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"Auer Colitis" in Rabbits Induced by Intrarectal Antigen.* (29362)

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In 1920, Auer(1) produced a severe inflammation at the site of a previously induced, non-specific irritation, by injection of a large dose of the specific antigen to a sensitized animal. Previous studies from this laboratory have demonstrated an "Auer" inflammatory reaction in the distal colon ("colitis") of rabbits sensitized to crystalline egg albumin, after prior mild irritation of the bowel with dilute formalin; followed by intravenous or intraperitoneal administration of the specific antigen(2). Histologically, the Auer colitis was characterized by superficial ulceration and hemorrhage, infiltration with polymorphonuclear leukocytes, plasma cells and lymphocytes. Circumstantial evidence suggested the implication of a specific antigen-antibody reaction, facilitated by increased capillary permeability, induced by the exposure to mild formalin. Kraft and his associates(3) recently have provided direct evidence of the antigen-antibody nature of the Auer reaction in the colon. By immuno-

fluorescent techniques, localization of the specific antigen and antibody was demonstrated only at the site of the "colitis" and not in the uninvolved colon or in the colon of sensitized and non-sensitized controls.

The present study was undertaken to determine if direct intrarectal instillation of the challenging antigen also would initiate an "Auer colitis."

Methods and materials. Sensitization. Adult New Zealand white rabbits, weighing 2 to 3 kg, and maintained on a diet of Purina Rabbit chow and water, were used exclusively. Sensitization was accomplished with 5 times crystallized egg albumin (Pentex) as the antigen, by the multiportal method described by Wissler *et al*(4). One ml of a 2% solution was injected into each of 5 portals (intravenous, intramuscular, intraperitoneal, subcutaneous and intradermal) daily, for a total of 100 mg. This schedule was repeated 3 days each week for 3 successive weeks. Occasionally, during the final week of sensitization the intradermal injections produced positive Arthus reactions; and sensitization was discontinued in these ani-

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TABLE I. Response of Rabbit Colon to "Rectal" Auer Procedure.

Test group	No. of animals	Gross colon findings			Severity of colitis			Negative
		Hyper- emia	Granu- larity	Erosions ulcerations	Mild	Mod- erate	Severe	
"Auer colitis"	18	16	15	15	0	4	11	3
Saline enema								
Sensitized rabbits	6	3	2	1	1	0	0	5
Unsensitized rabbits	6	1	1	0	0	0	0	6
Formalin enema								
Sensitized rabbits	6	4	5	3	0	2	1	3
Unsensitized rabbits	6	0	2	1	1	0	0	5
Antigen enema only								
Sensitized rabbits	6	3	2	0	0	0	0	6
Unsensitized rabbits	6	0	1	0	0	0	0	6
Auer procedure								
Unsensitized rabbits	6	5	0	1	1	0	0	5

mals, to avoid anaphylaxis. Invariably, all sensitized rabbits demonstrated strongly positive Arthus reactions; and this response served as the index of sensitization. Adjuncts were not used.

Auer procedure. A standardized, "chronic" Auer procedure was performed as follows: the first day, 1 ml 0.5% formalin was instilled into the distal colon by enema. During the next 4 days, instillation of 1 ml of 0.5% formalin was followed in 45 minutes by a challenging "antigen enema," consisting of 10 ml of a 10% suspension of crystalline egg albumin in isotonic saline; this schedule was repeated for a second week. Thus, the colon was challenged on 8 occasions, each with one gram of antigen, for a total of 8 grams of crystalline egg albumin. To instill the enema, the rabbit was placed in a special proctoscopic rack, with head down at a 45° angle. A lubricated 14 French catheter was inserted gently to a depth of 10 to 15 cm, and the enema was permitted to flow into the colon by gravity. After the catheter was withdrawn, the rabbit was maintained in the inclined position briefly to ensure retention of the fluid.

At the conclusion of the "chronic" Auer procedure, the rabbits were sacrificed with sodium nembutal intravenously. The colon was examined, and representative sections were taken for histologic study. Proctoscopic examination facilitated periodic inspection of the distal 10 cm of bowel.

Controls. Control studies were made in groups of 6 sensitized and 6 unsensitized rabbits in each of various categories. These rabbits were treated concurrently with a "test" group and were sacrificed at the same time (Table I). In the absence of gross changes, sections were obtained from similar areas of colon as in the test animals. The control groups included (a) saline enema only—10 ml of isotonic saline solution were substituted for the 10 ml of 10% egg albumin enema; and formalin was not instilled. (b) formalin enema only—1 ml of 0.5% formalin enema was given daily; antigen was not administered. (c) antigen enema only—10 ml of 10% egg albumin enema were administered, as in the test group, but without prior instillation of formalin. (d) unsensitized "test"—the procedure was the same as in the sensitized "test" groups, but performed in unsensitized rabbits.

Serum proteins. Several ml of blood were drawn from each rabbit before sensitization (control), on completion of the sensitization, and after conclusion of the Auer or control procedures. Serum was collected and the proteins were separated by the electrophoretic technique of Jenks *et al*(5). The strips were stained with bromphenol blue in methanol; alpha, beta and gamma globulins and albumin were identified and the percentage of each fraction was calculated.

Results. Gross observations (Table I).
"Test" Auer rabbits. Proctoscopic examina-

TABLE II. Average Serum Proteins (%).

Test group	No. of animals	Control				Post sensitization				Post reaction			
		γ globulin	β	α	Albu-min	γ globulin	β	α	Albu-min	γ globulin	β	α	Albu-min
Auer procedure	16	11.4	10.8	11.5	66.3	15.7	11.1	10.9	62.3	13.0	13.9	12.3	60.8
Sensitized controls	17	11.0	10.2	10.6	68.2	14.4	10.6	10.6	64.4	11.4	10.4	10.9	67.3
Unsensitized controls	12	11.1	10.4	11.3	67.2					11.2	10.2	11.0	67.6

tion demonstrated hyperemia, and the presence of blood; the bleeding, as noted at autopsy, originated in areas proximal to the proctoscopic level, in the area of exposure to the antigen enema. A "colitis" was demonstrated in 15 of the 18 test rabbits; 11 of these were classified as severe. Bleeding occurred usually from punctuate erosions, and from superficial ulcerations.

Control rabbits. Only mild hyperemia and granularity of the distal colon were noted occasionally at autopsy in rabbits given saline enemas, antigen enemas and in unsensitized rabbits given formalin enemas or subjected to the "Auer" procedure. Of the 6 rabbits sensitized to egg albumin and receiving formalin enemas, 3 developed reactions judged as moderately pronounced; and consisting chiefly of clusters of tiny erosions in the distal colon; hemorrhages or ulcerations were not observed.

Microscopic observations. Auer test rabbits. Histologic examination of areas of ulceration (8 of 18 animals) demonstrated destruction of mucosa, ulcers occasionally pene-

trating the muscularis propria, and infiltration with round cells (Fig. 1). In areas without ulceration, the most frequent feature was infiltration of the mucosa and submucosa with polymorphonuclear leukocytes, eosinophiles or plasma cells, in varying degree. The serosa usually was thickened by fibrinous exudate.

Controls. In the 3 sensitized rabbits developing tiny erosions after exposure of the colon to formalin, the findings included edema, increased cellularity of the lamina propria and mucosa, and slight fibrosis. No ulcers were seen; and the mucosa was intact.

In summary, most of the rabbits sensitized to crystalline egg albumin, and subjected to the Auer test procedure, developed an hemorrhagic and ulcerative reaction in the distal colon, approximately in the area of exposure to the direct instillation of specific antigen. The response was similar in type, extent and degree to that observed when the challenging antigen was introduced intravenously or intraperitoneally(2). Control rabbits, with the exception of the sensitized-formalin enema group, manifested only mild to moderate edema; and, occasionally minimal cellular infiltration.

Serum proteins (Table II). The data on serum proteins were divided into several groups: (a) (Auer procedure and sensitized controls): Sensitizing rabbits to crystalline egg albumin resulted in an expected increase in the gamma globulin. (b) (Sensitized controls): The serum proteins of sensitized control rabbits returned to normal levels after discontinuation of the injections of albumin. (c) (Unsensitized controls): The serum did not change in unsensitized control rabbits after the various control procedures. (d)

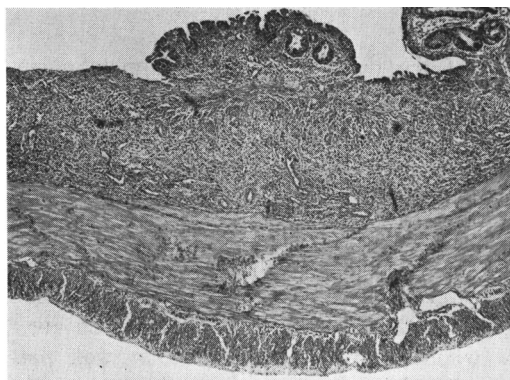


FIG. 1. Ulceration and chronic inflammation of rabbit colon involved in "Auer colitis." $\times 65$

(Auer procedure): The development of an "Auer colitis" in sensitized rabbits was accompanied by a temporary elevation of the beta globulin in 12 of 16 Auer test rabbits; this trend was not observed in sensitized or unsensitized rabbits.

Discussion. The responsiveness of the colon to immunologic reactions has been well documented; and immunofluorescent techniques have provided evidence for an antigen-antibody reaction as the mechanism of the "Auer colitis." The present data indicate that an "Auer colitis" also may be induced by direct intrarectal instillation of specific antigen. An advantage of the rectal over the intravenous method is that it may be repeated in the same animal for long periods, with improved reproducibility and with less likelihood of generalized anaphylaxis.

The colon reaction in the sensitized rabbits receiving formalin enemas, though not as severe as in the "Auer colitis," requires explanation. In earlier control experiments, formalin enemas alone, repeated daily for as long as 6 months in unsensitized rabbits, caused only slight to moderate hyperemia and granularity of the distal colon. Also, in

these studies, scattered punctate hemorrhages were noted in 6 of 48 sensitized animals after administration of formalin enemas. The possibility exists that the reaction may have resulted from an antigen-(egg albumin) antibody reaction, facilitated by the increased capillary permeability of the distal colon in sensitized rabbits, induced by exposure to the mild formalin solution.

Conclusion. The intrarectal instillation of specific antigen (crystalline egg albumin) in sensitized rabbits may induce a "colitis" in the distal colon, previously exposed to a mild formalin solution, to increase capillary permeability locally. The "Auer colitis" develops presumably on the basis of an antigen-antibody reaction.

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Change in Serum Haptoglobin Type Following Human Liver Transplantation.* (29363)

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(Introduced by D. W. Talmage)

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The haptoglobins constitute a population of serum proteins characterized by their ability to form a stable bond with hemoglobin. The molecular heterogeneity of these hemoglobin-binding proteins, demonstrable by starch gel electrophoresis, is under genetic control(1). The 3 major haptoglobin types have been designated 1-1, 2-1 and 2-2.

The opportunity to study a patient before and after liver transplantation provided a unique circumstance in which to evaluate the

role of the liver in haptoglobin metabolism. The patient and the donor had different haptoglobin types; and we report here the temporary appearance of haptoglobin of the donor type in the serum of the recipient after liver transplantation.

Materials and methods. The subject of this investigation was a 29-year-old Caucasian female with a primary carcinoma of the liver in whom a liver transplantation was performed. A preliminary operation was done on Oct. 2, 1963, and hepatectomy and trans-

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