An Attempt at Production of Autoimmunity to Tissue of the Gastrointestinal Tract. (26337)

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The existence of autoimmunity as a factor in human disease has been demonstrated in cases of Hashimoto's struma(14,11,1). The production of disease in animals by technics of immunization utilizing tissue antigen to produce disease of the corresponding tissue has been successful in the case not only of thyroid but also of the uveal tract(3), brain (8,6), testes(5) and adrenals(4). The lesions induced have been inflammatory reactions of the tuberculin type(13). This type of reaction, and the rather special tissues in which it has occurred, suggests a relationship with the phenomenon of homograft rejection. It is as though the tissue involved becomes for the first time immunologically "recognized" through the mechanism of the immunization procedure and is then rejected(13). It has been tempting to apply such mechanisms to other human diseases characterized, as in Hashimoto's struma, by chronic inflammation in the absence of demonstrable pathogenic organisms. Among such conditions regional enteritis and ulcerative colitis in particular have been considered by many workers(8,12), as being produced by immune processes. Colitis has been induced in rabbits by producing repeated responses of the Auer or Arthus type(7), and in patients with chronic ulcerative colitis circulating antibody to a polysaccharide antigen obtained from colon has been demonstrated(2,9). We are not aware, however, that a serious attempt has been made to produce autoimmunity to material from the gastrointestinal tract using the technics which have been successful in the case of thyroid, eye, brain and testes. In this communication, we are reporting an attempt in which tissue from 3 levels of the gastrointestinal tract, made up as a whole tissue antigen in Freund's adjuvant, was used in immunization by intradermal injection of several animal species.

Materials and methods. Animals. New Zealand white female rabbits (5-6 lb) Western white female rats (200 g), laboratory-bred white A-strain mice (20 g) and white Hartley strain guinea pigs (300 g) were used in this study. All animals were kept in wire cages and fed chow and water *ad libitum.*

Antigens. Normal animals of each species were sacrificed to provide antigen. The entire digestive tract was dissected free and portions of stomach, small bowel, and large bowel were removed, opened and washed free of contents. They were immediately chilled in ice water and ground in a high speed Virtis grinder at 45,000 rpm with a sufficient amount of saline to give a fluid suspension. Portions of these saline suspensions were emulsified in 2 volumes of light mineral oil-Arlacel* mixture (8.5 to 1.5) containing 10 mg/ml of killed mycobacterium tuberculosis. Final concentrations of tissue in emulsion for each preparation are given in Table I.

Injections. All animals were given approximately 0.02 ml of the appropriate emulsion into each food-pad (or several toe pads on each foot in the case of the rabbits). In addition, rabbits, guinea pigs and rats were given 2-3 intradermal injections of 0.02 ml of adjuvant in the flanks.

An additional 3 rabbits were injected every 2 weeks with the rabbit colon emulsion for a period of 10 weeks, when they were sacrificed.

All animals were weighed periodically and carefully watched for signs of fecal blood and general distress. At 2 weeks, 4 weeks and 8 weeks, pairs of animals from each group were sacrificed and the gastrointestinal tract was dissected free and examined for gross pathology. Then several portions of stomach, small and large intestine were removed, fixed in formalin and sectioned for

* Obtained from Atlas Powder Co., Wilmington, Del.

TABLE I. Animals and Antigens Used for Immunization Studies.

Animals	No.	Antigen used	Conc. of tissue in adjuvant mixture, mg/ml	ml inj. /animal
Rabbit	6	Rabbit stomach	50	.2
"	"	" small bowel	40	.2
"	"	" large "	50	.2
G. pig	"	G. pig stomach	40	.14
"	"	" small bowel	50	.14
"	"	" large "	50	.14
Rat	"	Rat stomach	30	.14
"	"	" small bowel	40	.14
"	"	" large "	50	.14
Mouse	"	Mouse stomach	3	.08
"	"	" small bowel	50	.08
"	"	" large "	60	.08
Rabbit	3	Rabbit large bowel	220	.2 ml repeated 4 times

histologic examination with haematoxylin and eosin stain.

Results. Table I summarizes the data on antigen preparations used and amounts injected in the various species. None of the animals developed gross signs of pathology. All gained weight normally during the experiment and showed no evidence of blood in the stools either visually or by benzidine test. Grossly, the gastrointestinal tracts looked normal with no evidence of ulceration, hemorrhage or inflammation. Histologically, no signs of pathology such as necrosis, infiltration or hemorrhage were apparent. In the small bowel of all guinea pigs were large masses of monocytes but these appeared to be consistent with normally distributed lymphoid tissue (Peyer's patches) and were not infiltrating.

In the present experiment, therefore, no evidence of pathology was found in rabbits, guinea pigs, rats and mice injected with homologous stomach, small intestine and large intestine incorporated into Freund's adjuvant.

Discussion. This negative result cannot of course be construed as final evidence that autoimmunization to tissue from the gastrointestinal tract cannot be produced experimentally. Quantity of antigen, frequency of injection and length of observation period could all be varied with conceivably different results. The many reports in the literature, however, that autoimmunity to brain, thyroid and testes, as well as other tissues, may be produced readily in at least one of the species studied by this method and route of immunization, makes it appear more likely that fundamental immunologic restrictions rather than shortcomings in technic account for the present negative results.

It has been suggested(10) for example, that the tissues towards which autoimmunity has been succesfully produced seem to have in common some degree of separation by more or less impermeable barriers from the general vascular bed. Thus dissemination of the antigen to sites of immune response would be inhibited by the pia-glia membrane of the central nervous system, the capsule of the lens of the eye, the basement membrane of the seminiferous tubules and the follicle epithelium of the thyroid. In this event, tolerance to these tissues would not be achieved in the young animal, and subsequent exposure to them would produce autoimmunization. It is doubtful whether this would hold true for the elements of the gastrointestinal tract.

Summary and conclusions. Immunization of rabbits, guinea pigs, rats, and mice with antigen in Freund's adjuvant prepared from homologous stomach, small intestine and colon was not found to be pathogenic to the animals as to production of gastrointestinal disease.

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Histochemistry LXIV. Succinic Dehydrogenase System in Isolated Mast Cells from the Rat.* (26338)

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Growing recognition of the important physiological role of mast cells prompted this study on a succinic dehydrogenase system which could reflect certain metabolic conditions in the cells as a function of the influence of various states and agents. Previously, in a study of mast cells isolated from peritoneal washings from the rat, *in vitro* effects on the cell morphology of agents, known or suspected of having physiological influences on these cells, were reported(1). This investigation is concerned with effects of age, environmental stress (cold), and subcutaneous injections of ACTH, *E. coli* endotoxin, histamine, serotonin, and compound 48/80 ("histamine liberator") on the activity of the enzyme system in the isolated cells.

Methods. Male albino rats, Sprague-Dawley (Holtzman, Madison, Wis.), were housed singly or in pairs in a constant climate room (25° ± 1°C, 30-50% relative humidity, controlled illumination, lights on 6 a.m. and off 6 p.m.) for at least 4 days prior to use. All rats, except those employed for study of age effects, were approximately 3 months old and weighed about 300 g. They received Purina Fox Chow and tap water *ad libitum,* and were killed instantly between 11:00 and 11:30 a.m. by a single blow on the head.

Suspensions of pure mast cells in Hanks' solution were obtained from peritoneal washings as described by Glick *et al.*(2) using a sucrose density gradient. After this work was started, Ficoll (Pharmacia, Uppsala, Sweden), a dextran that provides solutions of the required density with relatively low viscosity, became available, and its value for density gradient separations was shown(3). Mast cells isolated in a Ficoll gradient are not subjected to as great an osmotic strain and they retain their histamine which is lost to some degree during separation in sucrose (4). Comparison of the activity of the succinic dehydrogenase system in cells isolated in either medium showed no difference however (Table I). Apparently the enzyme in the mitochondria is unaffected or influenced to the same degree by either procedure, although greater morphological damage was observed in cells separated in the sucrose gradient.

After isolation, washing the cells in Hanks' solution had no morphological effect, but in a Versene-phosphate buffer (0.067 M phosphate, 5×10^{-4} M Versene), of the pH (7.7) required for optimal enzyme activity, the cells were all disrupted, resulting in marked increase in activity (Table I).

The activity of the succinic dehydrogenase

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