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# HISTOLOGIC AND IMMUNOHISTOCHEMICAL FEATURES OF THE AUER "COLITIS" IN RABBITS

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Auer [1] in 1920 demonstrated that massive inflammation could be produced at the site of a previously mild, local, nonspecific inflammatory reaction, by the parenteral administration of a large dose of antigen to a specifically sensitized animal. He produced severe inflammatory reactions and necrosis in the ears of rabbits sensitized to horse serum, by gently rubbing the ears with xylol and then re-injecting horse serum, usually intraperitoneally. He attributed the resultant inflammatory response to a local anaphylactic reaction. Subsequent workers [2] confirmed his observations regarding the attraction of mildly irritated tissue for circulating antigen and antibody, and emphasized the importance of increased capillary permeability in the selected concentration of antigen and antibody at these sites. Colloidal dyes have been employed to suggest indirectly the localization and concentration of circulating antibodies in areas of inflammation,[3] while regional antibody titers have been increased after the preliminary instillation of a mild irritant in sensitized animals.[4] To our knowledge, however, there has been no previous direct demonstration of specific antigen or antibody in the identical sites of the cytotoxic reaction characterizing the Auer phenomenon, except as briefly reported by us.[5,6]

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In the application of the Auer technique to the colon, Kirsner and associates [7,8] previously reported an experimental "colitis" in rabbits sensitized to crystalline egg albumin (CEA) and receiving very small rectal instillations of dilute formalin, followed by additional "challenging" doses of the antigen by a variety of routes. While these experimental findings were compatible with the concept of an antigen-antibody reaction as the basis of the "colitis," objective evidence of antigen or antibody localization was lacking and the evidence for an immunologic classification was primarily circumstantial. The present study expands the earlier observations and demonstrates a method which results in increased reproducibility of the Auer "colitis." Specific antigen and antibody have been identified in the identical sites of the cytotoxic reaction by fluorescent immunohistochemical methods, and therefore the earlier assumption of the participation of these immune substances in the tissue damage characterizing the Auer "colitis" is strengthened. The principles would seem to involve sensitization to a soluble antigen; a generalized antibody response; the localization of circulating antigen and antibody in a nonspecifically mildly irritated tissue on the basis of an increased vascular permeability in these areas; and the resultant antigen-antibody reaction leading to tissue injury.

## Material and Methods

### *Animals*

The animals used in these experiments were albino hybrid rabbits of both sexes, weighing from 2 to 3 kg., obtained from a single local breeder. The animals were maintained on a diet of Purina rabbit pellets and water *ad libitum*.

### *Antigen*

Five times crystallized egg albumin (CEA; Pentex, Inc., Kankakee, Ill.) in a 2 per cent solution of distilled water was utilized as the antigen in all animals, both for sensitization and challenging injections. A multiple portal method of immunization was employed; this involved intravenous, intraperitoneal, intramuscular, intradermal and subcutaneous injections without the use of an adjuvant.[9] The period of immunization was from 4 to 6 weeks. The immunized status of the rabbits was evidenced by subsequent positive Arthus skin tests employing the intradermal injection of 0.1 ml. of 0.5 per cent CEA and, in the few animals tested, by the appearance of specific precipitin blood titers. Others [10,11] similarly have found a definite parallelism between the precipitin content of the serum and the intensity of the Arthus reaction.

### *Nonspecific Rectal Irritant*

The experimental animals and appropriate controls received 1 ml. 0.4 per cent formalin per rectum by soft rubber catheter inserted through an infant-sized proctoscope, according to the schedule described below. The proctoscope was removed prior to instillation of the rectal irritant at a depth of approximately 9 cm., and invariably the animals retained this small fluid volume. Previous studies [7] employing simultaneous rectal instillation of gentian violet had shown the cephalad flow of this enema into the descending colon for a total depth of 20 to 30 cm.

### *Observation*

The rabbits remained under careful clinical surveillance and were observed particularly for evidence of diarrhea, rectal bleeding or obvious toxicity. Prior to the start of the experimental period and each day of the 11-day test period, the rectum was inspected initially, using the infant proctoscope introduced to a level of 9 cm. above the anal ring. Daily observations were made as to the color, consistency and quantity of stool, as well as notations on a 1+ to 4+ basis as to the presence of granularity, increased vascularity, hyperemia, ulceration and polypoid mucosal elevations. Proctoscopy did not necessarily reveal the full extent of the "colitis" when present, for the more intense reactions frequently were found at necropsy in the area from 15 to 30 cm. The gross and microscopic features of the rectum and colon thus were utilized rather than the proctoscopic gradations in characterizing the Auer "colitis."

### *Histologic and Immunofluorescent Study*

The rabbits were killed with intravenous sodium pentobarbital either 3 or 24 hours following the final "challenging" antigen injection. Representative areas of reactive and normal colon were quick-frozen, sectioned in a cryostat and fixed in 95 per cent ethanol in preparation for fluorescence microscopy. Comparable adjacent sites also were fixed in 10 per cent formalin for appropriate staining and examination by conventional microscopy. In some animals sections of Arthus skin reactions were similarly sampled and fixed. In a few instances sections of grossly normal stomach and kidney also were obtained. Efforts were made to cut the unfixed frozen sections as rapidly as possible, usually within 24 to 48 hours, although sometimes the fluorescent immunohistochemical staining was delayed for several days, in which case the slides were stored in a deep freeze.

*Antiserum.* Anti-CEA globulin was obtained by ammonium sulfate precipitation of the pooled serums from CEA-sensitized rabbits other than those used for the present experiment. The immunologic activity of this antiserum was tested by the demonstration of positive precipitin ring tests at the interface between this globulin and known amounts of the specific antigen.

*Fluorescein Isothiocyanate.* A crystalline preparation (Lot No. 110623) of fluorescein isothiocyanate, from Baltimore Biological Laboratories, was used in the present study to prepare the fluorescent anti-CEA globulin.

*Conjugation of Antiserum.* The protein content of the globulin solution was determined employing the biuret test and adjusted to a 1 per cent concentration. The pH of the globulin solution was adjusted to 9.0 with 0.5 M carbonate-bicarbonate buffer. The fluorescein isothiocyanate powder (0.05 mg. per mg. protein) was added to the constantly stirring refrigerated (0 to 4° C.) globulin solution. Conjugation was carried out for approximately 18 hours in a cold room.

*Dialysis of Conjugate.* The conjugate was placed in a dialysis sack tied to a magnetic stirring bar over a 500 cc. graduate. The conjugate was dialyzed against pH 7.6 phosphate buffer saline (0.01 M phosphate) until the dialysate was grossly free of fluorescein. Dialysis was carried out at 4° C. with frequent changes of buffered saline over a period of 2 to 4 days. The conjugate was removed from the dialysis sack and placed in the refrigerator for storage, or frozen in small amounts if the staining experiment was delayed. For the immunohistochemical demonstration of antibody, a commercially obtained solution of fluorescein isothiocyanate conjugated with sheep anti-rabbit globulin (Sylvana Chemical Company, Lot No. 509601) was employed and a sephadex G-50 column used for the removal of fluorescent dialyzable products by the method of Goldstein, Slizys and Chase.[12] Earlier efforts to demonstrate the specific antibody in the colon reaction sites involved the use of fluoresceinated CEA.

*Absorption of Conjugated Antibody.* For most of the fluorescent antibody studies

the conjugated anti-CEA globulin was absorbed with whole lyophilized mouse liver powder in the proportion of 100 to 150 mg. of liver powder to 1 ml. of fluoresceinated antibody. Absorption took place in plastic centrifuge tubes for 1 hour at room temperature followed by centrifugation at 5,000 to 10,000 r.p.m. The supernatant then was collected and re-centrifuged at slower speeds. The conjugate also was passed through a Dowex 2-X4 ionic exchange resin column. In case the immunofluorescent study was not performed on the same date, usually a second absorption with mouse liver powder was undertaken on the date of the staining experiment.

*Fluorescent Staining.* In general, the techniques employed were those of Coons and Kaplan.[13] Prior to staining, the cryostat-cut sections were incubated for 15 minutes in Coplin jars containing pre-heated (37° C.) 95 per cent ethanol, and they were then removed, drained on edge and dried for another 30 minutes. The fixed slides were rinsed for 10 minutes in constantly circulating buffered saline (pH 7.6), excess saline was removed, and a capillary pipette employed to overlay the tissue sections with the appropriate stain. Staining usually took place over a 30-minute period followed by a similar 10-minute rinse in buffered saline. Excess saline again was removed and the slides were mounted in buffered glycerol. The cover slip was sealed with nail polish to minimize drying and fluorescent fading prior to microscopic study.

*Staining Controls.* The following specificity controls were used: (a) application of fluoresceinated normal rabbit serum; (b) pre-incubation with unlabeled antibody prior to fluorescent antibody application; (c) staining with "deactivated" conjugated antibody after antigen adsorption on the basis of optimum proportion ring test studies; (d) testing with heterologous fluorescent antibody stains, e.g., fluoresceinated anti-Ehrlich ascites tumor gamma globulin; (e) testing with buffered saline alone.

*Models.* Control "models" for the immunofluorescent staining reactions were prepared by submucosally injecting normal rabbit colon at the time of autopsy with 0.05 cc. of 1:1 mixture of 2 per cent CEA and 12 per cent gelatin prior to quick freezing. Such a control was useful initially to indicate the apple-green color of the fluorescent antibody in relation to the auto-fluorescence of colonic tissue. The use of such a model for each staining experiment also insured against deterioration in the immunologic potency of the fluorescent antiserums.

*Fluorescence Microscopy and Photomicrography.* The basic apparatus consisted of a conventional light microscope modified by the use of a dark-field condenser (E. Leitz), and a 200 watt source of ultraviolet radiation (Osram HBO-200). A Schott UG-2 exciter filter was employed. A Wratten 2-B barrier filter was inserted above the microscope objective. For photography, exposures were made on 35 mm. high speed Ektachrome daylight film with a Leitz (Leica) camera. Exposure time was invariably 4 minutes and was controlled with an automatic timer. Cargille's type B low fluorescence nondrying immersion oil was employed.

### *Experimental Protocol*

The current report is based upon an experimental group of 43 rabbits and an additional 17 controls. The experimental animals were sensitized to CEA by multi-portal routes over a period of 4 to 6 weeks as referred to above. Each animal subjected to the full Auer procedure received an enema of 1 ml. 0.4 per cent formalin on experimental days 1 through 4 and 8 through 11. On experimental days 2 through 4 and 9 through 11, the enema was followed 45 minutes later by the intravenous administration of 1 ml. 2 per cent CEA, for a total of 6 "challenging" antigen injections over the 11-day experimental period. Controls among the sensitized animals included animals subjected to no procedures, those receiving challenging antigen without rectal irritation, and those receiving rectal irritation but saline rather than antigen challenges. Controls among the nonsensitized animals consisted of animals undergoing no procedures, those receiving intravenous antigen injection without rectal irritation, those receiving rectal irritants as the only procedure and those receiving both rectal irritation and intravenous CEA injections over the 11-day schedule.

*Observations*

*Gross.* In general, the gross colonic observations at the time of necropsy were those listed above in reference to the proctoscopic evaluations.

*Microscopic.* The formalin-fixed, hematoxylin and eosin-stained sections of normal and inflamed colonic mucosa were carefully studied with special reference to the mucosal, submucosal and related changes noted in the previous studies of the Auer colon reaction. In the mucosa these included: fibrinopurulent exudate, atrophy, ulceration, hemorrhage, inflammatory "pseudo-polyps," granulomas, and infiltration with eosinophils, pseudo-eosinophils and mononuclear cells. The submucosa was scrutinized for evidence of similar perivascular cellular infiltration, edema and dilatation of blood and lymphatic vessels.

## RESULTS

### *Gross Features of the Auer "Colitis"*

Gross necropsy abnormalities of varying severity occurred in all 43 animals of the experimental Auer group and included hyperemia, hemorrhages, ulceration, thickening of the bowel wall, and patchy areas of denuded mucosa (Fig. 1). The colons of those control animals receiving the rectal irritant varied from normal to minimal hyperemia. The colons in the other sensitized and nonsensitized controls were grossly normal at necropsy, except for occasional scattered areas of proctoscopic trauma. The colon more proximal to 50 cm. from the anus, and the remainder of the gastrointestinal tract and other abdominal viscera in all instances were grossly normal.

### *Histologic Features of the Auer "Colitis"*

Hematoxylin and eosin-stained microscopic sections of the reactive colonic sites revealed: submucosal edema; mucosal hemorrhage and ulceration; mucosal-submucosal infiltration by mononuclear cells, eosinophils and pseudo-eosinophils; perivascular round cell accumulations; dilated lymphatics; and engorged blood vessels. The histologic findings in the Auer colitis were similar or identical to those described earlier from our laboratory.[7,8] Submucosal edema was prominent even in nonulcerated inflammatory areas (Fig. 2). Microscopic study of the colons of control animals receiving dilute formalin enemas showed mucosal-submucosal vascular congestion, rare superficial mucosal ulceration and a slight increase in round cells in the lamina propria. There was little or no submucosal edema; eosinophils and pseudo-eosinophils were seldom present in increased numbers; and in no instances were perivascular cellular infiltrates observed. The colons of controls not subjected to dilute formalin irritation, as well as the colons of sensitized and nonsensitized animals receiving no procedures, were histologically within normal limits. In all animals the colon more proximal than 50 cm. from the anus was microscopically normal. The sections of the Arthus skin reactions, usu-

ally of 24 to 48 hours' duration at the time of necropsy, demonstrated acute angiitis; many pseudo-eosinophils, eosinophils and mononuclear cells; tissue necrosis; edema; and vascular thrombi.

*Immunohistochemical Localization of Specific Antigen*

Specific antigen was demonstrated immunohistochemically at the sites of the Auer colon reactions and not in the colons of the sensitized or nonsensitized control rabbits. The antigen was found perivascularly in the edematous submucosa, especially in the areas of cellular infiltrations adjacent to the dilated blood and lymphatics vessels (Fig. 6). In some instances, the apple-green fluorescent staining of this soluble antigen was in close proximity to the vascular endothelium of submucosal arterioles and capillaries. Outside these vessels, the antigen could often be visualized diffusely in the edematous submucosa or lower mucosa; or, in some instances, it appeared to surround mononuclear cells of the inflammatory exudate (Figs. 6 and 7). The antigen was best demonstrated in those areas of the Auer "colitis" which showed only a moderate degree of activity. In the more advanced hemorrhagic and ulcerated colon lesions, it has been our experience that considerable cellular disruption and tissue necrosis occurred, resulting in much autofluorescence and an increased degree of nonspecific fluorescent staining. The latter also occurred in tissues that had not been well preserved, with degeneration prior to fixation and staining. When present, this nonspecific staining invariably included the cytoplasm of the mucosal glandular cells.

To correlate each fluorescent observation with conventional light microscopy, histologic sketches were drawn of hematoxylin-eosin stains of a serial frozen section. Efforts then were made under the dark-field microscope to study and photograph the same serial sites in the various experimental and control sections. Observation of fluorescent antibody-treated sections which were then stained with hematoxylin and eosin afforded useful information regarding the specific sites of antigen localization (Figs. 3 to 5). In such preparations the antigenic loci often appeared as slightly more eosinophilic, diffuse or localized aggregates in the edematous layers of the submucosa and mucosa. In those instances where the antigen was localized in close association with the inflammatory cells among the mucosal glands and in adjacent edematous areas, the more cellular organization of the fluorescence suggested the possibility that the antigen so visualized might have reacted with antibody bound to cells since antibody formation has been allied to small lymphocytes and plasma cells.[14] The reddish brown autofluorescence of muscle, the bluish purple autofluorescence of mucosal glands, and the bluish white, bright autofluorescence of elastic tissue also were helpful in evaluating the lo-

calization of the specific apple-green fluorescence under dark-field microscopy.

The fluorescent staining of specific antigen was not visualized in other tissues of these animals or in the colons of the sensitized or nonsensitized control rabbits. Fluorescence was not taken to represent specific antigen unless the comprehensive staining controls were appropriately negative.

*Immunohistochemical Localization of Antibody*

Efforts to demonstrate the tissue localization of the specific antibody (ACEA) directly, employing fluoresceinated CEA as an immunohistochemical stain, were not successful. Some evidence was obtained that this might be attributed to the formation of soluble antigen-antibody complexes, since fluorescent aggregates were noted in certain staining experiments employing tissues from immunized animals. These aggregates, however, were seen diffusely over the slide, often seemed to be on a different microscopic plane than the tissue, and bore no relationship to cellular structure. Conceivably, a reduction in the protein concentration in these conjugated antigen solutions and an alteration in the fluorescein:-protein ratio [12] might have obviated the earlier difficulties.

Recent studies employing conjugated sheep anti-rabbit globulin as the immunohistochemical stain, with an adequate reduction of the fluorescein:protein ratio, enabled us to demonstrate rabbit globulin, presumably antibody in the Auer "colitis" lesions. The increased tissue globulins were localized in the same areas as the specific antigen and correlated with the sites of the perivascular submucosal and mucosal edematous and inflammatory reactions. The apple-green fluorescence of the presumed antibody in these studies was also found in association with the mononuclear cells in the lower layers of the mucosa and in the perivascular areas of the submucosa. In some instances it appeared on the surface of these cells, and often it was demonstrated in the cytoplasm, as the nonfluorescent nucleus of these cells was quite apparent. More frequently, however, there was a small pool of fluorescence and occasionally this appeared to be an aggregate of fluorescent globulin emanating from a cluster of mononuclear cells (Fig. 8). Specificity controls for these fluorescent studies again included inhibition, "de-activation" and heterologous fluorescent antibody techniques. Some fluorescence was seen in the more proximal normal colon areas of the Auer "colitis" animals and some antibody could be observed within occasional submucosal arterioles in the colons of some of the immunized control rabbits, but there was a virtual absence of fluorescence outside the vascular tree, except in those instances where a combination of increased capillary permeability and antigen sensitization co-existed.

## Discussion

The studies reported herein demonstrated the reproducibility of the Auer "colitis" as previously reported by Kirsner, Elchlepp and their coworkers.[7,8] The histologic findings in the lesion included edema; mucosal hemorrhage; cellular infiltration in the mucosa and submucosa with polymorphonuclear cells, plasma cells, lymphocytes, monocytes and eosinophils; dilated lymphatics; and occasional perivascular infiltrates. Our results were quite similar and with the modified 11-day experimental dosage schedule, the reproducibility of the Auer reaction was increased to 100 per cent of animals so tested. It had been postulated that the Auer procedure involved (a) a generalized antibody response to sensitization with a soluble and diffusible antigen; (b) artificially increased capillary permeability in the lower bowel, facilitating localization of antigen and antibody in this area; and (c) their combination, inducing a reaction constituting a "colitis." [15]

The evidence for formulating such a pathogenetic mechanism was circumstantial in the colon. Indeed, there has been no previous objective evidence for the presence of either specific antigen or antibody in association with the histologic lesions of the Auer reaction, as has been demonstrated for the Arthus reaction.[16–18] The predominantly perivascular nature of the early Auer reaction, the prevalence among the inflammatory elements of plasma cells, eosinophils and pseudo-eosinophils, and the resemblance to the histologic appearance of the classic Arthus reaction all pointed to an antigen-antibody combination participating in the cytotoxicity of the Auer "colitis." The immunohistochemical demonstration of the specific antigen by the direct technique, coupled with the subsequent demonstration of increased tissue globulins have now provided, for the first time, objective evidence that an antigen-antibody reaction was indeed present in the "colitis" areas. Gross and microscopic lesions were not seen, and neither antigen nor antibody could be demonstrated in animals that had not been sensitized (i.e., no circulating antibody), had not been mildly, nonspecifically traumatized (i.e., no increased capillary permeability), and had not received challenging specific antigen doses (i.e., antibody *and* antigen both required for the reaction).

The similarity of the antigen and antibody localization in the Auer colitis reaction to that of the Arthus reaction [16–18] is highly significant. In the Arthus reaction, immunofluorescent techniques demonstrated antigen localization in the subendothelial region of blood vessels, and antigen-antibody complexes have been demonstrated in adjacent tissues, sometimes in large aggregates within and between the necrotic muscle

fibers, in the cytoplasm of cells, and mixed with erythrocytes and necrotic debris.[18] Others have studied the Arthus-type immediate hypersensitivity reaction in the rabbit mesentery, using a combination of electron and fluorescence microscopy, and have similarly demonstrated antigen-antibody interaction in the lumen and walls of small blood vessels and in the surrounding interstitial connective tissue.[19] The antigen in the present study was found on the endothelial surface of small arterioles and also in adjacent tissues in a diffuse form or in association with the cytoplasm of inflammatory cells. Re-staining of these fluorescent slides with hematoxylin and eosin indicated that while some antigen was localized to structures of the type cited, in other instances it was represented as an amorphous and occasionally more eosinophilic substance. The possibility existed that in such instances unbound antigen accumulated at the site of an antigen-antibody complex.

With respect to whether actual antigen-antibody union was responsible for the cytotoxic reaction, final objective proof has not been demonstrated as yet. In this regard, one possible modification of these experiments would be to employ double fluorochromes in the immunohistochemical studies.[20] Also, in recent years, there has been an increasing interest in the immunogenic properties of antigen-antibody complexes,[21,22] and such studies in relation to the Auer reaction in the colon are in progress. There is evidence, however, that the antigen-antibody complexes themselves may not be toxic, but elicit an inflammatory response via other cellular and chemical mediators. For example, the Arthus vascular inflammation has been prevented by depletion of polymorphonuclear leukocytes which seem to participate actively in the hemorrhage and necrosis of such lesions.[17] There also is evidence that antigen-antibody complexes may produce injuries to certain tissues by virtue of their capacity to fix and activate complement, perhaps with a resultant activation of certain permeability factors.[23] Tissue mast cells may also be involved in the pathogenesis of the cytotoxicity associated with antigen-antibody union.[24]

The Auer "colitis" demonstrated in these studies provided further evidence of the ability of the colon to participate in hypersensitivity reactions. The development of modifications of the Auer technique reported herein for the production of more chronic reactions, coupled with serial immunohistochemical and histologic correlations, may well be an important step in gaining a better understanding of the reaction of the colon to injury in general and may shed more light on the pathogenetic mechanisms involved in the changes in the large and small bowel in patients with ulcerative colitis and other disorders attributable to hyperimmune mechanisms.[25,26]

## Summary

The present investigation has provided corroboration of earlier studies, indicating that an experimental "colitis" in rabbits may be produced by a modification of the Auer reaction. The principles would seem to involve sensitization to a soluble antigen, a generalized antibody response, an "attraction" of nonspecifically mildly irritated tissue for circulating antigen and antibody on the basis of an increased vascular permeability in these areas, and a resultant antigen-antibody reaction leading to tissue injury by mechanisms which have not been fully clarified. The histologic features of the Auer "colitis" include submucosal edema, perivascular round cell accumulations, mucosal-submucosal infiltrations of inflammatory cells, and ulceration. The direct immunofluorescent technique has been used to demonstrate specific antigen and antibody in the identical sites of this cytotoxic reaction characterizing the Auer phenomenon.

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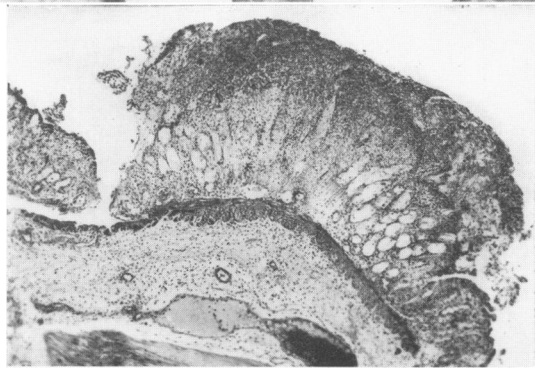
## Legends for Figures

FIG. 1. A continuous strip of a necropsy specimen of Auer "colitis," with the distal rectum at the bottom left. Hyperemia, hemorrhage, ulceration, and thickening of the bowel wall are most marked in the central area (approximately 15 cm. proximal to the anus). × 2.

FIG. 2. A section of the specimen shown in Figure 1 at the top edge of the intense central inflammatory area. The features of the Auer "colitis" demonstrated include submucosal edema, mucosal hemorrhage and ulceration, mucosal-submucosal cellular infiltration (see text), and engorged blood vessels. Hematoxylin and eosin stain. × 30.

1



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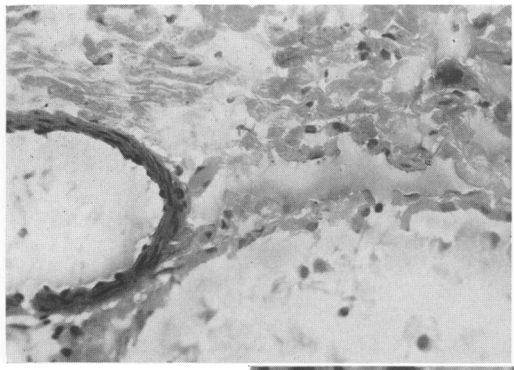

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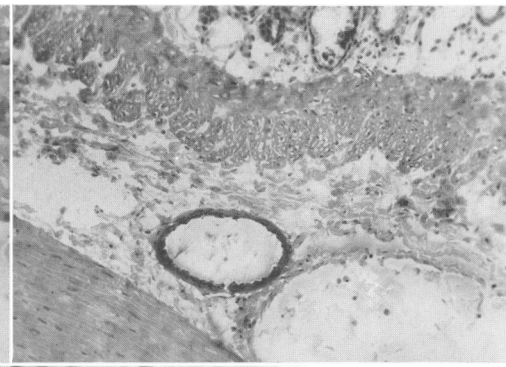

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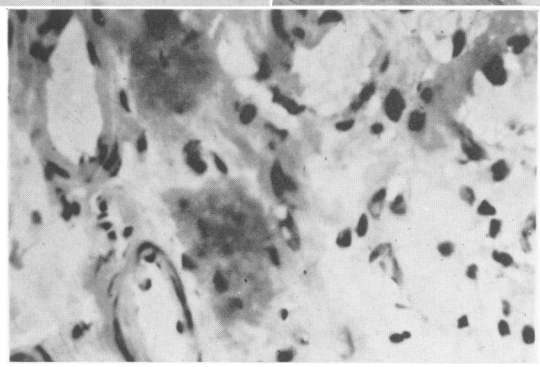

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FIG. 3. The same field shown in Figure 6 after restaining with hematoxylin and eosin. The cellular detail is less distinct than in comparable formalin-fixed tissue but is quite adequate for immunohistochemical correlations. × 230.

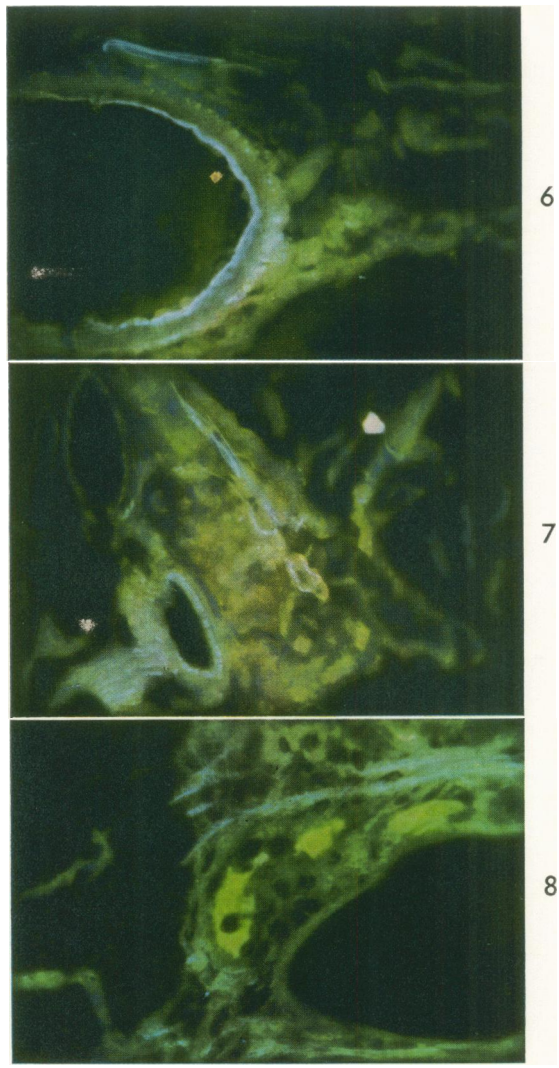
FIG. 4. The same slide used for Figures 3 and 6 shows the lower mucosa, submucosa and muscularis. The arteriole seen in the previous figure is located in the edematous submucosa. × 90.

FIG. 5. The field shown in Figure 7 after re-staining with hematoxylin and eosin. The fluorescent staining of the antigen in Figure 7 is localized either in homogeneous areas of edema or in close proximity to the cytoplasm of mononuclear cells in the inflammatory exudate. × 250.

FIG. 6. Frozen section of Auer "colitis" fixed with 95 per cent ethanol and exposed to fluoresceinated-ACEA globulin. The apple-green fluorescent staining of the antigen is seen adjacent to the endothelium of a submucosal arteriole as well as diffusely throughout the edematous perivascular area. × 230.

FIG. 7. Auer "colitis," fixed with 95 per cent ethanol and exposed to fluoresceinated-ACEA globulin. Specific apple-green fluorescence is seen perivascularly in the submucosa and scattered within the muscularis mucosa. The bluish white autofluorescence of elastic tissue and the reddish brown autofluorescence of muscle is evident. A tissue eosinophil in the lower mucosa appears white. × 250.

FIG. 8. Immunofluorescent view of the lower mucosa and submucosa of a section of Auer "colitis" exposed to fluorescein-labeled sheep anti-rabbit globulin. The characteristic apple-green fluorescence is shown in the two lighter-colored "pools" in the center, near the base of the glandular lumen. Appropriate controls strongly suggest that these areas of increased tissue globulins represent specific antibody. The more generalized darker green color is nonspecific fluorescence. × 250.



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