking effect on the structure and function of the lymphoid system induced experimentally by a hormone has been reported by Mark and Schlesinger (1964). These workers produced in suckling mice a fatal wasting syndrome, closely resembling "runt disease", by the subcutaneous administration of a single dose of 0.1 to 0.25 mg. of cortisone acetate per gm. of body weight. The process could be moderated by reducing the amount of cortisone injected. At autopsy there was marked reduction in the weights of thymus and spleen. The microscopical appearance in lymphoid organs was said to be one of lymphocyte depletion and distortion of structure. In this experiment therefore injection of cortisone into neonate mice had a

similar effect to that produced by thymectomy. In runt disease evoked by grafting lymphoid cells from a donor into a genetically non-identical recipient the antigens of the latter are reacted on by foreign immunologically-competent cells. It seems reasonable to deduce in the wasting disease appearing as the result of thymectomy or of a relatively enormous, single, injected dose of cortisone in neonatal mice that there is a failure on the part of surviving lymphoid cells to recognise as self large numbers of the body's antigens. It is true that a number of workers have shown that cells from the lymphoid organs of neonatally-thymectomised mice are less capable of producing signs of graft versus host activity in appropriate recipients than the same number of lymphoid cells from normal mice (Miller, 1963a). It is also true that most neonatally-thymectomised mice fail to reject homografts or heterografts of skin (Miller, 1963b). However, weak immunological reactivity which was ineffective against a small portion of foreign skin might have very marked effects if acting throughout the body against a multiplicity of antigens regarded as foreign. Thymectomy in adult rodents has no dramatic effects but I think it not unreasonable to suggest that the gross quantitative effects produced in the thymus and lymphoid structures of adults by dysfunction of the thyroid gland and adrenal cortex may be paralleled by disorders of function of the lymphoid system.

2. Metabolic dysfunction

a. Renal tubular acidosis.

One cannot be certain that endocrine function, particularly of the parathyroid gland, is normal in renal tubular acidosis of adults. It seems clear however that metabolic upset is the prime and important abnormality present. There are, no doubt, many who would dispute that the results, reported in Section I, of serological investigation in three examples of this condition prove that auto-allergic disorders were present. This can certainly be said of case 1 if indeed pernicious anaemia with parietal cell antibody comes into that category. This is a theory with substantial support and it will be discussed at a later stage. We enter into an interesting but more controversial field when case 2, and more so, case 3 are considered. The former was a typical case of rheumatoid arthritis and I would contend that case 3 was an example, in latent form, of the same kind of connective tissue disease. Dunkel (1964) gave the most helpful, concise summary on the state of knowledge in connective tissues generally when he stated:-

> ".....rheumatoid arthritis remains essentially an enigma, despite the fact that the link to clear immunologic disorders is difficult to ignore. A reasonable working hypothesis is that there exists a basic genetic diathesis common to the whole group of connective tissue disorders which involve immunological mechanisms. Environmental stress, particularly infections, may lead secondarily

to the characteristically variable clinical patterns observed."

It appears therefore that there is sufficient common ground between pernicious anaemia and rheumatoid arthritis to make it likely that the gross metabolic upsets occurring in renal tubular acidosis initiate or hasten the emergence of these conditions. In cases 2 and 3 there was gross, chronic potassium deficiency. It is accepted that chronic potassium deficiency can cause damage to renal tissue (Lancet, 1964b) so that production of tissue damage in the gastric mucosa and elsewhere by this means is perfectly feasible. The role of a hereditary predisposition to develop these diseases is not denied. Case 1 had a family history of pernicious anaemia and although there was no family history of rheumatoid arthritis in the other two cases the disease is common - 4% incidence - in women of their age group (Lawrence, 1964) and still more common in older women. Many other factors may play a part in the pathogenesis of auto-allergy. I suggest that in these examples metabolic dysfunction has played some part.

b. Diabetes mellitus.

Upset of carbohydrate metabolism is by definition a feature of diabetes mellitus. The younger the diabetic the more marked generally speaking is this metabolic upset and, as described in Section I, it was in the younger diabetic subject that evidence of associated thyroid and gastric auto-allergic disease was most apparent. The interest of this

observation is increased by the findings of electron microscopy in the diabetic kidney reported by Sabour, McDonald and Robson (1962). Even when no abnormality could be detected in the renal biopsy by light microscopy shortly after clinical onset of diabetes, thickening of basement membrane of tubules and glomerular capsules was demonstrated by electron microscopy. It is possible but unlikely that this lesion develops rapidly as a result of the upset in carbohydrate metabolism. It seems more probable that the basement membrane/vascular components of the disease and the carbohydrate disturbance arise from some common cause, perhaps a different kind of metabolic upset. The detection of parietal cell antibody in 2 young males within a couple of weeks of the clinical onset of diabetes (Moore and Neilson, 1963a) was a finding still less likely to be secondary to carbohydrate upset. In one of these patients the titre of antibody (1/40) was substantial and there is every reason to suppose that chronic atrophic gastritis is a slowly progressive lesion and that the appearance of parietal cell antibody is secondary.

c. Iron Deficiency.

Chronic atrophic gastritis is common in iron-deficiency anaemia (Joske et al., 1955; Coghill and Williams, 1955; Davidson and Markson, 1955; Badenoch et al., 1957) but the relationship of one to the other is not clear. Iron deficiency can be produced readily in immature animals

by feeding them a diet deficient only in iron. This is not so in the case of adult animals where resort to iron depletion by bleeding is required. Such a manoeuvre necessarily causes depletion of substances other than iron. In human subjects iron deficiency anaemia is encountered mainly in females of child-bearing age as a result, usually, of two adverse circumstances:-

(1) an intake of iron in the food sufficient only for basal needs, and

(2) loss to the body of a number of substances, of which iron is one, because of blood loss related to menstruation or of demands of the foetus during pregnancy.

The study of anything related to iron deficiency in the human subject is made difficult by a number of things, amongst which are the possible occurrence of tissue deficiency of iron without anaemia and the tendency for iron-deficiency to present in episodic fashion, each episode being treated in a rather arbitrary fashion and then forgotten. It is therefore seldom possible for the investigator to have a clear idea of the history of a particular patient in respect of tissue deficiency of iron, quite apart from any concomitant deficiency.

Valberg, Taylor and Witts (1961) were unable to produce gastric changes in young rats by feeding iron-deficient diets. This may have been because the deprivation was insufficiently prolonged, as they themselves suggested,

or because, as I think, other factors operating in the naturally-occurring human disorder were lacking in this particular experiment. The tissue changes occurring in iron-deficiency anaemia (Beutler, 1964) are inconstant or ill-defined and of doubtful significance. It therefore seems likely that any effect iron deprivation might have on gastric auto-allergy would not be simple or direct. Better simulation of the human disorder in animal experiments and studies of gastritis and gastric antibodies in relatives of patients with iron-deficiency anaemia may clarify some of the problems mentioned.

IV

The Relation of Chronic gastritis to Diabetes and Malignancy

1. Chronic gastritis in Malignant Disease.

Any supposed fact pertaining to human cancer must be considered in relation to the enormous accumulation of knowledge on cancer or at least, in this instance, to that part of it bearing on the modest findings reported in Section I, as well as to the current interpretations of that knowledge.

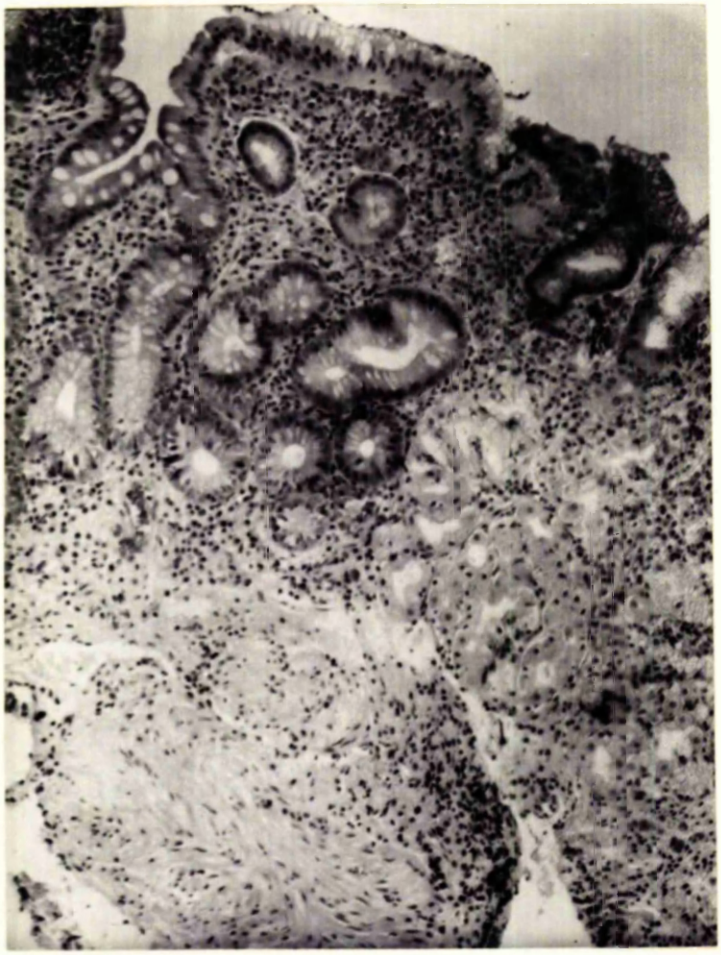
First, however, the strength of the evidence of a relation between chronic gastritis, and chronic thyroiditis, and certain cancers should be assessed. The evidence

consists of serological findings only and the abnormalities described were detected in relatively small groups of patients and in only a minority of these. No biopsies of stomach or thyroid were obtained. There is however some independent evidence bearing on the occurrence of gastritis in malignant diease. Joske et al., (1955) reported that, of the 1000 gastric biopsies studied by them, 13 were from patients with malignancy of a site other than the stomach. No details were given about the site or nature of the tumours concerned. The biopsy appearances were normal in only 4 of these patients. The remainder showed moderate or severe superficial gastritis (2), superficial gastritis with atrophy (3), severe atrophic gastritis (3), and gastric atrophy (1). Amongst the controls used by colleagues investigating gastric biopsy appearances in pernicious anaemia and iron-deficiency anaemia (Markson and Davidson, 1956; Davidson and Markson, 1955) were 3 patients with malignant disease not affecting the stomach. Particulars of these patients were as follows:-

(1) 63-year-old male with bronchial carcinoma:

(2) 66-year-old male with well-differentiated epidermoid carcinoma of left upp r bronchus: the tumour was considered to be inoperable and X-irradiation was administered:

(3) 73-year-old female with carcinoma of head of pancreas confirmed at autopsy 2 months after gastric biopsy.



Atrophic gastritis in adult male with bronchial carcinoma.

H. & E. x 80.

Patient (1) had a normal gastric mucosa. The biopsy in patient (2) (illustrated opposite) showed atrophic gastritis and intestinal metaplasia and in the third patient there was atrophic gastritis of moderate degree. Scrutiny of the history of cases (2) and (3) demonstrated the difficulty of assessing all the factors involved. Patient (2) was not anaemic but he had a pneumonic episode 9 months previously and was cachectic at the time the biopsy was taken. Patient (3) suffered from a pancreatic type of steatorrhoea for several months before admission and not long after her biopsy was having gross obstruction to the common bile duct. Such abnormalities would have a very marked effect, particularly in a woman of 73. In the course of the same investigations Markson and Davidson found atrophic gastritis in the biopsy specimen from a patient suspected of having pernicious anaemia. This patient had been admitted on account of an acute exacerbation of chronic bronchial asthma. She gave a history of having mild anaemia at the age of 50 which was treated in Glasgow Royal Infirmary with iron and extract of liver. Liver therapy was given intermittently until the age of 58. At this point she had the gastric biopsy mentioned. Glossitis and histamine-fast achlorhydria but no anaemia were present at this time. There was in addition a history of hysterectomy and unilateral salpingo-oophorectomy at the age of 52 on account of endometrial cancer. The only example

of associated pernicious anaemia and malignant disease personally encountered, which association may of course have been coincidental, occurred in a patient under the care of Dr. J. B. Bonnie. This was a woman in her early sixties who developed simultaneously signs of renal adenocarcinoma and subacute combined degeneration of the cord. Megaloblastic anaemia was present and responded satisfactorily, as did the cord lesion, to vitamin B 12 therapy.

It seems very unlikely that chronic gastritis where present could have any influence on the genesis or course of cancer. If it were accepted that malignant and auto-allergic disease may be sometimes significantly associated, one of a number of possible explanations might be that endocrine dysfunction facilitated the development of both. In the case of malignancy this could operate partly by an effect on immune responses and partly by stimulation of the epithelium of endocrine glands or target organs.

a. Hormones and Cancer.

Although hormones exert an important influence in some human cancers, cure of an established cancer seldom results from hormone therapy and any regression so produced is usually temporary. In animals cancers may be induced by administration of hormones or by other manoeuvres causing prolonged over-stimulation by endogenous hormone. Hyperplasia of the target tissue ensues, sometimes with cycles of

exhaustion and regenerative hyperplasia of epithelial cells. This seems to set the stage for the qualitative change or series of changes to neoplasia. Prostate, endometrium and thyroid provide examples of this kind of change in human subjects. In the transition from hyperplasia to neoplasia hormonal imbalance may or may not play a part, along with other influences such as viruses, carcinogens, and inherited susceptibility of the tissue concerned: hormones do not themselves act as carcinogens in the accepted sense. Hormones may also, on the analogy of animal experiments, play a part in inducing some human cancers, but it should be remembered of animal cancers induced in this way that the degree of hormonal imbalance required is often extreme and that the animals used have often been selected for proneness to cancer (Foulds, 1964). Of such cancers Foulds observes that:-

> "In general hormones evoke neoplasia only in their recognised target tissues but there are exceptions to the rule: oestrogens induce leukaemia in mice and kidney tumours in hamsters, and hormones modify the effects of chemical carcinogens on the breast and on the liver."

In established human cancers endocrine changes unrelated to therapy are observed occasionally (Lancet, 1964a) and it has been established that in certain instances the tumour itself secretes hormones or hormone-like substances. A

more common occurrence is for the growth, invasiveness, and dissemination of certain tumours to be influenced by the normal endocrine changes associated with pregnancy, lactation and the menopause.

b. Immunity and Cancer.

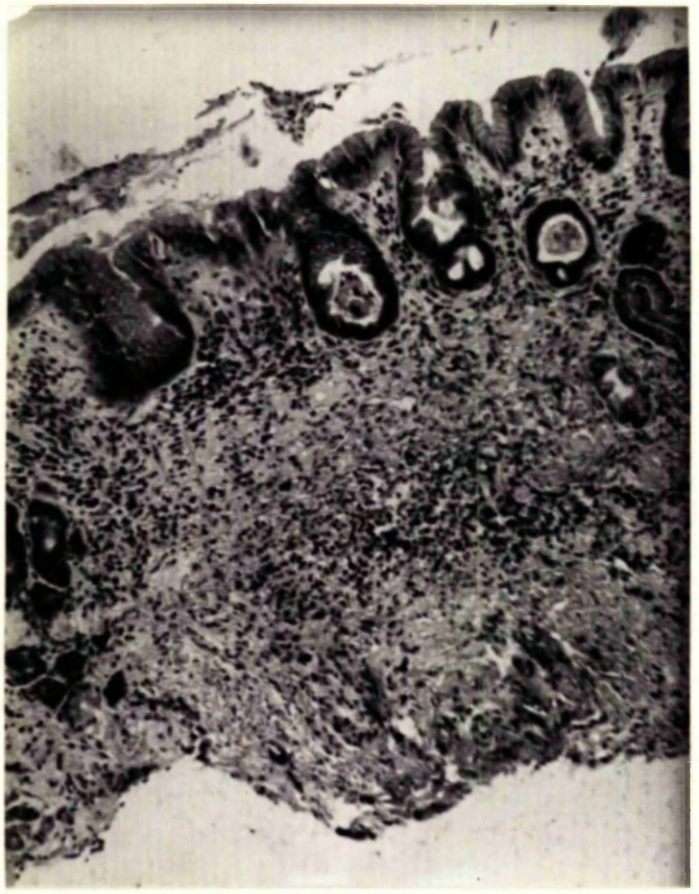
It has been considered by many writers that immunity may be of importance in cancer. Amongst the facts suggesting this are (1) the rare but authenticated spontaneous regression or cure of certain human cancers, notably neuroblastoma, hypernephroma, choriocarcinoma, and malignant melanoma (Everson, 1964), (2) the sometimes prolonged survival after incomplete removal of carcinoma, (3) the occurrence in a few cancers of a long period of latency succeeded by simultaneous, rapid growth of metastases in different parts of the body (Rao and Moore, 1961), (4) the relatively frequent failure of metastases to survive (Moore et al., 1957), (5) the tendency for prolonged survival in cancer patients to be associated with lymphoid infiltration of tumours and sinus histiocytosis of regional lymph nodes (Sutherland, 1960). At the same time the more dramatically malignant tumours clearly grow without check of any kind, but in relation to neoplasia of all kinds this type may constitute only a small proportion of all tumours.

Green (1958) has put forward the idea that carcinogens alter the identity of cytoplasmic "tissue-specific antigens" sufficiently for an immune reaction to be provoked. This

in turn either kills the abnormal cells or results in an adaptive deletion of the new antigen. In respect of the antigen deleted normal controls depending on antigen recognition no longer operate and the cancer cells are able to grow without hindrance from this type of control. Lieger (1961) examined this idea critically and concluded that, although there was other evidence for loss of an important protein constituent of cytoplasm in the cells of some cancers it was quite insufficient to warrant characterising this phenomenon as the mechanism underlying carcinogenesis whether this was expressed in immunological or any other terms. Further work showing that cancer cells differ from normal cells in cytoplasmic content of potentially antigenic substances (Goudie, 1963) can be criticised similarly even if Green's explanation is in fact the correct one. As a tiny contribution to a consideration of this impasse I will describe one sketchy experiment, or prelude to an experiment, carried out on a resection specimen of stomach removed from a 68-year-old woman because of gastric polyposis. Seven simple, pedunculated polyps were present and there was marked, chronic atrophic gastritis in the intervening, macroscopically normal mucosa although parietal cell antibody was not detected in the serum. An extract of body mucosa, excluding polyps, was made in the usual way (Technical Appendix) on the hypothesis that the mucosa was in a pre-malignant state (Roxburgh, 1962).

on testing by complement-fixation it was found that the extract contained parietal cell antigen but in a concentration substantially less than that found in extracts made from the body mucosa of stomachs from patients with duodenal or stomal ulcer. The serum from 7 patients with gastric carcinoma, from 4 patients who did not have gastric disease, and that of the patient herself, all gave negative results in complement-fixation tests with both normal and polyposis extracts. In the case of 2 other patients with gastric carcinoma I was seropositive with both extracts and the other with the polyposis extract only. It was not possible to investigate the matter further at the time. If this could be confirmed with adequate numbers of sera in a variety of examples of a lesion accepted as pre-malignant, one might feel a little more partial to the idea of antigenic change being an essential prelude to, and not a by-product of, the development of malignancy.

Green (1964) drew attention in an interesting brief review to certain aspects of immunity in cancer. These were (1) the alterations in immune responsiveness, mainly cellular (depression of delayed skin test and homograft responses), seen particularly in patients with lymphomatous disease, (2) the appearance of circulating antibody to some antigenic components or products of their tumour in the serum of a few patients, (3) the failure of patients with advanced cancer to accept autografts of their tumours in a majority of cases,



Atrophic gastritis in 16 year old male with Hodgkin's disease.

H. & E. x 80.

(3) immunological reaction in the form of dermatomyositis by a few cancer patients to some component or product of their tumour as demonstrated by positive immediate type skin test responses to antigens prepared from the patient's own tumour. It has been estimated (Curtis et al., 1961) that 3% of cases of dermatomyositis occurring in patients over cancer age were associated with malignant disease. Points (1) and (4) above may be considered applicable to 2 cases encountered in the course of investigating malignant disease but the mechanisms suggested by Grace seem inappropriate. The first patient (case no. 4, Section VI) was a 16-year-old youth with Hodgkin's disease who had chronic atrophic gastritis (see opposite) and parietal cell antibody in his serum. The second patient (case no.5, Section VI) was a woman aged 54 who had been under observation on account of very chronic disseminated lupus erythematosus for 15 years prior to the development of myelofibrosis. Depression of delayed hypersensitivity reactions could not in my opinion have led to the very early development of chronic gastritis seen in the first patient (see also Waksman, 1962) nor would it seem likely that in the second patient disseminated lupus erythematosus was a form of hypersensitivity reaction to a component or product of whatever process underlies myelofibrosis. Pelner (1959) has observed the apparent activation of malignant disease in some patients treated with corticoids for one type or another of connective tissue disease. The second patient described

above did not have corticoid therapy for the lupus erythematosus.

These 2 cases illustrate some of the difficulties encountered when trying to assess what place, if any, immunity has in cancer. All sorts of immune reactions may be involved - host against tumour or tumour product, tumour against host - and all sorts of factors may influence these. The findings described in cancers of breast, ovary, and endometrium are probably related to factors influencing auto-allergic reactions unless chronic thyroiditis and chronic gastritis occurring in association with cancers are reactions to tumour products. Ovarian cancer is the only one of the three which is sometimes associated with entities like dermatomyositis and haemolytic anaemia, but even this is uncommon.

There is a little morphological evidence that an immune reaction may occur in certain ovarian tumours. Black and Speer (1960) have shown that, in carcinomas of breast and stomach, lymphoid infiltration and degree of nuclear differentiation in the tumour, and also the presence of sinus histiocytosis in regional lymph glands could be correlated with biological behaviour. Stone et al.. (1963) assessed the first two of these factors in a large series of cases of carcinoma of ovary. The recognised hormone-secreting tumours such as the coma and granulosa cell tumour were excluded because they are known to have a relatively good prognosis.

The tumours in 10 patients surviving for more than 5 years were all of papillary-serous or sero-anaplastic type and in 3 of these regional metastases were present at the time of the original diagnosis. In this long-survival group the tumours showed ".....a tendency towards nuclear differentiation or lymphoid infiltration to a degree not found in a series of similar types of ovarian carcinoma in which the patients died within 2 years of diagnosis". Absence of lymphoid reaction carried a bad prognosis although its presence was neither a guarantee of long survival nor essential to it. This suggests that in tumours showing lymphoid infiltration the body's immunologically competent were reacting with antigens foreign to them in and around the tumour cells, although not necessarily to the body's advantage in all cases.

c. Breast Cancer and Chronic Thyroiditis.

Although this is not directly relevant to what has gone before, I believe that the evidence for such an association obtained as described in Section I merits a brief discussion at this point.

There is substantial information available on the relation between thyroid disease and breast cancer (Repart, 1952; Loeser, 1954; Adelstyn, 1958) and, even more so, on general endocrine dysfunction in that disease (Sutherland, 1960; Bonser et al., 1961; Foulds, 1964). The importance of

endocrine factors in at least some patients with breast cancer is shown by the increased frequency of this cancer in nulliparous, pre-menopausal women. It was for advanced carcinoma of the breast that Beatson introduced ovariectomy at the end of the nineteenth century and it is now standard surgical practice to ablate the ovaries of pre-menopausal women undergoing surgery for breast cancer, although ablative treatment to pituitary and adrenals has had only meagre success. The whole position of this kind of therapy in breast cancer is being reconsidered with a view to selecting those most likely to benefit from its use (Bonser et al., 1961). The results described in Section 1 suggest that one factor possibly requiring attention is the presence of chronic thyroiditis. The same is probably true of chronic gastritis also for, although the overall figure for parietal cell antibody in women with breast cancer was not high (12%), it was detected more frequently in those with thyroid antibody than in those without, 4/19 (20%) compared with 3/39 (7.7%).

2. Chronic Gastritis and Diabetes

The literature on diabetes mellitus is so extensive and the complexity of the subject so great that I intend to discuss only those facets of diabetes which might explain the co-existence of chronic gastritis and diabetes. I consider insulin to be the entity most requiring attention in this connection because of (1) its hormonal nature and (2) its

antigenicity.

(a) Endocrine Function and Diabetes.

A diabetic state can be produced experimentally in animals by a variety of methods. One of the best known examples of experimental diabetes is that resulting from the injection of growth hormone (Young, 1936), but there seems to be no general agreement that these artificially-induced states are comparable to the human disease. The pituitary gland does have some influence on the naturally-occurring disorder in that (a) human growth hormone causes a marked exacerbation of diabetes in patients who have undergone hypophysectomy, (b) it is rare for patients with hypopituitarism to develop diabetes, and (c) juvenile diabetics tend to be above average height for their age. Sprague (1962) reviewed the literature concerning the effect on diabetic retinopathy of hypophysectomy and hypophseal stalk section. He concluded that in some cases these procedures alleviated this complication but did not alleviate the complications of nephropathy and neuropathy. The danger to the patient of ablative therapy of this type and the lack of striking advantage in control of carbohydrate upset or nephropathy made its general use undesirable in his opinion.

Adreno-cortical dysfunction has an effect in diabetes somewhat similar to that resulting from pituitary dysfunction. Hypercorticism tends to produce a not very severe diabetes, as does acromegaly, and injection of glucosteroids such as cortisone

accentuates existing or latent abnormalities of carbohydrate metabolism. The combination of Addison's disease and diabetes is rare (McNicol and McNicol, 1960) but Addison's disease is itself uncommon and there is reason to think that it is complicated by diabetes with increased frequency. That this is not a straightforward endocrine matter is shown by the occurrence of a triad of diabetes, Addison's disease, and lymphocytic thyroiditis. Out of 15 cases of combined thyroid and adreno-cortical insufficiency (Schmidt's syndrome) active or latent diabetes was present in 10 and there was also striking evidence in the group of a history of allergic reactions (Carpenter et al., 1964). One of the reactions mentioned was recurrent urticaria and this was a feature in childhood of a case of combined pernicious anaemia and diabetes occurring in a young woman with an interesting heredity (Moore and Neilson, 1963a). Bloodworth et al. (1954) showed that lymphocytic thyroiditis was present in Schmidt's syndrome and that no abnormality was detectable in the pituitary. Lentle and Thomas (1964) briefly reviewed the literature on adrenal function and diabetes with particular reference to the complications of diabetes. Because it has been reported that the adrenal glands are heavier and adreno-cortical adenomata more common in diabetes without complication, Lentle and Thomas investigated 15 diabetics and found that the former category had higher levels of plasma cortisol throughout the day than

either healthy persons or patients with uncomplicated diabetes. From this rather meagre evidence and from the reported results of long-continued administration of corticosteroids to alloxan-diabetic animals they concluded that the adrenal cortex was over-active in patients with complicated diabetes. Sprague (1962) on the other hand reviewed the literature on adrenalectomy in diabetic subjects and concluded that it had been of no value in palliating retinal or any other complications of diabetes.

Thyroid dysfunction has an effect on established diabetes. Thyrotoxicosis aggravates and hypothyroidism alleviates the defect in carbohydrate metabolism.

I conclude from the foregoing that endocrine dysfunction modifies established diabetes to a variable extent but that evidence of its place in the pathogenesis of the naturally occurring condition is scanty and difficult to assess. The striking drop in requirements of injected insulin in diabetics in whom there is loss of pituitary or adreno-cortical function and the dramatic fall in insulin requirements produced in some insulin-resistant diabetics by injections of cortisone may be explained in terms of indirect endocrine effects on immunity, but other explanations are possible.

(b) Insulin and Immunity in Diabetes.

The disturbance of carbohydrate metabolism in diabetes is complex but it is probable that most variants depend on the

availability to the appropriate tissues of insulin or an equivalent. Insulin is produced in the beta cells of the pancreatic islets of Langerhans and is stored in the cytoplasm of these cells as aggregates, the beta granules, which are visible with light microscopy. It passes in a non-aggregated form to the bloodstream and, if unhindered, becomes bound to tissue - mainly liver and muscle. Lacy and Hartroft (1959) pointed out that 3 membranes and 5 spaces separate the beta granules from the bloodstream and that it was possible, in theory at least, for alterations at any of these sites to interfere with the liberation of insulin. Even when discharged into the blood insulin is subjected to a myriad of modifying, antagonising and controlling influences, normal or pathological, and on occasion its action is mimicked wholly or in part by other substances. It is therefore not to be wondered at that no single morphological abnormality has been detected consistently in the pancreatic islets of diabetic subjects although this conclusion is based on a study of autopsy material and the pancreas is liable to undergo autolytic changes post-mortem. It may be that electron microscopic studies of biopsy material will reveal a lesion in the pancreatic islets pathognomonic of diabetes but this seems unlikely.

The more difficult part of the diabetic problem is to determine what place, if any, insulin has in the vascular/basement membrane complications of diabetes since these now

constitute the main difficulty in therapeutics. Insulin consists of dissimilar polypetide chains A and B and has a molecular weight of 6000. Insulin prepared from pancreas of pig, sheep, and ox is antigenic when injected intramuscularly for more than a week or two into normal or diabetic human subjects. Antibody to heterologous insulin so provoked is detectable by a variety of methods and can be shown to cross-react with human insulin. Many elderly patients of course do not require insulin injections for control of mild diabetes but some workers claim that antibody to insulin is detectable even in cases where no insulin has been administered. Fav et al. (1963), using a complement consumption test, demonstrated antibody reacting with heterologous insulin in 30% of diabetics who had never received injections of insulin and in 4% of non-diabetic controls. Mancini et al., (1963) obtained positive immunofluorescence of fresh human pancreatic islets when these were treated in the presence of complement with fluorescein-conjugated gamma globulin derived from an elderly diabetic who had never been treated with insulin. In their view this finding demonstrated the presence in this patient's serum of antibody to human insulin, provoked perhaps by an abnormal insulin. The curious feature here is that none of the sera from diabetics who had been treated with heterologous insulin reacted positively in an immunofluorescent test with islets of beef and pork pancreas, as one would have expected them to do in at least some instances. The human pancreatic tissue

used as substrate by these workers was obtained at autopsy within 1 hour of death. Material obtained 24 hours after death was useless. This may account in part for negative results obtained by me in complement-fixation tests on diabetic sera using extracts of neonatal human pancreas taken at autopsy 5 hours post-mortem.

To determine if insulin or antibody to insulin was detectable in diabetic renal lesions Blumenthal et al., (1962) used the immunofluorescent technique to test 3 groups of patients - (1) 25 diabetics with nodular glomerulosclerosis, (2) 16 diabetics without nodular glomerulosclerosis, (3) 56 subjects who were normal or who suffered from other kinds of renal disease. They treated formalin-fixed sections of renal tissue obtained at autopsy with fluorescein-conjugated beef insulin. Evidence of binding at sites staining positively with F.A.B. was obtained with almost all of the kidneys of group (1) patients, with a minority of those from group (2), and with none from group (3) patients. Pre-treatment of the sections with non-conjugated insulin prevented the development of the reaction. The kidneys of 4 patients were tested for the presence of insulin by treating sections with conjugates of animal antiserum to beef insulin and of human antiserum to exogenous insulin. A positive reaction was obtained with the renal tissue of 2 patients from group (1) and a negative reaction with those of 1 patient from each of groups (2) and (3). The authors

suggested that the P.A.S. positive lesions in diabetic kidneys, i.e. in the glomerular nodules, the periphery of hyalinised crescents, the basement membrane of the tubules, and the fibrillary material in the proliferative vascular lesions, contained an insulin/anti-insulin complex. There is some dispute about the nature of the anti-insulin in this situation. Some workers have shown fluorescence in these lesions using conjugated anti-human globulin but Dixon (1961) could not confirm this. Blumenthal et al., in the paper already mentioned, tested renal tissue from their first two groups with conjugated anti-human globulin and obtained positive results in 48% of group (1) cases and in 12.5% of group (2) cases. The latter figure includes 2 of the 3 cases in this group who reacted positively with conjugated insulin. It seems likely that the insulin binding shown in this investigation was effected by insulin antibody in some instances and in others perhaps by a different antagonist of insulin.

For the purpose of relating the presence of chronic gastritis to the foregoing, the concept which seems to fit most readily is that insulin/anti-insulin complex, however brought into being and whatever the nature of the anti-insulin present, damages the small blood vessels and basement membrane around the gastric glands and so liberates a suitable quantity of gastric antigen in circumstances appropriate for the initiation of a delayed hypersensitivity reaction. If

this idea is correct, and there are many other possibilities, then chronic gastritis should be regarded as a complication of diabetes of the same nature as nephropathy. The corollary of this view is that a secondary delayed hypersensitivity reaction may contribute to tissue damage in diabetic glomerulosclerosis. Disease of larger blood vessels which can also be considered as a major complication of diabetes has little relevance to the present discussion since a different pathological entity appears to be involved.

An alternative mechanism for the production of chronic gastritis in diabetes is that suggested in Section III, namely, that some as yet undefined metabolic disturbance precedes all the features of diabetes and is their cause. However, this is speculation as yet, and must await the results of studies on healthy persons who, on genetic grounds, are likely to develop diabetes (Camerini-Davalos et al., 1963).

V

The Nature of Chronic Atrophic Gastritis

Australian workers, e.g. Joske et al., (1955), maintain that chronic atrophic gastritis gives rise to symptoms whereas others, e.g. Coghill (1960), find it to be asymptomatic. Tests of gastric function are at present mainly of auxiliary value although this may change. Gastroscopic appearances are not a satisfactory guide to the state of the

mucosa. For these reasons the appearance of the gastric mucosa as determined by gastric biopsy has become the main criterion of the presence of gastritis. Several workers, e.g., Taylor and Truelove, 1962, have suggested that chronic atrophic gastritis of the type described in Section II includes a variety of conditions indistinguishable on morphological grounds. Investigations on patients who have gastritis accompanied by parietal cell antibody indicate that this sub-group is also heterogeneous since, if the view already expressed is correct, the gastritis occurring in diabetics is started off by the harmful action of insulin/anti-insulin complexes on the gastric glands. It seems likely therefore that chronic atrophic gastritis accompanied by parietal cell antibody represents the reaction to a variety of noxious influences of a stomach or individual in whom there is some kind of inherited peculiarity.

It has been suggested in Section III that metabolic and endocrine abnormalities may sometimes begin or hasten the development of the type of gastritis being studied, but it is evident that only a minority, and possibly only a small minority, of patients with this lesion suffer from very gross endocrine dysfunction and in some of these it is difficult to be sure that this does not result from allergic or auto-allergic damage to the endocrine gland in question. In addition, even if the suggestion is sound, the influence of other factors cannot be ignored.

1. Noxious physical factors

(a) Heat.

Edwards and Edwards (1956) reported that, by measuring the temperature of ingested hot beverages, they found that patients drinking fluids of a higher than average temperature were more liable to have chronic gastritis than were those drinking less hot fluids.

(b) X-irradiation.

Palmer (1954) distinguished between the severe, irreparable damage to the whole thickness of stomach wall occurring in the direct path of a beam of super-voltage irradiation and the diffuse but milder atrophic gastritis produced by smaller amounts of irradiation. the latter, in his experience, lasted only a few months. Joske et al., (1955), reviewed the results of the apparently once-fashionable treatment of duodenal ulcer by irradiation, and concluded as follows:-

> "In general the X-ray irradi tion produced a moderate superficial gastritis, frequently with some glandular atrophy. The effects were greatest 2 to 3 months after irradiation, but changes were noted within a month. There was subsequently a radual return to normal, and 6 months after irradiation changes were slight in the majority of cases. .....There was no evidence to suggest that X-irradiation in these doses (150-200r

over a period of 3 weeks, J.M.M.) would produce permanent changes in the gastric mucosa, nor did gastric atrophy develop in any case."

It should perhaps be mentioned in passing that Joske et al. found a low incidence of chronic gastritis in duodenal ulcer except where antral resection had been carried out and an increased incidence in gastric ulcer. This explains the finding of Doniach and Doitt (1964) that there was a 22% incidence of parietal cell antibody in gastric ulcer and a normal incidence in duodenal ulcer.

2. Nutritional factors

Floch and Thomassen (1963) and Floch et al., (1963), reported that chronic atrophic gastritis was common in Puerto Ricans suffering from schistosomiasis, hookworm disease, or tropical sprue. The sprue patients did not have iron-deficiency anaemia but this was a common feature in the other 2 conditions. Multiple nutritional deficiencies are common in Puerto Ricans and their vulnerability to attacks of rheumatic fever when they migrate to the slums of New York may reflect this. It therefore seems likely that nutritional deficiency is responsible to some extent for gastritis being common in Puerto Rico. Floch et al. made the interesting comment that gastric carcinoma is the second-commonest form of malignancy in males in that country. Scott (1960) in a general practitioner survey found that there were regional differences in the incidence of

pernicious anaemia, the disease being more common in the north and less common in the South-East of England. L. J. Witts suggested that the areas of high incidence corresponded to those affected adversely by the economic depression of the nineteen thirties. If his deduction was correct, deficiency of iron would be one of the deficiencies prevalent in those areas at that time. The occurrence of gastritis in malignant disease might be influenced by the poor state of nutrition in many patients, possibly (Womack, 1964) because of the selective uptake of nutrients by the tumour. Chronic gastritis is relatively common in alcoholics (Joske et al., 1955) but as Palmer (1954) pointed out it is very difficult to determine how much of this is attributable to poor nutrition and how much to hepatic cirrhosis and resulting circulatory changes in the stomach. The final piece of evidence on the role of nutritional factors in chronic gastritis relates to vitamin B 12 and consists of a report by Siurala, Iramaa, and Nyberg (1963) that superficial atrophic gastritis was found in patients with Diphyllobothrium anaemia and that this cleared when the worms were expelled.

3. Undetermined factors

The increased frequency of chronic gastritis in chronic disease of the pancreas and gall bladder (Joske et al., 1955) is unexplained and has not to my knowledge been investigated.

Auto-allergy and Chronic Atrophic Gastritis

The resemblance of the lesion seen in the gastric mucosa in chronic gastritis to that found in the thyroid gland in Hashimoto's disease, has sometimes been suggested as evidence that at least some examples of the condition are auto-allergic in nature. However a chronic atrophic gastritis indistinguishable histologically from other kinds is present commonly in syphilis (Joske et al., 1955) and presumably represents an immune reaction to treponemal antigen because a quite markedly gastritic mucosa can be restored to normal within 3 weeks of commencing anti-syphilitic treatment (Mitchell et al., 1962). Any atrophy resulting from chronic syphilitic gastritis might then result from the chronic inflammation of infection.

One point in favour of the idea that chronic atrophic gastritis may in some instances be an auto-allergic reaction emerges from a consideration of gastric parietal cell antigen as a possible 'secluded' antigen. It has hitherto seemed to me a little far-fetched to think that body components other than cartilage, ocular lens, and comparable structures could be secluded in the immunological sense since normal cell breakdown would surely have produced a constant liberation of cellular components. However there are cells, and they include the cells of the gastric lands, which never divide

under normal circumstances but undergo periodic auto-rejuvenation (Popoff, 1941; Shorter and Creamer, 1962; Gastroenterology, 1964). This contrasts very sharply with what happens in the case of the superficial mucosal cells of the stomach and of the remainder of the gastro-intestinal tract, which are renewed approximately every 3 days. Under abnormal circumstances the parietal and chief cells are replaced by the inward growth of mucous neck cells and their subsequent differentiation. This functional peculiarity of parietal and peptic cells under physiological conditions suggests that some components of their cytoplasmic contents could be 'secluded' antigens. This type of cell renewal also suggests how florid atrophic gastritis could progress to total mucosal atrophy unaccompanied by cellular infiltrate and with intestinal metaplasia, since total destruction of mucous neck cells by severe inflammation would entirely eliminate parietal cell antigen and halt formation of the surface mucosa.

If the inflammatory reaction in chronic atrophic gastritis is sometimes of an auto-allergic nature, the occurrence of pernicious anaemia in cases of hypogammaglobulinaemia (Larsson et al., 1961) points to the mechanism being one of delayed hypersensitivity. The absence of parietal cell antibody from the one case reported in which it was looked for (de Vilde et al., 1964) and the presence of a vigorous lymphocytic reaction in the gastric mucosa in this case is good evidence

that formation of parietal cell antibody is a secondary phenomenon. That being so its absence in the serum from a patient with chronic gastritis does not permit one to conclude that the lesion is not auto-allergic in nature. From the example of Hashimoto's disease one is inclined to think that a presumed auto-allergic disorder related to it should show hypergammaglobulinaemia. The serum proteins in 8 cases of pernicious anaemia tested for me by Dr. J. W. Chambers were normal, which supports the findings of Wührman and Wunderly (1957). I think the difference in Hashimoto's disease is probably due to the qualities of thyroglobulin as antigen.

If a delayed hypersensitivity reaction produces the variety of chronic gastritis accompanied by parietal cell antigen and others not so accompanied, the sensitising agent or agents presumably being cytoplasmic components of parietal or peptic cells, then it should be possible in theory to transmit the disease by transferring lymphocytes or lymphoid tissue from an affected to a non-affected person and, conversely, to fail to transmit it by infusing serum containing parietal cell or intrinsic factor antibody. This cannot be attempted in human subjects and there is as yet no experimentally-produced gastritis in animals comparable to the human disease. Smith et al., (1960) and Hennes et al., (1962), from the same laboratory, have claimed to be able to do just this by injecting suitably processed gastric juice in adjuvant into dogs, but

th purely atrophic lesions devoid of round-celled infiltrate which they obtained are very different from the gastritic lesion of humans. It may be that there are greater difficulties than in the case of experimental thyroiditis, but a better experiment of the type used by the above-mentioned workers would be to use adjuvant plus crude gastric mucosal extract, parietal cell microsomes, intrinsic factor, or, still better, purified gastric antigens prepared by the methods of Kapp et al., (1964). Such endeavours would be worthwhile but of greater potential value in my opinion would be the study of naturally-occurring disease of appropriate type in animals and an attempt to produce auto-allergic disease in animals by manipulation of heredity and the internal environment. An example of the former is the diabetes which affects the hamster (Reier and Yerganian, 1960). Of the latter there are no examples known to me but the principal and only virtue of the emphasis placed in other sections on endocrine and metabolic dysfunction as possible factors in the pathogenesis of chronic atrophic gastritis is that the idea can be readily tested, a desirable attribute of any hypothesis. The other hypothesis mentioned, namely that chronic atrophic gastritis in human subjects is frequently auto-allergic in nature, provides a useful working basis but its correctness will only be proved when a self-perpetuating, auto-immune gastritis can be produced in animals.

VI

Case histories

1. A.J. Renal tubular acidosis. The disease was diagnosed at the age of 41 although the patient gave a history of muscle weakness dating back 6½ years. 4 years previously she had 2 episodes of generalised flaccid paralysis for which she was admitted to a hospital and treated with potassium without the nature of the disease being recognised. At the age of 40 the muscle weakness increased and she developed a rather waddling gait. Eventually and not surprisingly she became depressed and was admitted to the psychiatric wards of Dublin Hospital where the physicians realised that she was suffering from a physical disorder. Investigation revealed osteomalacia (pseudofractures of femoral neck and generalised rarefaction of bone), appropriate blood changes (low potassium, phosphate, and alkali reserve; normal calcium and urea), and absence of aminoaciduria and glycosuria. A loading test with ammonium chloride reduced the urinary pH to only 6.4. The patient derived great benefit from treatment with citrate and potassium although it took some time for the bones to become normal. Pernicious anaemia was recognised at the age of 48 and she remains well on appropriate therapy 1½ years later.

2. C.F. Renal tubular acidosis. This patient was admitted at the age of 48 to Auchill Infectious Diseases Hospital on account of weakness and what was suspected to be paralytic ileus associated with poliomyelitis. The patient was deaf and she had suffered from rheumatoid arthritis for 9 years so that it was rather difficult to determine accurately the duration of muscular weakness but it was estimated that this had been present for at least 7 months. She gave a history of having 3 attacks of pleurisy, the last 5 years previously, and of "inflammation of the kidney" 7 years previously. She was married and had 3 children and the menopause came on at the age of 45. There was no skeletal disease and no nephrocalcinosis. Examination of the blood revealed low values for alkali reserve and phosphate and a very low potassium level. A loading test with ammonium chloride failed to depress the urinary pH in the normal way. Treatment with alkalis and potassium restored her to normal health. Appropriate therapy cleared up a gross pyuria and Proteus bacilluria present at the time of admission, and she remains in good health, apart from her rheumatoid arthritis, 3 years later.

3. E.G. Renal tubular acidosis. This patient was admitted to Stobhill Hospital at the age of 46 with an extraordinary degree of generalised flaccid paralysis, being unable to raise her head voluntarily. She was dehydrated

although she had not been vomiting and was mentally alert. She gave a history of thirst, polyuria and weakness of several months duration although on reflection stated that she had not felt well since the birth of her youngest child 7 years previously. The investigations disclosed a biochemical condition and clinical state similar to that found in case 2, and treatment with potassium and alkali had an equally beneficial effect which has been maintained in the ensuing 5½ years.

4. S.O'S. Hodgkin's disease. This patient developed Hodgkin's disease of cervical and mediastinal glands at the age of 15 and this was accompanied by fever, anaemia, and weight loss. There was no relevant family history and no previous illnesses other than that requiring tonsillectomy in childhood. Radiotherapy was administered to the affected glands of left side of neck (3000r) and to the mediastinum (6270r) with good effect and he has remained well in the succeeding 2½ years. The serological tests reported were carried out at intervals during this period. The gastric biopsy was taken 11 months after the diagnosis of Hodgkin's disease and free acid was present in the gastric juice at this time.

5. [illegible] Chronic disseminated lupus erythematosus and myelofibrosis. This patient had 9 pregnancies, one of them resulting in an abortion at 3 months. 2 children died, 1 of an accident at the age of 2 and the other of gastro-enteritis in infancy. At the age of 35 she developed redness of the hands, forearms, and soles of the feet. There was also crusting of the skin - across the bridge of the nose and on one ear. She was treated at this time in Glasgow Royal Infirmary, with some success, as a case of "lupus erythematosus". At the age of 44 she received treatment with chloroquine in Stobhill Hospital for what was labelled "wides read discoid or chronic disseminated lupus erythemato us". She developed pleurisy on several occasions and on one occasion was treated for a small pulmonary embolism and pneumonia secondary to bilateral "white leg". At the age of 54 she was admitted to Stobhill Hospital with a history of feeling chronically listless and tired for years, of increasing pallor, and of incidents of pleurodynia 6 weeks and haemoptysis 2 weeks previously. On examination she showed pallor and a facial purpuric rash on the lower limbs. There were minimal erythemato-scaly lesions on the dorsal skin of the distal phalanges of the fingers. There was no palpable enlargement of spleen or lymph glands and a pancytopenia (Hb 9.4 gm/100 ml; platelets 14,000/cmm; bc 4000/cmm with 63% neutrophils, was present. Radio-chromium studies demonstrated a haemolytic process and the direct Coombs test o the patient's red cells

was positive. Marrow aspiration failed and a biopsy of the iliac crest showed myelofibrosis and osteosclerosis. Radiography of the skeleton revealed gross sclerotic changes in pelvis, spine, and skull. The latex nucleoprotein (Wyland) and immunofluorescent tests for anti-nuclear factor were positive but L.E. cells were not found by the haematologists in the buffy coat of 2 specimens of defibrinated and incubated blood. It was concluded that the patient had co-existing myelofibrosis and chronic disseminated lupus erythematosus. Treatment with prednisolone brought the blood values back to normal and the patient appeared well 6 months later.

Technical Appendix

Complement-fixation Tests.

The technique used in the first 2 investigations of Section 1 were as described by Markson and Moore (1962b) and Moore & Wilson (1963a and b). The technique used subsequently differs in minor details and is described below. It is a variant of standard technique and no originality is claimed for it.

All tests of this type - gastric mucosal (G.M.C.F.), thyroid microsomal (T.M.C.F.), and auto-immune non-specific (A.I.C.F.) were carried out identically the same way except that the antigenic extracts used differed.

1. Preparation of extracts.

a. Stomach. The body mucosa was dissected off an unfixed resection specimen of stomach obtained the same day at operation on a patient with duodenal or stomal ulcer. The mucosa was mopped clean with gauze and any bruised tissue discarded.

b. Thyroid. Unfixed thyroid tissue from a patient with thyrotoxicosis was used as received on the day of operation. The best extracts were obtained from substantially-enlarged glands.

c. Kidney and liver. Tissues were obtained from a freshly-killed and exsanguinated guinea pig.

Each of these tissues was frozen at -15°C. and on

the following day was sliced thinly and shaken on a throttle-controlled Gawsley pipette shaker for 1 hour at 4°C. with twice the volume of chilled phosphate-buffered saline (p 6.8). Thereafter the extracts were centrifuged at 4,000 r.p.m. for 15 minutes. The supernatant fluid was placed in 1.5 - 2.0 ml. volumes in 2" x ⅜" stoppered glass tubes and stored at -15°C. until required. The extracts were tested against known positive and negative sera in titrations and compared with potent extracts in current use. The working dilutions in non-buffered saline most often used were 1/15 for gastric extracts, 1/6 for thyroid extracts, and 1/4 for extracts of kidney and liver. These were made up just before using in tests.

2. Tests.

Sensitised red cells were prepared on the day of use by mixing equal volumes of a haematocrit-standardised 5% suspension in saline of washed sheep red cells and saline containing an appropriate volume of glycerinated rabbit anti-sheep red cell haemolytic serum (Cayne) and incubating for 30 minutes at 37°C. in a water bath.

The source of complement was fresh guinea pig serum stored at -15°C. and used within 1 or 2 days. In titration of complement, dilutions were incubated at 37°C. for 30 minutes before and 10 minutes after adding sensitised cells. The end reading was taken as the tube with the highest dilution giving complete haemolysis.

sera to be tested were inactivated for 30 minutes at 57°C. on the morning of testing. They were first tested at a dilution of 1/4 in saline and, if positive, at dilutions of 1/4, 1/10, 1/20, 1/40, 1/80, 1/160.

5 burner tubes were used, 1 being a control tube in which saline was substituted for antigen, and the other tubes for extracts of gastric mucosa, thyroid, kidney, and liver respectively. 0.1 ml. of each of diluted test sera, 2 ... of complement, and antigen, or saline, were added to each tube. The tubes were shaken and incubated in a water bath at 37°C. for 30 minutes. The racks were removed and to each tube was added 0.1 ml. of the suspension of sensitised sheep red cells. Incubation was continued for 10 minutes at 37°C. The results were read visually, any tube showing an estimated 50% haemolysis or less being regarded as positive. A positive result with kidney or liver antigen or both was regarded as a positive A.I.C.F. reaction.

Tanned red cell Agglutination Test.

Thyroglobulin-sensitised and non-sensitised cells (Burroughs Wellcome & Co.) were used and the tests were carried out in perspex trays, exactly as recommended by Burroughs-Wellcome, except that a single dropping pipette rinsed thrice in saline between dilutions was used.

Immunofluorescent test for parietal Cell Antibody and Antinuclear factor.

Tissues used were as follows:-

1. Portions of gastric mucosa from the body of a resected stomach were quick-frozen on to microtome chucks which were then wrapped in plastic sheeting and stored in the cryostat chamber for up to 8 weeks.

2. Portions of liver from a newly-killed guinea pig were treated in the same way.

Glass slides were washed in hot water containing macro-solclearing agent and rinsed very thoroughly by shaking in a rack in several changes of water and then allowing the rack and slides to dry in an incubator. On the day of testing 4 u sections were cut by Mr. I. Macsie from appropriate blocks and stood unfixed on slides at 4°C. until required.

Reagents used were as follows:-

1. Sorbitone-buffered saline of pH 7.2 was prepared on the day of testing by dissolving Complement-fixation test diluent Tablets (Oxoid) in de-ionised water.

2. Fluoresceim-labelled anti-human globulin (Burroughs Wellcome Co.) was absorbed on the day of testing by shaking with dried liver powder of pig (Burroughs Wellcome Co.) for half-an-hour at room temperature twice in succession and then spun in very thoroughly in the centrifuge to eliminate all particles.

test. The test was carried out by covering the appropriate tissue section with test serum diluted 1/4 with barbitone buffered saline, incubating without agitation for 30 minutes at 37°C. in moist chambers, washing the slides, incubating with diluted conjugate at 37°C. for 30 minutes, washing again, and then mounting in buffered glycerine. Washing was carried out by placing 2 slides in a Coplin jar containing barbitone buffer and placing them on the rotary Buckham shaker for 3 minutes. The preparations were examined on the same day although they stored well if kept at 4°C. The U.V. apparatus used was that of Leitz with a dry, dark ground substage condenser.

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