and Norwich Hospital; Northampton General Hospital; Northern General Hospital, Edinburgh; Orpington Hospital; Princess Louise Hospital; Queen Elizabeth Hospital, Birmingham; Queen Mary's Hospital, Sidcup; Radcliffe Infirmary, Oxford; Redhill County Hospital, Surrey; Royal Alexandra Hospital, Rhyl; Royal Infirmary, Cardiff; Royal Infirmary, Manchester; Royal National Hospital for Rheumatic Diseases, Bath; Royal Sussex County Hospital, Brighton; St. Alfege's Hospital, Lewisham; St. Asaph Hospital, Flints; St. David's Hospital, Cardiff; St. Martin's Hospital, Bath; St. Mary's Hospital, Paddington; St. Thomas's Hospital, London; University College Hospital, London; Western General Hospital, Edinburgh; Westminster Hospital, London; Willesden General Hospital, London.

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HYPOGAMMAGLOBULINAEMIA IN CHRONIC LYMPHATIC LEUKAEMIA

BY

G. HAMILTON FAIRLEY, M.A., D.M., M.R.C.P.
Leverhulme Research Scholar, Royal College of Physicians of London

AND

RONALD BODLEY SCOTT, M.A., D.M., F.R.C.P.
Physician, St. Bartholomew's Hospital, London

It has been known for nearly fifty years that patients with chronic lymphatic leukaemia may be incapable of forming antibodies against *Salmonella typhi* either in response to infection or after inoculation with typhoid vaccine (Moreschi, 1914; Howell, 1920). This immunological inadequacy is now fully documented (Bernstein, 1934; Weinstein and Fitz-Hugh, 1935; Larson and Tomlinson, 1953; Shaw *et al.*, 1960; Barr and Fairley, 1961); it differs, moreover, from that observed in certain other malignant diseases of lymphoreticular tissue such as Hodgkin's disease, acute leukaemia, and chronic myeloid leukaemia. In these disorders the ability to form antibodies on primary immunization is impaired, but on re-immunization is normal or but slightly defective; in chronic lymphatic leukaemia the antibody response both to primary immunization and to re-immunization is depressed (Barr and Fairley, 1961).

The impaired formation of circulating antibodies in chronic lymphatic leukaemia is often accompanied by an abnormal gamma-globulin content of the serum. Reduced levels, or even absence, have been recorded (Brown *et al.*, 1948; Brem and Morton, 1955; Jim and Reinhard, 1956; Wall *et al.*, 1956; Jim, 1957; Sunderman and Sunderman, 1957; Creyssel *et al.*, 1958; Prasad, 1958; Teitelbaum *et al.*, 1959; Ultmann *et al.*, 1959; Hudson and Wilson, 1960; Onat and Cooper, 1960; Shaw *et al.*, 1960; Videbaek, 1960). In five of the largest series the frequency of hypogammaglobulinaemia has been variously reported as 19% of 47 (Videbaek, 1960), 36% of 50 (Jim, 1957), 50% of 61 (Creyssel *et al.*, 1958), 64% of 36 (Shaw *et al.*, 1960), and 68% of 40 (Hudson and Wilson, 1960). These discrepancies are partly explained by the different criteria of normality accepted by different observers.

Other authors record an increase in the gamma-globulin concentration of the serum (Neely and Neill, 1956). In one series this was noted in 24% of 50 patients (Jim, 1957), although Creyssel *et al.* (1958) believe it to be of rare occurrence and Hudson and Wilson (1960) met it in none of their 40 patients. In the majority of such cases paper electrophoresis shows a diffuse increase in the gamma-globulin fraction, but there have been reports of abnormal localized bands similar to those found in myelomatosis (Rundles *et al.*, 1954; Teitelbaum *et al.*, 1959). These could be explained by an excess of macroglobulin, for ultracentrifugation was not undertaken and the association of chronic lymphatic leukaemia and macroglobulinaemia has been noted in a few instances (Mackay *et al.*, 1957; Glenchur *et al.*, 1958; Braunsteiner and Sailer, 1960).

The frequency of infection in chronic lymphatic leukaemia has long been recognized and the suggestion is often made that it is related to the hypogammaglobulinaemia and the inability to form circulating antibodies (Wintrobe and Hasenbush, 1939; Osgood and Seaman, 1952; Hougie, 1956; Scott, 1957; Dameshek and Gunz, 1958; Reinhard *et al.*, 1959). This paper reports estimations of the quantity of gamma-globulin in the sera of 110 patients with this disease; it attempts to relate to it the frequency of bacterial infection and it describes the prophylactic use of gamma-globulin in cases of hypogammaglobulinaemia.

Selection of Cases

The gamma-globulin concentration was estimated in the sera of 110 patients with chronic lymphatic leukaemia and of 55 controls.

A diagnosis of chronic lymphatic leukaemia was accepted only when the peripheral lymphocyte count before treatment was constantly above 9,000/c.mm. or when the bone-marrow contained more than 40% of lymphocytes. These figures exceed by 100% the upper limits of normal (Osgood *et al.*, 1939; Scott, 1939; Dacie, 1956): by adopting such criteria some cases of chronic lymphatic leukaemia may have been overlooked, but none will have been wrongly included.

The controls were chiefly healthy blood donors, although some patients, convalescent from fractures but otherwise well, were included to widen the range of ages.

Methods

Gamma-globulin estimations were made by two methods.

Paper Electrophoresis (Flynn and de Mayo, 1951).—0.02 ml. of serum was applied to a strip of Whatman

No. 1 paper 7.5 cm. wide and the separation performed in barbiturate buffer (*p*H 8.6 ; ionic strength 0.05) for 16 hours at 2.5 volts per cm. length of strip. The strips were dried for 20 minutes at 100° C., stained with light green, and washed repeatedly in 2% acetic acid until a white background was obtained. After drying, the strips were made translucent by immersion in oil (Grassmann *et al.*, 1951) and passed through an electrodensitometer (Laurence, 1954) to obtain a tracing of the protein pattern. The relative concentration of each fraction was obtained by measuring the area under each curve by planimetry and the absolute values calculated from the total protein concentration determined by the biuret method (Gornall *et al.*, 1949). The normal range of gamma-globulin obtained by this technique was 1.10–1.75 g./100 ml. of serum with a mean of 1.38 g. (Fig. 2). This is similar to the results of other workers using the same method (Gilliland *et al.*, 1956 ; Sunderman and Sunderman, 1957).

Immunological Techniques. — (*a*) Agar diffusion method: This was carried out by Dr. J. F. Soothill using the technique of Gell (1957). (*b*) Gamma-globulin neutralization method: The technique used was that described by Mollison (1956), and depends upon the neutralization by human gamma-globulin of the reaction between Rh-positive sensitized red cells and anti-human globulin serum. The anti-human globulin serum used for this test was supplied by Dr. A. E. Mourant from the Blood Group Reference Laboratory.

These two methods give similar results. The normal range is 0.80–1.20 g. of gamma-globulin per 100 ml. of serum, with a mean of 1.00 g.

Results

Four basic gamma-globulin patterns were found on electrophoresis of the sera from the 110 patients with chronic lymphatic leukaemia. In 29% the level was within the normal range, in 67% it was reduced, in 3% it was raised without an excess of macroglobulin, and in 1% (one patient) there was macroglobulinaemia (Fig. 1).

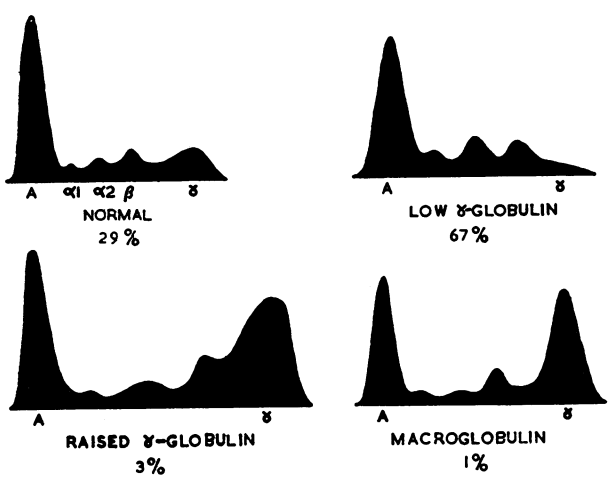

FIG. 1.—Four basic gamma-globulin patterns in the sera of 110 patients with chronic lymphatic leukaemia, showing the percentage with each pattern.

The distribution of the gamma-globulin levels determined by electrophoresis in these patients is shown in Fig. 2. In 12% the figure was below 0.5 g./100 ml. ; in the three patients with a raised concentration, but no excess of macroglobulin, the levels were 1.84, 2.33, and 3.55 g./100 ml. ; and for the single patient with macroglobulinaemia 3.75 g. In this case ultracentrifugal analysis, carried out by Professor N. H. Martin, showed an excess of macroglobulin with a sedimentation constant of S_{20w}19.5 (Fig. 3).

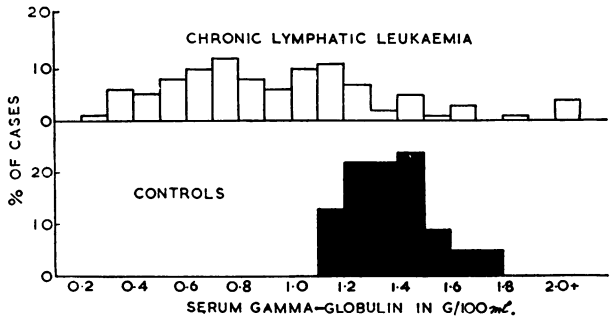

FIG. 2.—Serum gamma-globulin concentrations in 110 patients with chronic lymphatic leukaemia compared with those in 55 controls.

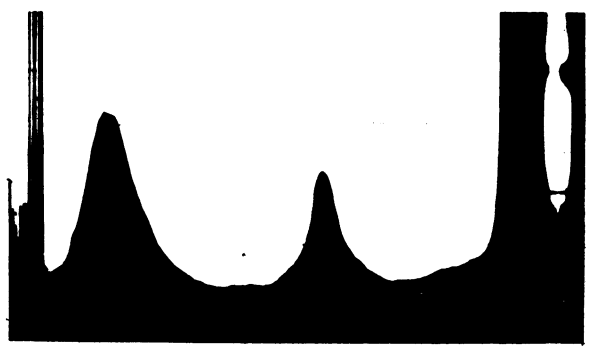

FIG. 3.—Ultracentrifugal analysis of serum from man aged 49 with chronic lymphatic leukaemia, showing excess of macroglobulin with a sedimentation constant of S_{20w}19.5.

Clinical Significance of Hypogammaglobulinaemia

The importance of hypogammaglobulinaemia lies in its frequent association with troublesome and recurrent bacterial infection. Fig. 4 shows the number of proved infective episodes occurring in each patient during the past year compared with the serum gamma-globulin concentration determined by paper electrophoresis. In all who suffered more than three infections in the year

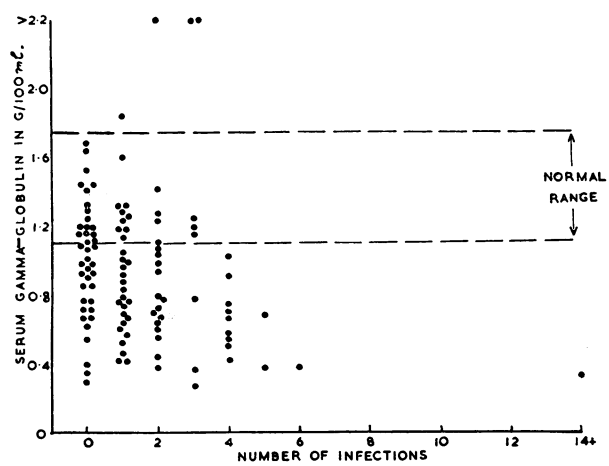

FIG. 4.—Relationship between serum gamma-globulin level determined by electrophoresis, and number of infections occurring during preceding year in 110 patients with chronic lymphatic leukaemia.

the level was reduced, and this takes no account of two patients, both with low gamma-globulin concentrations, in whom the first infection was fatal.

Measurement of the gamma-globulin concentration by paper electrophoresis is inaccurate with low levels (Gell, 1957), and it was in these patients that immunological methods proved valuable. We have followed the standards laid down for the M.R.C. Hypogammaglobulinaemia Therapeutic Trial in regarding values of under 0.2 g./100 ml. obtained by immunological methods as dangerously low (Squire, 1960).

Three patients with levels as low as this have been treated with gamma-globulin as used in the M.R.C. trial: it was prepared by the method of Kekwick and Mackay (1954) and supplied by the Blood Products Laboratory of the Lister Institute. The gamma-globulin was given by intramuscular injection, starting with a loading dose of 0.15 g./kg. body weight given over the first week, followed by a maintenance dose of 0.025 g./kg. weekly.

Case 1.—A woman, now aged 65, was found to have chronic lymphatic leukaemia in 1955. In 1956 she began to suffer from infections in the form of boils, paronychia, otitis media, bronchitis, and pneumonia, which increased in severity and frequency until treatment with gamma-globulin was started in April, 1959. The number of infective episodes before treatment was: April, 1956, to March, 1957, 6; April, 1957, to March, 1958, 11; April, 1958, to March, 1959, 18. During treatment the number was: April, 1959, to March 1960, 4; April, 1960, to March, 1961, 3. During the first three months of treatment two severe infections occurred, possibly because she received only a maintenance dose from the outset owing to difficulties in giving her a loading dose as an out-patient. In the last 21 months she has had only five mild episodes of infection. Treatment has thus reduced the incidence of infections from 18 to between 3 and 4 a year. She has received 1.5 g. of gamma-globulin in a volume of 15 ml. weekly by two intramuscular injections of 7.5 ml. each. The injections are unquestionably painful, but she is convinced that she feels better and that the treatment is worth while. She has gained weight, and even when an infection occurs it is neither so long nor so severe as those she had previously. Before treatment the serum gamma-globulin concentration was 0.16 g./100 ml. by the agar diffusion method and 0.19 g. by the neutralization test. It is being maintained at 0.28–0.5 g./100 ml.

Case 2.—A woman, aged 70 at the time of her death in 1959, underwent splenectomy for hypersplenism in 1950. The excised organ showed changes regarded as lymphosarcomatous. Later, lymphocytic infiltration of the bone-marrow and peripheral lymphocytosis established the diagnosis of chronic lymphatic leukaemia. During 1958 she suffered from six bacterial infections: three boils, one attack of pneumonia, and two abscesses. One gluteal abscess lasted three months. In 1959 her serum gamma-globulin concentration was found to be 0.14 g./100 ml. by the agar diffusion method and 0.10 g./100 ml. by the neutralization test, and gamma-globulin treatment was started. After three months the level had risen to 0.32 g./100 ml., but although she felt better she declined further treatment because the injections were so painful. Her condition deteriorated; she lost weight and died three months later after a succession of infections, including conjunctivitis, boils, pneumonia, and cystitis.

Case 3.—A woman, aged 67 at the time of her death in 1960, was found to have chronic lymphatic leukaemia in 1943. With the exception of pneumonia in 1947 she had suffered from no bacterial infections until her last admission to hospital in 1960. At this time an infection of the upper respiratory tract was followed by pneumonia and she was then found to have a serum gamma-globulin level of 0.08 g./100 ml. by the agar diffusion method and 0.07 g. by the neutralization test. In spite of full doses of gamma-globulin and the appropriate antibiotics she died a week later. Two days after the start of treatment the serum gamma-globulin concentration had risen to 0.32 g./100 ml.

Factors Responsible for Hypogammaglobulinaemia

In a search for possible causes of the hypogammaglobulinaemia in these patients an attempt was made to assess the importance of the duration, severity, and extent of the disease, the treatment received, and the age and sex of the patient. The first three are difficult to measure and arbitrary criteria have been used. The duration of the disease has been taken as the interval between diagnosis and testing and the severity as indicated by the interval between testing and death. The extent of the disease is expressed by the number of anatomical sites involved at the time of testing. The patients were placed in 10 grades, grade 10 indicating that disease was present in 10 or more sites.

The level of circulating gamma-globulin determined by paper electrophoresis was clearly related to the duration of the disease (Fig. 5). The correlation coefficient $r = -0.239$ ($P<0.05$). All patients known to have had chronic lymphatic leukaemia for five years or more had hypogammaglobulinaemia.

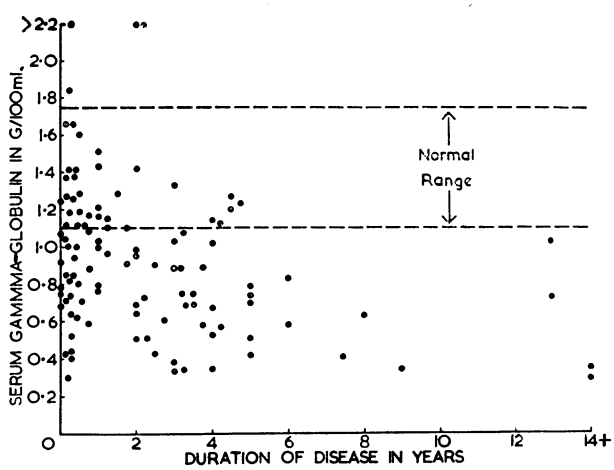

FIG. 5.—Relationship between serum gamma-globulin level, determined by electrophoresis, and duration of disease in 110 patients with chronic lymphatic leukaemia ($r = -0.239$; d.f. = 108; $P<0.05$).

There appeared to be no correlation between the level of gamma-globulin in the serum and the subsequent duration of life. Two patients with normal levels died within a week of testing, while two survived a year with reduced levels. However, only 10 of the 110 patients have died; thus the importance of this factor cannot be accurately assessed.

No correlation could be found between the extent of disease and the serum gamma-globulin concentration (Fig. 6). The correlation co-efficient $r = +0.058$. Of the 21 patients in grades 8–10, 13 (62%) had hypogammaglobulinaemia, compared with 28 (64%) of 44 in grades 1 and 2.

The Table shows that treatment was not responsible for the abnormal gamma-globulin levels. There was no significant difference between the treated and untreated nor between those who received different forms of treatment.

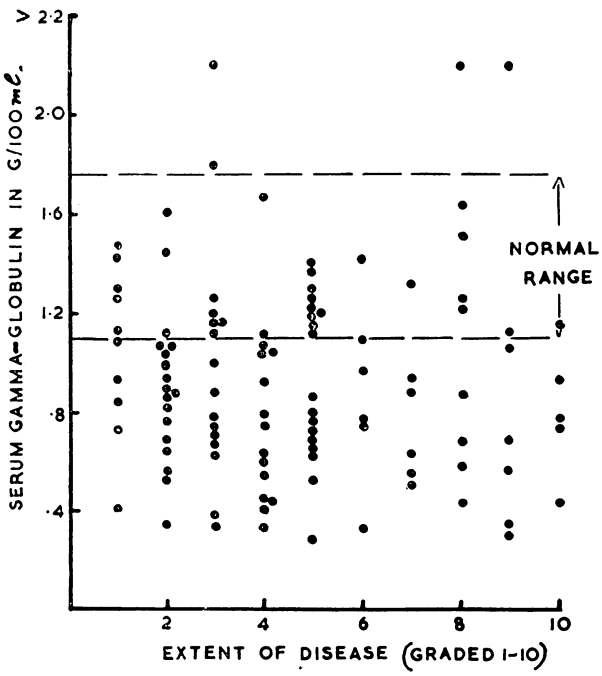

FIG. 6.—Relationship between serum gamma-globulin level, determined by electrophoresis, and extent of disease in 110 patients with chronic lymphatic leukaemia ($r = +0.058$, which is not significant).

Serum Gamma-globulin Levels in Relation to Treatment in Chronic Lymphatic Leukaemia

	Total Patients	Reduced Level	Normal Level	Raised Level
Treated group:				
Steroids only	3	2	1	0
Radiotherapy only	11	8	3	0
Cytotoxic drugs only	14	8	5	1
More than one form of treatment	26	20	5	1
Total	54	38 (70%)	14 (26%)	2 (4%)
Untreated group	56	36 (64%)	18 (32%)	2 (4%)
Both groups	110	74 (67%)	32 (29%)	4 (4%)

No correlation was found between the age of the patient and the gamma-globulin concentration ($r = -0.15$), nor was there any difference between the sexes.

In these patients, therefore, the gamma-globulin concentration of the serum may have been related to the duration of the disease, falling with the advance of time; but no association could be established with any of the other variables considered.

Discussion

From this and other published series it is seen that hypogammaglobulinaemia is a common occurrence in chronic lymphatic leukaemia. It is not due to such extraneous causes as age, sex, or treatment, and must therefore be regarded as an essential product of the disease. There is no doubt that in these patients the gamma-globulin concentration of the serum tends to fall with the progress of time: not only is there a statistically significant correlation between the duration of the disease and the gamma-globulin level, but in individual patients tested at regular intervals the values have become progressively lower. This association with the duration of the disease has been noted by others (Ultmann *et al.*, 1959; Hudson and Wilson, 1960).

The deficiency can be made good by intramuscular injection of gamma-globulin, and the results of such treatment in three patients are reported in this paper. The first patient proved that the treatment was effective enough to make the discomfort of the injections worth tolerating, the second established the danger of stopping treatment, and in the third disaster might have been averted if treatment had been started earlier.

The results indicate that in all patients who have had chronic lymphatic leukaemia for more than four years the serum gamma-globulin concentration should be measured at least every six months. If the level is dangerously low intramuscular injections of gamma-globulin should be started at the first indication of infection. Benefit from such injections has been recorded by others (Shaw *et al.*, 1960). However, the report of a hypersensitivity reaction (Diamond and Miller, 1961) enjoins caution, although such an event has not been noted with gamma-globulin prepared in this country (Squire, 1960).

Patients with acute leukaemia are also liable to infection, but probably from a different cause. In this disease the gamma-globulin levels may be raised (Corsini and Manfredi, 1957; La Grutta, 1957; Fahey and Boggs, 1960), but hypogammaglobulinaemia is rare and never more than slight (Teitelbaum *et al.*, 1959; Fahey and Boggs, 1960). Antibody formation, too, is only slightly impaired, normal, or even increased (Weinstein and Fitz-Hugh, 1935; Larson and Tomlinson, 1953; Silver *et al.*, 1960). There is no correlation between the frequency of bacterial infections and the serum gamma-globulin level (Fahey and Boggs, 1960) or the degree of antibody response (Silver *et al.*, 1960). Presumably in acute leukaemia infection results from a failure in the cellular defences of the body rather than of the humoral immunity mechanisms, and treatment with gamma-globulin would be of little benefit.

Summary

The serum gamma-globulin concentration has been determined by paper electrophoresis in 110 patients with chronic lymphatic leukaemia. In 29% the level was normal, in 67% reduced, in 3% raised with no excess of macroglobulin, and 1% had macroglobulinaemia.

The level of serum gamma-globulin was related to the duration of the disease, tending to fall with the passage of time, and all those known to have had chronic lymphatic leukaemia for five years or more had low levels.

In one patient treatment with intramuscular injection of gamma-globulin has reduced both the number and the severity of bacterial infections. This treatment has the disadvantage that the injections are painful and need to be given once a week.

It is suggested that the serum gamma-globulin level should be measured at frequent intervals and that patients in whom it is below 0.2 g./100 ml. by immunological methods should be treated promptly with gamma-globulin if bacterial infection develops.

Our thanks are due to Professor J. R. Squire and Dr. W. d'A. Maycock for supplies of gamma-globulin and much useful advice; to Dr. P. L. Mollison, Dr. J. F. Soothill, Dr. A. B. Anderson, Dr. I. McIntyre, Mr. M. P. Curwen, Dr.

A. E. Mourant, and Professor N. H. Martin for their help; and to many colleagues for permission to study their patients.

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"In recent years I have noted with interest the almost complete disappearance of the word *before*. In medical circles it is supplanted by the more cumbersome pseudo-elegant 'prior to.' In case presentations I have frequently heard the expression 'prior to' used from 20 to 50 times before the good old Anglo-Saxon word *before* was used. I have no idea what to make of this important observation." WILLIAM B. BEAN, in *Arch. intern. Med.*, 1961, **108**, 4.

CIRCULATING ANTIBODIES TO MILK PROTEINS IN ULCERATIVE COLITIS

BY

K. B. TAYLOR,* D.M., M.R.C.P.

AND

S. C. TRUELOVE, M.D., F.R.C.P.

Nuffield Department of Clinical Medicine, The Radcliffe Infirmary, Oxford.

During the last few years some patients with ulcerative colitis have been found to improve greatly when cow's milk has been totally excluded from the diet. In several of these patients, milk was reintroduced into the diet, and in every instance this was followed in the course of a few days or weeks by a frank attack of ulcerative colitis (Truelove 1961). These observations prompted us to look for the presence of antibodies to cow's milk proteins in that disease.

Materials Tested.—The chief proteins of milk—casein, α-lactalbumin, and β-lactoglobulin—were made available to us in a highly purified form by Dr. R. Aschaffenburg, of the National Institute for Research in Dairying, who has described the methods of preparation (Gunther *et al.*, 1960).

Tests Performed.—Sera were collected from patients with ulcerative colitis in all stages of the disease, from severe symptoms to prolonged freedom from symptoms. The sera of healthy subjects were collected from laboratory staff and from the healthy relatives of general medical in-patients. These sera were tested for the presence of antibodies by the coated-tanned-red-cell technique of Boyden (1951) as modified by Witebsky and Rose (1956), by the gel-diffusion method of Ouchterlony (1948), and, in some cases, by the passive cutaneous anaphylaxis reaction in guinea-pigs (Ovary, 1958).

Results

Ouchterlony Gel-diffusion Test.—The sera from 75 patients with ulcerative colitis and from 50 healthy subjects were tested against solutions of casein, lactalbumin, and lactoglobulin. The tests were repeated under a variety of conditions, varying the strengths of the milk proteins and of the sera. All the results were negative. In other words, this test failed to show any evidence of precipitating antibodies to individual milk proteins in either ulcerative colitis or in health.

Passive Cutaneous Anaphylaxis Test.—This test was performed on guinea-pigs by intravenous injection of 1% lactoglobulin, together with 1% Evans blue, four hours after intradermal injection of a number of sera which had shown either negative or strongly positive reactions to lactoglobulin by the coated-tanned-red-cell technique. The results were negative in all cases. It is known, however, that human sera do not always give strong reactions with this test (Ovary, 1958).

Tanned-red-cell Test.—The sera of 75 patients with ulcerative colitis and of 50 healthy subjects were tested against each of the three milk proteins. For each subject the serum was tested in serial dilution at concentrations of 1/20, 1/200, 1/2,000, and 1/20,000. It was found by experiment that optimal results were obtained by using each of the three milk proteins in a concentration

*Member of the M.R.C. Gastroenterology Research Unit.