IN VITRO STUDIES OF ULCERATIVE COLITIS

II. Cytotoxic Action of White Blood Cells from Patients on Human Fetal Colon Cells

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In the preceding paper (1) it was reported that no damage of human colonic cells *in vitro* was observed from exposure to sera from children with ulcerative colitis. Such negative results do not preclude an immunological concept of the pathogenesis of ulcerative colitis. Humoral antibodies may contribute to tissue damage without being cytotoxic (2). Moreover, many of the extracolonic symptoms so frequently seen in juvenile ulcerative colitis might be due to hypersensitivity of the delayed type (3). Cellular immunity is thought to play an important role in experimental autoimmune disease, which can be transferred with lymphoid cells but not by serum from the diseased animals (4–7). The positive results of transfer experiments indicate the presence of sensitized cells that may, directly or indirectly, produce cytotoxic lesions. It was therefore of interest to determine whether or not sensitized cells were present in the blood of patients with ulcerative colitis.

In preliminary experiments a significant fraction of monolayer cultures of human fetal colon cells died within 24 hours when treated with patients' white cells (8). This suggested the presence of sensitized white blood cells capable of attacking human colon cells. This paper contains the results of a more detailed study in which this *in vitro* cytotoxic reaction was investigated using release of radioactive isotopes from the colon cells as an indicator of cellular damage (1). A preliminary account of some of the results has been given elsewhere (8).

Materials and Methods

Tissues.—Samples of colon, small intestine, or kidney were obtained from human fetuses (legal abortions) (1). Two established strains of tissue culture cells were also used: *viz.*, Earle's L strain (mouse connective tissue) and Chang's (human adult liver) cultivated in suspension in Eagle's complete medium (9) supplemented with 10 per cent of heat-inactivated horse serum.

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Preparation and Labeling of the Tissue Cells.—For labeling, the fetal organs or tissue cultures were suspended in phosphate-free Eagle's medium containing ^{32}P-orthophosphate (carrier-free, see reference 1), or in Krebs-Henseleit's medium (10) containing ^{14}C-algal protein hydrolysate (specific activity 88 μc/mg; Amersham, Buckinghamshire, England). After incubation, the tissues were cut into small pieces and trypsinized. The cell suspensions were centrifuged (5 minutes, 500 R.P.M.) and the resuspended sediments filtered through gauze and finally washed 3 times in medium containing unlabeled phosphate or amino acids. For details see reference 1 and legends to figures and tables. Samples containing less than 80 per cent of viable cells (11) were discarded.

Preparation of White Blood Cells.—20 ml of blood were drawn either from healthy individuals or from children with ulcerative colitis in an active phase. The blood was collected in heparinized test tubes and allowed to sediment for 30 minutes. The relatively clear plasma layer was pipetted off and centrifuged at 800 R.P.M. for 3 minutes. The supernatant was pipetted off and centrifuged at 1500 R.P.M. for 5 minutes. The sedimented white cells were resuspended in a small volume of Eagle's medium and washed once by centrifugation for 5 minutes at 1500 R.P.M. The entire procedure was carried out at 4°C. In some experiments the cells were used within 1 to 2 hours (*fresh*). In others they were *aged* by gentle stirring overnight (12 to 18 hours) in Eagle's medium containing heat-inactivated human serum. They were then washed by centrifugation at 1500 R.P.M. (5 minutes) and counted after staining with lissamine green (11). In some experiments, control white cells were, before washing, incubated with patients' serum (Fig. 1 *a* to *d*, see legend).

Cytotoxicity Tests.—In the experiment of Figs. 1 and 3, aliquots of 2×10^6 ^{32}P-labeled colon cells, suspended in 0.3 ml of Eagle's medium, were placed in a series of test tubes. To each tube was added 10^7 white cells, suspended in 0.6 ml of Eagle's medium, 0.3 ml of fresh guinea pig serum (Fig. 1), and Eagle's medium, giving a final volume of 1.8 ml. The tubes were incubated at 37°C. Following brief centrifugation, samples of 0.6 ml were taken at different times, and the volume of 1.8 ml restored as described (1). *The medium used throughout this and the following experiments contained 10 per cent of heat-inactivated human serum.*

In the other experiments, tissue cells, labeled with ^{32}P or ^{14}C, were incubated at 37°C with white cells as described in the legends. After incubation, cells and supernatants were separated by centrifugation (1500 R.P.M., 6 minutes). In some experiments, both cells and supernatant were fractionated into a fraction soluble in ice-cold 5 per cent TCA (trichloroacetic acid), nucleic acids (soluble in 5 per cent TCA at 90°C), lipids (soluble in alcohol and ether), and proteins (TCA-insoluble) (12).

^{32}P was measured after drying and plating of small aliquots, or with a liquid-counting Geiger-Müller tube. ^{14}C was monitored, at infinite thinness, after drying and plating with a windowless Geiger-Müller tube in an automatic gas-flow counter. All values in Tables II to VII are expressed as the total radioactivity (C.P.M.) present in cells + medium. The relative release represents the radioactivity in the medium as per cent of that in cells + medium. In the kinetic experiments, the amounts of isotope released from the cells into the medium were calculated as described in the previous paper (1). The capitals N and U.C. in the legends to the tables refer to the origin of the white cells from normal individuals or ulcerative colitis patients, respectively. The subscripts are the initials of these persons.

RESULTS

The Action of Patients' and Normal White Cells on Colon Cells.—In the experiments of Fig. 1, ^{32}P-labeled colon cells were exposed to white cells and the release of isotope into the medium was measured as a function of the time of incubation. It will be seen that a rapid release of the total ^{32}P occurred in all

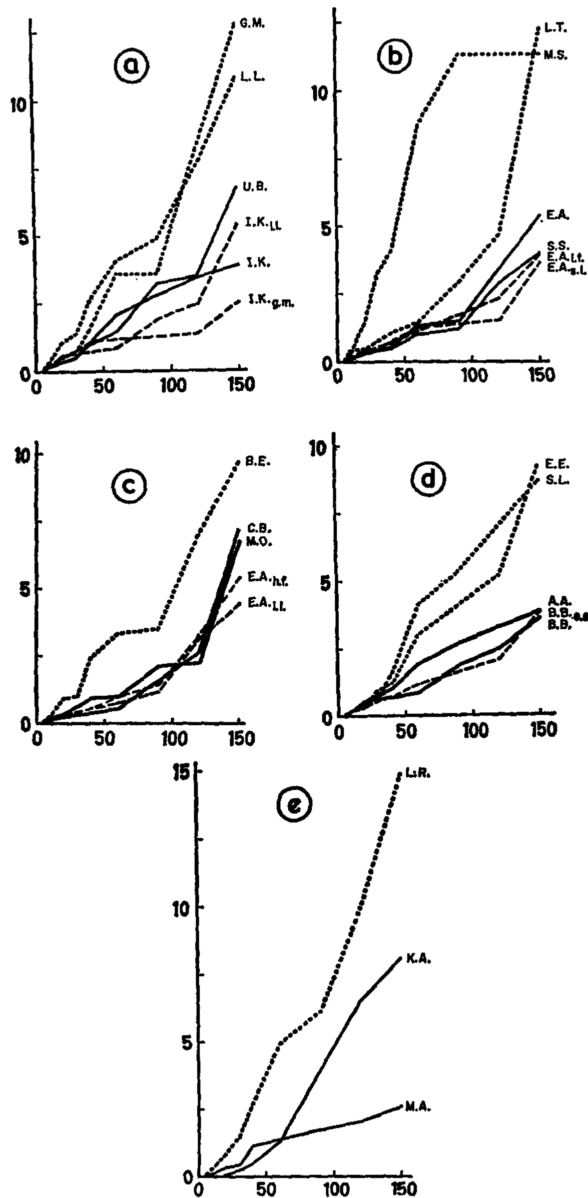

Fig. 1. Release of ^{32}P from colon cells upon incubation with *aged* white blood cells from patients with ulcerative colitis (dotted lines) or healthy individuals (solid lines). Broken lines: The white cells from healthy individuals were preincubated with patients' sera (10^7 cells/2 to 3 ml, 2 hours, 37°C) before they were added to the colon cells. Fig. 1 *a* to *e* represents 5 experiments, made with colon from different fetuses. The initials of the white-cell donors are designated with capital letters. The subscripts give the initials of serum donors. 100 μc of ^{32}P in 1 ml of medium were used for labeling the colon tissue in each experiment.

Ordinates: Cumulative values of radioactivity (C.P.M., $\times$ 10^{-5}) found in the medium. *Abscissae:* Time of incubation of colon cells with white cells (minutes).

samples from the beginning of incubation. However, a significantly higher release of isotope appeared in the samples treated with patients' white cells than in the controls.

The total radioactivity of the colon cells in the different experiments varied a great deal owing to losses of isotope during washing (13). However, even *within* each experiment there was a considerable variability of the total radioactivity present in the different mixtures ($\pm$ 15 per cent), mainly due to an unequal distribution of colon cells to different tubes. Therefore, the release of isotope into the medium was expressed as the per cent of the total ^{32}P present in

TABLE I

Relative Release of Isotope from Colon Cells into Medium after Different Times of Incubation with White Blood Cells

Time of incubation	U.C.			N			*P*
	$\bar{X}$	*s*	*n*	$\bar{X}$	*s*	*n*	
min.							
5	0.21	*0.06*	*8*	0.15	*0.3*	*10*	>0.3
10	1.8	*0.5*	*8*	1.3	*0.4*	*9*	<0.05
20	5.3	*2.7*	*8*	2.8	*1.0*	*10*	<0.05
30	8.8	*5.7*	*8*	3.9	*1.3*	*8*	<0.05
40	14.9	*7.1*	*8*	5.7	*2.0*	*9*	<0.01
60	28.5	*14.4*	*8*	9.5	*2.7*	*9*	<0.01
90	34.1	*18.9*	*8*	15.7	*3.5*	*8*	<0.02
120	50.1	*15.0*	*8*	24.1	*9.7*	*9*	≪0.01
150	75.7	*10.9*	*8*	39.5	*15.1*	*10*	≪0.01

$\bar{X}$, Mean values of the relative release (per cent) of isotope, calculated from the experiments of Fig. 1. U.C., N, release obtained after incubation of the colon cells with white cells from patients with ulcerative colitis and from healthy individuals, respectively.

s, standard deviation; *n*, number of incubation mixtures on which the calculation of $\bar{X}$ and *s* are based. The probability values (*P*) for significance of difference between the means were obtained by Student's t test.

each sample. The mean values of the relative release at different times, calculated from the experiments of Fig. 1 are given in Table I demonstrating again that the release of isotope was highest in the samples which were treated with patients' white blood cells. After 150 minutes of incubation, almost twice as much isotope was found in the medium of the patients' samples as compared with the controls.

Longer periods of incubation led to an increase of isotope release in all samples. Usually, after 4 to 5 hours of incubation, all the isotope was found in the medium in both patients' samples and controls.

As also illustrated in Fig. 1, attempts to demonstrate a specific cytotoxic effect by exposing colon cells to normal white cells previously treated with patients' serum have so far failed (Fig. 1 *a* to *d*).

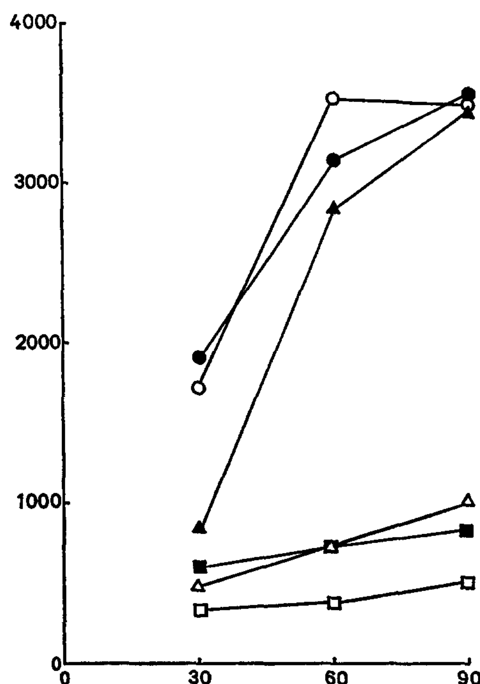

FIG. 2. Release of ^{14}C from colon cells upon incubation with *aged* white blood cells from a patient with ulcerative colitis (O. L., filled symbols) and a healthy person (P. C., empty symbols).

For labeling, colon from a 14 week old fetus was suspended in 2 ml of medium containing 5 μc of ^{14}C-amino acids.

Approximately 3×10^6 viable colon cells were incubated with 8.5×10^6 of *aged* white cells/tube. Final volume of incubation mixture 2 ml, containing 10 per cent of fresh guinea pig serum. After 30, 60, and 90 minutes of incubation, the tubes were centrifuged and 0.8 ml of the medium were taken off for extraction and measurement of radioactivity. The same amount of medium + guinea pig serum was immediately added to the cells for further incubation.

Ordinate: Cumulative values of radioactivity (C.P.M.) recovered in the different fractions of the medium (the values obtained at 60 and 90 minutes are corrected for the C.P.M. removed for the previous measurements). *Abscissa:* Time of incubation of colon cells with white cells (minutes). Circles, C.P.M. in protein; triangles, in alcohol-ether–soluble fraction; squares, in acid-soluble fractions (including nucleic acids, which, however, were only insignificantly labeled).

Chemical Nature of the Isotopic Material Released from the Cells.—Fig. 2 shows the results of an experiment with ^{14}C-labeled colon cells. The total radioactivity recovered at the end of the incubation was 10,184 C.P.M. for the ulcerative colitis sample and 9779 C.P.M. for the control. In the tube with the patients' white cells, 77 per cent of the isotope was found in the medium after 90 minutes. In the control, this value was 51 per cent. However, after 60 or 90 minutes of incubation, the radioactivity of the alcohol-ether–soluble fraction, containing

mainly radioactive amino acids combined with lipids, in the medium of the colitis samples was about 2 to 3 times higher than that of the controls. Similarly, a significant difference was also apparent in the acid-soluble fractions containing the bulk of free amino acids and low molecular peptides. In contrast, the high radioactivity of the TCA-insoluble protein in the medium was practically identical in both cases.

TABLE II

Action of White Blood Cells on Tissue Culture Cells

For labeling, 5×10^6 Chang cells or 1×10^7 L cells were suspended in 1 ml of medium containing 5 μc of ^{14}C.

Approximately 3×10^5 viable Chang cells or 2×10^5 L cells were incubated for 90 minutes with 1×10^7 *fresh* white cells/tube. Final volume of incubation mixtures 1 ml, containing 10 per cent of fresh guinea pig serum.

Soluble fractions: combined cold and hot TCA-soluble, and alcohol-ether–soluble fractions.

Incubation mixture	Soluble fractions		Protein	
	C.P.M.	Per cent release	C.P.M.	Per cent release
Chang cells —*	11,485	7.2	50,906	1.4
" " + N_K	9,361	10.9	33,920	2.0
" " + N_H	9,104	8.9	46,310	1.5
" " + $U.C._{P.N.}$	12,558	10.5	48,800	1.4
" " + $U.C._{B.L.}$	10,440	9.5	35,909	2.3
L cells —*	3,592	36.0	11,580	21.7
" " + $N_{A.P.}$	3,516	36.1	13,170	30.2
" " + $U.C._{P.N.}$	2,571	31.1	12,200	32.1
" " + $U.C._{B.L.}$	1,964	37.6	13,120	31.4

* No white cells added.

Confirmatory results were obtained in similar experiments using ^{32}P-orthophosphate as the label, although this gave only an unsignificant labeling of the TCA-insoluble protein. Examples of such experiments will be found in Tables III and VI.

Specificity of the Reactions.—Table II shows the results of two independent experiments, performed with 2 different strains of ^{14}C-amino acid–labeled tissue culture cells. In the experiment with the human liver cells, no difference between the action of the patients' white cells and that of the controls was noted. This was the same in the experiment with the L cells, although here the non-specific injury of the test cells was much higher.

Table III presents the results of a similar experiment, using a suspension of cells freshly isolated from fetal small intestine and labeled with ^{32}P-orthophos-

phate. Here again, there was no significant difference, in any of the fractions, between the samples treated with either patients' or normal white cells.

The limited supply of white blood cells and colon cells made it impracticable to include the latter as controls in the experiments recorded in Tables II and

TABLE III

Action of White Blood Cells on Cells of Small Intestine from Human Fetus

For labeling, small intestine from a 20 week old fetus was suspended in 1.5 ml of phosphate-free medium containing 10 μc of ^{32}P.

Approximately 2×10^5 viable cells were incubated for 120 minutes with 1×10^7 *fresh* white cells/tube. Final volume of incubation mixture 1 ml, containing 10 per cent of fresh guinea pig serum.

Incubation of small intestine cells with	Acid-soluble fraction		Lipids		Nucleic acids	
	C.P.M.	Per cent release	C.P.M	Per cent release	C.P.M.	Per cent release
—*	2186	44.7	386	27.0	1158	21.7
+ $N_{K.H.}$	2132	47.0	332	33.8	1335	30.8
+ $U.C._{K.S.}$	1210	48.3	214	34.6	1235	31.9
+ $U.C._{G.G.}$	1522	44.0	330	33.1	1399	31.2

* No white cells added.

TABLE IV

Action of White Blood Cells on Human Colon or Kidney Cells from the Same Fetus

For labeling, colon and kidney from a 24 week old fetus were separately suspended in 2 ml of phosphate free Eagle's medium containing 10 μc of ^{32}P.

Approximately 5×10^5 viable colon or kidney cells were incubated for 120 minutes with 2×10^6 *aged* white cells/tube. Final volume of incubation mixtures 2 ml, containing 20 per cent of fresh guinea pig serum.

Incubation mixture	Radioactivity	
	C.P.M.	Per cent release
Colon cells + $U.C._{L.T.}$	9039	36.5
" " + $No._{H.}$	9575	23.3
Kidney cells* + $U.C._{L.T.}$	13,981	29.3
" " + $No._{H.}$	25,087	27.4

* The number of kidney cells used in this tube was only 3×10^5.

III. However, white blood cells from the same patients were also active on colon cells when tested on other occasions (Tables V and VII). The results of a typical experiment with ^{32}P-labeled colon or kidney cells from the same fetus are shown in Table IV. In the case of the colon cells, the release of isotope ob-

tained with the patients' white cells was consistently higher than that obtained with the control white cells. In contrast, the release of isotope from the kidney cells was the same in both samples.

Desensitization of the Patients' White Cells.—In a limited number of experiments, the release of radioactive material from labeled colon cells was measured after exposure to white blood cells pretreated with colon antigen. In Experiment 1, Table V, two lyophilized colon extracts (14, 8, 15) were used for the pretreatment of some of the white cells. Colon extract A did not seem to contain antigen since sheep red blood cells coated with this material were not agglutinated by patients' sera (14). Neither did it inhibit hemagglutination of red cells coated with active preparations (8). In contrast, preparation B was highly potent in these respects. Table V demonstrates that pretreatment of the patients' white cells with preparation B also suppressed their cytotoxic action, whereas pretreatment with preparation A had no effect.

TABLE V

Effect on White Blood Cells of Pretreatment with Antigen

For labeling, colons from a 22 week old (Experiment 1) and a 19 week old (Experiment 2) fetus were suspended separately in 1.5 ml of phosphate-free medium containing 10 μc of ^{32}P.

Approximately 3×10^5 viable colon cells were incubated for 120 minutes with 1×10^7 *fresh* white cells/tube. Final volume of incubation mixture 1 ml, containing 10 per cent of fresh guinea pig serum.

Prior to incubation with colon cells, the white cells $N_{M.I.}$, $U.C._{G.G.}$, and $U.C._{B.L.}$ were pretreated with phenol-water extracted preparations A, B, or C obtained from human colon. To each sample of 1×10^7 fresh white cells suspended in 0.3 ml of Eagle's medium were added 2 mg of colon extract. The mixtures were incubated at 4°C for 2 hours. In Experiment 1, the pretreated white cells were then washed twice by centrifugation in 3 ml of medium. In Experiment 2, the mixture of white cells and colon extract was added to the colon cells without washing. For further details see text.

Experiment No.	Incubation of colon cells with	Radioactivity	
		C.P.M.	Per cent release
1	$N_{M.I.}$ pretreated with colon extract A	5184	32.5
	" " " " " B	6024	32.0
	$U.C._{G.G.}$ " " " " A	4650	53.3
	" " " " " B	5326	32.1
	$N_{K.H.}$	6326	37.1
	$U.C._{K.S.}$	3852	48.8
2	—*	7569	22.4
	$N_{K.}$	8513	24.5
	$U.C._{B.L.}$	7864	54.3
	$U.C._{B.L.}$ pretreated with colon extract C	6310	18.3

* No white cells added.

Confirmatory results were obtained in Experiment 2, Table V, made with colon extract C, also of high potency in the hemagglutination test. In this experiment, both the pretreated white cells and the incubation mixture were added to the colon cells. In spite of this, the release of isotope from the latter remained at a low level.

The Dependency of the Cytotoxic Effect on a Heat-Labile Serum Factor.—Fig. 3 shows the results of an experiment where ^{32}P-labeled colon cells were exposed to the white cells of a patient either in the presence or absence of fresh guinea pig serum. The release of isotope was consistently higher when guinea pig serum was present. After 150 minutes of incubation, the relative release was

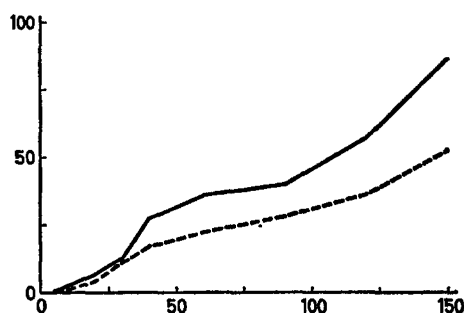

FIG. 3. Relative release of ^{32}P from colon cells upon incubation with *aged* white blood cells from a patient (L. L.) with ulcerative colitis in the presence (solid line) or absence (broken line) of 0.3 ml of fresh guinea pig serum. The experimental conditions and the calculation of the relative release of isotope were the same as those used in the experiments of Fig. 1 and Table I. (See Materials and Methods.)

Ordinate: Amount of total ^{32}P in the medium, as the percentage of the total ^{32}P present in the cells at the beginning of the experiment. *Abscissa:* Time of incubation of colon cells with white cells (minutes).

86.2 per cent in this case, whereas it was 52.8 per cent in the control. (Another batch of white cells from the same patient was used in the experiment of Fig. 1 *a*. In this case the release after 150 minutes was 83.6 per cent.)

The non-specific serum factor required for the cytotoxic action was heat-labile. In the experiment of Table VI, 40 per cent of the total ^{32}P was given off by the cells incubated with patients' white cells and fresh guinea pig serum. In the sample containing heat-inactivated guinea pig serum, the release was 22.9 per cent and in the control 24.6 per cent. Table VI shows that consistent differences were found in all 3 chemically separated fractions.

The data reported in Table VII show the results of the one experiment made thus far in which guinea pig serum was replaced by fresh human serum in addition to the 10 per cent of heat-inactivated human serum always present in the medium. The data suggest that fresh human serum is equally as effective as guinea pig serum in mediating the cytotoxic action of the cells.

TABLE VI

Effect of Fresh Guinea Pig Serum on the Release of Isotope

For labeling, colon from a 19 week old fetus was suspended in 1.5 ml of phosphate-free medium containing 10 μc of ^{32}P.

Approximately 3 $\times$ 10^5 of viable cells were incubated for 120 minutes with 1 $\times$ 10^7 *fresh* white cells/tube. Final volume of incubation mixture 1 ml containing 10 per cent of guinea pig serum. In one of the tubes the guinea pig serum was inactivated by heating to 56°C for 30 minutes.

Incubation of colon cells with	Acid-soluble fraction		Lipids		Nucleic acids	
	C.P.M.	Per cent release	C.P.M.	Per cent release	C.P.M.	Per cent release
Fresh guinea pig serum*	3349	18.6	342	9.4	3878	27.0
N_K. + fresh guinea pig serum	3890	24.3	239	33.9	4384	24.3
U.C.$_{P.N.}$ + " " " "	3310	35.2	268	54.9	2681	43.8
" + heat-inactivated guinea pig serum	3944	22.8	259	27.8	3975	22.8

* No white cells added.

TABLE VII

Effect of Fresh Human Serum on the Release of Isotope

For labeling, colon from a 20 week old fetus was suspended in 1.5 ml of phosphate-free Eagle's medium containing 2 μc of ^{32}P.

Approximately 3 $\times$ 10^5 viable colon cells were incubated for 120 minutes with 1 $\times$ 10^7 *fresh* white cells/tube. Final volume of incubation mixtures 1 ml, containing 10 per cent of human serum in addition to that supplied to the medium (see text). In two of the tubes the separately added serum was inactivated by heating to 56°C for 30 minutes.

Incubation of colon cells with	Radioactivity	
	C.P.M.	Per cent release
$N_{I.L.}$ + fresh human serum	740	33.8
" + heat-inactivated human serum	816	33.7
U.C.$_{K.G.}$ + fresh human serum	800	43.0
" + heat-inactivated human serum	650	28.6

DISCUSSION

The results presented in this paper are based on experiments made with white blood cells from 14 patients and 18 healthy individuals. The release of isotope into the medium from colon cells treated with patients' white cells was consistently higher than that occurring in the controls. The values for the relative release after comparable times of incubation were surprisingly similar despite certain variations in the experimental conditions. For example, the

results were similar when the excess in number of white cells over colon cells was only 3 or 4 (Figs. 1 to 3, Table IV) or 30- to 50-fold (Tables II, III, V to VII). Thus, the number of sensitized white cells was not the limiting factor in the system.

An evaluation of the present data in quantitative terms would be complicated. Most likely, only a certain fraction of the colon cells was reactive in the cytotoxic system (see references 1, 16). The release of isotope was probably also accentuated by repeated centrifugations of the cells. On the other hand, some isotope may have escaped detection in the medium because of uptake by white cells. However, the main complication was the non-specific action of white cells as revealed by a comparison of the present controls with those in the preceding paper (1). Although the handling of the samples was similar, more than twice as much isotope was given off from the colon cells when white cells were present. Most likely, the excessive cell concentrations and the ensuing change in the medium contributed significantly to these results.

Treatment of animal cells with cytotoxic serum antibodies, in the presence of complement, leads to alteration of the cell surface, changes in the osmotic equilibrium and rapid release of inorganic ions as well as of low molecular substances such as nucleotides and amino acids (17). Provided the protein concentration of the medium is not too high, the cells also lose a significant portion of their macromolecular constituents (18). Similar results were obtained in the present study. No reliable values for the specific release of protein were obtained, since this was masked by non-specific protein loss in all samples. The non-specific release of protein was probably due to damage done to the cells by trypsinization and centrifugations (13).

It has recently been found that skin exudates obtained from patients with ulcerative colitis are rich in basophilic granulocytes and tissue mast cells (19). However, no increased incidence of such cells was found in the peripheral blood of the patients, neither by the aforementioned authors nor by ourselves. Moreover, the present experiments showed that patients' white cells are specifically sensitized toward colon cells. This indicates that the cytotoxic reaction was immunologically specific.

Two different types of white cell preparations were employed in the present study. In the experiments of Tables II, III, V to VII, the preparations were fresh and 30 to 70 per cent of the cells were polymorphonuclear leucocytes. In the experiments of Figs. 1 to 3 and Table IV, the white cell suspension had been aged and the final suspensions, regardless of whether of patients' or of normal origin, consisted of 60 to 70 per cent of lymphocytes and 20 to 30 per cent of large mononuclear cells. Nevertheless, the specific cytotoxic effect obtained with the different samples was essentially the same in all cases. This makes it unlikely that polymorphs were responsible for the cytotoxic damage of the colon cells.

Attempts to sensitize normal white cells by pretreating them with patients' serum had no effect (Fig. 1 *a* to *d*). This would seem to speak against the possibility of a passive sensitization. It is, of course, possible that the antibody concentration of the sera was not sufficient for this purpose. Moreover, a passive sensitization of white cells *in vivo* by means of "cytophilic" antibodies (20) cannot be excluded. However, the cells retained their cytotoxic potency even after ageing and several washings in the presence of 10 per cent of normal human serum.

From the rather limited number of desensitization experiments (Table V), it appears that the antigen reacting in the cytotoxicity test is present also in the colon extract reacting in the hemagglutination test (14, 8). However, more experiments are needed for determining the specificity of the antigenic determinants reacting in the cytotoxic system.

The reaction *in vitro* between sensitized cells and antigen does not represent an unusual finding. In tuberculin hypersensitivity and related bacterial allergies, sensitized cells may be damaged or even stimulated when exposed to antigen (21–24). These reactions are slow and do not seem to require complement. In contrast, circulating lymphocytes, granulocytes, and large mononuclear cells from hypersensitive animals are lysed within a few minutes when reacting with antigen *in vitro* (25, 21). Complement is necessary for the reactions and immune plasma may sensitize normal lymphoid cells. In tuberculin or diphtheria toxoid hypersensitivities, a short *in vitro* contact time of sensitized rabbit cells with antigen deprives them of the capacity to transfer hypersensitivity to normal recipients (26). Desensitization is combined with a release of pyrogenic substances (27). A specific reaction *in vitro* between immune lymph node cells and extracted antigen has also been observed in a homograft system of the mouse (28). Whether or not desensitization in the present study was combined with specific injury of the sensitized white cells cannot yet be stated with certainty.

Cell mediated immune reactions are assumed to play an important role in tissue destruction (29). In cases where immune serum seems to be without effect, transfer of delayed hypersensitivity (30), homograft reactivity (31), or experimental autoimmune disease (4–7) can still be accomplished with lymphoid cells from various sources, including the peripheral blood (32, 33). However, although there is some evidence for a direct interaction *in vivo* of the transferred immune cells with antigen-bearing target cells in tuberculin hypersensitivity (34, 35), transfer may involve proliferation and differentiation of the transferred cells or their interaction with cells of the host's own immune apparatus (29, 36, 37). Therefore, the results of the present study cannot easily be reconciled with those obtained in transfer experiments.

Only a limited number of *in vitro* studies is available for direct comparison with the present investigation. Several attempts to reproduce homograft destruction *in vitro* by means of tissue fragments have given negative results (38–

40). Recently, however, Weaver (41) reported that mouse tumor cells were destroyed *in vitro* by peritoneal lymphocytes from immunized mice of another strain. Merril *et al.* (42) noted a slight cytotoxic effect when incubating peritoneal leucocytes of donor rabbits with those of recipients, grafted with donor skin and also challenged intraperitoneally with donor material. Govaerts (43) reported a slight cytotoxic effect, appearing after 24 and 48 hours, of thoracic duct lymphocytes from dogs homografted with kidney, on cultures from cells of the donor's second kidney. This was seen neither with cells from normal dogs nor with serum from grafted dogs. However, when immune cells and heat-inactivated recipient serum were combined, marked cytotoxic damage of the kidney cells was observed. Addition of complement (guinea pig serum) further increased this cytotoxic effect. Stuart (44) exposed fresh monolayer cultures of HeLa cells to spleen cells from normal or immune rats. In this heterologous system, cells from normal rats also had a cytopathic effect. However, immunized spleen cells produced lesions more rapidly (noticeable within 3 hours). Only living cells were cytotoxic and the reaction was said to require complement. Finally, Rosenau and Moon (45) studied the action of sensitized splenic lymphocytes from mice on homologous donor cells (Earle's L strain fibroblasts) in a similar way. Most of the tissue culture cells were destroyed within 48 hours. No antibody was found in the sera of the immunized animals and no heat-labile serum factor was required.

The positive results referred to above were probably achieved because of close contact between lymphoid cells, present in excessive numbers, and target cells. Such favorable conditions were also prevalent in the present investigation. Whether the cytotoxic mechanisms were the same in the various studies remains to be elucidated. That similarities may exist is apparent from our preliminary experiments with monolayer cultures (8). On the other hand, the data regarding the need of a heat-labile serum factor are conflicting.

The mechanism involved in the cellular activities reported here are unknown. (For discussion, see reference 37.) Briefly, it may involve cell-cell interaction between sensitized white cells and antigen-bearing colon cells. Alternatively, sensitized cells may release antibody locally or react with antigen given off from the colon cells (46). Participation of complement might then result in damage either of the colon cells or the white cells. In the latter case, the injury of the colon cells could ultimately be brought about by products released from the damaged white cells (22, 27, 37). However, the negative results of the desensitization Experiment 2 of Table V do not favor this explanation. Of particular interest is the requirement of a heat-labile serum factor. It is believed that such a factor, probably complement, is necessary for many allergic reactions of the immediate type (47) and even for certain homograft destructions (48, 49). There is, however, no evidence for complement dependency in delayed hypersensitivity. If it can be proved conclusively that the present reaction is

complement dependent, a complement fixing γ-globulin should play an important role in the present cytotoxic system.

This investigation has provided some evidence that colon-specific hypersensitivity reactions may be important for the formation of tissue lesions in ulcerative colitis. This is not meant to imply that immune reactions of the type described would be exclusively, or even primarily, responsible for the tissue damage observed in the diseased colon. It is realized, that the *in vitro* conditions applied here may have artificially accentuated an otherwise weak reactivity of the sensitized white cells. Be this as it may, it is also evident that a variety of immune and non-immune reactions will contribute to the final pathological condition in the ulcerated colon (2, 29).

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SUMMARY

Freshly isolated fetal human colon cells were labeled with ^{32}P-orthophosphate or ^{14}C-amino acids and exposed to white blood cells from children with ulcerative colitis or from healthy controls. Exposure of the colon cells to patients' white cells led to a rapid isotope release, significantly higher than that obtained with normal white cells. After 150 minutes of incubation, 75 per cent of the total isotope present was found in the media of the colitis samples but only 40 per cent in those of the controls. Consistent results were obtained with white blood cells from 14 patients and 18 healthy individuals. Similar results were obtained with either fresh white cells or with white cells aged for 12 to 18 hours and consisting to 60 to 70 per cent of lymphocytes and to 20 to 30 per cent of large mononuclear cells.

No specific cytotoxic activity could be conferred onto normal white cells by pretreating them with patients' serum containing antibodies against colon antigen. The cytotoxic action of the patients' white cells was immunologically specific, since no difference from the controls was found in the isotope release when cells from other organs or animals were similarly treated. Preliminary experiments suggested that the patients' white cells could be desensitized by pretreating them with colon extract. For obtaining a significant cytotoxic effect of the patients' white cells, the presence of 10 to 20 per cent of fresh guinea pig or human serum in the incubation medium was required.

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