REVIEW ARTICLE

Organ culture of gastrointestinal mucosa—a technique for the study of the human gastrointestinal tract

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Organ culture developed about 50 years ago, mainly for the study of morphogenesis in embryological tissue. Fell and co-workers in Cambridge studied many embryological events *in vitro* (Fell, 1940), and organ culture came to be defined as that type of culture where complete rudiments or fragments of organs were cultivated with a minimum of outgrowth and enlarged as a whole (Fell, 1953; Parker, 1961). Organ culture thus provided a means of preserving normal histological structure *in vitro* and of supporting normal embryological development.

Trowell successfully applied the technique to adult animal tissue, reporting his definitive method of organ culture in 1959. He successfully maintained pieces of several adult rat organs *in vitro* for up to 10 days. He did not attempt to culture gastrointestinal tissue, stating that he thought the problem of infection would be too great (Trowell, 1961).

It was not until 1969 that Browning and Trier reported a method for maintaining adult human intestinal mucosal biopsies *in vitro* for a 24-hr period using Trowell's technique. Since then several groups of workers have used the technique to study human gastrointestinal mucosa. It seems an ideal tool with which to study diseases of the gastrointestinal mucosa such as coeliac disease or ulcerative colitis. Using such a technique of culture, studies can be performed with control over various factors affecting the tissue. Although care should be exercised in extrapolating conclusions from *in vitro* experiments to the *in vivo* situation, such cultures are useful for the study of disease where there is no animal model or naturally occurring animal disease with which to compare it.

Katz and Grand (1979) summarized the potential of organ culture of gastrointestinal tissue by stating "it is clear that intestinal organ culture offers an opportunity to characterize specific cellular abnormalities induced by various types of mucosal injury, to study the effects of offending antigens on intestinal structure and function, and to identify abnormalities

in local immune function induced by such *in vitro* exposure". It is hoped to show in this review that some of this potential has been realised, particularly in relation to the study of coeliac disease.

The technique of organ culture for gastrointestinal mucosa

Browning and Trier (1969) described a method which has been used, with minor modifications, by several groups of workers, including the present author (Falchuk *et al.*, 1974a; Jos *et al.*, 1975; Fluge and Aksnes, 1981; Howdle *et al.*, 1981a). Usually mucosal biopsies are obtained for culture using an intestinal mucosa biopsy instrument. Biopsies are cut into pieces of about 3 mm in diameter and placed in the culture system within 5 min of excision. The pieces of mucosa are orientated villous or mucosal surface uppermost on a sterile stainless-steel wire grid and placed in the central well of a sterile culture dish (Fig. 1). The central well is filled with culture medium so as just to touch the under surface of the mucosa, a thin layer of medium being drawn over the mucosal surface by capillary action. The outer well contains a felt pad saturated with saline, and the whole dish is kept at 37°C in an atmosphere of 95% O₂: 5% CO₂ for the culture period. The culture medium consists of commercially available prepared media of known constitution together with an added biological medium, such as fetal calf serum.

Other investigators have modified the technique to the extent of using a roller tube apparatus (Mitchell, Mitchell and Peters, 1974; L'Hirondel, Doe and Peters, 1976) or a rocker platform (Autrup *et al.*, 1978; Autrup, 1980).

However, Trowell's main principles for the technique were to support the tissue in contact with, but not submerged in, suitable culture medium, in an oxygen-enriched atmosphere, and these principles have been utilized in all the methods described for this type of culture.

FIG. 1. Diagrammatic representation of organ culture system.

Assessment of cultured tissue

A recurring problem for investigators using this technique has been one of assessing the cultured tissue. The most obvious method has been to compare uncultured histological sections with those from cultured biopsies, and there are several reports of such a subjective comparison being made where mucosa has been "reasonably well-maintained" (Browning and Trier, 1969) or "well-preserved" (Mitchell *et al.*, 1974; Falchuk *et al.*, 1974a; Jos *et al.*,

1975). Such descriptions are a useful means of assessment. Examples of well-preserved mucosa after 24 or 48 hr of culture are shown in Figs. 2 and 3. However, if the technique were to be used to investigate a particular disease, there was a need to define the behaviour of normal and abnormal tissue during culture using more objective criteria. To this end biochemical/functional and morphological measurements have been made on mucosa before and after culture. These types of measurements have mainly been restricted to the study of small intestinal mucosa *in vitro*.

Browning and Trier (1969), for example, showed that ^3H -thymidine was taken up by undifferentiated crypt cells in normal jejunal mucosa and that the cells then migrated normally up the sides of the crypts during culture. They also showed that after culture the absorptive enterocytes could still take up labelled oleic acid and incorporate it into newly synthesised triglyceride (Trier, 1974). Eastwood and Trier (1973a) similarly showed epithelial cell proliferation in cultured rectal mucosa, and Trier and his colleagues went on to show steady state synthesis and secretion over 24 hr of culture of proteins and

FIG. 2. (a) Normal jejunal mucosa preculture ($\times 40$); (b) Mucosa from same patient after 24 hr culture ($\times 40$).

FIG. 3. (a) Normal rectal mucosa preculture ($\times 40$); (b) Mucosa from same patient after 48 hr culture ($\times 40$).

glycoproteins (MacDermott, Donaldson and Trier, 1974).

In 1974 Falchuk and colleagues published their first report of a series of experiments aimed at studying the pathogenesis of the mucosal abnormality in coeliac disease using the organ culture technique (Falchuk *et al.*, 1974a). In coeliac disease the small intestinal mucosa is damaged by the gluten component of wheat, the damage being predominantly sustained by the surface epithelial cells (enterocytes). Damage to the brush border region of the enterocytes is therefore a sensitive sign of gluten toxicity, which can be shown morphologically (Shiner, 1974) or biochemically by measuring the activity of brush border enzymes (e.g. alkaline phosphatase, γ -glutamyl transpeptidase, α -glucosidase (Peters, Jones and Wells, 1978)). Falchuk *et al.*, (1974a) decided to assess cultured tissue biochemically by measuring brush border enzyme activity. They measured alkaline phosphatase activity in whole homogenates of mucosa before and after culture. Such activity increased during culture in both normal and coeliac tissue, but there was a much greater increase in untreated coeliac mucosa than in normal or treated coeliac mucosa. This increase in activity was inhibited in untreated coeliac mucosa by the presence of gluten peptides. Thus they had defined the behaviour of normal jejunal mucosa during culture in terms of alkaline phosphatase activity and used this as a reference for the behaviour of coeliac mucosa under different experimental conditions. This type of assessment has been used by Jones, L'Hirondel and Peters (1981, 1982).

As already mentioned above, the surface epithelial cells bear the brunt of the damage in coeliac disease, becoming cuboidal and disorganised with a loss of cell height. They are known to respond quickly *in vivo* to the withdrawal of gluten from the diet (Stewart, 1974). Objective morphological measurement of jejunal mucosa was therefore suggested by Howdle *et al.* (1979) as being a useful means of assessment. They measured the height of the enterocytes in jejunal mucosa before and after culture. The enterocyte height fell during culture in normal mucosa and treated coeliac mucosa, but increased in untreated coeliac mucosa, this increase being inhibited by the action of gluten peptides. Such means of assessment of cultured tissue enabled the organ culture technique to be used to provide an *in vitro* model of coeliac disease (Howdle *et al.*, 1981b). Other workers confirmed these results using similar morphometric measurements (Fluge and Aksnes, 1981; Bramble, Watson and Record, 1981; Olives *et al.*, 1981).

Morphometry has proved to be a useful means of assessing cultured tissue, particularly since there has been some disagreement about the biochemical

changes initially reported by Falchuk *et al.* (1974a), others not finding them so reproducible (Hauri *et al.*, 1978; Howdle *et al.*, 1981a; Fluge *et al.*, 1982). Morphological assessment would also seem to be more appropriate since mucosal diseases, such as coeliac disease, are almost universally diagnosed using morphological criteria.

Cultured tissue has therefore been assessed either subjectively or objectively, by comparing post-culture findings with those in uncultured biopsies. The subjective observations, mainly of the preservation of histological structure, have been used by most workers. Objective criteria have more recently been developed in an attempt to use the organ culture technique as a reliable and reproducible means of studying particular diseases *in vitro*.

Human gastrointestinal mucosa cultures

Oesophagus/stomach

The organ culture technique has been orientated towards the study of diseases which affect the mucosa, such as coeliac disease or ulcerative colitis. There have therefore been few reports of the culture of mucosa from oesophagus or stomach. Another reason why there are few reports of oesophageal or gastric mucosal culture is that such mucosae have been less well-preserved in organ culture. Recently, however, it has proved possible to culture oesophageal mucosa for several weeks (Hillman *et al.*, 1980), raising the possibility of testing oesophageal carcinogens *in vitro*. Bachir, Collis and Joffe (1982) have demonstrated the toxicity of bile salt conjugates on oesophageal mucosa *in vitro*. Organ culture of human gastric mucosa has not been very satisfactory since, although antral mucosa can be successfully cultured, fundal mucosa shows degenerative changes within 6 hr of culture (Trier, 1976; Donaldson and Kapadia, 1980). Interestingly, rabbit gastric fundal mucosa has been cultured satisfactorily (Sutton and Donaldson, 1975; Kapadia and Donaldson, 1978).

Large intestine

As already referred to, rectal mucosa has been successfully cultured using this technique (Eastwood and Trier, 1973a; MacDermott *et al.*, 1974). Eastwood and Trier (1973b) showed there were increased rates of proliferation and migration of epithelial cells in active ulcerative colitis compared with normal when rectal mucosa was studied in organ culture. The same group of workers also showed that active ulcerative colitic mucosa incorporated increased amounts of labelled glucosamine into glycoproteins and secreted glycoproteins more

rapidly into the culture medium during organ culture than did normal mucosa (MacDermott *et al.*, 1974).

Alpers *et al.* (1980) and Serafini, Kirk and Chambers (1981) studied mucosa from chronic ulcerative colitis patients using the organ culture technique. The former investigators demonstrated an alteration in the control of DNA synthesis in this tissue, and the latter an increased rate of epithelial cell proliferation, similar to that in regenerating or "pre-cancerous" mucosa. The suggestion from these studies is that such abnormalities predispose to the increased incidence of carcinoma in ulcerative colitis.

Other investigators have shown that actively inflamed ulcerative colitic mucosa produces increased amounts of prostaglandin E₂ during organ culture, this production being inhibited by the presence of prednisolone, sulphasalazine and flufenamic acid in the culture medium (Sharon *et al.*, 1978; Rachmilewitz *et al.*, 1978; Hawkey and Truelove, 1981). The relevance of these *in vitro* observations needs careful evaluation since, for example, the treatment of ulcerative colitis with prostaglandin synthetase inhibitors has been unsatisfactory (Gilat *et al.*, 1979; Campieri *et al.*, 1980).

An exciting development in the technique of organ culture comes from the work of Autrup and colleagues (1978, 1980). They have succeeded in producing satisfactory preservation of human colonic mucosa for up to 28 days by careful manipulation of the culture conditions, including the use of a rocker platform. They hope this will be of use in identifying potential carcinogens and in assessing chemotherapeutic agents.

Small intestine

Reference has already been made to the initial report of organ culture of jejunal mucosa (Browning and Trier, 1969). Since then most of the gastrointestinal work in the field of organ culture has been in studying small intestinal mucosa. This is perhaps because, as suggested above, mucosal diseases are eminently suitable for the application of this technique. The common mucosal disease, coeliac disease, has been studied in detail in this way. The remainder of this review will describe how organ culture has been used to study coeliac disease, and the way in which the results have contributed towards the further understanding of the disease.

Organ culture and coeliac disease

Coeliac disease is that disease in which there is an abnormality of the small intestinal mucosa provoked by the gluten fraction of wheat (Scott and Losowsky, 1977). This implies that the mucosal abnormality has to be related to the presence or absence of gluten in the diet and that biopsies on at least two different

occasions are needed. Two other basic problems in the understanding of the disease are first, what is the precise nature of the toxic component within gluten which is responsible for provoking the mucosal abnormality and, second, how does this toxic component produce the abnormality. In using organ culture to provide an *in vitro* model of coeliac disease, investigators have addressed themselves to these problems.

An in vitro model of coeliac disease

It was first necessary in such investigations to show that a model of coeliac disease had indeed been established. Falchuk *et al.* (1974a), using changes in alkaline phosphatase activity as a means of assessing cultured jejunal mucosa, as already described, showed that untreated coeliac mucosa during culture behaved differently in the presence of Frazer's gluten fraction III (Frazer *et al.*, 1959), the expected increase in enzyme activity, believed to represent the normal maturation process, being inhibited by the presence of gluten. They showed that the inhibition of the rise in alkaline phosphatase activity in untreated coeliac mucosa was specific for gluten since it was not produced by the inclusion of casein in the culture medium. They also showed that the inhibitory effect of gluten was specific for coeliac mucosa since the increase in alkaline phosphatase activity occurred in the presence of gluten in mucosa which was abnormal for reasons other than coeliac disease. These authors used the model in a prospective manner to make a definite diagnosis of coeliac disease, using the initial biopsies (Katz and Falchuk, 1978). Forty patients who had an abnormal jejunal mucosal lesion were assessed; 26 were eventually diagnosed as having coeliac disease, having shown a mucosal response to a gluten-free diet and deterioration on gluten challenge. Twenty-two of these 26 eventual coeliac patients exhibited gluten sensitivity *in vitro* in that the rise in alkaline phosphatase activity was inhibited by the presence of gluten peptides in the culture medium—thus there were four out of 26 (15%) coeliac patients who gave false-negative results. On the other hand, of the 14 patients with an abnormal mucosa who were eventually shown not to have coeliac disease, 13 of these did not show *in vitro* gluten sensitivity, a false-positive rate of one in 14 (7%). These *in vitro* results were therefore very encouraging, demonstrating that the organ culture model is potentially useful in the differential diagnosis of an abnormal jejunal mucosa.

In vitro testing of possible toxic fractions in coeliac disease

This model of coeliac disease has also been used in the search for the toxic moiety of gluten. The toxicity

is known to lie within the gliadin fraction, that is the ethanol soluble part of gluten or wheat flour. The gliadin fraction of wheat has been shown to be a complex mixture of components (Evans and Patey, 1974) and gliadin is subdivided into four somewhat arbitrary fractions (α , β , γ and ω gliadins) with respect to their mobility on starch gel electrophoresis (Jones, Taylor and Senti, 1959; Kasarda, Bernadin and Nimmo, 1976). There is a widespread belief that the toxic component for coeliac patients lies within the α -gliadin fraction but there are difficulties in interpreting the results supporting this belief (Howdle *et al.*, 1984). It has therefore proved very useful to be able to test the toxicity of various gliadin fractions upon untreated coeliac mucosa during organ culture, particularly since only small amounts of these fractions are available owing to the difficult fractionation procedures. *In vivo* testing would not only need much larger amounts of various fractions to be available, but would entail a lengthy process of oral challenge in patients in remission, with the need for multiple jejunal biopsies.

Jos *et al.* (1978) were the first to use this technique to study the effects of different gliadin fractions on untreated coeliac mucosa. They prepared peptic-tryptic digests of α , β , γ and ω gliadins and assessed their *in vitro* effect by light microscopy after 48 hr of culture, using a grading system which noted either "reparation, no reparation, impairment, or extensive necrosis" of the mucosa. No objective measurements were made. However, their results revealed undoubted toxicity of α and β gliadins, less toxicity being evident for γ gliadin, and ω gliadin being considered to be "completely harmless".

In 1981 Jos and colleagues began to perform morphological measurements (Olives *et al.*, 1981) and in 1982 (Jos *et al.*, 1982) used these to assess the *in vitro* toxicity of various gliadin digests. They showed that a similar fraction of molecular weight 7–8,000 obtained from each of α , β , γ and ω gliadin was toxic for untreated coeliac mucosa. Howdle *et al.* (1981c) in the meantime had tested the *in vitro* toxicity of well-defined preparations of the four gliadins, assessing the mucosa by measurement of pre- and post-culture enterocyte height. They showed that all the four were toxic to untreated coeliac mucosa and concluded that a common component within all the gliadins is responsible for their toxicity in coeliac disease (Howdle *et al.*, 1984). Ciclitira *et al.* (1984) obtained evidence in two treated coeliac patients, after intraduodenal instillation of the same α , β , γ and ω gliadins, that all were toxic, thus supporting the *in vitro* results. In 1982 Wieser *et al.* also showed that peptic-tryptic digests of the four gliadins (α – ω) were toxic in organ culture when tested on mucosa from one untreated coeliac patient,

using changes in alkaline phosphatase activity as the means of assessing the mucosa during culture.

The *in vitro* evidence suggests that all gliadins are toxic to coeliac patients and that the toxic moiety is common to all the gliadin polypeptides. Following this line of argument, Wieser *et al.* (1983) have prepared, for *in vitro* testing in organ culture, a peptide from whole (unfractionated) gliadin which consists of 53 amino acid residues, although the full amino acid sequence has not yet been worked out. Such a compound can easily be tested for toxicity in coeliac disease using the organ culture technique, and perhaps valuable information obtained as to the precise nature of the toxic component within gluten which is responsible for the disease.

In vitro studies in the pathogenesis of coeliac disease

As suggested above, the other basic problem in coeliac disease is concerned with pathogenesis. How does the toxic component in gluten produce the abnormality in the small intestine? The organ culture technique has been used to investigate this, particularly in the study of the immunological theory of causation. This area of investigation was the main one pursued by Falchuk and colleagues in their use of organ culture. As stated above, they assessed tissue by changes in alkaline phosphatase activity, the expected increase during culture being prevented in untreated coeliac mucosa by the presence of gluten peptides. The gluten had no inhibitory effect, however, on the rise in alkaline phosphatase activity in treated coeliac mucosa. This implied to the workers that gluten is not directly toxic to coeliac mucosa but that some host or endogenous effector mechanism must first be triggered to produce the damage to the mucosa. In untreated (active) coeliac mucosa this endogenous mechanism would presumably be activated by the gluten in the diet. Falchuk *et al.* (1974a) believe that the endogenous effector mechanism could be antigluten antibody produced by 'primed' active coeliac mucosa in the presence of gluten, tissue in remission (i.e. treated) being unable to produce significant anti-gluten antibody for damage to ensue. These authors went on to produce further evidence towards this hypothesis. They reported a series of ingenious experiments in 1974 (Falchuk, Gebhard and Strober, 1974b) involving the simultaneous culture of tissue from untreated and treated coeliac patients. They showed that the rise in alkaline phosphatase activity seen in treated mucosa during culture could be inhibited by the presence in the same culture dish of both untreated coeliac mucosa and gluten peptides, the effect being specific for these conditions. These results they felt strongly supported the concept that a humoral substance was produced by untreated tissue under the stimulation of gluten

which affected the treated tissue, the humoral substance being postulated to be antiglutin antibody. These authors (Katz *et al.*, 1976) went on to show that the presence of cortisol in the culture medium appeared to provide some protection from the *in vitro* effect of gluten, suggesting that it is acting via an immunosuppressant effect. More recently they also showed that untreated coeliac mucosa was more sensitive to the effects of gluten *in vitro* if the patient were HLA-B8 positive (Falchuk *et al.*, 1980). This provided a link with the effector arm of the immune response by suggesting that the HLA-B8 antigen on the enterocyte could form part of the receptor to which gluten (or its toxic moiety) may combine in order to become antigenic and stimulate an immune reaction.

It has to be stated that some of these results obtained by Falchuk and co-workers have not been reproducible by other workers and that therefore the hypothesis built up upon their work has still to be viewed with caution. For example, not all workers have found it possible to detect gluten sensitivity in coeliac mucosa *in vitro* by measuring changes in alkaline phosphatase activity (Hauri *et al.*, 1978; Howdle *et al.*, 1981a; Fluge *et al.*, 1982), neither has the relationship between *in vitro* gluten sensitivity and HLA-B8 been confirmed (Howdle *et al.*, 1981a, b; Arnaud-Battandier *et al.*, 1983). There are various reasons why this might be, such as differences in culture methods, gluten fractions, types of patients, or biochemical methods, so that the findings of Falchuk and colleagues, although the subject of some disagreement, may still provide important evidence as regards the pathogenesis of coeliac disease.

Other immunological investigation involving the organ culture technique has been directed at cell-mediated immune mechanisms. In an important preliminary communication in 1975, Ferguson *et al.* showed that untreated coeliac mucosa, under the stimulation of α -gliadin, produced a factor which was probably a lymphokine. This finding was confirmed by Howdle, Bullen and Losowsky (1982) using gluten fraction III as antigen. They also showed that production of this putative lymphokine ceased after the patients had been treated with a gluten-free diet. The finding of lymphokine production by coeliac mucosa under the stimulation of gluten during organ culture was some of the more direct evidence available that cell-mediated immune mechanisms may be involved in the pathogenesis of the mucosal abnormality in coeliac disease. Of course other explanations are possible as to the nature and role of this factor detected by Ferguson *et al.* and these possibilities are summarized by Howdle *et al.* (1982).

From this brief review of the use of the organ culture technique as applied to the study of coeliac disease, the importance of the technique can be seen.

Several authors have shown that an *in vitro* model of the disease has been established, and this model has then been used to investigate the two outstanding questions in this disease, i.e. what is the toxic moiety in gluten and how does this produce the typical mucosal abnormality? The potential of the technique is therefore apparent.

Conclusions

Despite the important observations which have been made, the technique is still in its infancy, and there are few published systematic studies designed to establish optimal growth requirements for the culture of mucosa from various areas of the gastrointestinal tract. Recently, the technique has been improved in this regard for the culture of colonic mucosa (Autrup, 1980). There are obvious limitations of the method, particularly in terms of relating *in vitro* findings to the *in vivo* situation. The type of culture may be too insensitive for the study of some intercellular mechanisms, although it would seem likely that to be able to study a whole piece of tissue in a controlled way, with the tissue components still in their normal relationship, would eventually prove to be an important tool in biological research.

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(Received 14 March 1984)