ENTRY FOR THE WIGHTMAN PRIZE IN CLINICAL MEDICINE

"The Immune System and Disease: a presentation of five cases"

Alexander M. Pirie

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INTRODUCTION

Autoimmune phenomena have been found to occur in an expanding catalogue of disease, and clinical interest is growing as new therapeutic interventions become available. In this entry for the Wightman Prize in Clinical Medicine, five patients are presented in each of whom the immune system made some contribution to their illness. Each of these patients were clerked by the author in the Spring of 1988, while attached to wards 32 and 33 of the Royal Infirmary of Edinburgh.

Case 1: A proximal myopathy due to Grave's disease
Case 2: Haematemesis: Primary Biliary Cirrhosis
Case 3: A self-limiting Haemolytic Anaemia
Case 4: Pernicious Anaemia
Case 5: Diabetic Ketoacidosis

A PROXIMAL MYOPATHY

Mrs L was a 61 year old housewife who lived alone, admitted with a complaint of increasing leg weakness. Three days prior to admission, she had bent down to pick up the milk bottles from her doorstep when her legs had given way and she was unable to get to her feet again. She managed to crawl into the house but could not reach the telephone, so unable to get to the toilet and with nothing to eat or drink, she lay on the floor for nearly 3 days, until her neighbours contacted the police. The fall was not associated with dizziness, loss of consciousness or tongue biting, and she said the weakness affected both legs equally, with no pain. She had not injured herself on falling.

On admission, she was initially confused and dehydrated with loss of skin turgor and a dry tongue. She was slightly pyrexial at 37.5°C and had a cough productive of green sputum. Chest examination revealed bilateral basal expiratory crepitations, and as there were no signs of cardiac failure, she was treated with intravenous fluids for dehydration, and her chest infection was treated with Ampicillin.

As her mild confusional state resolved, neurological examination found wasting of both vastus medialis muscles with no fasciculation. The legs were a little flaccid and power was reduced to 3-4/5 at the hips and knees with normal power at the feet and upper limbs, the patient being unable to stand. All reflexes were brisk with downgoing plantars and a negative Hoffman's although there was persistent clonus at both knees. Cranial nerve examination revealed no abnormality of the facial, bulbar or ocular muscles, and radiology of the lumbar spine and sacrum showed mild osteoarthritic changes with osteoporosis but no fractures. The signs were consistent with a proximal myopathy.

Over the course of her admission, it emerged that this lady had not been well for several weeks. She had ascribed her symptoms to worry about her handicapped son and the fact that her husband had recently left her. For the previous 2-3 weeks, she had felt increasingly tired and emotionally labile and was unable to sleep at night. She experienced fast, regular palpitations in bed and felt hot and sweaty, having to sponge herself down with cold water. Her hands would tremble on occasions, and she thought she had lost about 7kg in the previous 3 weeks, although she described her appetite as being "ravenous".

She had never experienced angina or orthopnoea, but her ankles had been swelling

in the early evenings and she had mild exertional dyspnoea when climbing the stairs to her bedroom. Mrs L had beenmildly constipated since she had started taking Co-dydramol several years ago, for pain in her knees and her back. Six years before this admission, she had been diagnosed as having osteoarthritis of both knees and spondylosis, and she had attributed her increasing fatigue to this. Recently, she had been taking Mianserin and Diazepam for her symptoms of anxiety but was on no other medications. She gave no family history of note.

Mrs L did not smoke or take alcohol, and she lived alone in her one bedroomed house. She had been married for 30 years to a welder, but 6 months ago, they had become separated. She had 2 sons in their twenties, one of whom was handicapped, and she looked after him until he went into care 5 years ago. Her other son lived in Glasgow, but she coped domestically on her own, doing her own shopping and cooking.

Further examination found Mrs L to have a diffuse painless goitre with a soft bruit and no thrill. There was no retrosternal extension and the trachea was central. She had moist, warm hands with erythemaof the medial palms and a fine tremor but no clubbing. She had a resting tachycardia of 120 beats per minute which was regular with a good volume. The blood pressure was 150/90 and there was no evidence of cardiac failure. She had mild bilateral exophthalmos with lid retraction and lag, but no periorbital oedema or ophthalmoplegia and the fundi were normal. Her skin was not unusually pigmented with no vitiligo, pretibial myxoedema or apparent hair changes, but there was evidence of previous obesity with recent, rapid weight loss.

Initial biochemistry gave evidence of dehydration and demonstrated an elevated thyroxine level at 230nmol/l (N65-145) and a suppressed TSH at < 0.1 mU/l (0.3-0.5). A diagnosis of thyrotoxicosis with thyroid myopathy was made. The full blood count was unremarkable other than a white blood cell count of 14 (4-11 x $10^9 l^{-1}$) with 72% neutrophils, consistent with her chest infection. ESR was moderately elevated at 45 (0-10) and the pl. calcium was high at 2.97m.mol/l (2.12-2.62) with normal albumen. Hypercalcaemia is present in 10% of cases of hyperthyroidism and is thought to be related to the osteodystrophy. Her early morning cortisol was measured as hyperthyroidism reduces the half life of cortisol and may produce relative adrenal insufficiency. However, it was found to be normal at 438 nmol/l (N 190-550).

Radio iodine uptake scanning showed that the goitre was diffuse with no toxic nodules and uptake of radio iodine was increased at 57% in 4 hours (N 10-40%). Routine autoantibody profile demonstrated the presence of antithyroid antibodies in the serum. In view of the diffuse goitre and exophthalmos it was concluded that this lady's thyrotoxicosis was due to Graves' Disease. Since Mrs L was over 40 and her radio iodine uptake was more than 20%, she was offered radio iodine therapy. She was given an oral dose of 400mBq of I^{-131}. Three days later, she was started on Carbimazole for 6 weeks until the radio iodine took effect. Symptomatic treatment with Propranolol was contraindicated as it was felt she had a degree of COAD.

Within 3 weeks, Mrs L's symptoms had gradually dissolved. With physiotherapy and encouragment from the staff, she could stand easily on her own and walk around the ward, although her confidence was low. She was also a little perturbed by her increasing weight. However, she gradually became euthyroid with normal calcium levelsand she was discharged to a convalescent home and then back to her own house.

Relapse occurs in 25% of patients after their first dose of radio iodine at 4 months, and unfortunately this happend to Mrs L. Although her walking was much improved, she again felt fatigued, was hot and sweaty and experiencing palpitations. She was both clinically and biochemically hyperthyroid again, and it was planned to give her 600mBq dose of radio iodine, followed again by Carbimazole. At the time of writing, Mrs L is optimistic about future results.

DISCUSSION

Although fatigue and reduction of muscle power occurs in all patients with hyperthyroidism, this case is unusual in that the myopathy was severe, and as such, it was the main presenting feature. Thyroid myopathy is usually milder, with a more insidious onset, and it can precede the onset of the other symptoms of thyrotoxicosis.

On examination, tremor may be mistaken for fasciculation although the latter is not a feature of thyroid myopathy. In contrast with other metabolic myopathies, the tendon reflexes tend to be brisk. It is usually associated with some degree of atrophy, but the biochemical pathogenesis of thyroid myopathy is poorly understood. It almost always resolves with treatment of the hyperthyroidism.

The pathogenesis of Grave's Disease is interesting and of clinical relevance. It is well established that thyroid stimulating immunoglobulins (TSI) are directed

against the TSH receptors and activate the adenylate cyclase system mimicking the action of TSH. The discepancy between the size of a goitre and its toxicity and the occurence of non-toxic goitres in the families of those with thyrotoxicosis led to the suggestion that there may also be thyroid growth-stimulating immunoglobulins (TGI) causing thyroid hyperplasia with or without TSI causing thyrotoxicity. The presence of such TGI was established in 1980 using a laborious cytochemical assay. Blocking antibodies have also been demonstrated in the serum of patients with Grave's disease. Different combinations of blocking and stimulating antibodies may explain the varied clinical courses of Grave's disease. For example, some patients have rapid and complete remissions whereas others persistantly relapse. TSI can now be specifically and sensitively assayed by its ability to stimulate cAMP production in a clone of immortalised rat thyrocytes (FRTL-5). TGI can be assayed as it increases the mitotic index of the FRTL-5 line. The development of this assay may lead to exciting discoveries in the near future.

Why are antibodies to TSH receptors generated in the first place? There is a recognised genetic predisposition to Grave's disease, it is seven times commoner in women and it is associated with the HLA haplotype DR3. It is emerging that some thryrocytes in Grave's disease express MHC class II molecules on their surface. These proteins are normally only expressed by certain cells, particularly antigen presenting cells such as the macrophage. It has been shown that thyrocytes aberrantly expressing MHC class II activity can present antigen and stimulate the prolifweration of clones of autoreactive T-lymphocytes. It has been speculated that in Grave's disease an initiating factor such as a virus causes aberrant expression of MHC class II activity in genetically predisposed individuals. This may result in presentation of the TSH receptor to T-lymphocytes. This would generate an immune response to produce blocking and stimulating antibodies to the receptor. Then the function of T-suppressor cells would determine the outcome of this event. If suppression was deficient, the autoimmune condition would develop.

Another speculation involves the notion of anti-idiotypy. According to this theory, the initial immune response is against the 28kDal TSH molecule itself. The idiotype of the anti-hormone antibody then induces the production of a regulatory anti-idiotype antibody to the anti-hormone antibody. The idiotype of the anti-idiotype antibody will resemble the ligands of the original TSH hormone, and as such could autoreact with the TSH receptor.

These hypotheses are presently being examined by prospectively following immunological events in the unaffected members of families with thyroid disease. An understanding of the initial events in autoimmunity could well result in the evolution of new

therapies such as early immunosuppression or even antigen-specific suppression. Meanwhile, the case of Mrs L illustrates both the success and the limitations of present day treatment.

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HAEMATEMESIS

Mrs M was a 64 year old retired shop manageress admitted as an emergency, with a 12 hour history of haematemesis and melaena.

She was well until 5 weeks before admission when she had an episode of abdominal and ankle swelling which resolved within 2 weeks on a diuretic (Diazide). This left her feeling more lethargic than usual, but was otherwise well until the morning of admission when she wakened feeling nauseated and vomited a significant quantity of fresh unaltered blood. She also passed copious quantities of melaena stool.

She gave no history of anorexia or weight loss, and did not complain of dysphagia, abdominal pain, previously altered bowel habit or pruritus.

She gave no previous history of peptic ulcer or liver disease, was not on non-steroidal anti-inflammatory drugs or anticoagulants, and consumed minimal amounts of alcohol. She had had no surgical operations, had never received blood products, and had never travelled abroad.

Her only medications on admission were Quinine Sulphate for night cramps and topical Betnovate and Alphasol for mild psoriasis, which she had on both knees.

Her sister had psoriasis and a goitre but there was no other family history of relevance.

She lived with her husband, a retired joiner, and was visited regularly by her daughter and 2 grandchildren. She smoked occasionally and gave no other history of note.

On examination, Mrs M was pleasant and undistressed with normal speech and orientation. She was mildly icteric with brittle nails and a brownish pigmentation to her skin. She had no clubbing or lymphadenopathy and there was no pallor of the conjunctivae or oral mucosa. There were no further stigmata of chronic liver disease or chronic anaemia and she had no tattoos or surgical scars.

She was afebrile and haemodynamically stable with warm peripharies, pulse 75 and blood pressure 130/60.

Her abdomen was slightly distended with minimal shifting dullness. A smooth non-

tender liver edge was palpable 5cm below the costal margin but there was no bruit, no splenomegaly and no other palpable masses. Bowel sounds were active and the rectum contained fresh malaena.

There was no peripheral oedema and the rest of the examination was unremarkable. Dipstick analyses of the urine was positive for blood and urobilinogen. Chest x-ray and electrocardiogram showed no abnormality.

Initial biochemistry and haematology are shown on table 1. Grossly deranged liver function tests showed features of hepatocellular damage and obstruction. Plasma protein turnover was impaired with low total protein and albumen. Prothrombin time was slightly prolonged suggesting a significant deficiency of clotting factors made by the liver and this was confirmed by a full clotting screen. Plasma urea was slightly high with normal creatinine, presumably due to degradation and absorption of blood from the gut. Total calcium was depressed because of hypoalbuminaemia. Haematology showed a mildly macrocytic anaemia with normal serum B12, folate and ferritin and a blood film demonstrated target cells, altered red blood cells seen in thalassaemia and liver disease. White cell count was at the upper limit of normal and platelet count was low.

These findings were consistent with liver disease and a diagnosis of an upper GI bleed secondary to oesophageal varices seemed likely. Haemorrhage from an ulcerating lesion could not be excluded.

As the patient was not actively bleeding, her initial management consisted of bed rest and fasting, intravenous fluids via large venflons, and hourly observation of temperature, pulse and blood pressure with monitoring of the urinary output via an urimeter. She was given intravenous Ranitidine to aid healing of a putative ulcer and to minimise the gastric erosions often associated with varices. In view of her mild anaemia and the fact that haemodilution takes up to 12 hours to occur after haemorrhage, 3 units of red cell concentrate were transfused overnight.

Endoscopy in the morning showed blood in the oesophagus, stomach and duodenum, and a clot was observed at the gastro-oesophageal junction on the greater curvature, over a small linear tear. There was no apparent ulceration of the mucosa, and the appearances were consistent with either Mallory-Weiss syndrome or oesophageal varices. Re-endoscopy with a view to injection sclerotherapy was recommended.

That evening, Mrs M had a dramatic haematemesis and became encephalopathic.

On examination, she was markedly jaundiced with hepatic foetor but no asterixis, and she had become drowsy and disorientated with slurred and muddled speech. Her abdomen was now distended with obvious shifting dullness and active bowel sounds. Although her peripheries were warm, her pulse was rising and her blood pressure had dropped to 90/70.

The circulation was stabilised with plasma protein substitute followed by 3 units of whole blood and 3 units of red cell concentrate under central venous pressure monitoring to prevent overload. The prothrombin time had risen to 21 seconds and this was treated with intravenous Vitamin K and 2 units of fresh frozen plasma to replace deficient clotting factors.

The hepatic encephalopathy was managed by protein restriction and oral lactulose syrup to minimise absorption of psychotropic nitrogenous compounds from the gut, plus neomycin to decrease the proliferation of nitrogenous organisms. Ranitidine was continued to minimise the risk of further haemorrhage from gastic erosions.

Urgent endoscopy demonstrated erythematous mucosal spots of the gastropathy seen in portal hypertension, and confirmed the presence of 3 large varices traversing the gastro-oesophageal junction with one just behind the junction in the greater curvature. These were sclerosed by injection of 3% STD.

The diagnosis was now acute decompensation of a chronically diseased liver. This was probably precipitated by variceal haemorrhage and subsequent absorption of blood from the gut. The metabolic load and associated hypotension would synergistically compromise hepatic function. Management was now monitoring and treatment of the potential complications of liver failure, namely hypoglycaemia, acid base balance disturbances, hyponatraemia, arrythmias, renal failure, infection, and cerebral oedema.

After sclerotherapy, Mrs M had no further bleeding problem and her encephalopathy began to resolve as evidenced by her diminishing constructional apraxia copying a star and a clock face (see figure 1). Her brightened mental state and hopes of her family were short lived however, as her illness progressed through the following complications:

1 Acute Gastric Dilatation and Paralytic Ileus: After sclerotherapy her abdomen became distended and tympanitic with absent bowel sounds and abdominal x-ray showed gas in the small bowel with a large bubble in the stomach. Surgical opinion confirmed the diagnosis of Ileus and as plasma potassium magnesium and amylase were all normal, it was thought to be a reaction to

scelotherapy. This settled with nasogastric aspiration and intravenous fluids.

2 The white cell count increased to 26 x $10^9/l$ with 89% neutrophils. Serial blood cultures produced no growth, catheter specimens of urine produced E-coli and abdominal paracentesis demonstrated spontaneous coliform peritonitis. These infections are recognised complications of liver failure. They were treated with intravenous Cefuroxime and Metronidazole after consultation with the bacteriologist.

3 Diminished air entry and dullness to percussion over the right base was confirmed by chest x-ray to be a right basal pneumonia, the lobe most commonly involved by aspiration.

4 Shifting dullness and distension due to ascites became worse and the urine output began to fall below 30mls per hour while plasma urea, creatinine and potassium steadily rose as the kidneys began to fail. Advanced cirrhosis may be associated with functional renal failure with sodium and water retention, a urinary sodium of less than 10 mmol per day and osmolality 1½ times that of the plasma. This is known as hepatorenal syndrome and has a poor prognosis even though the failure is functional and the kidneys structurally intact. Acute tubular necrosis is also associated with advanced cirrhosis.

Management consisted of careful fluid balance with daily weights, sodium, potassium, and fluid restriction, intravenous Frusemide and Spironolactone to stimulate natriuresis and diuresis and salt poor albumen was transfused to expand the plasma compartment.

5 As the liver glycogen stores became deplete and hepatic glycogenolysis and gluconeogenesis failed, continuous infusion of 10% Dextrose was required to prevent hypoglycaemia and BM-stix were checked every 2 hours. Glucagon is obviously ineffective in the absence of hepatic glycogen stores.

6 Finally, on day 14, Mrs M became drowsy and agitated, her pulse rising to 100, BP falling to 90/60 and her urine output was less than 5mls per hour. Her pupils were still equal and briskly reactive to light and there was no focal neurological deficit. The prothrombin time was now 110 seconds and a full clotting screen indicated gross deficiency of all factors. She had a metabolic acidosis with minimal respiratory compensation. At least some of this acidosis was due to lactate release from hypoxic glycolytic tissues not being metabolised by the liver to glucose. Hypoglycaemia was being managed with

pulses of 50% Dextrose as well as the 10% Dextrose infusion. This seemed to represent an acute exacerbation of liver failure and although it seemed that she had re-bled, nasogastric aspiration produced only bile stained secretion. Although she was apyrexial, her white cell count had risen to 31 x $10^{9/1}$ and FDP's were elevated at 140mg per litre. This implied an element of disseminated intravascular coagulation probably secondary to endotoxin challenge from her gram negative infections. She was given fresh frozen plasma but Mrs M died that night. There was no post mortem examination.

CAUSE OF CIRRHOSIS

As Ultrasound confirmed the presence of a small diffusely echogenic liver with no dilated ducts, no focal lesions and no gallstones, the pathology underlying Mrs M's liver failure was likely to be cirrhosis. An alcoholic or viral aetiology seems unlikely in view of her history (corroborated by her family) and radio-immunoassay for hepatitis B surface antigen was negative and antibodies to the core antigen were absent. Haemochromatosis was excluded as serum ferritin was normal, Wilson's disease was excluded by a normal serum caeruloplasmin, and autoimmune chronic active hepatitis was unlikely in the absence of anti-smooth muscle antibodies and a normal immunogobulin profile. She did not have alpha-1-antitrypsin deficiency and her normal alphafoetoprotein levels were not consistent with hepatocellular carcinoma. However, anti-mitochondrial antibodies were present at a high titre, and in view of the patient's age and sex, her cholestatic liver function tests with very high alkaline phosphatase, the diagnosis of Primary Biliary Cirrhosis was likely.

DISCUSSION

Primary Biliary Cirrhosis (PBC) is an uncommon chronic disease of unknown aetiology in which there is progressive destruction of interlobular bile ducts.

The typical case of PBC is female (90% of cases), aged between 55 and 70 although she is usually in her 5th or 6th decade. The point prevalance is 2 - 5.4 cases per 100,000 population in the UK but all nationalities, races and socio-economic classes are affected.

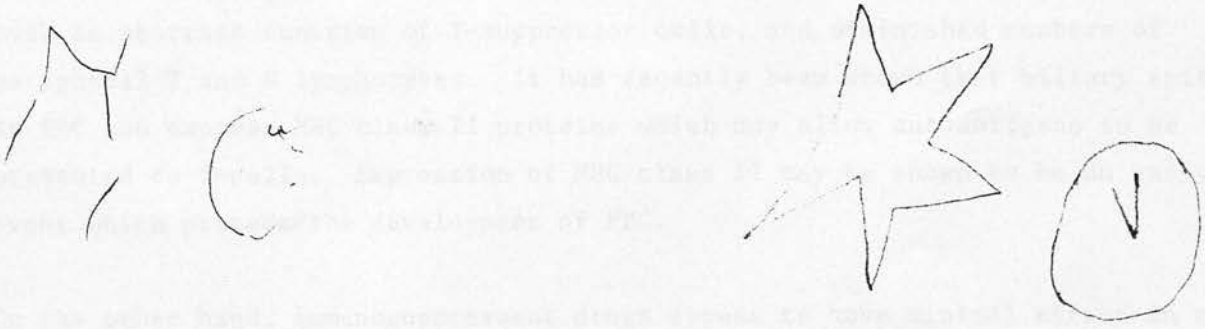
Sixty per cent of cases present with pruritis and mild fatigue followed months to years later by jaundice. A minority (12%) present like Mrs M with variceal haemorrhage. Many patients are asymptomatic. Steatorrhoea resulting from mal-absorption and parasthesiae due to lipid infiltration of the peripheral nerves can occur. Bone pain or fractures due to osteoporosis (hepatic osteodystrophy)

Table 1: Urea, Creatinine and Liver Function Tests

Day	Urea	Creat	Bili	ALT	ALP	GGT	TP	ALB	PTT
0	18	109	42	77	555	335	58	26	19
2	16	89	37	45	298	191	42	24	21
4	20	124	70	59	193	124	45	25	20
6	17	119	97	63	205	124	50	27	20
8	20	133	99	46	212	84	53	26	19
10	22	159	144	68	228	70	54	24	22
12	31	239	172	50	217	53	53	22	--
14	39	417	203	321	196	44	48	19	110

Urea 2.5-6.6mmol/l
Creat = Creatinine 55-150μmol/l
Bili = Bilirubin 2-17μmol/l
ALT = Alanine aminotransferase 10-40 U/l
ALP = Alkaline Phosphatase 40-100 U/l
GGT = Gamma-glutamyl transferase 5-35 U/l
TP = total protein 60-80g/l
ALB = albumin 36-47g/l
PTT = Prothrombin time 11-15s

Figure 1: Assessment of Encephalopathy with a star and clock



Day 2

Day 4

occurs and osteomalacia due to calciferol malabsorption is less common. Physical findings include hepatomegaly (70%) and splenomegaly (35%), darkening of the skin (66%), xanthelasma (19%), palmar erythema and spider naevi. Features of the commonly associated diseases Sjogrens, CREST and hypothyroidism may be present.

Laboratory findings include a very high alkaline phosphatase. 95% of patients with PBC have high titres of circulating antibodies to mitochondria. They are found in 30% of cases of autoimmune chronic active hepatitis and 3% of patients with connective tissue disorders, although in these cases it is generally in lower titres However, the autoantigen M2 is said to be specific for PBC and has recently been shown to be the lipoate acetyltransferase (E2) component of the pyruvate dehydrogenase complex located on the inner mitochondrial membrane.

Liver biopsy can confirm the diagnosis of PBC and permits histological staging, but needle biopsy is limited by the patchy nature of the lesions.

Differential diagnosis includes other obstructive disorders such as duct calculi, carcinoma of the head of the pancreas, cholangiocarcinoma and sclerosing cholangitis, thus ERCP or abdominal ultrasound is required. Sarcoidosis can be excluded by the absence of extrahepatic features such as bilateral hilar lymphadenopathy, erythema nodosum and a negative Kveim test. Chronic active hepatitis is not usually cholestatic although there is a recognised overlap syndrome with PBC.

The association of PBC with known autoimmune conditions and the resemblance of the histological lesion to Graft versus Host Disease suggests an immunological basis. Known alterations in humoral immunity in PBC include hypergammaglobulinaemia (although many cases like Mrs M have normal Igs), the presence of autoantibodies against mitochondria (also anti-thyroid and anti-nuclear antibodies) and markers of complement hypercatabolism. There are also alterations in cellular immunity such as aberrant function of T-suppressor cells, and diminished numbers of peripheral T and B lymphocytes. It has recently been shown that biliary epithelium in PBC can express MHC class II proteins which may allow autoantigens to be presented to T-cells. Expression of MHC class II may be shown to be an early event which precedes the development of PBC.

On the other hand, immunosuppressant drugs appear to have minimal effect on the disease process, and unlike other autoimmune diseases, there is no obvious association of PBC with major histocompatibility antigens. Thus it may be that the immune phenomena represent

the host response to an enviromental aetiology rather than a primary defect in the immune system.

TREATMENT

Immumosuppression with azathioprine, cyclosporin, corticosteroids, and chlorambucil have been tried as has inhibition of collagen synthesis with colchicine. None of these therapies has yet been shown to be useful. As hepatic copper levels are very high in PBC, the copper chelating agent penicillamine has been tried. Although this drug is efficacious in Wilson's disease, it is of little proven value in PBC, probably because the copper accumulation is secondary to cholestasis and is not a cause of the cirrhosis. Side effects of all these drugs are often the limiting factors. For example, the osteoporotic effects of steroids exacerbates hepatic osteodystrophy.

Management of PBC therefore consists of treating the complications. Pruritus can be treated with the bile acid chelating agent Cholestyramine, an anti-histamine such as Terfenadine, and the use of ultraviolet light. Steatorrhoea is managed by fat restriction, medium chain triglycerides and parenteral replacement of fat soluble vitamins, such as D and K with calcium supplementation. Oral hydroxyapatite has been used for osteoporosis but has little effect.

The prognosis for PBC is about 5 to 10 years depending on the stage at which the disease is diagnosed. Ultimately, chronic liver failure supervenes and death from complications such as bleeding varices ensues.

The pathogenesis of PBC is still unclear and its elucidation should lead to more effective stategies for treatment or prevention. Meanwhile, liver transplantation is the only available "cure" for suitable patients with end stage disease. Mrs M may have benefited from early detection and monitoring with a view to a well timed transplant.

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A SELF-LIMITING HAEMOLYTIC ANAEMIA

M was a 16 year old schoolboy with a lymphoma undergoing allogeneic bone marrow transplant. He had initially presented with a painless submandibular lymph node which had suddenly enlarged, and biopsy showed it to be a high grade T-cell lymphoma of the convoluted cell type. Characteristically, T-cell neoplasms have a high incidence of multiple abnormalities at chromosome 14. However, M's lymphoma had a single cytogenetic abnormality - an inversion at chromosome 14. Single defects in B-cell lymphomas may carry a better prognosis, and it was thought that the same might apply to M's T-cell lymphoma. However, there was evidence that his disease was progressing.

Pre-operatively, M was in good condition. He was given cyclophosphamide and total body irradiation to ablate his existing marrow, and then donor marrow was injected from his HLA-identical sister. She was blood group O rhesus negative and M was A rhesus negative.

The new marrow evidenced its recovery with an increasing haemoglobin, white cell count and platelet count over the first week. On the 9th day post-operatively, however, M began to look mildly jaundiced. His haemoglobin had dropped from 12.8g/dl to 8.7g/dl in 48 hours. The plasma bilirubin concentration had risen to 49μmol/l (N 2-17) but his other liver function tests were stable within the reference range. His reticulocyte count had risen to 4.4%, his serum haptoglobin was low at 0.12g/l (N 0.3-2) and his urine contained haemosiderin. His jaundice and anaemia were concluded to be due to haemolysis.

The cause of the haemolysis was established when the indirect antiglobulin test demonstrated the presence of IgG in M's serum directed against his own red cell A-antigens. Cross-matching showed that the new marrow was producing group O red cells thus M had changed his blood group to that of his sister. It appeared that the graft was producing anti-A allo-antibodies which were attacking the remaining host red cells.

As his urea, electrolytes, coagulation screen and urinary output were undisturbed, the only treatment required was transfusion with O rhesus negative blood. Within days, the bilirubin had fallen and his haemoglobin concentration began to rise again.

Recovery was further complicated by a mild diarrhoea associated with Clostridium difficile which resolved on vancomycin, and a mild pyrexia which resolved with

azlocillin and netilmycin, though no causative organisms were isolated from the blood, sputum, urine or Hickman line. He also developed a mild pruritic maculopapular rash, but skin biopsy did not show the lymphocytic infiltrate or epithelial degeneration associated with Graft versus Host disease.

Three weeks post-operatively, a bone marrow aspirate confirmed that the new marrow was proliferating with all 3 cell lines being present with a female karyotype , XX. As his haemoglobin, white cell and platelet counts were improving, he was discharged feeling well and tolerating modest amounts of food. At his most recent follow-up (106 days post-BMT) his weight was increasing and his hair was growing back. He was cycling and playing tennis and said he felt well.

DISCUSSION

Haemolysis whether intravascular or extravascular (ie in reticuloendothelial cells) is associated with an unconjugated hyperbilirubinaemia with urobilinogen in the urine. There should be no bilirubin in the urine because unconjugated bilirubin is relatively insoluble and is tightly bound to albumin.

When red blood cells are haemolysed intravascularly, the liberated haemoglobin is bound to the alpha-2-globulin haptoglobin which is subsequently catabolised in the liver. Thus a diminished concentration of haptoglobin is characteristic of haemolysis although it can also be congenital. Once all the haptoglobin has bound haemoglobin, free haemoglobin passes into the renal tubular fluid. This is reabsorbed by the epithelium of the renal tubules where the haemoglobin is degraded and the iron stored as haemosiderin. With turnover of the tubular cells, the haemosiderin appears in the urine, and its presence always indicates intravascular haemolysis. The laboratory features of a haemolytic anaemia are well illustrated in this case.

The haemolysis arose because the graft marrow mounted an immune attack on the original host red cells, and as such, the process was self-limiting as compatible red cells were being produced. The HLA haplotype is coded on chromosome 6 whereas the red cell antigens are coded on chromosome one, and so HLA and ABO compatibility are not genetically linked.

A more serious manifestation of graft reactions to the host produces dermatitis, hepatitis, cholestasis and diarrhoea. It can be diagnosed by rectal or skin biopsy and is known as Graft versus Host Disease (GVHD). Although low grade GVHD may be advantageous in terms of survival it can severely impair the quality of life. At his last follow-up, M had no evidence of GVHD.

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PERNICIOUS ANAEMIA

Mr B is a 78 year old retired engineering inspector, who was admitted via the Accident and Emergency Department. He had been increasingly tired over the last 6 weeks and recently he had been "tripping over his own feet" although he normally walked with a zimmer. For the last year, he had been experiencing tingling sensations in both his feet, and now he was getting similar paraethesiae in his hands. His wife explained that he slept for most of the day, and he had had episodes of confusion in the last fortnight.

Mrs B maintained that she provided a wholesome diet, but that Mr B's appetite had waned of late, and he had lost about 12kg in one year.

He gave no history of oral symptomatology, dysphagia, dyspepsia or abdominal pain and there was no incontinence of blood or mucus. His bowel motions were regular and normal in colour and consistency, and although he had symptoms of mild prostatism, there was apparently no haematuria. The remainder of the systems enquiry was unrevealing.

He had a previous history of chronic obstructive airways disease for which he took Prednisolone and Ventolin, and had had a CVA 8 years previously. This had left him with a mild left hemiparesis and he had to walk with a zimmer. Significantly, he had had colonic polyps removed some years ago, prior to which he had suffered from iron deficiency anaemia. There was no history of travel abroad and to his knowledge neither he nor his family had ever suffered from thyroid disease, diabetes or Addison's Disease. He had never had gastric surgery and he was not on non-steroidal anti-inflammatory drugs or anticoagulants.

He drinks only occasionally and previously smoked 20 cigarettes a day. He and his wife live in a ground floor flat and have 3 married sons and 8 grandchildren. His wife is fit and copes domestically.

On examination, Mr B had a somewhat cachectic appearance with white hair and blue eyes. His conjunctivae and oral mucosa were strikingly pale and there was no inflammation or atrophy of the tongue. There was no finger clubbing, lymphadenopathy, angular stomatitis or koilonychia. Cardiovascular examination showed no evidence of failure while chest examination was consistent with his chronic obstructive airways disease. Abdominal examination revealed only a smooth enlarged prostate and there was neither fresh nor occult blood in the stools.

Neurological examination found Mrs B to be a somewhat morose man but orientated with normal speech and mental state. Fundi could not be seen due to bilateral cataracts and there was some deafness on the right. There were signs consistent with his old upper motor neurone lesion in the left arm and leg with 4-5/5 power, mild spasticity, brisk reflexes and an upgoing plantar However, dorsiflexion of both feet was symmetrically weak (4/5) and there was bilateral absence of the ankle jerks. The right plantar response was equivocal. There was sensory loss over both feet in a stocking distribution for light touch and pin prick and there was distal impairment of vibration and position sense in the lower limb. There was no calf tenderness. These findings are consistent with a peripheral neuropathy plus some degree of loss of the long corticospinal tracts and dorsal colums (subacute combined degeneration of the cord).

Dipstick urine analysis was positive for urobilinogen, while ECG showed sinus rhythm of 80 with normal axis and partial left bundle branch block.

In viewof the patient's history, appearance, neurological signs and urin-analysis, a provisional diagnosis of Pernicious Anaemia (PA) was made. Hypo-thyroidism, gastric carcinoma and a recurrence or neoplastic change of colonic polyps would also have to be considered, but the latter seemed unlikely in the absence of both altered bowel habit and foecal occult blood.

Pernicious anaemia results from the failure to absorb vitamin B_{12} due to auto-immune damage to gastric parietal cells. These cells normally synthesise intrinsic factor (IF), a glycoprotein which carries B_{12} to its site of absorption in the terminal ileum. B_{12} deficiency results in a failure of DNA synthesis which affects all cells but is initially apparent in the rapidly dividing cells of the haemopoietic system. The diagnosis of PA was further substantiated by the following investigations (summarised in table 2):

1 Haematology showed a severe macrocytic anaemia with haemoglobin of 4.5 g/dl and MCV 124fl. The red blood cells were well haemoglobinised (high MCHC) but diminished in number with marked variation in shape (poikilocytosis) and size (anisocytosis). Absolute reticulocyte count was decreased and the neutrophils showed hypersegmentation (right shift) with a normal white count. The platelet count was slightly reduced.

A pancytopenia is often present in pernicious anaemia but Mr B's blood picture is still consistent with an underlying megaloblastic marrow.

2 Bone marrow aspiration confirmed megaloblastic anaemia with the presence of megaloblastic red cell precursors and giant metamyelocytes (white cell precursors). Theselarge and mis-shapen cells reflect the failure of DNA synthesis in B_{12} or folate deficiency. Megaloblastosis results from any of the causes of B_{12} or folate deficiency, antimetabolite drugs and rare congenital enzyme disorders. Megaloblastosis need not produce a macrocytic blood film, for example, in the presence of thalassaemia. Furthermore, macrocytic anaemia can occur in the presence of a normoblastic marrow in normal pregnancy, hypothyroidism, alcohol abuse and liver disease.

3 Biochemistry showed normal electrolytes with normal liver and thyroid function . Plasma lactate dehydrogenase was massively increased at 2488 units per litre (N 100-300). This urea stable isoenzyme of LDH is present in red blood cells and myocardium and its elevation in the presence of a normal plasma aspartate aminotransferase (another "cardiac" enzyme) is probably due to the destruction of abnormal red cells by macrophages in the marrow and spleen rather than myocardial damage.

4 A good index of the total body reserve of vitamin B_{12} is the serum B_{12} and this was low at <50ng/l (N 220-750).

5 Acute folate deficiency is unlikely in view of Mr B having a normal serum folate at 18µg/l (N 2-20). Red cell folate is a more sensitive index particularly for long standing deficiency because the folate is locked into the red cell before it leaves the marrow and it is there for the cell's life span of 120 days. Red cells contain 30 times more folate than the serum. This assay was not performed on Mr B.

6 Iron deficiency is unlikely in the absence of hypochromatic red cells in the blood film. Further more, the serum ferritin level is within the reference range and this iron storage protein (which is also an acute phase protein) has been shown to be a better index of total body iron stores than serum iron, total iron binding capacity or transferrin levels.

7 Routine autoantibody profile demonstrated the presence of antibodies to IF but not to gastric parietal cells or thyroid cytoplasm. Fifty-six per cent of patients with PA but less than one per cent of normal individuals have antibodies to IF. Eighty-four per cent of cases of PA have gastric parietal cell antibodies, but so do up to 10% of normal individuals and 32% of those with thyroid disease. Fifty-five per cent of cases of PA have antithyroid antibodies compared with 5% of normal individuals. Thus the presence of anti IF

adds considerable weight to the diagnosis of PA.

8 A Schilling Test demonstrated a failure of B_{12}absorption due to lack of IF. Mr B was fasted and given 1μCi of cobalt labelled vitamin B_{12} orally and 1000mg of unlabelled B_{12} intramuscularly. The injected B_{12} saturates the transcobalamin and R-binder in the blood such that the labelled B_{12} being absorbed from the gut is lost in the urine. His urine was collected for 24 hours and radioactivity of the collection measured.

A normal response is to excrete >15% of the administered radiolabel. It is good practice to check the radiolabel in a sample of blood as the test is invalidated by incomplete urine collection which is often the case with the elderly. The test is repeated with IF and if this corrects the deficient absorption, then the diagnosis is likely to be IF deficiency. If large quantities of IF are required to correct malabsorption , this may be due to the presence of IF antibodies in gastric juice . If IF is ineffective, the terminal ileum should be investigated for malabsorption such as in Crohn's disease.

Mr B excreted only 1.9% of the radiolabel in the first part of the Schilling test and this was increased to 6% by intrinsic factor, and so a definitive diagnosis of PA was made.

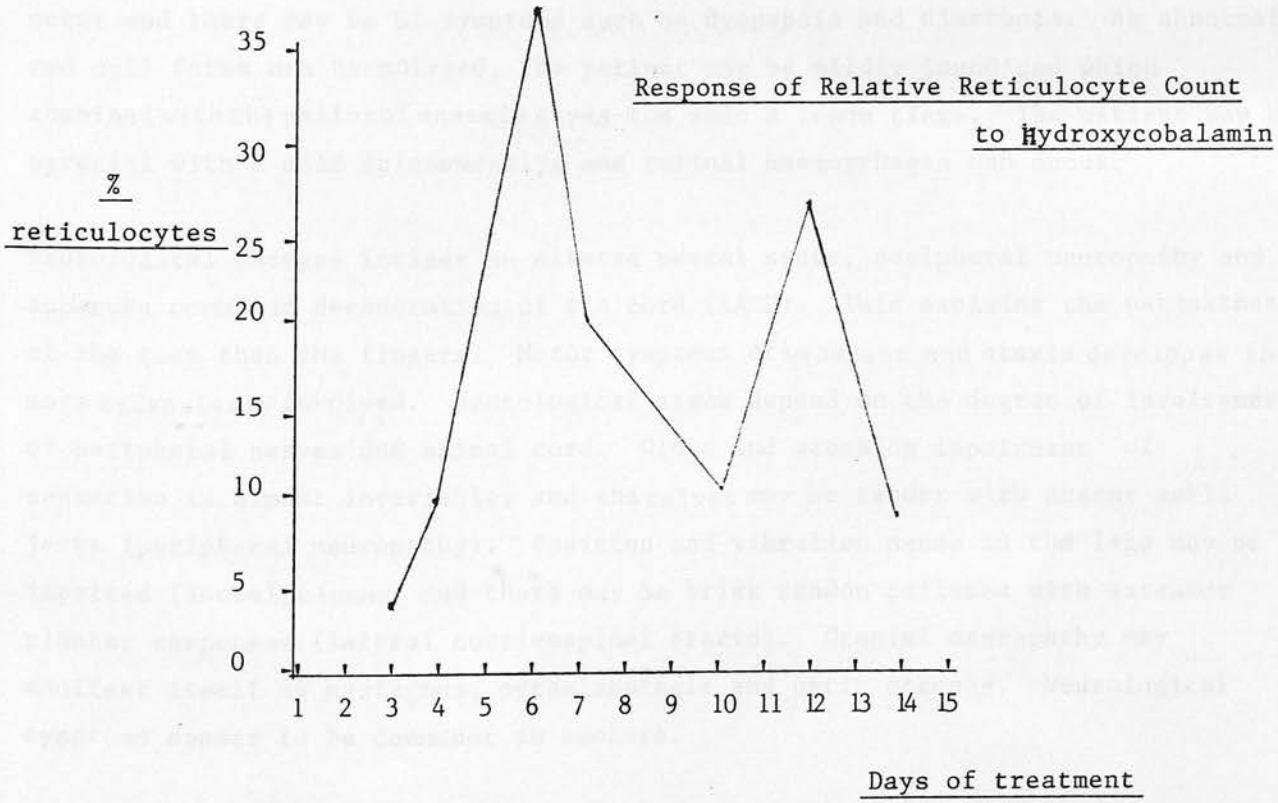
TREATMENT

Transfusion is not indicated in PA except in the presence of cardiac failure, extreme debility, infection or a haemoglobin <4g/dl. Some authorities argue that transfusion is absolutely contra-indicated. However, if transfusion is necessary, red cell concentrate should be given slowly with Frusemide. Co-existing infection should be treated as it may impair the marrow's response to treatment.

As hydroxycobalamin is relatively non-toxic, high doses may be given. Mr B was given 1mg of intramuscular hydroxycobalamin daily for one week to replenish his body stores. As treatment becomes established, iron is incorporated into the haemoglobin and potassium into red cells. Indeed Mr B's ferritin and potassium levels began to fall and oral ferrous sulphate and Slow K were required. Response is heralded by a dramatic reticulocytosis beginning within 3 days and peaking from the 5th to the 10th day. Mr B responded with a reticulocytosis of 38% (n< 2%) on the 6th day. His LDH fell steadily while his haemoglobin&platelet count increased. The time course of these events is shown on table 2.

Table 2: Response to treatment in PA

Day	Hb	RBC	MCV	MCH	Bili	LDH
3	4.5	1.09	124	41	15	--
4	5.0	1.26	125	39	11	--
5	---	---	---	36	9	2486
6	5.8	1.59	121	36	--	2221
7	5.8	1.62	115	33	--	1912
10	6.4	1.95	114	32	--	1066
12	7.5	2.37	110	31	--	860
14	7.9	2.52	108	--	--	--



On the ward, Mr B brightened becoming more alert and conversational. With the aid of the physiotherapist, he once again began to mobilise with his zimmer and after 12 days, he went home to the delight of himself and his family.

DISCUSSION

Addisonian PA is rare before the age of 30, occurs mainly between the ages of 40 to 80 and is commoner in women. It is more frequent in Scandinavians, Swedes and American Negroes, and is very rare in orientals. It has a recognised association with blood group A, gastric carcinoma and autoimmune diseases such as Graves' Disease, Hashimoto's thyroiditis, vitiligo and adrenalitis and there is an increased familial incidence.

It is said that patients are often fair haired and blue eyed with premature greying. Because the liver stores 2 to 5000μg of B_{12} against a daily requirement of 1 to 2μg, deficiency after gastric atrophy takes about 3 years to develop, and so the onset of symptoms is highly insidious. The symptoms include those of anaemia with weakness and fatigue, palpitation, light headedness and shortness of breath and high output cardiac failure may be present. Paraesthesiae of the fingers and toes, a smooth atrophic tongue and, less commonly, a sore red tongue can occur and there may be GI symptoms such as dyspepsia and diarrhoea. As abnormal red cell forms are haemolysed, the patient may be mildly jaundiced which combined with the pallor of anaemia gives the skin a lemon tinge. The patient may be pyrexial with a mild splenomegaly, and retinal haemorrhages can occur.

Neurological changes include an altered mental state, peripheral neuropathy and subacute combined degeneration of the cord (SACD). This explains the paraesthesiae of the toes then the fingers. Motor symptoms of weakness and ataxia develop as the cord's more extensively involved. Neurological signs depend on the degree of involvement of peripheral nerves and spinal cord. Glove and stocking impairment of sensation is almost invariable, and the calves may be tender with absent ankle jerks (peripheral neuropathy). Position and vibration sense in the legs may be impaired (dorsal columns) and there may be brisk tendon reflexes with extensor plantar responses (lateral corticospinal tracts). Cranial neuropathy may manifest itself as nystagmus, opthalmoplegia and optic atrophy. Neurological symptoms appear to be commoner in smokers.

Diagnosis of PA depends on finding a megaloblastic marrow, low serum B_{12} and an abnormal Schilling test corrected by IF. These investigations were described in relation to Mr B but other tests include demonstrating pentagastrin-fast achlorhydria (gastric ph >3.5), high levels of circulating gastrin, and methyl-

malonate aciduria. Endoscopy is required to exclude gastric carcinoma in view of the recognised association .

Pathologically, the gastric mucosa is thin and atrophic. The bone marrow is cellular and hyperplastic with megaloblastic changes in erythocyte, granulocyte and thrombocyte precursors. Such change is usually more apparent in the erythroid cells but megaloblastic granulocytopoiesis becomes apparent when there is a call for a white cell response as in infection. The spinal cord shows variable and often asymmetrical demyelination of the dorsal and lateral colums, and cranial and spinal nerves are similarly affected.

The pathogenesis of PA is thought to involve autoimmune atrophy of the gastric mucosa, and this theory is supported by the presence of antibodies and association with other autoimmune diseases. In addition, glucocorticoids have been shown to permit some regeneration of the gastric mucosa with increased synthesis of intrinsic factor and it is interesting that Mr B was on low dose Prednisolone for his COAD. The presence of IF antibody is significant in that it may neutralise the trace of IF being produced by an atrophic fundal mucosa.

The effect of B_{12} deficiency on DNA synthesis is explained by the formate starvation hypothesis which augments the earlier Methyltetrahydrofolate trap hypothesis. This is explained as follows: THF carries carbon through 3 levels of reduction in the cell - formate, methylene and methyl. At the Methyl level, $-CH_3$ is passed via an organometallic complex with the co-enzyme B_{12} to homocysteine to make methionine. S-adenosyl methionine forms, which is then taken through a series of reactions to produce the formate which can be reduced to methylene. Thus deficiency of B_{12} results in a lack of formate and methylene, and as formate is necessary for purine synthesis and methylene for thymidine synthesis, DNA synthesis becomes defective. Thus, megaloblastosis can also be produced by folate deficiency, methotrexate (an inhibitor of DHF - reductase), azathioprine (inhibitor of purine synthesis), 5-flurouracil (inhibitor of thymidylate synthesis) etc.

The neurological changes in B_{12} deficiency are more difficult to explain but may involve impaired DNA synthesis affecting dividing myelin producing cells.

Vitamin B_{12} replacement in pernicious anaemia rapidly results in a normoblastic marrow with a more gradual improvement in the haemoglobin concentration. Sensory neurological changes take longer to improve and other neurological changes may be irreversible depending on the degree of involvement.

As in the case of Mr B, response to treatment can be quite gratifying, and he is now receiving 1 to 3 monthly injections of B_{12} to maintain a normal blood count. Although there is an increased incidence of gastric carcinoma, if a normal blood count is maintained, the patient may have a normal expectancy of life. Indeed, early diagnosis and treatment can make pernicious anaemia, a once fatal disease, quite "unpernicious".

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DIABETIC KETOACIDOSIS

Miss C had been an insulin-dependent diabetic from the age of fourteen. She was now twenty-five years old and unemployed. She presented to casualty complaining of abdominal pain and breathlessness which had started about twenty-four hours before. The pain had began retrosternally but was now epigastric and aching in nature and it appeared to radiate diffusely over the entire abdomen. She could not keep her food down and had vomited twice that morning. She had passed no bowel motions for two days. There was an associated feeling of dyspnoea and she admitted to excessive thirst and polyuria increasing over the previous two days. It emerged that her symptoms had started after a family argument had induced her to stop taking her insulin properly.

On examination, Miss C was distressed but fully conscious with normal orientation and speech. She was dehydrated with a dry mouth and persistant requests for fluids. She had Kussmaul's respirations and her breath smelled sweetly of acetone. She had a tachycardia of 130 bpm but her blood pressure was normal at 120/80 and her peripheries were well perfused. She was apyrexial but had diminished air entry over the right base. Her abdomen was diffusely guarded with no rebound tenderness and normal bowel sounds. Neurologically, the reflexes were sluggish but there was no papilloedema and no signs of focal disturbance or meningeal irritation. Urinalysis confirmed the presence of large amounts of ketones and glucose in the urine.

Initial biochemistry showed her glucose to be 47mmol/l (N4-6), urea 10mmol/l (N2.2-6.6), sodium 120mmol/l (N132-144), potassium 5.2mmol/l (N3.2-4.7), and total CO_2 <9mmol/l (N24-30). Metabolic acidosis was confirmed by arterial blood gases which showed a hydrogen ion concentration of 126nmol/l (N36-44) which corresponds to a pH of 6.9. Arterial HCO_3^- was 2.9 mmol/l (N22-26), $PaCO_2$ was 2.0kPa (N4.8-6.0) and PaO_2 was 17kPa (N11-13) on 60% oxygen. Serum amylase was within the reference range. It was later noted that the glycosylated haemoglobin fraction, HbA_1, was 20.4% (N<6%) indicating poor diabetic control over the preceding three months.

Full blood count showed a slightly elevated haematocrit at 0.49 (N0.35-0.47) and a leucocytosis of 33 x 10^9 l^{-1} with 88% neutrophils. Platelet count was elevated at 449 x 10^9 l^{-1}.

ECG showed a sinus tachycardia with slightly tall T-waves. Radiology showed clear lung fields with no pneumomediastinum (in view of retrosternal pain) but there was a large gas shadow over the stomach.

The diagnosis was clearly diabetic ketoacidosis with acute gastric dilatation. Management consisted of rapid infusion of 0.9% saline (1L in the first 30 minutes, 1L in the next hour, then 500ml per hour until the blood glucose has fallen to 10mmol/l) followed by 5% dextrose to replace the water and electrolyte deficits. To protect the heart from hypokalaemia, 10 to 20mmol of potassium chloride was added to each bag, depending on the plasma levels. A loading dose of 2 units of unmodified insulin (actrapid) was given intravenously followed by a pumped infusion of 4 units per hour.

Vital signs, urine output and ECG were regularly monitored and potassium and glucose levels were checked hourly. Blood, urine and sputum specimens were sent for culture and sensitivities. Nasogastric intubation and urinary catheterisation were considered unnecessary as the patient was fully conscious and no longer vomiting.

Blood glucose concentration should fall at 3-6mmol/l/hour. However, within four hours, Miss C's glucose had fallen from 47mmol/l down to 17mmol/l. The rate of infusion of insulin was therefore reduced to 2 units per hour. At this point, the patient had developed a dry cough associated with pain over the right side of her chest, and fine crepitations could be heard over the right base. Treatment was commenced with Amoxycillin.

Two hours later, the patient was still distressed by her air hunger as it was causing her discomfort to breathe. She was still severely acidotic with a hydrogen ion concentration of 133nmol/l and bicarbonate 2.7mmol/l. It was decided to give her 500ml Of 1.26% of sodium bicarbonate with potassium over one hour and to increase the infusion rate of insulin to 3 units per hour. However, the acidosis persisted with a hydrogen ion concentration of 99nmol/l and bicarbonate 6mmol/l. A further 500ml of 1.26% bicarbonate plus 6 units of insulin were given. The acidosis settled at H^+ 54nmol/l and HCO_3^- 13mmol/l. As the blood glucose was now approaching normal, the saline infusion was replaced with 5% dextrose with potassium. The hyperventilation was easing off and the patient settled for the night.

The following day, Miss C was much better. She no longer had pain in her abdomen or chest, although her cough was still present. Chest X-ray showed no abnormality and cultures and serology were all returned negative.

On the third day after admission, all of Miss C's symptoms had resolved, and the drip was taken down. She was given small frequent meals preceded by subcutaneous insulin, and Miss C was discharged the following day with a prescription to finish the course of Amoxycillin.

DISCUSSION

Before the discovery of insulin, ketoacidosis killed more than half of all diabetics, and yet today, this complication is still associated with almost 2% of diabetic deaths.

The pathophysiology of diabetic ketoacidosis (DKA) is a consequence of absolute deficiency of the polypeptide hormone insulin. This deficiency impairs glucose transport across many cell membranes and the resulting threat of intracellular energy and glucose deficiency is met by an increase in the activity of the "diabetogenic" hormones, glucagon, cortisol and growth hormone.

These hormones activate adenylate cyclase in hepatocyte membranes to generate an increase in the concentration of the intracellular messenger, cAMP. In turn, this activates a protein kinase which can regulate the activity of intracellular enzymes by phosphorylating them. A more recently discovered intracellular messenger is fructose-2,6-bisphosphate, and its concentration falls in the presence of glucagon. It seems to antagonise the effects of cAMP in the cell. The overall result is activation of the enzyme fructose-1,6-bisphosphatase and inhibition of phosphofructokinase, and so the synthesis of glucose from substrates such as pyruvate, lactate and amino-acids (gluconeogenesis) is stimulated while the rate of glycolysis falls. Glycogen phosphorylase is also activated producing glycogenolysis, while inhibition of the synthase suppresses glycogenesis.

In effect, the hepatic biochemistry perceives intracellular starvation and responds by generating glucose and delivering this to the blood. However, the glucose cannot enter many of the extrahepatic tissues in the absence of insulin, and so hyperglycaemia develops. When the blood concentration of glucose exceeds the renal threshold, glycosuria results and produces a heavy osmotic diuresis. Both water and electrolytes are lost in the urine, producing the characteristic dehydration and hypovolaemia seen in DKA. Large quantities of potassium are lost, but the extracellular loss is offset by mobilisation of intracellular potassium which accompanies the efflux of amino-acids. Thus severe depletion of total body potassium can occur in the face of a normal or paradoxically raised plasma concentration. When insulin is given, fatal hypokalaemia can rapidly follow.

Diabetogenic hormones also activate adenylate cyclase in adipose tissue. The consequent increase in cAMP activates the enzyme triglyceride lipase. This catalyses the hydrolysis of triglyceride into free fatty acid and diglyceride which is further hydrolysed to free fatty acids and glycerol. The free fatty acids are transported to the liver as lipoprotein where the fatty acyl chains are esterified to carnitine before entry to the mitochondria. There they undergo beta-

oxidation to form acetyl units linked to co-enzyme A. The acetyl units would normally be oxidised to CO_2 and H_2O by combining them with oxaloacetate to form citrate which is subsequently catabolised round the tricarboxylic acid cycle. However, the cycle's capacity is overwhelmed by the appearance of acetyl units from beta-oxidation and so acetyl co-enzyme A accumulates. This acetyl co-A cannot be stored by making fatty acyl chains because the already high levels of free fatty acids and the diabetogenic hormones act to inhibit the enzyme Acetyl Co-A Carboxylase.

The accumulating acetyl co-A is condensed in the liver to form acetoacetic acid which can be reduced to beta-hydroxybutyrate or decarboxylated to acetone. Because of the low vapour pressure of acetone, it evaporates at the alveolus to give the breath a characteristic sweet odour. The acids, however, being beta-substituted, dissociate readily in the plasma to produce a metabolic acidosis. The H^+ are buffered by bicarbonate to form carbonic acid which decomposes in the presence of red cell carbonic anhydrase to from CO_2 and H_2O. The H^+ stimulate the respiratory centre in the medulla oblongata to increase the rate and depth of ventilation (Kussmaul's respiration). This drives the buffering reactions forwards by blowing off the CO_2, and the concentration of bicarbonate falls even further.

Some of the acidosis in DKA is due to lactic acid. This accumulates in the plasma because the insulin deficiency and high level of acetyl co-enzyme A act to inhibit Pyruvate Dehydrogenase, the enzyme which oxidises pyruvate to acetyl co-A. Thus more pyruvate is made available to the Lactate Dehydrogenase for reduction to lactic acid. Lactic acid is also produced by anaerobic respiration of glucose in muscle tissue. This tissue respires anaerobically if peripheral perfusion is compromised by hypovolaemia. It is possible that Miss C had developed a degree of lactic acidosis when she required bicarbonate considering that she was severely acidotic but not particularly ketotic. However, her lactate level and anion gap were not measured.

This complex chain of physiological events is adirect consequence of a primary deficiency of insulin. Evidence is accumulating that insulin-dependent diabetes mellitus (IDDM) may have an autoimmune aetiology. For example, virtually all cases of IDDM have the HLA haplotypes DR3 or DR4. Islet cell antibodies have been demonstrated in the serum around the time of onset of the disease, and it was recognised that their presence may be secondary to islet damage rather than causative. However, prospective studies of the unaffected members of diabetic families found them to be present before the onset of the disease. Examination of the pancreas of a diabetic who died shortly after the onset of the disease found evidence of an aggressive immune attack with cytotoxic T-cells, immune complexes and complement activation, directed against beta-cells. There was no evidence of mumps, coxsackie or other common viruses. Furthermore, when healthy

pancreatic tissue from an unaffected twin was transplanted into his identical twin brother who had become diabetic twenty years before, the transplanted tissue rapidly developed an insulitis specifically involving the beta-cells. Recently, pancreatic beta-cells in IDDM have been shown to be capable of expressing MHC class II proteins. If this occurs, the cells could conceivably be capable of presenting their own antigens to lymphocytes.

Recent trials have suggested that immunosuppression with cyclosporin may increase the rate and length of remissions in IDDM of recent onset. Clearly, a broader understanding of the pathogenesis of diabetes is emerging, and this may lead to efficient stratagems for its prevention and control.

Meanwhile, regardless of how much we understand about the pathogenesis and biochemistry of diabetes, patients like Miss C will continue to have difficulty coping with diabetic control. It transpired that this was her third admission for ketoacidosis, and her life-threatening illness had resulted from an argument at home: her mother, herself a diabetic, had locked her daughter out of the house and refused to allow her any insulin.

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CONCLUSION

The spectrum of disease in which the immune system contributes to the patient's illness is illustrated by the five cases presented here. Each condition could have been diagnosed clinically on the basis of the history, examination and simple urialysis, but the diagnoses were each substantiated by laboratory investigations which are largely based on our understanding of the disease process. A more profound understanding of the molecular and cellular events underlying these diseases may allow us to evolve novel therapeutic approaches in the future, as it has done for the once fatal diseases diabetes and pernicious anaemia in the past. For example, the goal of antigen-specific immunosuppression becomes closer with our emergent understanding of regulation in the immune system. However, as in the case of Miss C, illnesses are not just a consequence of deranged physiology or an internal threat from the immune system within: they are related to patients' interactions with their families and the people around them. Responding to this is also part of the challenge of Clinical Medicine.

* * * *

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