Immunological reactions in gastrointestinal disease: a review

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All the major diseases of the gastrointestinal tract occurring in a temperate country like Great Britain are of unknown aetiology. Efficient public health measures and the development of sulphonamides, antibiotics, and vermifuges have sharply reduced the amount and severity of disease directly caused by the infections and infestations. Side by side with this improvement, chronic duodenal ulcer, regional enteritis, idiopathic steatorrhoea, appendicitis, diverticulosis, and ulcerative colitis have been much more widely recognized during the present century than previously. In some cases this is doubtless due to improved diagnosis but in others there is strong evidence of a true increase in incidence, a notable example being chronic duodenal ulcer (Craig, 1948).

In all the diseases which we have just mentioned it is usual to find that several theories of aetiology have been advanced and that they are the same theories for each disease, namely, infective, nutritional, allergic, and psychosomatic. With the single possible exception of coeliac disease and idiopathic steatorrhoea, nothing conclusive has been added to our knowledge of causation during the past 20 years and textbooks of medicine continue to reiterate the same four general possibilities without substantial change.

Recently, the role of immunological processes has excited interest far outside the sphere of infective disease, to which it was largely confined for many years, and much of this interest has been focused on the possible ways in which immunological hypersensitivity may play a part in causing or perpetuating disease.

Immunological hypersensitivity may be defined as an immunological response of the subject to substances which provoke no visible or a much smaller response in normal individuals, or a response of the hypersensitive subject to much smaller quantities of such a substance than would provoke response in normal individuals (Boyd, 1956). Hypersensitivity may express itself as an immediate-response type of reaction, characterized by rapidly appearing skin reactions following intradermal injection of the

appropriate antigen and often associated with the presence of circulating antibody to the antigen, or as a delayed-type hypersensitivity, characterized by slowly developing skin responses of the tuberculin type, not necessarily associated with circulating antibody and transferable by living cells and not by serum. The difficulties of demonstrating and quantitating the latter are well recognized today and much effort is being directed to this problem, so far with little success. Skin tests have proved of little value except in certain diseases such as tuberculosis. However, more sensitive methods of detecting circulating antibodies have been devised (see Boyden, 1959) and such improvements have stimulated a broadening interest in immunological processes. Another stimulating factor is the escape from the concept that the body does not respond immunologically to any of those of its own constituents which might act as antigens in other animals. Although this view, which was advanced strongly by Ehrlich, was early challenged by Widal and others, who suggested that certain cases of haemolytic anaemia might be due to autohaemagglutinins in the patient's own sera, the phenomenon of autoimmunization was not generally recognized until the last 10 to 15 years. A great deal of experimental work has shown that animals may be sensitized to their own tissues or extracts of their own tissues, and that such sensitization may, to a variable degree, be both organ and species specific. Techniques of sensitization usually, but not always, involve the use of special adjuvants of the Freund type (Freund and McDermott, 1942). Circulating antibodies to the sensitizing antigen and inflammatory changes in the target organ, the two not necessarily showing correlation, have resulted. In such affected organs characteristic lesions appear. Using such techniques, experimental thyroiditis (Rose and Witebsky, 1956), adrenalitis (Colover and Glynn, 1958), encephalitis (Rivers, Sprunt, and Berry, 1933), hepatitis (Behar and Tal, 1959), to name only a few, have been produced. Another point which may be relevant is that circu-

lating auto-antibodies may be produced experimentally by using haptens. Thus Ackroyd (1955) elegantly demonstrated that auto-antibodies were responsible for the purpura associated with taking the drug Sedormid, the patient's own platelets acting as the protein moiety and the drug as the hapten moiety of the sensitizing antigen. The importance of such observations in relation to the part both exogenous proteins and haptens, such as drugs, may play in the development of hypersensitization will be referred to later.

However, two aspects of the experimental work deserve special mention. First, the tendency is for those inflammatory changes induced in an organ to be somewhat short-lived and rarely chronic, and secondly, attempts to produce models of human disease of the gastrointestinal tract in animals by such means have not been successful (Taylor, 1959a; Coghill, 1960) with the single possible exception reported by Wolf and his colleagues (Hennes, Sevelius, Llewellyn, Woods, Joel, and Wolf, 1960) of the production of gastric atrophy in dogs; these results have not yet been confirmed.

In man the finding of circulating auto-antibodies in disease and in apparent health is becoming almost commonplace and yet it is still quite unclear what significance such antibodies have in relation to the causation of lesions in any disease. That circulating antibodies against tissue constituents may cause organic lesions gains no support from experimental attempts to produce such lesions by transfusion of sera containing high concentrations of antibody activity from immunized to non-immunized animals of the same species, and the only way of transferring experimental auto-immune disease is by adoptive transfer of living lymph nodes or lymphocytes, a manoeuvre which may also transfer delayed-type hypersensitivity. Nevertheless, Pulvertaft, Doniach, Roitt, and Hudson (1959) have demonstrated cytotoxic activity of human anti-thyroid sera *in vitro*, and in the acquired haemolytic anaemias and thrombocytopaenic purpura there is a strong relationship between circulating antibody and destruction of red cells and platelets respectively. In disseminated lupus erythematosus circulating anti-nuclear factors are well recognized but that these factors reach the nuclei of cells and damage them *in vivo* seems rather unlikely. The Hargraves L.E. cell is only produced *in vitro* after imposing some damage on white cells. At present it is easier to suppose that circulating antibodies have occurred as a result of damage to tissue, resulting in the release of antigenic material previously quarantined from the immunologically competent mechanisms of the body, so that immunological tolerance to such material has not been acquired. Thus after acute myocardial infarction

auto-antibodies to heart muscle may be found (Dornbusch, 1957).

There is at present a tendency to give far more attention, in disease, to auto-immune processes than to possible immune responses to exogenous antigens or to the foreign hapten-protein complexes mentioned above.

The gastrointestinal tract is repeatedly exposed to a welter of foreign substances contained in food, many of which may conceivably have an antigenic action in appropriate circumstances. The colon possesses a rich bacterial flora, which, though not normally pathogenic in that site, can possibly become so. In a variety of diseases, this flora may be altered and it may also be changed by various medical treatments, such as antibiotics. Bacterial disease, either locally in the gastrointestinal tract, or elsewhere in the body, may be harmful to the digestive tract through immunological mechanisms. These various factors may inter-react.

Food proteins can act as antigens and allergic responses to many different foods are known to occur. The reaction may take the form of a skin rash, asthma, migraine, or other manifestations outside the gastrointestinal tract and such occurrences are conventionally classed as examples of alimentary allergy. Alternatively, the symptoms may be wholly gastrointestinal in nature or both types of symptom may occur together. When the gastrointestinal tract is the site of disturbance the term 'gastrointestinal allergy' is employed. The distinction does not seem to us to be of any fundamental importance although there may be a certain convenience in having the terms for the purpose of clinical description. We shall here consider only those diseases of the gastrointestinal tract in which immunological reactions to foods may be causal or perpetuating.

SJÖGREN'S DISEASE

This was the first disease involving any part of the gastrointestinal tract in which auto-antibodies were demonstrated. The full clinical picture, which is not always seen, is of kerato-conjunctivitis sicca, with dryness of the mucosal surfaces of the mouth and pharynx, intermittent swelling of lacrimal and salivary glands, and chronic polyarthritis of the rheumatoid type. Cardell and Gurling (1954) first drew attention to the similarities between the histological appearances of the salivary and lacrimal glands seen in this condition and those of the thyroid in Hashimoto's disease, the most striking feature being the lymphocytic and plasma cell infiltration, a phenomenon common to many of the other diseases we shall be considering later. Jones (1958) found in the sera of patients with Sjögren's syndrome circu-

lating antibodies to extracts of salivary and lacrimal tissue, though these antibodies were not organ specific. This observation was confirmed by Anderson, Gray, Beck, and Kinnear (1961), who found that nine of 29 different sera tested reacted with factors present in salivary and lacrimal tissue and the same sera also reacted with extracts of a number of other tissues. Heaton (1959) found a positive L.E. test in a third of patients with the syndrome and recently antibodies to thyroglobulin have been demonstrated in 27% of cases (Bunim, 1961) and in 42% of cases (Anderson *et al.*, 1961). Though, clinically, a significant association between Sjögren's syndrome and thyroiditis has not been established, these immunological results do suggest that these diseases may have some factors in common, but all that can be said at present is that some patients with Sjögren's syndrome possess unduly responsive immunological mechanisms capable of forming antibodies which have not been fully characterized.

APHTHOUS ULCERATION OF THE MOUTH

Ulceration of the mouth may occur as one manifestation of a number of diseases, such as acute leukaemia, agranulocytosis, pellagra, the Stevens-Johnson syndrome, and Behçet's disease. The familiar aphthous ulcers are very common in their usual, minor form. Severe, recurrent aphthous ulceration is not rare in persons who are otherwise healthy, and, in addition, it is a feature of certain gastrointestinal diseases. In tropical sprue it occurs frequently (the word 'sprue' comes from the Dutch word *spruw* meaning aphthous disease (Dubois and van den Berghe, 1948)). In idiopathic steatorrhoea, also, it is common (Cooke, Peeney, and Hawkins, 1953).

Crohn (1949) states that three of 36 patients with ileojejunitis had aphthous stomatitis, and in ulcerative colitis clinical impression suggests a higher incidence of severe aphthous ulceration than would be expected as a chance coincidence (Rice-Oxley and Truelove, 1950; Samitz and Greenberg, 1951; Kelley, 1962).

The causes of recurrent aphthous ulcers are not known. Many authors regard them as an allergic manifestation. For example, Andresen (1953) stated that, 'in the mouth aphthous ulcers (canker sores) are the most frequent manifestations (of gastrointestinal allergy)'. Vaughan and Black (1954), in a detailed analysis of the symptoms in 150 cases of gastrointestinal allergy due to food, found that aphthous ulceration occurred in 13%.

While these and a number of other clinical allergists have regarded recurrent aphthous ulceration

as frequently allergic in origin, basing their opinions either on skin tests or on the effect of elimination diets, this view is far from generally held and, for example, receives little attention in the study of Sircus, Church, and Kelleher (1957) of 120 examples of the condition. Recently, Ship, Merritt, and Stanley (1962) have made intensive studies of six patients with severe recurrent aphthous ulceration and found no evidence of food allergy as a responsible factor. The view that aphthous ulcers frequently have an allergic basis must therefore be regarded as tenuous, although we and our colleagues have found that an excessive proportion of subjects with severe recurrent aphthous ulceration possess high titres of circulating antibodies to one or more of the proteins of cow's milk or to a gluten fraction (unpublished observations). However, the significance of these findings is at present uncertain.

DISEASE OF GASTRIC MUCOSA

The cause of chronic gastritis, gastric atrophy, and gastric ulcer, and the relationship between them, remain obscure. The development of gastritis and gastric atrophy must be symptomless in many, if not the majority, of cases (Coghill, 1960), making studies of the natural history of these conditions very difficult. Although gastric ulcer may be more eloquent and permit a reasonably good assessment of its incidence, such knowledge has shed little light on its causes: neither has the real fall in its incidence in England and in the United States in recent years.

Acute gastritis may be produced by the consumption of alcohol, or irritant drugs, by intercurrent infections, or by other factors. A remarkable feature is the rate of recovery of the mucosa in the large majority of patients (Palmer, 1954), a fact consistent with the rapid regeneration of the gastric mucosa in dogs after surgical excision (Milton, Maxwell, and Finckh, 1960). However, a follow-up study of patients who received x-irradiation to the stomach to suppress acid production (Ricketts, Kirsner, Humphreys, and Palmer, 1949) showed that some subjects developed mucosal atrophy. Another fact supporting the suggestion that repeated trauma may produce chronic gastritis (Faber, 1935) is the observation by Edwards and Edwards (1956) of a relationship between the heat of ingested fluids and the incidence of chronic gastritis. If in some individuals repeated minor damage can lead to the development of chronic gastritis, the factors ordinarily responsible remain unknown. Figure 1 is a hypothetical scheme, showing the possible factors concerned. Coghill (1960) and Taylor (1961) have discussed more fully some of these factors.

Gastric biopsy studies in patients with pernicious

FIG. 1.

anaemia have rendered untenable the earlier view that the mucosal changes in this disease are specific. In the rarely occurring juvenile pernicious anaemia the gastric mucosa may be histologically normal and only its capacity to secrete adequate amounts of Castle's intrinsic factor seems to be impaired. Published observations of the disease in the adult make it clear that structural abnormalities of the mucosa always occur. These consist of severe, atrophic gastritis or of a condition known as mucosal atrophy. The term gastric mucosal atrophy is applied when the gastric glands virtually disappear, the surface epithelium shows a marked degree of intestinal metaplasia, and the lamina propria is thin and does not show the heavy infiltration with plasma cells and lymphocytes which is seen in atrophic gastritis. The work of Wood and his colleagues showed that atrophic gastritis was often found in patients with pernicious anaemia (Doig and Wood, 1950; Joske, Finckh, and Wood, 1955). This finding is at variance with the previously held view that gastric mucosal atrophy is the specific lesion in pernicious anaemia (Magnus, 1952). The fact that many subjects have severe atrophic gastritis but do not show impaired absorption of vitamin B₁₂ suggests that chronic gastritis is a heterogeneous condition.

So far, immunological studies of gastritis have been confined to pernicious anaemia. Human and pig intrinsic factor preparations are capable of acting as antigens when injected into animals, with the

production of circulating antibodies, demonstrable by a biological test involving inhibition of intrinsic factor activity (Taylor and Morton, 1958; 1959). In the human disease, the sera of some patients who have been treated with oral vitamin B₁₂ preparations containing partly purified hog intrinsic factor have the capacity of inhibiting hog, human, and rat intrinsic factor activity (Schwartz, 1958). As in these patients the ability to respond to oral hog intrinsic factor becomes impaired but they can still respond to oral human intrinsic factor, it seems likely that two different, though possibly related, mechanisms may be operating.

Taylor (1959b) showed that the sera of some untreated patients with pernicious anaemia and some treated solely with parenteral vitamin B₁₂ inhibit intrinsic factor and suggested that the development of the gastric lesion in pernicious anaemia might be associated in some cases with auto-immunization. These observations have been confirmed by Schwartz (1960) and by Abels, Jansz, Woldring, Arends, and Nieweg (1961). The inhibitory activity is present in the globulin fraction of the serum and corticosteroids depress its activity, which is of interest as there are several clinical reports of enhanced absorption of vitamin B₁₂ by patients with pernicious anaemia given corticosteroid therapy (Frost and Goldwein, 1958; Gordin, 1959; Kristensen and Friis, 1960). Jeffries, Hoskins, and Slesinger (1962) have produced evidence that the inhibition of intrinsic factor activity is due to the presence of a circulating anti-

body by showing that the electrophoretic mobility of a vitamin B₁₂-intrinsic factor complex is significantly inhibited by appropriate sera. This has been confirmed by Taylor, Roitt, Doniach, Couchman, and Shapland (1962), who have also demonstrated the antibody nature of the inhibitor by means of a co-precipitation technique, using a labelled vitamin B₁₂-intrinsic factor complex. Recently Irvine, Davies, Delamore, and Wynn Williams (1962) and later Taylor *et al.* (1962) have demonstrated in the sera of a high percentage of patients with pernicious anaemia complement-fixing antibodies to extracts of human gastric mucosa. This antibody is not the same as the intrinsic factor inhibitor and it is present in a much higher percentage of pernicious anaemia sera than the intrinsic-factor-inhibiting factor. Taylor *et al.* (1962) have found that the complement-fixing antibody can be demonstrated by the immunofluorescent technique using gastric mucosal sections, in which most pernicious anaemia sera stain the cytoplasm of parietal cells specifically.

An interesting association is that between thyroid disease and pernicious anaemia (Tudhope and Wilson, 1960; 1962; McNicol, 1961). Auto-immune processes are known to occur in thyroid disease (Roitt, Doniach, Campbell, and Hudson, 1956) and it is conceivable that the same immune processes are responsible for changes in both organs. Irvine *et al.* (1962) have found that 37% of the sera of patients with pernicious anaemia contain complement-fixing antibodies to a saline extract of thyroid gland. Doniach, Roitt, and Taylor (1962) have examined the immunological relationship between pernicious anaemia and thyroid disease using a battery of all the accepted tests for thyroid antibodies. They have found that 55% of patients with pernicious anaemia over the age of 60 have positive reactions with one or more of these tests, whereas a control group matched for age and sex had a much lower incidence of positive reactions. Such correlative immunological studies may reveal unexpected links between diseases which at present we regard as totally unrelated.

COELIAC DISEASE AND IDIOPATHIC STEATORRHOEA

In coeliac disease, the clinical observations of a Dutch paediatrician during the Second World War suggested that removal of gluten from the diet resulted in marked improvement (Dicke, 1950). That wheat and rye gluten were harmful in the majority of cases was confirmed by Weijers and van de Kamer (1950) and by Anderson, Frazer, French, Gerrard, Sammons, and Smellie (1952).

Since that time, general experience has shown that children with coeliac disease maintained on a gluten-free diet are capable of normal growth and

development, although serial biopsy of the jejunum suggests that complete reversal of the histological changes is slow, if it does occur at all. Many adults with idiopathic steatorrhoea likewise benefit when treated with a gluten-free diet (French, Hawkins, and Smith, 1957) and such cases are sometimes referred to as adult coeliac disease. Two theories have been advanced to explain the injurious effects of gluten in the coeliac disease of infants and adults (Weijers and van de Kamer, 1950; Frazer, 1960). The Dutch workers considered that there was some direct toxic action by a component of gluten on the small-intestinal mucosa and they postulated an enzymic deficiency in the mucosa with resulting incomplete digestion of gluten and the accumulation of a glutamine-containing polypeptide in amounts sufficient to damage the mucosa. Weijers and van de Kamer (1960) have shown that when coeliac children are given gluten by mouth, blood glutamine levels are considerably higher than those found in healthy subjects under identical conditions. The harmful effect of gluten is retained when gluten is partly digested by pepsin and trypsin with the production of a soluble fraction known as fraction III (Frazer, Fletcher, Ross, Shaw, Sammons, and Schneider, 1959). Complete proteolytic digestion renders gluten harmless.

The second theory considers that coeliac disease is an allergic response. Berger (1958) reviewed the literature and showed that after the administration of gluten to a few children with coeliac disease changes occurred in the levels of circulating antibodies to gluten as measured by complement-fixation which were of a different order from those occurring in normal children. Taylor, Thomson, Truelove, and Wright (1961) compared the serological reactions in 24 children with coeliac disease and 50 healthy children and also compared those occurring in 60 adult patients with steatorrhoea and 60 healthy adults. They were unable to confirm Berger's observations employing complement-fixation tests. They were likewise unable to demonstrate precipitins by the Ouchterlony gel-diffusion technique, using fraction III. (Subsequent observations using a saline extract of gliadin have also given negative results although positive results have been reported by Heiner, Lahey, Wilson, and Peck, 1961.) The coated tanned red cell test yielded a sharp difference between coeliac disease and idiopathic steatorrhoea patients and their controls, with a heavy preponderance of high-titre reactions to gluten fraction III among the patients. By contrast, a small group of patients with pancreatic steatorrhoea behaved like normal subjects. Among the patients with idiopathic steatorrhoea, there was no appreciable difference between the reactions in those with a

history going back into childhood (adult coeliac disease) and the remainder.

A conspicuous finding was that the liability to high-titre serological reactions in coeliac disease and idiopathic steatorrhoea was not confined to gluten but also occurred with cow's milk proteins. The reactions to milk proteins were independent of the reactions to gluten and their biological significance remains uncertain. The greatly increased titres may be an indication of enhanced absorption of antigenic protein through a diseased mucosa or may represent an abnormal degree of specific response to one or more dietary proteins associated with hypersensitivity of the mucosa to these same proteins. The dissociation between the reactions to fraction III and to milk proteins is a point against regarding excessive absorption of antigenic protein as sufficient explanation. Furthermore, in ulcerative colitis the serological titres to fraction III resemble those in normal subjects and contrast with the findings with milk proteins. In spite of these objections to increased absorption of protein being responsible for the higher levels of circulating antibodies to dietary proteins in coeliac disease, idiopathic steatorrhoea, and ulcerative colitis, the issue must be regarded as unsettled because the abnormal absorption of one dietary protein compared with another may depend on their molecular sizes and rates of digestion and on whether the mucosal lesion is located high or low in the intestine.

Another approach to the problem of the effect of gluten on the intestinal mucosa in coeliac disease and idiopathic steatorrhoea has been made by Rubin and his colleagues (Rubin, Brandborg, Flick, Parmentier, Phelps, and van Niel, 1960). They took multiple ileal biopsy specimens in two patients with temperate sprue in remission and maintained on a gluten-free diet, before and during the instillation of wheat flour into the ileum. These subjects showed acute clinical and biochemical relapse and changes in the mucosa characteristic of the disease, in the vicinity of the site of instillation where the mucosa had been normal previously. None of these changes occurred in control subjects. The speed of response is compatible with an immunological reaction, but no immunological observations were made.

Corticosteroids have a beneficial action in coeliac disease and idiopathic steatorrhoea, which is manifest both clinically and biochemically. The response to corticosteroid therapy is more rapid but usually less complete than that obtained with a gluten-free diet (Finlay and Wightman, 1956). This effect of corticosteroids is readily explicable if immunological mechanisms play an important part in this disease but is difficult to explain if the underlying mechanism is a deficiency of one or more mucosal

enzymes. In forms of steatorrhoea due to faulty digestion, such as pancreatic and post-gastrectomy steatorrhoea, corticosteroid therapy has no effect (Drenick, Hvolboll, and Halsted, 1955).

ULCERATIVE COLITIS AND CROHN'S DISEASE

It is conventional to regard Crohn's disease and ulcerative colitis as two distinct diseases, because of differences in their histological pictures and in the different distribution of their lesions. In ulcerative colitis, the typical early mucosal changes consist of infiltration of the lamina propria with plasma cells, lymphocytes, and eosinophils, dilatation of the small blood vessels of the mucosa with diapedesis of red blood cells, and alterations in the epithelial cells which become enlarged with big nuclei possessing an abnormal pattern of chromatin. In addition, a supposedly distinctive lesion, the 'crypt abscess', is frequently found. Involvement of the muscular coat is a feature only of advanced disease and may not occur even in patients showing severe clinical manifestations. In Crohn's disease, the distinctive lesion is a microscopic granuloma composed of epithelioid cells frequently containing giant cells. The whole thickness of the intestinal wall is usually involved, as also are the regional lymph nodes, and the lymphatics are dilated. Probably related to these pathological features is the liability of Crohn's disease to form adhesions and fistulae, no matter where the primary lesion occurs, whereas in ulcerative colitis fistulae are much less common and are almost always pararectal when they do occur.

In spite of these histological and clinical differences, there are some reasons for regarding the two conditions as separate bands of the spectrum of a single type of disease process. Many of the complications remote from the intestinal tract are similar, such as arthritis, ankylosing spondylitis, iritis, conjunctivitis, and erythema nodosum. Epidemiologically, the two conditions appear to occur in parallel. When multiple cases occur in families, one member of the family may have classical ulcerative colitis while another has typical Crohn's disease. In the same patient, one may observe the pathological picture of Crohn's disease in the small intestine coupled with ulcerative colitis. Occasional patients have disease of the large intestine with histological features of both Crohn's disease and ulcerative colitis: for example, we have seen a colon with the entire mucosa showing the typical changes of ulcerative colitis while the muscular coat was greatly hypertrophied and contained many annular clefts composed of epithelioid granulomata with giant cells.

Our knowledge of the aetiology of both these conditions is fragmentary. Considerably more work

has been done in ulcerative colitis than in Crohn's disease and, in view of the similarities between them, it might be profitable to make studies of Crohn's disease in parallel with those which have yielded results in ulcerative colitis.

ULCERATIVE COLITIS

Immunological responses to three different types of antigen may play a part in the causation or perpetuation of ulcerative colitis. The antigens may be alimentary (foods, chemicals, or drugs), bacterial, or derived from the patient's own tissues.

ALIMENTARY ANTIGENS In 1925, an American physician, Andresen, described a case of ulcerative colitis which he considered to be due to food allergy. He continued to study ulcerative colitis from an allergic viewpoint and he subsequently reported that among 50 patients with ulcerative colitis no less than 33 were examples of food allergy, with milk as a responsible allergen in the majority while other foods incriminated were wheat, tomatoes, oranges, potatoes, and eggs (Andresen, 1942). Rowe (1942) reported somewhat similar views, basing his judgment on the effect of elimination diets.

This view gained little general support, probably because convincing experimental data are hard to obtain. We ourselves only became interested in this approach after finding that some patients not merely showed a favourable clinical response to a milk-free diet but also that these same patients would relapse soon after the reintroduction of milk into the diet (Truelove, 1961). This led us to compare the serological reactions to purified cow's milk proteins in ulcerative colitis and in health. Tests for precipitins, complement-fixation, and passive cutaneous anaphylaxis were uniformly negative but the coated tanned red cell test showed the presence of circulating antibodies to the milk proteins. High-titre reactions were much more common in ulcerative colitis than in health (Taylor and Truelove, 1961). Confirmatory findings have been reported by Gray (1961) and by Davidson (1961).

The exact significance of these findings cannot yet be assessed. Analysis of the data in relation to age, sex, radiological extent of the disease, activity of the disease, corticosteroid therapy, and diet yielded no clear-cut clues; the one positive finding was a significantly higher incidence of high-titre reactions to casein in patients with chronic disease as compared with those in a first attack. One way of assessing the aetiological significance of high levels of circulating antibodies to specific dietary proteins is to carry out a controlled therapeutic trial of various exclusion

diets and to relate the clinical responses to the initial antibody titres; such a trial is in progress.

Another approach to the experimental study of the relationship between food and ulcerative colitis has been adopted by Rider, Moeller, Devereaux, and Wright (1960). They have carried out intramucosal injections through a proctoscope of dilute preparations of egg, wheat, milk, and other food substances in patients with ulcerative colitis and in normal persons. In normal persons the rectal mucosa showed only mild, non-specific responses, whereas patients with ulcerative colitis showed sharp local reactions to one or more of the injected food proteins. The authors state that the patients showed a favourable clinical response when they were put on diets excluding the particular foods to which they had shown positive mucosal responses. It must be remarked, however, that full clinical details are not given of the subsequent course of the patients and, as we have already suggested in relation to circulating antibodies, it is imperative to check any experimental findings by clinical trial carried out under the most formal conditions. The approach of Rider and his colleagues has the virtue of dealing with reactions of the target organ of the particular disease and it may well prove to be valuable, in contradistinction to skin testing which is virtually useless.

If dietary proteins are important in the genesis of ulcerative colitis, there are clinical grounds for supposing that they do not necessarily exert their effect through being present in the lumen of the colon. When simple ileostomy without removal of the colon was the standard surgical approach to the treatment of ulcerative colitis, it was observed that the colon left *in situ* but unconnected with the upper gastrointestinal tract could show recurrences of the disease, even after long periods of apparent quiescence. Recently, when making use of double-barrelled ileostomy to facilitate topical treatment to the colon, we have occasionally observed recurrence of disease in an isolated colon which had previously become quiescent. If these recurrences bore any relation to ingested proteins, the effect must have been mediated by systemic spread via the blood or lymph. There are many analogous situations in allergic disease, because ingested protein may cause reactions in target organs outside the gastrointestinal tract, such as the skin and the joints.

This raises the question whether whole proteins, or at least antigenic moieties, can be absorbed from the healthy gastrointestinal tract. Anderson, Schloss, and Myers (1925) showed by precipitin tests and complement fixation that there was evidence that human infants do absorb dietary proteins. A later study by Lippard, Schloss, and Johnson (1936) suggests that, while the α -lactalbumin of cow's milk is absorbed

into the blood stream in detectable amounts in early infancy, this no longer occurs after a few months. The way in which the gastrointestinal tract becomes virtually impervious to dietary proteins is unknown but it is possible that immunological mechanisms are partly or solely responsible. It has been found that circulating antibodies to cow's milk proteins and to gluten fraction III can be demonstrated, at any rate in low titre, in almost all healthy subjects (Gunther, Aschaffenburg, Matthews, Parish, and Coombs, 1960; Wright, Taylor, Truelove, and Aschaffenburg, 1962). It is possible that the blood travelling through the intestinal mucosa bathes the tissues in antibodies and that these combine with any dietary proteins entering the tissues and prevent them from gaining access to the blood, at any rate in an antigenic form.

In view of the finding that young infants absorb cow's milk protein in an antigenic form the possibility arises that early artificial feeding may set in train a variety of immunological responses which have some bearing on later liability to specific diseases. It is therefore relevant that Acheson and Truelove (1961) found a significant excess of ulcerative colitis in patients who had been weaned from the breast during the first weeks of life compared with a control group matched for age and sex. Suggestions have been made that a number of other diseases, of which disseminated sclerosis is one, are especially common in subjects who were artificially fed in early infancy, but we do not know of any studies in which the evidence is sufficiently precise to be accorded any scientific weight.

The mechanism by which early artificial feeding may predispose to a specific disease much later in life is totally unknown. At first sight it might be supposed that the newborn baby is not immunologically competent to deal with any dietary proteins other than those of maternal milk. However, Wright *et al.* (1962) have found that the cord blood of newly delivered babies contains circulating antibodies to cow's milk proteins and to gluten fraction III and that the titres in the cord blood are frequently higher than those of the corresponding maternal blood. It is presumed that these antibodies reach the foetus by transplacental transfer from the mother since it has been established that this is the important route of transfer of antibodies in primates (Bangham, Hobbs, and Terry, 1958; Dancis, Lind, Oratz, Smolens, and Vara, 1961). The pattern of circulating antibodies to dietary proteins after delivery has yet to be studied in detail in relation to infant feeding.

AUTOGENOUS ANTIGENS Recently there has been some evidence that auto-immune processes may operate in ulcerative colitis. Broberger and Perlmann (1959) found evidence of circulating antibodies to

human colon, liver, and kidney in the sera of some children suffering from this disease and also in the sera of a few patients with rheumatoid arthritis and acute nephritis. In the case of ulcerative colitis sera, they obtained 28 out of 30 significantly positive results using the colonic extract and about half the number in the case of the liver and kidney extracts. Available evidence suggested that the reacting antigens in the three extracts were identical and subsequent work (Broberger, 1961) tends to confirm this hypothesis. They found it necessary to employ foetal colon to make their antigen extract. Subsequently, Asherson and Broberger (1961) reported that positive results were also obtained in some patients with diffuse lupus erythematosus and some other diseases so that the reaction may not be specific for ulcerative colitis. Perlmann and Broberger (1960) have demonstrated auto-antibodies against antigen derived from colon in the microsomes of regional colonic lymph nodes in human ulcerative colitis. These antibodies were not present in regional ileal lymph nodes.

Polcak and Vokurka (1960), using collodion particles coated with an extract of normal human colon, obtained positive precipitin tests with the sera of 30 patients with ulcerative colitis, while a large control group of normal subjects and patients with other diseases gave uniformly negative results. On the other hand Maratka (1961), using a similar technique, found that about 30% of sera from patients with diseases other than those of the gastrointestinal tract gave positive reactions with extracts of adult colon.

Some workers have found it impossible to reproduce the results of Broberger and his colleagues but they may have failed to do so for technical reasons. For example, Gray, Walker, and Thompson (1961) have reported negative findings in 50 subjects with ulcerative colitis but, instead of the untanned cells used by the Scandinavian workers, they used tanned cells which do not adsorb polysaccharides efficiently.

Some years ago, we ourselves looked for precipitin reactions in the sera of patients with ulcerative colitis, using a saline extract of human colonic mucosa and employing both tube and gel-diffusion techniques. Positive reactions were obtained with the sera of some patients with ulcerative colitis but similar reactions also occurred with some of the control sera. We therefore discounted these results as being of any significance in ulcerative colitis. In retrospect, the positive findings may have represented serological reactions to bacterial antigens included in our extract, a possibility which Broberger and Perlmann have emphasized. We also attempted to show uptake of human serum globulin by sections of fresh-frozen human colonic mucosa using a

fluorescein-conjugated anti-human globulin; the reactions were uniformly negative in the sera of 24 adult ulcerative colitis patients so tested. Recently, Broberger and Perlmann (1962) found that six out of 13 sera from children with ulcerative colitis gave positive results by the same technique.

Another approach to the problem of auto-immune processes in ulcerative colitis has been the search for antinuclear factors in the sera of patients with this disease. Calabresi, Thayer, and Spiro (1961) have demonstrated the presence of such a factor, which appears from its uptake by calf thymus to be different, however, from the antinuclear factors present in diffuse lupus erythematosus.

BACTERIAL ANTIGENS Bacteria which damage the gastrointestinal tract usually cause acute disease which appears to undergo complete resolution. There have been numerous attempts to find an infective cause for ulcerative colitis, but without success. Nevertheless, there is some evidence that bacterial infection may play a role in the disease. Felsen (1936) studied the subsequent fate of 122 persons who were involved in an outbreak of bacteriologically proven bacillary dysentery in Jersey City and found that 10 (8.2%) of them subsequently developed ulcerative colitis. It is also notable that three patients (2.5%) subsequently developed regional ileitis. Any physician with much experience of ulcerative colitis will have seen patients who were normal until they were involved in an outbreak of infective enteritis, when they developed chronic ulcerative colitis although all the remaining sufferers from the enteritis were back to normal health in a few days. Such patients are not examples of chronic bacillary dysentery because the organisms are not present in the faeces and treatment with antibiotics and sulphonamides does not bring rapid relief. The mechanism whereby a short-lived infection can induce a chronic disease remains obscure but at least four possibilities can be advanced as potential explanations. First, by damaging the intestinal mucosa, the infection may set in train auto-immune processes which are self-perpetuating. Secondly, non-pathogenic bacteria normally living as harmless commensals in the lumen of the bowel may be enabled to invade the intestinal wall and thus sensitize the host to one or more of the antigens of his own intestinal flora; or these bacteria may react with the components of the damaged mucosa to form complex antigens. Thirdly, there may be immunological cross-reactions between components of certain strains of micro-organisms and colonic or other gastrointestinal tissues, as between human heart tissue and group A streptococci (Kaplan and Meyeserian, 1962). Fourthly, the damaged mucosa

may allow the entry of antigenic dietary protein which then induces a hypersensitivity state which becomes chronic.

A well-recognized feature in ulcerative colitis, Crohn's disease, and idiopathic steatorrhoea is the exacerbation of the disease which commonly follows any intercurrent infection. In ulcerative colitis it is commonplace for patients in complete remission to be thrown into acute relapse by a trivial upper respiratory tract infection. No explanation for these occurrences is immediately forthcoming but an analogy might be drawn with the anamnestic response in which the titre of circulating antibodies to previous infective agents may rise sharply during an infection with another organism. It is conceivable that a similar enhancement of delayed hypersensitivity reactions may also accompany infections, a possibility which deserves experimental study.

Although the target organ in ulcerative colitis is by definition the colon, other organs are frequently affected in this disease. Pyoderma gangrenosum, erythema nodosum, arthritis, ankylosing spondylitis, hepatitis, and iritis are among the complications of the disease and several of these are now regarded as manifestations of a hypersensitive state. One result has been that some students of the disease regard it as a generalized reaction of the whole body in which the colonic lesion is only one part of the process. However, this hypothesis will not stand up to critical appraisal. When total colectomy is performed for ulcerative colitis, the extracolonic manifestations of the disease cease to occur. Therefore they must be regarded as secondary to the inflamed colonic mucosa and not as an integral part of the disease in its pure form. Although this is so, the mechanisms by which these secondary lesions are produced are quite likely to include immunological reactions but at present we know of no convincing work to substantiate our views. In view of the severity of some of the extracolonic lesions, which may sometimes so dominate the clinical picture that they dictate our approach to treatment, this is an aspect of the disease which deserves scientific study.

In the case of Crohn's disease, it is impossible to know whether removal of the affected part of the intestine is followed by a cessation of the remote complication which it shares with ulcerative colitis. The difficulty is that, whereas with ulcerative colitis it is possible to remove the entire colon so that future return of the disease is eliminated, with Crohn's disease it would be necessary to remove the whole of the gastrointestinal tract before being certain of a permanent cure. The persistence or onset of systemic complications in Crohn's disease already treated by resection usually implies that a fresh intestinal lesion is present.

GASTROINTESTINAL LESIONS IN THE COLLAGEN DISEASES

The group of diseases commonly known as the collagen diseases are thought to be wholly or in part produced by immunological mechanisms. In all of them the gastrointestinal tract may be involved. For example, polyarteritis nodosa, which has been mimicked in animals by Rich (1942) by repeated injection of heterologous serum, frequently produces gastrointestinal lesions (Mowrey and Lundberg, 1954; Pugh and Stringer, 1956; Rose and Spencer, 1957). Likewise, in diffuse lupus erythematosus, in which auto-immune processes have been demonstrated, gastrointestinal involvement is common (Dubois, 1953; Harvey, Shulman, Tumulty, Conley, and Schoenrich, 1954). In diffuse systemic sclerosis (scleroderma), the part of the gastrointestinal system to be commonly involved is the oesophagus but less frequently the small or large intestine may also be affected (Rosenthal, 1957). Gastrointestinal ulceration is a common manifestation of dermatomyositis (Domzalski and Morgan, 1955). Giant-cell arteritis not infrequently affects the visceral vessels and produces gastrointestinal lesions. In Henoch-Schönlein purpura, gastrointestinal involvement is usual, with symptoms ranging from colicky abdominal pain to frank haemorrhage or obstruction due to pseudo-tumour formation.

The fact that these generalized diseases which may have an immunological basis can produce gastrointestinal lesions is chiefly important in showing the types of pathological change which may result from immunological mechanisms. For example, Warren and Sommers (1949) consider that in a proportion of subjects with ulcerative colitis the primary lesion is an arteritis of the vessels of the colonic submucosa, the vascular lesions resembling those of polyarteritis nodosa or allergic vasculitis (McCombs, Patterson, and MacMahon, 1956).

OBSERVATIONS IN ANIMALS

Repeated attempts have been made to produce a model of ulcerative colitis and of ileitis in experimental animals. A recent example is the study by LeVeen, Falk, and Schatman (1961) who injected dogs intravenously with sera obtained from rabbits or ducks previously injected with extracts of canine colon. The dogs developed a bloody diarrhoea after a single injection of 6 to 10 ml. of the heterologous serum and subsequent histological examination of the colon in some animals revealed a lesion which they regard as similar to that of human ulcerative colitis. Animals which were allowed to survive became entirely symptom-free. Similar lesions have

been produced in the ileum of the guinea-pig by a method analogous to that of LeVeen *et al.* (Bernier, Terris, and Lambling, 1961). Such failures to produce chronic disease are typical of the published experimental work. Undoubtedly, acute lesions in the colon and ileum can be produced by a variety of experimental procedures, including some with an immunological basis, but the animals either die or recover completely. Good accounts of the immunological procedures which have been utilized in the experimental production of gastrointestinal lesions are included in a review by Kirsner and Goldgraber (1960) and in a symposium edited by Kirsner (1961).

REFERENCES

- Abels, J., Jansz, A., Woldring, M. G., Arends, A., and Nieweg, H. O. (1961). Experiments on the nature of the intrinsic factor inhibiting substance in serum of patients with pernicious anaemia. "Vitamin B₁₂ und Intrinsic Factor-2nd. Europ. Symposium, 1961", pp. 623-8. Enke, Stuttgart.
- Acheson, E. D., and Truelove, S. C. (1961). Early weaning in the aetiology of ulcerative colitis. *Brit. med. J.*, 2, 929-33.
- Ackroyd, J. F. (1955). Platelet agglutinins and lysins in the pathogenesis of thrombocytopenic purpura, with a note on platelet groups. *Brit. med. Bull.*, 11, 28-35.
- Anderson, A. F., Schloss, O. M., and Myers, C. (1925). The intestinal absorption of antigenic protein by normal infants. *Proc. Soc. exp. Biol. (N.Y.)*, 23, 180-2.
- Anderson, C. M., Frazer, A. C., French, J. M., Gerrard, J. W., Sammons, H. G., and Smellie, J. M. (1952). Coeliac disease: gastro-intestinal studies and the effect of dietary wheat flour. *Lancet*, 1, 836-42.
- Anderson, J. R., Gray, K. G., Beck, J. S., and Kinnear, W. F. (1961). Precipitating autoantibodies in Sjögren's disease. *Ibid.*, 2, 456-60.
- Andresen, A. F. R. (1942). Ulcerative colitis—an allergic phenomenon. *Amer. J. dig. Dis.*, 9, 91-7.
- (1953). Allergic manifestations in the gastrointestinal tract. *Gastroenterology*, 23, 20-35.
- Asherson, G. L., and Broberger, O. (1961). Incidence of haemagglutinating and complement-fixing antibodies. *Brit. med. J.*, 1, 1429-33.
- Bangham, D. R., Hobbs, K. R., and Terry, R. J. (1958). Selective placental transfer of serum-proteins in the rhesus. *Lancet*, 2, 351-4.
- Behar, A. J., and Tal, C. (1959). Experimental liver necrosis produced by the injection of homologous whole liver with adjuvant. *J. Path. Bact.*, 77, 591-6.
- Berger, E. (1958). Zur allergischen Pathogenese der Cöliakie, mit Versuchen über die Spaltung pathogener Antigene durch Fermente. *Bibliotheca Paediatrica* [supplementa ad Annales Paediatrici (Basel)], No. 67, pp. 1-55.
- Bernier, J. J., Terris, G., and Lambling, A. (1961). Production chez le cobaye d'illéites par auto-anticorps. *Arch. Mal. Appar. dig.*, 50, 1259-63.
- Boyd, W. C. (1956). *Fundamentals of Immunology*, 3rd ed., p. 380. Interscience Publishers, New York and London.
- Boyden, S. V. (1959). Approaches to the Problem of Detecting Antibodies: Henry Ford Hospital International Symposium on Mechanisms of Hypersensitivity, pp. 95-116. Churchill, London.
- Broberger, O. (1961). Studies of the immunological relationship between the antigens from different organs in human ulcerative colitis. *Acta paediat. (Uppsala)*, 50, 577-80.
- , and Perlmann, P. (1959). Autoantibodies in human ulcerative colitis. *J. exp. Med.*, 110, 657-74.
- , — (1962). Demonstration of an epithelial antigen in colon by means of fluorescent antibodies from children with ulcerative colitis. *Ibid.*, 115, 13-26.
- Bunim, J. J. (1961). A broader spectrum of Sjögren's syndrome and its pathogenetic implications. *Ann. rheum. Dis.*, 20, 1-10.

- Calabresi, P., Thayer, W. R. Jr., and Spiro, H. M. (1961). Demonstration of circulating antinuclear globulins in ulcerative colitis. *J. clin. Invest.*, **40**, 2126-33.
- Cardell, B. S., and Gurling, K. J. (1954). Observations on the pathology of Sjögren's syndrome. *J. Path. Bact.*, **68**, 137-46.
- Coghill, N. F. (1960). The significance of gastritis. *Postgrad. med. J.*, **36**, 733-42.
- Colover, J., and Glynn, L. E. (1958). Experimental iso-immune adrenalitis. *Immunology*, **1**, 172-8.
- Cooke, W. T., Peeney, A. L. P., and Hawkins, C. F. (1953). Symptoms, signs, and diagnostic features of idiopathic steatorrhea. *Quart. J. Med.*, **22**, 59-77.
- Craig, J. D. (1948). The evolution of gastric and duodenal ulceration. *Brit. med. J.*, **2**, 330-4.
- Crohn, B. B. (1949). *Regional Ileitis*. Staples Press, London.
- Dancis, J., Lind, J., Oratz, M., Smolens, J., and Vara, P. (1961). Placental transfer of proteins in human gestation. *Amer. J. Obstet. Gynec.*, **82**, 167-71.
- Davidson, L. (1961). Milk proteins in ulcerative colitis. *Brit. med. J.*, **2**, 1358.
- Dicke, W. K. (1950). Coeliakie. M.D. thesis, Utrecht.
- Doig, R. K., and Wood, I. J. (1950). Gastric biopsy in pernicious anaemia. *Med. J. Aust.*, **2**, 565-8.
- Domzalski, C. A., and Morgan, V. C. (1955). Dermatomyositis: diagnostic features and therapeutic pitfalls. *Amer. J. Med.*, **19**, 370-82.
- Doniach, D., Roitt, I. M., and Taylor, K. B. (1962). Auto-immune phenomena in pernicious anaemia: II Correlation of gastric, thyroid, and other antibodies (awaiting publication).
- Dornbusch, S. (1957). The value of the gel-precipitation method for the study of auto-immunological problems. *Int. Arch. Allergy*, **11**, 206-13.
- Drenick, E. J., Hvolboll, E., and Halsted, J. A. (1955). Effect of ACTH and cortisone on fat absorption in steatorrhea of various causes. *New Engl. J. Med.*, **253**, 303-8.
- Dubois, A., and van den Bergh, L. (1948). *Diseases of the Warm Climates*, footnote on p. 390. Grune and Stratton, New York.
- Dubois, E. L. (1953). The effect of the L.E. cell test on the clinical picture of systemic lupus erythematosus. *Ann. intern. Med.*, **38**, 1265-94.
- Edwards, F. C., and Edwards, J. H. (1956). Tea-drinking and gastritis. *Lancet*, **2**, 543-5.
- Faber, K. (1935). *Gastritis and its Consequences*. Oxford University Press, London.
- Felsen, J. (1936). The relationship of bacillary dysentery to distal ileitis, chronic ulcerative colitis and non-specific intestinal granuloma. *Ann. intern. Med.*, **10**, 645-69.
- Finlay, J. M., and Wightman, K. J. R. (1956). Modern treatment of the malabsorption syndrome in adults. *Ibid.*, **45**, 191-206.
- Frazer, A. C. (1960). The present state of knowledge on the celiac syndrome. *J. Pediatr.*, **57**, 262-76.
- , Fletcher, R. F., Ross, C. A. C., Shaw, B., Sammons, H. G., and Schneider, R. (1959). Gluten-induced enteropathy: the effect of partially digested gluten. *Lancet*, **2**, 252-5.
- French, J. M., Hawkins, C. F., and Smith, N. (1957). The effect of a wheat-gluten-free diet in adult idiopathic steatorrhea. *Quart. J. Med.*, n.s. **26**, 481-99.
- Freund, J., and McDermott, K. (1942). Sensitization to horse serum by means of adjuvants. *Proc. Soc. exp. Biol. (N.Y.)*, **49**, 548-553.
- Frost, J. W., and Goldwein, M. I. (1958). Observations on vitamin B₁₂ absorption in primary pernicious anemia during administration of adrenocortical steroids. *New Engl. J. Med.*, **258**, 1096-8.
- Gordin, R. (1959). Vitamin B₁₂ absorption in corticosteroid-treated pernicious anaemia. *Acta med. scand.*, **164**, 159-65.
- Gray, J. G. (1961). Antibodies to cow's milk in ulcerative colitis. *Brit. med. J.*, **2**, 1265-6.
- , Walker, F. C., and Thompson, D. (1961). Autoimmunity in ulcerative colitis. *Lancet*, **2**, 51-52.
- Gunther, M., Aschaffenburg, R., Matthews, R. H., Parish, W. E., and Coombs, R. R. A. (1960). The level of antibodies to the proteins of cow's milk in the serum of normal human infants. *Immunology*, **3**, 296-306.
- Harvey, A. M., Shulman, L. E., Tumulty, P. A., Conley, C. L., and Schoenrich, E. H. (1954). Systemic lupus erythematosus: Review of the literature and clinical analysis of 138 cases. *Medicine (Baltimore)*, **33**, 291-437.
- Heaton, J. M. (1959). Sjögren's syndrome and systemic lupus erythematosus. *Brit. med. J.*, **1**, 466-9.
- Heiner, D. C., Lahey, M. E., Wilson, J. F., and Peck, G. A. (1961). Precipitins to wheat in steatorrhea. *Trans. Soc. Pediatr. Research, Amer. J. Dis. Child.*, **102**, 446-9.
- Hennes, A. R., Sevelius, H., Llewellyn, T., Woods, A. H., Joel, W., and Wolf, S. (1960). Experimental production of atrophic gastritis by a presumably immunologic mechanism. In Proceedings of thirty-third annual meeting of Central Society for Clinical Research. *J. Lab. clin. Med.*, **56**, 826-7.
- Irvine, W. S., Davies, S. H., Delamore, I. W., and Wynn Williams, A. (1962). Immunological relationship between pernicious anaemia and thyroid disease. *Brit. med. J.*, **2**, 454-56.
- Jeffries, G. H., Hoskins, D. W., and Slesinger, M. H. (1962). Antibody to intrinsic factor in serum from patients with pernicious anaemia. *J. clin. Invest.*, **41**, 1106-1115.
- Jones, B. R. (1958). Lacrimal and salivary precipitating antibodies in Sjögren's syndrome. *Lancet*, **2**, 773-6.
- Joske, R. A., Finckh, E. S., and Wood, I. J. (1955). Gastric biopsy. *Quart. J. Med.*, **24**, 269-94.
- Kaplan, M. H., and Meyersian, M. (1962). An immunological cross-reaction between group-A streptococcal cells and human heart tissue. *Lancet*, **1**, 706-10.
- Kelley, M. L. Jr. (1962). Skin lesions associated with chronic ulcerative colitis. *Amer. J. dig. Dis.*, n.s. **7**, 255-72.
- Kirsner, J. B. (1961). Editor of a symposium: New frontiers in ulcerative colitis. *Gastroenterology*, **40**, 287-369.
- , and Goldgraber, M. B. (1960). Hypersensitivity, autoimmunity, and the digestive tract. *Ibid.*, **38**, 536-562.
- Kristensen, H. P. Ø., and Friis, T. (1960). Effect of prednisone on B₁₂ absorption in pernicious anaemia. *Acta med. scand.*, **166**, 249-54.
- LeVeen, H. H., Falk, G., and Schatman, B. (1961). Experimental ulcerative colitis produced by anticolon sera. *Ann. Surg.*, **154**, 275-80.
- Lippard, V. W., Schloss, O. M., and Johnson, P. A. (1936). Immune reactions induced in infants by intestinal absorption of incompletely digested cow's milk protein. *Amer. J. Dis. Child.*, **51**, 562-74.
- Magnus, H. A. (1952). Gastritis. In *Modern Trends in Gastroenterology*, 1st ed., edited by F. Avery Jones, pp. 323-51. Butterworth, London.
- Maratka, Z. (1961). Recherches sur la participation de l'intestin grêle dans les affections digestives. Étude de la steatorrhée et des auto-anticorps antigrelé. *Arch. Mal. Appar. dig.*, **50**, 1263-7.
- McCombs, R. P., Patterson, J. F., and MacMahon, H. E. (1956). Syndromes associated with "allergic" vasculitis. *New Engl. J. Med.*, **255**, 251-61.
- McNicol, G. P. (1961). Thyrotoxicosis associated with pernicious anemia. *Amer. J. med. Sci.*, **241**, 336-42.
- Milton, G. W., Maxwell, G. A., and Finckh, E. S. (1960). Changes in gastric acidity and epithelial regeneration following excision of the gastric mucosa in the dog. *Brit. J. Surg.*, **47**, 562-6.
- Mowrey, F. H., and Lundberg, E. A. (1954). The clinical manifestations of essential polyangiitis (periarteritis nodosa) with emphasis on the hepatic manifestations. *Ann. intern. Med.*, **40**, 1145-64.
- Palmer, E. D. (1954). Gastritis: a revaluation. *Medicine (Baltimore)*, **33**, 199-290.
- Perlmann, P., and Broberger, O. (1960). Auto-antibodies against antigen derived from colon in the microsomes of regional colonic lymph glands in human ulcerative colitis. *Nature (Lond.)*, **188**, 749-51.
- Polcak, J., and Vokurka, V. (1960). Auto-immune reactions in the course of ulcerative colitis. *Amer. J. dig. Dis.*, n.s. **5**, 395-405.
- Pugh, J. I., and Stringer, P. (1956). Abdominal periarteritis nodosa. *Brit. J. Surg.*, **44**, 302-8.
- Pulvertaft, R. J. V., Doniach, D., Roitt, I. M., and Hudson, R. V. (1959). Cytotoxic effects of Hashimoto serum on human thyroid cells in tissue culture. *Lancet*, **2**, 214-6.
- Rice-Oxley, J. M., and Truelove, S. (1950). Complications of ulcerative colitis. *Ibid.*, **1**, 607-11.
- Rich, A. R. (1942). The role of hypersensitivity in periarteritis nodosa. *Bull. Johns Hopk. Hosp.*, **71**, 123-40.
- Ricketts, W. E., Kirsner, J. B., Humphreys, E. M., and Palmer, W. L. (1949). Effect of roentgen irradiation on the gastric mucosa. *Gastroenterology*, **11**, 818-32.
- Rider, J. A., Moeller, H. C., Devereaux, R. G., and Wright, R. R. (1960). The use of an intramucosal test to demonstrate food hypersensitivity in ulcerative colitis. *Acta allerg. (Kbh.)*, **15**, suppl. 7, pp. 486-94.

- Rivers, T. M., Sprunt, D. H., and Berry, G. P. (1933). Observations on attempts to produce acute disseminated encephalomyelitis in monkeys. *J. exp. Med.*, **58**, 39-53.
- Roitt, I. M., Doniach, D., Campbell, P. N., and Hudson, R. V. (1956). Auto-antibodies in Hashimoto's disease (lymphadenoid goitre). *Lancet*, **2**, 820-1.
- Rose, G. A., and Spencer, H. (1957). Polyarteritis nodosa. *Quart. J. Med.*, n.s., **26**, 43-81.
- Rose, N. R., and Witebsky, E. (1956). Studies on organ specificity: V. Changes in the thyroid glands of rabbits following active immunization with rabbit thyroid extracts. *J. Immunol.*, **76**, 417-27.
- Rosenthal, F. D. (1957). Small intestine lesions with steatorrhea in diffuse systemic sclerosis (scleroderma). *Gastroenterology*, **32**, 332-42.
- Rowe, A. H. (1942). Chronic ulcerative colitis—Allergy in its etiology. *Ann. intern. Med.*, **17**, 83-100.
- Rubin, C. E., Brandborg, L. L., Flick, A. L., Parmentier, C., Phelps, P., and van Niel, S. (1960). The effect of wheat instillation into the proximal ileum of patients with idiopathic sprue. *J. clin. Invest.*, **39**, 1023.
- Samitz, M. H., and Greenberg, M. S. (1951). Skin lesions in association with ulcerative colitis. *Gastroenterology*, **19**, 476-9.
- Schwartz, M. (1958). Intrinsic-factor-inhibiting substance in serum of orally treated patients with pernicious anaemia. *Lancet*, **2**, 61-2.
- (1960). Intrinsic factor antibody in serum from patients with pernicious anaemia. *Ibid.*, **2**, 1263-7.
- Ship, I. I., Merritt, A. D., and Stanley, H. R. (1962). Recurrent aphthous ulcers. *Amer. J. Med.*, **32**, 32-43.
- Sircus, W., Church, R., and Kelleher, J. (1957). Recurrent aphthous ulceration of the mouth. *Quart. J. Med.*, **26**, 235-49.
- Taylor, K. B. (1959a). An antibody to Castle's intrinsic factor. In Proc. VIIth int. Congr. Haemat., Rome, 1958. *Haemat. lat. (Milano)*, **2**, 181-6.
- (1959b). Inhibition of intrinsic factor by pernicious anaemia sera. *Lancet*, **2**, 106-8.
- Taylor, K. B. (1961). Chronic atrophic gastritis and pernicious anemia. *Gastroenterology*, **41**, 147-151.
- , and Morton, J. A. (1958). An antibody to Castle's intrinsic factor. *Lancet*, **1**, 29-30.
- , — (1959). An antibody to Castle's intrinsic factor. *J. Path. Bact.*, **77**, 117-22.
- , Thomson, D. L., Truelove, S. C., and Wright, R. (1961). An immunological study of coeliac disease and idiopathic steatorrhea: serological reactions to gluten and milk proteins. *Brit. med. J.*, **2**, 1727-31.
- , and Truelove, S. C. (1961). Circulating antibodies to milk proteins in ulcerative colitis. *Ibid.*, **2**, 924-9.
- , Roitt, I. M., Doniach, D., Couchman, K. G., and Shapland, C. (1962). Auto-immune phenomena in pernicious anaemia: I Gastric antibodies.
- Truelove, S. C. (1961). Ulcerative colitis provoked by milk. *Ibid.*, **1**, 154-60.
- Tudhope, G. R., and Wilson, G. M. (1960). Anaemia in hypothyroidism. *Quart. J. Med.*, **29**, 513-37.
- , — (1962). Deficiency of vitamin B₁₂ in hypothyroidism. *Lancet*, **1**, 703-6.
- Vaughan, W. T., and Black, J. H. (1954). *Practice of Allergy*, 3rd ed., p. 1067. London.
- Warren, S., and Sommers, S. C. (1949). Pathogenesis of ulcerative colitis. *Amer. J. Path.*, **25**, 657-79.
- Weijers, H. A., and van de Kamer, J. H. (1950). Fat absorption in normal and abnormal infants and children with special reference to coeliac disease. Centr. Inst. v. Voedingsonderzoek, T.N.O. Publ. No. 113, Utrecht.
- , — (1960). Some biochemical investigations into the cause of wheat sensitivity in celiac disease. *Gastroenterology*, **38**, 587-91.
- Wright, R., Taylor, K. B., Truelove, S. C., and Aschaffenburg, R. (1962). Circulating antibodies to cow's milk and proteins and gluten in the new-born. *Brit. med. J.*, **2**, 513-15.