Papers and Originals

Enterocyte in Coeliac Disease*—1

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Advances in knowledge of the pathogenesis of disease in man are often correlated with the ease by which biopsy specimens of relevant tissue may be obtained. Since biopsy of the blood simply requires a venepuncture and the bone marrow can be aspirated very readily, it is not surprising that haematologists have often been in the forefront of research in nutritional disease, autoimmunity, and genetically determined disorders of protein synthesis.

This lecture seeks to show that gastroenterology is fast approaching the same position. It has now become possible to obtain representative biopsy specimens of most parts of the gastrointestinal tract, and though methods of cell measurement and counting remain imperfect by haematological standards modern work has thrown considerable light on cellular structure and function in health and disease. This is particularly true of studies of the small-intestinal mucosa, and the intestinal biopsy techniques which have been developed in the past 10 to 15 years (Shiner, 1956; Crosby and Kugler, 1957; Baker and Hughes, 1960; Flick *et al.*, 1961) have greatly increased our understanding of disorders such as milk intolerance, intestinal lipodystrophy, tropical sprue, and coeliac disease.

In this lecture I propose to concentrate on coeliac disease, the most fascinating of these disorders. Since Dicke's (1950), classical observations in Holland it has been confirmed that this disorder, occurring both in children and in adults, is produced in some way by a normal dietary constituent, gluten, which is present in wheat and rye flour, and which damages the absorbing cells of the jejunum. The term "coeliac disease" is often used to describe a variety of malabsorption syndromes, but for the purpose of this lecture coeliac disease will be defined as the condition in which there is an abnormal jejunal mucosa that responds morphologically to treatment with a gluten-free diet.

A great deal of effort has been expended on seeking the toxic factor in gluten. It is known to be present after digestion of gluten by pepsin and trypsin, but toxicity is lost after further digestion by hog intestinal mucosa (Frazer *et al.*, 1959). It seems likely that it is an acidic polypeptide of molecular weight less than 1500 (Krainick and Mohn, 1959; Kowlessar, 1967). But how this toxic factor damages the intestinal mucosa, and why coeliac patients are unable to tolerate gluten when most individuals can eat bread with impunity, is uncertain. It seems likely, however, that there is some inherent abnormality in the absorbing cell of the small intestine, and this lecture describes a search for this defect. The anatomy of the normal absorbing cell of the small intestine (the enterocyte) will first be described and contrasted with the morphology of the intestinal cell in coeliac disease. The changes in cytoanatomy will then be correlated with histochemical studies. Two hypotheses which have been put forward to explain the pathogenesis of coeliac disease will be examined. Firstly, that the disorder is an inborn error of

metabolism due to an enzyme defect in the small-intestinal mucosa (Frazer, 1956). Secondly, that the disease may represent an immunological reaction to an unusual dietary protein (Malik *et al.*, 1964; Rubin *et al.*, 1965), a protein which has only been a major constituent of the diet of man since the neolithic revolution.

Normal Jejunal Mucosa

The absorbing cells of the surface of the villi are transparent, so that through them the capillary bed of the core of the villus can be seen, the microcirculation of the villus cascading down the basement membranes of the absorbing cells (Fig. 1). Only at the edge of each villus can the thin white line of columnar absorbing cells covering the villus be seen. The histology of the normal jejunal mucosa (Fig. 2) shows the three

Fig. 1.—Dissecting microscope appearance of normal jejunal mucosa ($\times 36$).
(From Stewart *et al.*, 1967.)

Fig. 2.—Histological appearance of normal jejunal mucosa. ($\times 30$.)

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main parts of the intestinal mucosa; firstly, the crypt zone or germinative epithelium, where the cells are formed; secondly, the villi—tall, slender finger-like processes projecting into the lumen of the gut; and thirdly, the lamina propria with its cellular infiltrate made up of lymphocytes and plasma cells. This mucosa is a tissue with the most rapid turnover of any in the body. The cells are formed in the crypts, then are pushed out on to the side of the villus, and they ascend the villus as if they were on a moving staircase to be extruded at the villus tip. This whole process may take less than five days in man (Shorter *et al.*, 1964) so that each columnar absorbing cell has a short and finite lifetime.

Doniach and Shiner (1960) first drew the analogy between this mucosa and the haemopoietic system. They compared the crypt cells in the germinative zone with the cells of the bone marrow, and suggested that the adult absorbing cells, which show no mitosis and have a limited life span, are similar to the peripheral blood cells. To pursue this analogy further, a nomenclature for these cells which is based on haematological principles has now been proposed. The adult absorbing cell can be called an "enterocyte," and the crypt cell is therefore an "enteroblast." The whole process of cell turnover can then be termed "enteropoiesis" (Booth, 1968). This nomenclature does more than simply provide names for the intestinal mucosal cells, for the concept of enteropoiesis implies that there may be an "enteropoietin" responsible for maintaining cell turnover in the intestine.

The enterocytes are normally tall columnar cells, and the blood vessels are closely applied to their basement membranes. Each cell at its luminal side has a well-defined brush border, which on electron microscopy is shown to be made up of microvilli covered by the "fuzzy coat" or glycocalyx.

Thus far the enterocytes have been described at relatively low magnification, or on examination by conventional cross-section. The new technique of scanning electron microscopy has made it possible to view the surface of the small intestine at very high magnification, and the outline of the individual enterocytes can be defined as well as their microvilli (Marsh *et al.*, 1968; Marsh and Swift, 1969). This technique reveals that the villus surface is, in fact, more complex than it appears on dissecting microscopy and it shows a series of convolutions. The outline of the individual enterocytes can be made out and they can be seen closely packed together on the surface of the villus (Fig. 3). Their hexagonal shape can

FIG. 3.—Tip of an individual villus viewed with the scanning electron microscope. The corrugated appearance of the surface of the villus and the hexagonal shape of the individual enterocytes can be recognized. (From Marsh and Swift, 1969.)

FIG. 4.—Scanning electron microscopy of the surface of a single enterocyte to show microvilli. (From Swift and Marsh, 1968.)

be recognized, providing another example of hexagons in nature as in the beehive. At high magnification the microvilli can be seen as individual structures (Fig. 4). Under normal circumstances, therefore, the enterocytes are tall columnar cells, hexagonal in shape, packed tightly together like bricks around the core of the villus.

Cytoanatomy of the Coeliac Enterocyte

In coeliac disease the appearances of the intestinal mucosa are strikingly different. There are variations in the severity of the lesion in different patients (Stewart *et al.*, 1967), but at low power under the dissecting microscope the mucosa is usually flat and devoid of villi. At higher power the mucosa is often seen to be divided up by grooves into a mosaic pattern (Fig. 5). The crypts of Lieberkuhn, not being hidden by villi, open directly on to the surface of the mucosa and their orifices can be seen. Histology of the flat mucosa reveals three main features (Fig. 6). The enterocytes are no longer columnar but are flat, cuboidal, and reduced in number. The enteroblasts are increased, however, the crypt layer being markedly thickened compared with the normal, and there is an infiltration of the lamina propria with lymphocytes and plasma cells.

There are two possible ways in which the mucosa may develop this appearance (Doniach and Shiner, 1960). Either there is direct damage to the enterocytes themselves, the

FIG. 5.—Jejunum mucosa from a patient with coeliac disease viewed under the dissecting microscope ($\times 36$). The mucosa is flat and the orifices of the crypts of Lieberkuhn can be clearly seen. (From Booth, 1965.)

FIG. 6.—Histological appearance of coeliac jejunal mucosa ($\times 78$). The enterocytes are abnormal but there is enteroblastic hyperplasia. (From Stewart *et al.*, 1967.)

FIG. 7.—Scanning electron microscopy of the orifice of a single crypt of Lieberkuhn showing enteroblasts emerging from the crypt and enterocytes heaped around the crypt orifice. (From Marsh *et al.*, 1970.)

FIG. 8.—Scanning electron microscopy of individual enterocytes in coeliac disease. There is variation in size and shape. (From Marsh *et al.*, 1970.)

hyperplasia of the enteroblasts then being compensatory in an attempt to make up for damage to the surface cells, a situation analogous to haemolytic anaemia, or some factor inhibits the maturation of the enteroblasts, the enterocytes then being reduced in number because of this maturation arrest, a situation similar to the hyperplastic bone marrow of pernicious anaemia.

All the evidence now suggests that the first of these hypotheses is correct, and there are three main reasons for this. Firstly, there is an increased mitotic rate in the enteroblasts (Padykula *et al.*, 1961); this merely suggests increased cellular activity and it could equally well be produced by a maturation arrest. Secondly, in the coeliac jejunal mucosa there is frequently an increased amount of aspartate carbamoyl transferase and dihydroorotate dehydrogenase, enzymes involved in pyrimidine biosynthesis, suggesting increased cell growth and turnover (Clark and Senior, 1969). Thirdly, intubation studies have shown that there is an increased amount of DNA in the gut lumen of coeliac patients compared with normal subjects (Croft *et al.*, 1968). The DNA in the lumen of the intestine is presumably derived from the nuclei of cells desquamated from the surface of the intestinal mucosa and indicates that there is probably an increased loss of cells into the lumen of the gut. It therefore seems likely that whatever is the basic abnormality in coeliac disease, the defect appears to involve damage to the enterocytes, the enteroblastic hyperplasia being compensatory to surface cell damage and loss. For this reason attention will be directed to the enterocyte rather than to the enteroblast in this lecture.

Coeliac enterocytes are flat, cuboidal, and irregular in size and shape, and there is an increased infiltration of mononuclear cells between the individual enterocytes (Fig. 6) (Doniach and Shiner, 1957; Rubin *et al.*, 1960). Scanning electron microscopy of the surface of the small intestine in untreated coeliac patients shows the mosaic pattern already described, with the crypts opening directly on to the surface. At higher power the individual crypt orifices can be seen opening on to the surface of the mucosa and the enteroblasts can be seen emerging from within the crypt (Fig. 7). The cells in this region probably take no more than an hour or two to cover the last few fractions of a millimetre from the interior of the crypt and on to the surface. Around the crypts the cells appear to be heaping up in rings, as if each crypt was pouring out cells in an attempt to keep pace with cell loss from the surface. At higher power (Fig. 8) the individual enterocytes can be seen end on, and they are different from normal. Instead of showing a regular hexagonal pattern the cells vary considerably in size and shape (Fig. 8).

The microvilli of these surface cells are often disorganized and irregular (Fig. 9), corresponding to the grossly abnormal microvilli that can often be seen on conventional electron microscopy (Trier and Rubin, 1965; Shiner, 1967). All the cells in the mucosa, however, do not show this grossly abnormal appearance. Cells sheltering just within the protection of a crypt of Lieberkuhn, for example, may show an entirely normal microvillus pattern.

So far as cytoanatomy is concerned, the enterocyte in coeliac disease is therefore abnormal. It is a flattened cuboidal cell, often with a grossly abnormal and disorganized brush border, and it may lose its regular hexagonal shape.

Response to a Gluten-free Diet

The appearances of the abnormal jejunal enterocyte may improve dramatically when a gluten-free diet is given, the response being more rapid and more striking in children than in adults. These observations refer to the jejunal enterocyte, for the ileum in this condition is either normal or less severely involved than the jejunum (Rubin *et al.*, 1960; Booth

FIG. 9.—Scanning electron microscopy of the microvilli of coeliac enterocytes showing disorganization and irregularity. (From Marsh *et al.*, 1970.)

et al., 1962; Stewart *et al.*, 1967), which would be expected if the intestine were being directly damaged by an orally ingested toxin. After institution of a gluten-free diet the individual enterocytes may become normal, and electron microscopic studies have shown that the improvement in cytoarchitecture is accompanied by a striking involvement in the appearances of the brush border of the enterocytes. Cytological changes are often the earliest signs of a response to a gluten-free diet and, as Yardley *et al.* (1962) have shown, they may occur within a week of starting treatment. In an adult coeliac patient treated with a gluten-free diet the mucosa may remain flat on dissecting microscopy for four weeks or more, but the surface cells often begin to recover their normal shape within a week, and within eight weeks they may return to normal (Fig. 10). All the evidence sug-

FIG. 10.—Jejunal mucosal response to treatment with a gluten-free diet in adult coeliac disease.

gests that the damage to the enterocyte is directly caused by gluten, for it can be reinduced in sensitive subjects by refeeding gluten.

Histochemical Studies

The anatomical changes already described are associated with abnormalities in the histochemistry of the intestinal cell. With a variety of histochemical techniques it is possible to study the activity of the different cellular organelles (Fig. 11). Enzymes such as alkaline phosphatase, adenosine triphosphatase, or leucine aminopeptidase are localized in the brush border membrane. Lysosomal enzymes include acid phosphatase, beta-glucuronidase, glucosaminidase, and E600 resistant esterase. There are other enzymes which are found in the mitochondria, the Golgi apparatus, or the endoplasmic reticulum. For the purpose of this lecture two subcellular organelles only will be considered; firstly, the brush border, and alkaline phosphatase and leucine aminopeptidase will be examined, and, secondly, the lysosomes for which acid phosphatase and lysosomal esterase have been used as markers (Riecken *et al.*, 1966).

FIG. 11.—Diagrammatic representation of an individual enterocyte showing the subcellular organelles and their respective enzymes. (From Riecken *et al.*, 1966.)

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Brush Border Enzymes

Alkaline phosphatase is not present in the brush border of the enteroblasts but it develops as the intestinal cell emerges from its parent crypt. Within the few hours that it takes for the cell to travel the short distance between the top of the crypt and the base of the villus, alkaline phosphatase develops. Since it is a brush border enzyme it is surprising that some histochemists have reported this enzyme to be normal in the grossly abnormal brush border of the coeliac enterocyte (Padykula *et al.*, 1961). Our own studies, however, have often revealed a reduction in the alkaline phosphatase (Riecken *et al.*, 1966). After treatment with a gluten-free diet, however, there may be a remarkable recovery in the cytoanatomy, particularly of the brush border. This may be reflected by recovery of alkaline phosphatase. Not all enzymes are so reduced, however. It has been shown that leucine aminopeptidase, another brush border enzyme, may be increased in the untreated coeliac patient. There may therefore be variability in the function of the brush border of the enterocyte in coeliac patients, regardless of the disorganized cytoanatomy (Riecken *et al.*, 1966), possibly as a result of compensatory increases in enzyme synthesis.

Lysosomal Enzymes

The lysosomes are discrete intracellular organelles which lie just beneath the brush border of the enterocyte (Fig. 11) (Riecken *et al.*, 1966). Lysosomes are surrounded by a lipid membrane which encloses a group of hydrolytic enzymes, and this membrane is sensitive to a variety of forms of damage (Gianetto and de Duve, 1955). It has been suggested that

when the membrane is damaged the lysosomal enzymes can leak out into the cell. The increased free lysosomal activity in the liver in hepatitis (Pagliaro *et al.*, 1964) and in hypervitaminosis A (Weissmann and Thomas, 1963) are possible examples of lysosomal disruption. A similar situation may arise in the untreated coeliac patient. In the untreated jejunal mucosa the lysosomal enzymes—for example, acid phosphatase and esterase—are not present in discrete organelles but are diffusely scattered throughout the disorganized enterocyte. As Wattiaux and de Duve (1956) suggested, it is possible that this diffusion of intensely hydrolytic enzymes into the cytoplasm may contribute to cell damage. The changes are not specific to coeliac disease, and probably simply reflect the gross damage that gluten causes to the susceptible enterocyte.

After treatment with a gluten-free diet there is a restoration of lysosomal integrity. Riecken *et al.* (1966) studied the histochemical changes in the jejunal mucosa of three patients before and at varying intervals after giving a gluten-free diet. Within four weeks of starting treatment the lysosomal bodies had reappeared as discrete organelles, and within 22 months there had been restoration of structural and functional normality. This sequence of changes was also shown with other lysosomal markers.

These observations suggest that the damage to the enterocyte in coeliac disease may possibly be related to a toxic effect of gluten on the lysosomal membrane. It may be that the toxic substance derived from gluten passes into the cell, rupturing the lysosomal membrane. This hypothesis would allow diffusion of the autodigestive enzymes of the lysosomes into the cytoplasm, the result being autolysis and destruction of the cell (Riecken *et al.*, 1966). Subsequent work by Dolly and Fottrell (1969a) *in vitro* suggests that lysosomes are in fact particularly sensitive to gluten peptides.

Response to Steroid Treatment

If this theory is correct it should be possible to restore the cytoanatomy in coeliac disease by treatment with steroids, since it is well known that steroids have a stabilizing effect on lysosomal membranes. It is also known that steroids may be beneficial in relieving steatorrhoea in coeliac patients (Adlersberg *et al.*, 1951; Lepore, 1958), but the effect of steroids on the fine anatomy and histochemistry of the enterocyte has received little attention. To investigate this response Wall *et al.* (1970) studied five patients with untreated coeliac disease and treated them for one month with oral prednisone in a daily dose of 30 mg. There was a striking response to the steroid treatment. The enterocytes in the jejunal biopsies obtained four weeks after steroid treatment became normal and the brush border showed similar improvement. Histochemically the most interesting feature was the lysosomal response. In the untreated coeliac patient the lysosomal enzymes were diffusely present throughout the abnormal enterocytes. After one month's treatment, however, there was restoration of lysosomal integrity. These results support the theory that gluten may act by rupturing lysosomal membranes, and the known action of steroids in stabilizing the lysosomal membrane may explain the steroid response. This is not a specific response, but it provides a further possible example of the relationship of lysosomal damage to cell destruction, similar to that which has been shown in other tissues of the body, particularly the liver. It suggests *how* the enterocyte may be damaged, but it does nothing to answer the fundamental question which remains—why is the coeliac enterocyte susceptible to this sort of damage?

Digestive Enzymes of the Enterocyte

In recent years it has become increasingly clear that the enterocyte plays a fundamental part in digestion as well as in

absorption. The last stages of digestion of carbohydrate and protein do not occur in the gut lumen but at the level of the enterocyte. The final splitting of disaccharides, for example, is now known to occur at the brush border region of the cell, and it is likely that polypeptides and dipeptides are cleaved either at the brush border or within the cytoplasm of the enterocyte.

Disaccharidases

In view of the abnormality of the microvilli of the jejunal enterocytes in coeliac disease, it is not surprising that the disaccharidase enzymes which are located in the brush border region may be markedly reduced. There is, for example, a generalized reduction in the concentration of lactase, sucrase, and maltase in intestinal mucosa from untreated adult coeliac patients (Plotkin and Isselbacher, 1964). This may be important clinically because occasionally a coeliac patient presents with milk intolerance, this intolerance being due to the deficiency of lactase. As with other enzymes, these disaccharidase deficiencies are clearly secondary, since they recover, though slowly, after treatment with a gluten-free diet.

Peptidases

Since gluten is a protein, enzymes concerned with protein digestion are of greater interest. It is necessary to establish how proteins such as gluten are digested, both in normal subjects and in patients with coeliac disease. Knowledge of protein digestion is very much less well founded than that relating to polysaccharides. It has been known for many years that there is relatively little dipeptidase activity in the lumen of the gut (Florey *et al.*, 1941) and that the digestion of dipeptides may take place *within* the enterocyte (Newey and Smyth, 1960). But precisely what size of peptide or polypeptide can enter the intestinal cell is not yet clear, and until recently little was known of where within the cell peptide digestion takes place. It seems likely that protein is first broken down by gastric and pancreatic proteases in the gut lumen, yielding a variety of peptides and small amounts of free amino-acids. Recent work using subcellular fractionation techniques in the guinea-pig indicates that some polypeptides, at least up to six amino-acids in length, may then be digested at the brush border of the cell (Peters *et al.*, 1969). Though small amounts of dipeptidase activity are found in the brush border region more than 90% appears to be present within the cytoplasm of the enterocyte (Robinson, 1963; Peters, 1968) and one can therefore envisage an enzymatic sieve (Fig. 12)

FIG. 12.—Proposed mechanism of protein digestion. (Peters, 1968; Peters *et al.*, 1969.)

through which the polypeptides and dipeptides pass, ultimately yielding amino-acids which are then transported into the blood stream, (Peters, 1970). This is a more complex scheme for protein absorption than for the simpler dietary carbohydrates.

Clinical Observations

The question then arises whether coeliac disease could be due to a specific deficiency of one of the enzymes or groups of enzymes involved with protein digestion. If there is a congenital enzyme deficiency there are two clinical features which should be present in the disease. Firstly, the disorder should persist through life and there should be no natural recovery in the absence of a gluten-free diet. Secondly, the disease should be genetically determined.

Persistence of Disease through Life

Biopsy studies have shown that the disease persists through life unless treated with a gluten-free diet. In a follow-up study of coeliac patients at intervals of 20 to 40 years after an initial episode of coeliac disease had been treated, but without a gluten-free diet, Mortimer *et al.* (1968) found that though these individuals had few, if any, symptoms, all had a flat jejunal mucosa (Table I). This evidence suggests that there is no natural recovery of the condition in the absence of a gluten-free diet.

TABLE I.—Follow-up Study of Untreated Coeliac Disease

Case No.	Sex	Age at Initial Diagnosis (years)	Gluten-free Diet	Age at Biopsy	Jejunal Biopsy
1	M.	1½	Never	27	Flat
2	F.	2 8/12	Never	40	"
3	F.	7 10/12	For 1 year at 8 years	23	"
4	F.	2½	Never	32	"
5	F.	2½	Never	30	"
6	F.	3	For 3 months	24	"
7	F.	1½	Never	30	"
8	F.	3½	Never	40	"
9	F.	2½	Never	28	"

(From Mortimer *et al.*, 1968).

Genetics

Genetic studies are in keeping with the hypothesis that there could be an enzyme deficiency. Only one really satisfactory study has been published on the incidence of coeliac disease in relatives of patients with the disorder. MacDonald *et al.* (1965) found that 11% of 96 symptomless relatives had a flat jejunal mucosa, an incidence far higher than would be expected in the general population. Individual family trees show that brother and sister may be involved, brother and sister and first cousin, and parent and child. These genetic findings are difficult to explain in classical Mendelian terms, but it has been suggested that the disorder may be inherited as a dominant gene with incomplete penetrance.

Peptidase Deficiency in Coeliac Disease

In view of these observations the possibility must be examined that coeliac disease may be due to a genetically determined enzyme deficiency (Frazer, 1956). The hypothesis that has been put forward is as follows: Gluten, like other proteins, is thought to be digested within the gut lumen to peptides and to a small amount of amino-acids. The peptides may then pass on to be digested by the intestinal cell. In coeliac disease it has been suggested that there may be a deficiency of an intracellular peptidase, allowing the accumulation within the coeliac enterocyte of a toxic peptide, which might then rupture the lysosomal membrane and produce damage to the cell. This condition could be inherited

as an incomplete dominant, and variable deficiency of the enzyme would lead to the varying degrees of abnormality seen in the intestinal mucosa of this condition.

This hypothesis can be tested. The toxic peptides can be obtained by peptic and tryptic digestion of gluten. Jejunal mucosa can be obtained from coeliac patients, and its capacity to release amino-acids in vitro from the peptide solution can be studied (Crabbé, 1964; Pittman and Pollitt, 1966). The results of a recent study have been reported by Douglas and Booth (1970). Homogenates of jejunal mucosa from untreated and treated patients with coeliac disease were incubated with a peptic and tryptic digest of gluten and the amounts of proline and glutamic acid, as well as of other amino-acids released, were measured (Fig. 13). In the untreated state the

FIG. 13.—Amount of proline and glutamic acid released from a peptic/tryptic digestion of gluten after incubation for 60 minutes with jejunal mucosa from control subjects, from patients with untreated coeliac disease, and after successful treatment with a gluten-free diet. (From Douglas and Booth, 1970.) Broken lines indicate 2 S.D. from mean; open circle = treated coeliac patients with normal mucosa.

amount of amino-acid released was less than normal, suggesting defective peptidase activity in the intestinal mucosa. If this were the basic abnormality in the coeliac enterocyte one would expect it to persist when the enterocytes return to normal following treatment with a gluten-free diet. However, after treatment with a gluten-free diet, when the intestinal morphology has improved, the amounts of amino-acid released return to normal levels (Fig. 13). This evidence suggests that though there are deficiencies of enzymes responsible for the digestion of gluten in untreated coeliac enterocytes, these deficiencies are probably secondary, since they return to normal after treatment with a gluten-free diet.

Deficiency of dipeptidase has also been demonstrated in untreated coeliac jejunal mucosa by Messer *et al.* (1961), by Lindberg *et al.* (1968), and by Dolly and Fottrell (1969b). Douglas and Peters (1970) studied the glycyl-glycine peptide hydrolase activity in normal subjects and in untreated and treated coeliacs and showed that there is a reduction of dipeptidase activity in untreated coeliac mucosa which returns to normal after treatment with either steroids or a gluten-free diet. Similar results were obtained for leucyl-leucine peptide hydrolase. The impairment of jejunal mucosal digestion of gluten and of certain dipeptides therefore appears to be a secondary phenomenon, and there is no support for the hypothesis that the coeliac enterocyte lacks an enzyme specially concerned with the digestion of gluten.

These secondary deficiencies of enzymes concerned with protein digestion, however, may be responsible for certain other observations in coeliac disease. Weijers and van de

Kamer (1955) showed in 50 children with coeliac disease that there was a greater rise in blood glutamine after oral gliadin than in control subjects. Similar observations have been made after feeding gluten to adult coeliac patients. Douglas and Booth (1969) studied nine control subjects and six patients with coeliac disease before and after treatment with a gluten-free diet. The amino-acids in the blood were measured by ion exchange chromatography before and after giving an oral load of 25 g. of gluten (Fig. 14). In the untreated coeliac patients there was a surprising and striking increase in the amino-acids above normal levels at two hours, but the pattern became normal after treatment with a gluten-free diet. This observation is difficult to explain, but it is possible that in untreated coeliac patients in whom there are secondary deficiencies of enzymes concerned with protein digestion peptides and polypeptides may escape digestion during absorption and pass on into the body relatively unchanged. They may then only be more slowly broken down to amino-acids by the liver, thus yielding high levels in the untreated state. The normal results after treatment may be particularly significant, since they suggest that the mucosa is functionally nor-

FIG. 14.—Plasma amino-acid curve after feeding 25 g. of gluten to control subjects and patients with coeliac disease before and after treatment. (From Douglas and Booth, 1969.)

mal so far as gluten is concerned after treatment. They provide no support for the hypothesis of a specific enzyme defect.

The conclusion of this lecture, with a list of references, will be published in our next issue.

Plasma Propranolol Levels in the Quantitative Assessment of β -adrenergic Blockade in Man

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Summary: Plasma propranolol levels associated with reductions in endogenous and exogenous cardiac β -stimulation were determined in normal people. The levels associated with a given degree of blockade of exercise-induced tachycardia were about three times greater after intravenous administration than after oral administration. This shows that an active metabolite of propranolol is formed only after the drug is taken by mouth. The greatest reduction in the tachycardia of strenuous exercise was associated with plasma levels of 40 ng./ml. with oral administration and 100 ng./ml. with intravenously administered propranolol.

The effect on isoprenaline-induced tachycardia following intravenously administered propranolol showed that the dose ratio for isoprenaline was about 30 with plasma levels of 100 ng./ml. and 10 with levels of 10-20 ng./ml. These plasma levels give 100% and 20-30% blockade of exercise-induced tachycardia. These findings suggest that some of the therapeutic effects of propranolol may be unrelated to β -adrenergic blockade.

Introduction

There is surprisingly little information about the quantitative aspects of β -adrenergic blockade in man. For example, it is not yet possible to predict the dose of drug required to produce a given degree of β -adrenergic blockade in a given patient. Furthermore, the dose used may be greater than that required for complete blockade of endogenous sympathetic stimulation, when another property of the drug might be

responsible for its therapeutic effect. In pharmacological terms an accurate knowledge of the dose/response relationship is required, but the precise measurement of both dose and response has proved difficult in practice.

The dose should be related to the concentration of the antagonist drug to which the target organ is exposed. In practice this requires a method for measuring the plasma levels of the drug, as the concentration of a drug in the plasma (and therefore reaching the target organ) after a fixed dose may vary widely among individuals, especially when the drug is given by mouth. A suitable test of β -adrenergic stimulation has also posed problems, for it should be capable of producing with safety a maximal effect which, if possible, involves an increase in sympathetic stimulation rather than withdrawal of vagal tone. All the simple tests available have some disadvantages, but the tachycardia produced by exercise appears to be the most satisfactory, for if the work is strenuous it does not seem to be mediated by change in vagal tone (Robinson *et al.*, 1953).

An exogenous stimulus, such as isoprenaline, can be used, but its effects have not yet been related to those of endogenous sympathetic stimulation, and it does not follow that, if the effects of a given dose of isoprenaline are abolished by an antagonist, those of sympathetic nerve stimulation will be affected to the same extent. The test procedure having been defined, a full range of doses of the antagonist, including those producing a maximal effect, should be investigated.

The present study was designed to define the plasma-concentration/dose-response relationship for propranolol by measuring the degree of blockade of the tachycardia produced by exercise at various work loads. The antagonism of an isoprenaline-induced tachycardia was then determined to compare the effects of the drug on endogenous and exogenous stimulation of cardiac β -receptors.

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