AUTOANTIBODIES IN HUMAN ULCERATIVE COLITIS

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(Received for publication, July 15, 1959)

The etiology of human ulcerative colitis is still completely unknown. Among other mechanisms, infection with various microorganisms (1), enzymatic digestion of the colonic mucosa, particularly by lysozyme (2), emotional disorder (3, 4), or food allergy (1) have been blamed for the disease. However, real evidence for these hypotheses has not been furnished. The clinical picture of ulcerative colitis, with frequent extra colonic manifestations, probably originating from a state of hypersensitivity, suggests that immunological mechanisms may be involved in the pathogenesis. Thus, skin involvement including erythema nodosum, urticaria, necrotic ulcerations, or angiomata, as well as conjunctivitis, arthritis, or renal damages are relatively common (5–7). A high incidence of complications in connection with transfusions, or in connection with drug reactions, is also encountered in this disease.

The pathologic features of human ulcerative colitis are not incompatible with an immunological concept. The process seems to start in the mucosal and submucosal layers, with diffuse cellular infiltration of lymphocytes, eosinophils, and plasma cells, associated with edema, vascular congestion, and rupture of cryptic abscesses (8). Extensive destruction of the basement membranes seems to occur uniformly and granulomas with or without giant cells are found in about 20 per cent of the cases (9). Vascular changes identical with those of active periarteritis nodosa (10) have been demonstrated and may possibly be looked upon as an Arthus reaction.

One may ask whether or not the human colon is capable of developing a state of hypersensitivity. A number of reports in the literature seem to favour a positive answer to this question. Thus, cases of ulcerative colitis arising in connection with pollen allergy have been reported (11). Moreover, upon introduction of the specific antigen into the bowel lumen, Gray and Walzer (12) observed an inflammatory reaction in the rectal wall of patients who had previously been given injections of serum containing reagin. Elshlepp and Kirsner (13, 14) and Goldgraber and Kirsner (15) succeeded in producing Auer or Arthus type of response or a typical Schwartzman reaction in the colon of rabbits sensitized to crystalline egg albumin. By producing a repeated Auer response in rabbits it was also possible to induce a colitis resembling ulcerative colitis in man.

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During the last years, "autoimmunization" has been demonstrated to occur in an increasing number of diseases, characterized by symptoms similar to those described above as typical for ulcerative colitis. Circulating antibodies against constituents of human tissue have been detected in disorders of the thyroid (16–19), lupus erythematosus disseminatus (20–23), Addison's disease (24), lupoid hepatitis (25, 26), acute nephritis (27, 28), the nephrotic syndrome (27), and in other diseases. In some of these disorders it has been possible to determine the chemical nature of the antigen capable of reacting with such antibodies. Thyroglobulin has been shown to be one of the antigens in disorders of the thyroid and desoxyribonucleic acid in lupus erythematosus disseminatus. However, in the majority of these disorders, crude saline extracts of tissue homogenates were used for testing and the chemical nature of the antigens is unknown.

The presence of circulating and complement-fixing antibodies against saline extracts of human tissue has been reported in cases in which ulcerative colitis was associated with liver disease (29). (Chronic ulcerative colitis is often associated with liver disease (30, 31). In some cases the liver damage precedes the colitis whereas in others several years may pass between the onset of the colitis and the first detectable signs of liver disorder (32).)

The beneficial effect of steroid therapy, noted in ulcerative colitis (33) would agree with the assumption that an autoimmune disorder could be involved. Thus it is possible that the adrenal steroids, in addition to their antiinflammatory effect, also inhibit the production of antibodies. Such an inhibition has been demonstrated in a well establised case of Hashimoto's disease, where steroid therapy alone led to a complete restoration of the function of the thyroid and a simultaneous, almost complete disappearance of the circulating antibodies against thyroglobulin (34).

It should also be noted that high levels of γ-globulin are often observed in sera from patients with ulcerative colitis (6). This may be a reflection of an enhanced production of antibodies against microorganisms, invading the destroyed bowel. On the other hand, it might indicate that antibodies are formed against constituents of the patient's own tissue.

Since many factors seemed to favour the assumption that autoimmune processes may be involved in ulcerative colitis, we were prompted to start an investigation of this question. In the studies to be reported it was possible to demonstrate that sera from children with chronic ulcerative colitis contain antibodies reacting *in vitro* with extracts of normal human tissue.

Materials and Methods

Antisera.—The investigation was based on an examination of sera from 30 children of various ages, all suffering from ulcerative colitis. In half of the cases extracolonic manifestations were present. These included disorders of the liver, arthritis, erythrocyteuria, stomatitis and involvement of the skin with erythema nodosum, necrotic ulcerations, and angiomata. Many of the patients presented a clinical picture similar to that of lupus erythematosus disseminatus. However, testing for LE cell phenomenon gave negative results in all cases. In addition, all sera were examined with regard to the occurrence of anti-deoxyribonucleic acid–antibodies, by means of diffusion techniques (35) and the method of passive hemagglutination as applied by Miescher and Strässle (23). Neither in the sera from patients with ulcerative colitis nor in the controls

was it possible to demonstrate precipitating or hemagglutinating antibodies. On the other hand, in 6 sera from patients with lupus erythematosus disseminatus such antibodies could be shown to occur in fairly high titers. (With these sera, hemagglutination was obtained in dilutions up to 1:1600.)

As additional sources of antibodies, saline extracts of regional lymphatic glands from the colon were also used. These glands were obtained from colectomy specimens, removed from 5 of the children with ulcerative colitis and were adjacent to areas of ulceration.

Controls.—The controls consisted of the sera from 70 healthy or diseased children of various ages. The pathologic sera, 32 in number, were obtained from children with disorders in which autoimmunization is known or suspected to participate (acute nephritis, nephrosis, rheumatoid arthritis, rheumatic fever, Hashimoto's disease, and idiopathic thrombocytopenia).

Source and Preparation of Antigen.—The test solutions were prepared from colon, liver, kidney, and spleen. In order to avoid gross contamination with bacteria, the organs were taken, under aseptic conditions within 1 hour after death, from children (blood group O), who had died, without feeding, during their 1st day of life. The organs were homogenized with saline for 3 minutes in a cooled Waring blendor and for a further 5 minutes in a cooled glass homogenizer. The homogenates were then centrifuged at 70,000 *g* for 30 minutes in a Spinco preparative ultracentrifuge and the supernatants used for the immunological tests. A number of the homogenates was also treated with trypsin, papain, lysozyme, or lecithinase A. After digestion the homogenates were centrifuged and the supernatant used as antigen.

In later experiments the test solutions were prepared by extraction with phenol-water at 65°C. according to the method of Westphal *et al.* (36). 30 gm. of tissue, suspended in water, was homogenized as described above, diluted to 300 ml. heated to 65°C. and was finally mixed with an equal volume of freshly redistilled phenol. After stirring for 30 minutes in a waterbath at 65°C., the mixture was cooled with ice water and centrifuged in the cold. The watery layer was pipetted off. The phenolic phase was once more mixed with 300 ml. of water and extracted as described. After cooling and centrifugation the watery layer was again removed and combined with the first one. The extract was dialyzed against distilled water, lyophilyzed, and resuspended in 200 ml. of distilled water. The solution was mixed with an equal volume of ethanol under gentle stirring. The precipitate formed was discarded and the supernatant was finally mixed with six to ten volumes of ethanol. The precipitate obtained was centrifuged off, suspended in 5 to 10 ml. of distilled water and was lyophilyzed. 30 gm. of colon gave approximately 100 mg. of this lyophilyzed dry powder, containing mainly lipopolysacharides as well as material absorbing light in the ultraviolet region (260 mμ). This was probably ribonucleic acid since the absorbing substance could be removed by incubation of the samples with ribonuclease and subsequent dialysis. For the immunological experiments, 10 mg. of the lyophilyzed powder were dissolved in 100 ml. of buffered saline (pH 7.4, phosphate).

Precipitin Reactions.—The techniques applied were those of gel diffusion according to Ouchterlony (35), using 1.5 per cent agar in physiological saline as medium and small plastic containers for the reagents (37). The basins were refilled several times. Before recording, the plates were kept at 37°C. for a week and in a refrigerator for additional 2 weeks. They were then dried and stained according to standard procedures.

Immunophoresis.—Immunophoretic studies on supporting glass plates, 5 × 8 cm. large, were carried out following the descriptions given by Grabar and Williams (38). The electrophoretic separations were performed in barbiturate buffer of pH 8.3 and $\mu = 0.05$. The immunological reagent was an antiserum (horse) against human serum, available commercially from The Pasteur Institute, Paris.

Hemagglutination.—In most experiments indirect hemagglutination was performed according to Middlebrook and Dubos (39, 40). The tests were carried out as follows. In order to inactivate complement, all sera were incubated at 56°C. for 30 minutes. Furthermore, before test-

ing, heterophile antibodies were removed by absorption with washed sheep erythrocytes. Sheep erythrocytes were washed once in physiological saline and three times in physiological saline, buffered with 0.15 M phosphate buffer (pH 8.0). For sensitization, 1 volume of the washed cells was incubated at 37°C. for 30 minutes with 3 volumes of the antigenic solution (*cf.* above). The cells were centrifuged off at 1500 R.P.M. (5 minutes), washed once in buffered saline and were finally suspended in saline to give a suspension of 0.25 per cent. Of the sera to be tested serial dilutions were made with buffered saline. 0.5 ml. of each dilution was mixed in a test tube with an equal volume either of sensitized cells or of only washed cells (controls). In addition, controls were set up, consisting of 0.5 ml. of sensitized cells mixed with an equal volume of buffered saline. For stabilization, 0.1 ml. of rabbit serum was added to each tube. The tubes were thoroughly shaken and incubated at 37°C. for 2 hours. They were then kept at room temperature overnight, shaken occasionally, and the degree of agglutination was finally recorded macroscopically against an illuminated background.

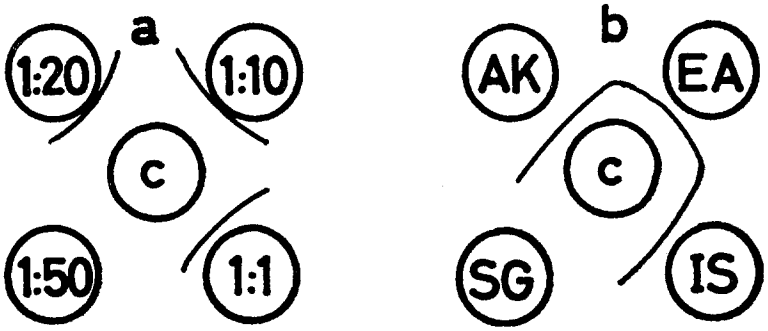

FIG. 1. Diagram of precipitin reactions in agar. (*a*) Saline extract of colon (C) reacted with 4 different dilutions of serum from a patient with ulcerative colitis (AK). (*b*) Aliquot of the same extract (C) reacted with undiluted samples of sera from patients with ulcerative colitis (AK, EA, IS, SG). No visible precipitate was obtained with the serum from the patient SG.

RESULTS

Precipitin Reactions.—In gel diffusion experiments only 3 of the 30 sera from children with ulcerative colitis gave precipitates when tested with saline extracts of homogenized colon. A number of positive tests are shown diagrammatically in Fig. 1. The precipitates formed with these extracts were rather distinct. However, they were only weakly stained by the conventional protein dyes, amidoblack or azocarmine B.

Incubation of the homogenates with trypsin, papain, lysozyme, or lecithinase A did not improve their reactivity in the precipitin tests. Moreover, experiments with saline extracts of homogenized liver, kidney, or spleen were entirely negative. This was also the case, when sera from healthy children or from children with unrelated diseases were tested with these saline extracts.

Since it could be assumed that the possible antigen might be only scarcely soluble in saline, the various tissues were extracted with phenol-water at 65°C. according to the description given by Westphal *et al.* (36). This procedure has

been used mainly for the extraction of lipopolysaccharides from Gram-negative bacteria. Table I shows a summary of the results which were obtained when such extracts were reacted with the various sera. As can be seen, 22 of the 30 sera from patients with ulcerative colitis now reacted positively when extracted colon was used as antigen. A photograph of some typical reactions is shown in Fig. 2. In addition, 4 of these sera now also gave precipitates with extracts from liver and 6 with extracts from kidney. In some experiments, where different extracts were reacted with the same serum (Fig. 3), continuous precipitates

TABLE I

Summary of Precipitin Reactions with Various Sera and Phenol-Water Extracts of Colon, Liver, and Kidney

Origin of sera	Total No.				No. of sera rendering positive precipitin reactions with extracts of		
					Colon	Liver	Kidney
Ulcerative colitis				30	22	4	6
Nephrosis	Controls	Diseased		5	0	2	2
Acute nephritis				2	0	0	1
Rheumatoid arthritis				9	0	0	0
Rheumatic fever				8	0	0	0
Idiopathic thrombocytopenia				3	0	0	0
Hashimoto's disease				5	0	0	0
				32			
Normal		Healthy		10	0	0	0

were obtained. This might indicate that it was the same antigen which was extracted from the different tissues. However, this point has not been studied in detail so far.

Table I also shows that no reactions occurred when the sera from healthy children were allowed to react with the various extracts. Of the sera from diseased children, 2 of the cases with nephrosis reacted with extracts from both liver and kidney (Figs. 4 and 5) and one case with acute nephritis with the extract from kidney.

These results indicate that the sera of the patients with ulcerative colitis contain antibodies capable of reacting with antigens, present in extracts of the tissues. It can be assumed that one site of production of such antibodies is the lymphatic glands in the colonic region. As described in the experimental part of this paper, in 5 cases of ulcerative colitis, fresh glands could be obtained

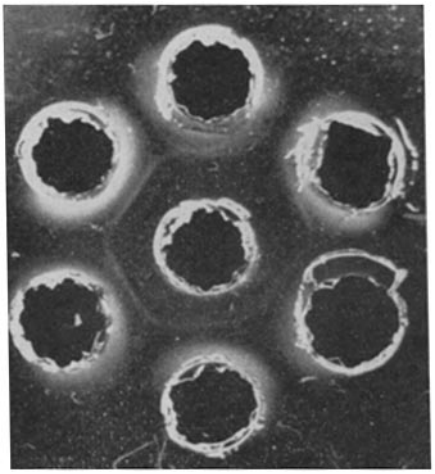

FIG. 2. Photograph (dark field) of precipitin reactions in agar. Phenol-water extract of colon was allowed to diffuse from the reservoir in the centre towards the sera from 6 patients with ulcerative colitis, diffusing from the peripheral reservoirs. The continuous precipitate appeared after 3 days incubation at 37°C.

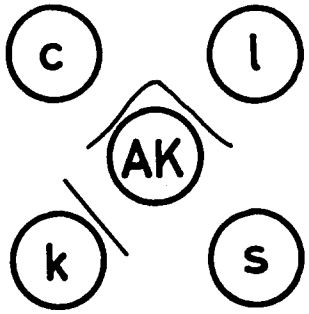

FIG. 3. Diagram of precipitin reactions in agar. Serum from a patient with ulcerative colitis (AK) was allowed to diffuse from the reservoir in the centre towards phenol-water extracts of colon (*c*), liver (*l*), kidney (*k*) or spleen (*s*), diffusing from the peripheral reservoirs. The continuous precipitate between (*c*) and (*l*) suggests an immunological identity of the antigens derived from these two organs. The absence of an interaction of the precipitates between (*c*) and (*k*) may be due to suboptimal concentrations of the reagents and does not allow any conclusions to be drawn as to the immunological relationship of the antigens.

after colectomy from the subserosal layer in the neighbourhood of the most ulcerated areas. When saline extracts of the glands were allowed to react with extracts from colon (phenol-water), distinct precipitates were obtained in the Ouchterlony plates. Whereas, when the sera were used, 4 to 5 days were neces-

sary for visible reactions, these precipitates appeared as early as the 2nd day after the onset of the experiments.

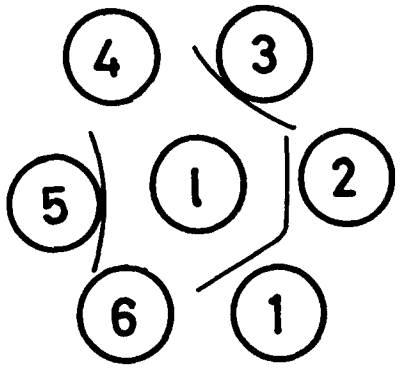

FIG. 4. Diagram of precipitin reactions in agar. Phenol-water extract of liver (*l*) was allowed to diffuse from the reservoirs in the center towards sera from patients with ulcerative colitis (1, 2, 4, 6) or nephrosis (3, 5), diffusing from the peripheral reservoirs. The precipitates formed between (1) and (2) show a reaction of identity. Again, the apparent absence of an interaction of the precipitates between (2) and (3) is not conclusive.

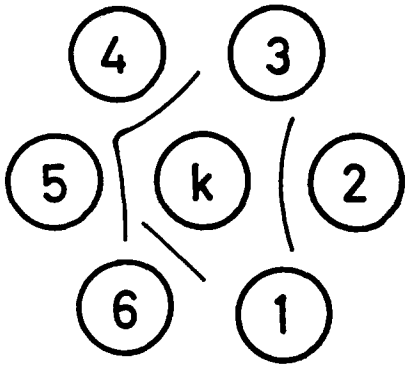

FIG. 5. Diagram of precipitin reactions in agar. Phenol-water extract of kidney (*k*) was allowed to diffuse from the reservoir in the centre towards sera from patients with ulcerative colitis (1, 2, 3, 6) or nephrosis (4, 5), diffusing from the peripheral reservoirs. The precipitate formed between (4) and (5) shows a reaction of identity. The absence of an interaction of the precipitates between (5) and (6) may be due to suboptimal concentration of the reagents.

Immunophoretic Studies.—Further evidence for the immunological nature of the precipitin reactions was obtained in immunophoretic experiments. Fig. 6 shows the diagram of an experiment of this sort in which the serum of a patient with ulcerative colitis first had been fractionated electrophoretically on agar. When a horse anti-human serum was added after completion of the electro-

phoresis, the immunological pattern typical for the serum proteins was obtained (41). Simultaneous addition of a phenol-water extract from colon on the opposite side gave a precipitate in the region of the γ-globulins. This indicates

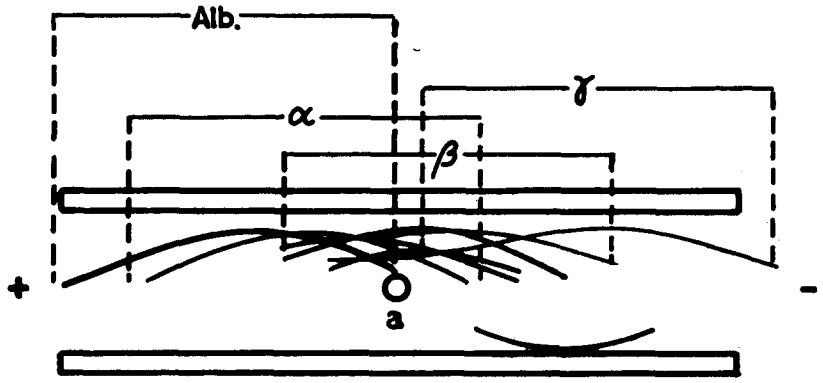

FIG. 6. Diagram of immunophoresis. Serum of a patient with ulcerative colitis was added at (*a*) and separated electrophoretically (5 hours, 1.5 ma./cm. cross-section, agar 2 mm. thick). A horse antihuman serum was subsequently added from the upper lateral channel and a phenol-water extract of colon from the lower lateral channel. *Alb:* immunological precipitate obtained with serum albumin. α: precipitates obtained with α-globulins. β: precipitates with β-globulins. γ: precipitate obtained with γ-globulin. For further explanations see text.

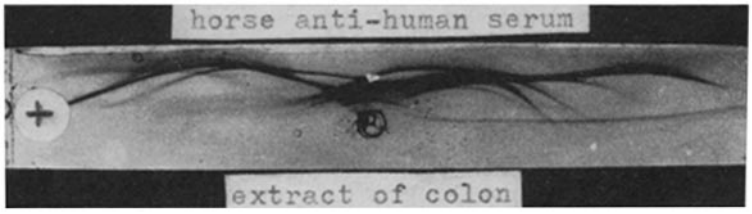

FIG. 7. Photograph of immunophoresis, taken after drying of the agar and staining with amidoblack. Electrophoresis of a saline extract of regional colonic glands. For conditions of electrophoresis see legend to Fig. 6. Horse anti-human serum was allowed to diffuse from the upper lateral channel and a phenol-water extract of colon from the lower lateral channel. For further explanations see text.

that the precipitating substance in the serum of the patients has the electrophoretic properties typical for antibodies in general.

Identical results were obtained with the saline extracts of the lymphatic glands. In addition to a certain amount of serum albumin, α- and β-globulins, these extracts also contained considerable amounts of γ-globulin. When the glandular extract was allowed to react with the colonic antigen, a precipitate appeared again in the γ-region (Fig. 7). (Owing to an excess of antigen in the

experiment of Fig. 7, the precipitate was formed exceptionally far from the basin containing the colonic extract.)

Passive Hemagglutinations.—In preliminary experiments, the method of passive hemagglutination according to Boyden (42) was used. However, no reproducible results were obtained with these techniques. This might be due to the fact that our antigen probably is not a protein. Assuming the antigen described above to be a polysaccharide, it could be expected that the hemagglutination method of Middlebrook and Dubos (39, 40) would offer better experimental conditions, since it is known that bacterial polysaccharides can be adsorbed on the surface of untreated erythrocytes. These expectations were verified and positive results were obtained when erythrocytes were sensitized with the phenol-water extracts described above. The adsorption of the antigen was brought about without heating or treatment with dilute alkali, found to promote adsorption in some cases (43).

TABLE II

Hemagglutinating Titers of Various Sera Tested with Erythrocytes from Sheep, Sensitized with Phenol-Water Extract of Colon

Origin of sera	Total No.			No. of sera rendering positive hemagglutination at highest dilution of						Total No. of sera rendering positive hemagglutination
				1/1	1/10	1/100	1/200	1/400	1/800	
Ulcerative colitis			30		2	11	5	8	2	28
Nephrosis	Controls	Diseased	5			2				2
Acute nephritis			2			1				1
Rheumatoid arthritis			9			1	1			2
Rheumatoid fever			8 (32)							0
Idiopathic thrombocytopenia			3							0
Hashimoto's disease			5							0
Normal		Healthy	38							0

Controls with the different sera + non-sensitized erythrocytes were negative in all cases.

The results of the experiments are summarized in Tables II–IV. Table II gives the results of the tests in which the colonic extract was used for sensitization. As can be seen, positive hemagglutination was obtained with 28 out of 30 sera from children suffering from ulcerative colitis. None of the sera from healthy children reacted positively in this series. Of the 32 controls representing

TABLE III

Hemagglutinating Titers of Various Sera Tested with Erythrocytes from Sheep, Sensitized with Phenol-Water Extract of Liver

Origin of sera	Total No.			No. of sera rendering positive hemagglutination at highest dilution of						Total No. of sera rendering positive hemagglutination
				1/1	1/10	1/100	1/200	1/400	1/800	
Ulcerative colitis			30		8	5				13
Nephrosis	Controls	Diseased	5			1	1			2
Acute nephritis			2							0
Rheumatoid arthritis			9			2	1			3
Rheumatic fever			8 (32)							0
Idiopathic thrombocytopenia			3							0
Hashimoto's disease			5							0
Normal		Healthy	38							0

Controls with the different sera + non-sensitized erythrocytes were negative in all cases.

TABLE IV

Hemagglutinating Titers of Various Sera Tested with Erythrocytes from Sheep, Sensitized with Phenol-Water Extract of Kidney

Origin of sera	Total No.			No. of sera rendering positive hemagglutination at highest dilution of						Total No. of sera rendering positive hemagglutination
				1/1	1/10	1/100	1/200	1/400	1/800	
Ulcerative colitis			30		4		6		2	12
Nephrosis	Controls	Diseased	5					2		2
Acute nephritis			2			1				1
Rheumatoid arthritis			9		1	2				3
Rheumatic fever			8 (32)							0
Idiopathic thrombocytopenia			3							0
Hashimoto's disease			5							0
Normal		Healthy	38							0

Controls with the different sera + non-sensitized erythrocytes were negative in all cases.

various diseases, positive hemagglutination was obtained with one serum from a child with acute nephritis, with two from children with nephrosis and with two from children with rheumatoid arthritis. However, the hemagglutinating titers of the sera from patients with ulcerative colitis were usually higher.

The results of experiments with extracts of liver are given in Table III and those of experiments with extracts of kidney in Table IV. Here, of the cases with ulcerative colitis, 13 hemagglutinated cells sensitized with liver, and 12 those sensitized with kidney. With a few exceptions, the sera giving positive reactions in these 2 series were aliquots of the same samples. The experiments with sera from normal children were negative throughout, whereas the controls from diseased children reacted to approximately the same extent with these cells as with the cells sensitized with colon. It should be pointed out that the positive controls were from the same patients in all three series.

In a series of tests performed with cells sensitized with a similar extract from spleen, no hemagglutination was obtained with any of the sera.

With sera from patients suffering from Hashimoto's disease, tested with thyroglobulin-coated erythrocytes, hemagglutination titers of 1:250,000 have been reported (18). This is appreciably higher than what was found in the present investigation. However, it does not necessarily imply that the degree of immunization is less in patients with ulcerative colitis than in patients with Hashimoto's disease. So far, we do not know the amount of antigen required for optimal sensitization of the erythrocytes nor do we know whether the chemical nature of the antigen at all allows that an adsorption on the erythrocytes can be brought about to an optimal extent. The concentration of the erythrocytes may also be of importance.

DISCUSSION

The results reported in this paper demonstrate that the sera of patients suffering from ulcerative colitis contain a precipitating and hemagglutinating factor reacting with one or several constituents of colon, liver, and kidney. It has also been shown that this factor is a γ-globulin. There is thus good evidence for the conclusion that we are dealing with real autoantibodies. Since these are present also, in considerable amounts, in saline extracts of regional colonic glands, this might indicate that a production of the antibodies takes place near the ulcerative lesions.

Although the colonic extracts were of the highest reactivity, positive reactions were also obtained with extracts from liver or kidney. The diffusion experiments gave certain indications that the same antigen was involved in these cases. However, from other results, it cannot be excluded that the antigens extracted from these organs are chemically and immunologically different.

The specificity of the antigenic extracts for ulcerative colitis is not absolute. In our rather limited material of controls from other diseases, a few sera from

children with nephrosis, acute nephritis, and rheumatoid arthritis contained precipitating and hemagglutinating antibodies against substances in the phenol-water extracts from both colon, liver, and kidney. In these diseases, the occurrence of circulating antibodies against antigens in tissue extracts has been reported by several authors. Thus, Liu and McCrory (27) could demonstrate precipitating and hemagglutinating antibodies in the sera of children with acute nephritis and the nephrotic syndrome when using saline extracts of kidney and, in some instances, also of liver. Even in the case of patients with rheumatoid arthritis, circulating antibodies against heart and other tissues have been found by a number of workers (44, 45). It would be reasonable to assume that the antigens in these saline extracts were the same as those present in the phenol-water extracts used in this study. It may be expected that these preparations are immunologically heterogenous. However, a more detailed knowledge of the chemistry of the extracts has to be gained before anything can be stated as to the identities of the antigens reacting with the antibodies occurring in the various diseases.

From the experimental results it is clear that an autoimmune disorder is involved in ulcerative colitis. The question then arises: are the demonstrated antibodies responsible for the destructive processes, or are they only immunological by-products of no pathogenetic significance? So far, a cytotoxic effect of circulating antibodies in hitherto known autoimmune disorders has not been established to full evidence.

In Hashimoto's disease, representing a thoroughly studied case, circulating antibodies are known against at least two antigens, thyroglobulin and an antigen present in the microsomal fraction of thyrotoxic thyroid tissue (46). However, no cytotoxic effect was found, when tissue cultures of human thyroid cells were exposed to sera from patients with Hashimoto's disease (18). The objections could perhaps be raised that experiments with tissue cultures cannot be regarded as a sure guide to reactions occurring in the human body. More promising in this connection are the results of Rose and Witebsky's studies in rabbits (47). One lobe of the thyroid of the rabbit was removed, thyroglobulin was prepared from it and injected into the foot-pad of the same animal. After 3 weeks, circulating antibodies could be demonstrated. When the remaining lobe was removed after another week, pathologic features suggestive of those typical for Hashimoto's goitre could be demonstrated.

It has also been suggested that in Hashimoto's disease the antibodies act by eliciting a state of delayed hypersensitivity (46). This concept would well agree with the findings of Buchanan *et al.* (48). These authors found that intradermal injections of extracts from thyroids into patients with Hashimoto's disease elicited local skin reactions which were absent in healthy individuals.

We have performed a number of preliminary experiments in order to find out whether or not cytotoxic effects could be obtained with sera from patients with ulcerative colitis. When cultures of colonic cells, derived from 20 weeks old

fetuses (legal abortions) were repeatedly exposed to such sera, in the presence of complement, no cytotoxic effect could be demonstrated. However, experiments with γ-globulin, labelled with fluorescent dye, indicated that the globulins from the sera of patients were specifically adsorbed onto cells in these cultures whereas the globulins from normal individuals were not. The presence of the antigen in this material could also be established by means of the *in vitro* techniques described in this paper. Further experiments in this field are going on and will be described later.

Regardless of the pathogenetic significance of the antibodies occurring in autoimmune disorders one may ask what mechanisms are responsible for their production. The mechanism underlying the formation of autoantibodies in general is very little understood.

It is often considered in connection with the reverse phenomenon of immunological tolerance (49, 50). This has been defined by Billingham *et al.* (51, 52) as "the state of specific immunological non-reactivity that may be brought about by confronting animals with antigenic substances before they have become immunologically mature." From studies of transplantation immunity it is known that tolerance to tissue antigens may be induced in fetal or newborn animals by injecting homologous cells derived from adult donors (51–53). It has been assumed that formation of autoantibodies can only be elicited by such tissue constituents which do not normally gain access to the antibody forming systems and which therefore can not produce a tolerant state. It is reasonable to assume that this could be true for antigens of the eye lens, the uveal tract (54, 55), the central nervous system (56, 57), or for spermatozoa (57, 58). However, it is doubtful whether this explanation holds for the production of antibodies to an antigen present in the human colon, which is highly vascularized early during embryonic life.

Infection precedes many of the suspected autoimmune disorders. For example, this is the case in rheumatic diseases and in nephritis. It has been proposed that some constituent from the microorganism in the host acts as hapten and thus introduces a new antigenic specificity, giving rise to the formation of antibodies. Hence, it is assumed that infection in these cases acts in the same manner as do drugs in drug allergy. In this context, Boyd (59) refers to the sedative, sedormid (allyl isopropylacetyl urea), which causes thrombocytopenic purpura in some individuals, probably by conjugating with the platelets of the patients. Antibodies are formed which are directed against the sedormid platelets, as sera from sensitized patients seem to lyse normal platelets only in the presence of sedormid (60, 61).

In the case of ulcerative colitis, it seems justifiable to exclude an immunological mechanism of the latter type. The specific antigen is present not only in the colonic tissue from newborn babies but also in colonic tissue from 20 weeks old fetuses. A conjugation of tissue cells with a hapten from microorganisms (or drugs) could not possibly have taken place.

Infection may be of importance in a different manner. Thus, some constit-

uent of the host's tissue may have antigenic groupings in common with an antigen of the invading microorganism. The invading microorganism will give rise to a production of antibodies some of which also will cross-react with the similar antigen of the host.

For example, from the studies of Heidelberger *et al.* (62, 63) it is known that antibodies to polysaccharides from certain strains of pneumococci will react with human glycogen. It has been demonstrated that the antigenic determinants of the polysaccharides contain glucose in linkages similar to those of glycogen (64). Moreover, as demonstrated by several authors (65, 66), antibodies to the type-specific polysaccharide of pneumococcus Type XIV are known to cross-react with the blood group substances of the A,B,O-system. Here, the antigenic determinants in common seem to be multiple galactosyl linkages and *N*-acetyl glucosamine groups (67–69). When the blood group substance is exposed to mild acid hydrolysis its blood group activity decreases whereas its capacity to react with antibodies against pneumococcus Type XIV increases (66). During this procedure, certain carbohydrate side chains are split off and new determinants, related to Type XIV, are exposed and hence are made available for reaction with the antibodies.

It seems not impossible that the production of circulating or even fixed autoantibodies in disease may at least be started through a mechanism of this type. Therefore, it would be of great interest to study the reactions between sera from patients with ulcerative colitis and antigens from the great number of microorganisms known to invade the tissue in this disease. As one example, Svartz (70) has found an abundant flora of enterococci in fecal cultures from patients with ulcerative colitis. In some untreated cases the enterococci occur in such large quantities that even in the first cultivation they appear to exist in a pure culture. In some cases coliform bacteria are entirely absent. An investigation of this question will be undertaken in the future.

SUMMARY

Sera from 30 children, suffering from ulcerative colitis, were examined for the presence of antibodies capable of reacting with antigens of normal human tissue. It was possible to demonstrate that most of the sera contained a precipitating and hemagglutinating factor, reacting with a constituent of human colonic tissue. This constituent was obtained, within 1 hour after death, from colonic tissue of newborn babies who had died without feeding. It could also be prepared from fetal tissue. The antigen can be extracted with phenol-water at 65°C. and seems to be a polysaccharide. The precipitating factor in the sera of the patients behaves electrophoretically as a γ-globulin.

Phenol-water extracts from liver and from kidney also reacted positively with the sera from certain patients. There are indications which suggest that the antigen obtained from these tissues is identical with that from colon.

Sera from 38 healthy children did not give any reactions with the extracts used. In additional controls, the sera of 32 children with various diseases, all of suspected autoimmune origin, were also tested. A few of these reacted positively with the phenol-water extracts from the organs mentioned above. Most likely, the antigen reacting in these cases is different from that reacting with the antibodies in the sera from patients with ulcerative colitis.

The possible role of the antibodies in the pathogenesis of ulcerative colitis and the mechanism of their formation are discussed.

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