Morphological and Histochemical Changes in the Rat Conceptus Following Administration of a Non-Ionic Detergent.* (31276)

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A previous study on the role of lysosomes in ethionine-induced resorption of the rat conceptus indicated a change in the size and distribution of the lysosomes of the placental tissues and no fluctuation in total amount of acid hydrolases in these tissues during resorption(1). A more direct approach to the determination of the involvement of lysosomes in resorption was the injection of Triton WR-1339 into pregnant rats. The non-ionic detergent has been shown to be a labilizer of lysosomes of other tissues *in vivo*(2). The effect of this compound on placental and fetal lysosomes was correlated with the maintenance of pregnancy.

Materials and methods. Pregnant Sprague-Dawley rats were injected intravenously (vena cava inferior) with single doses of Triton WR-1339 (Winthrop Lab.). The injection regimen was 200 mg Triton WR-1339/ml rat Ringer's solution on day 10 or 11 of pregnancy. Control rats were injected with equivalent amounts of rat Ringer's solution on the same days of pregnancy. All rats were autopsied 24 or 48 hours after injection, on day 12 of pregnancy.

Whole conceptuses were removed from the uterus, fixed in Pease's modification of Millonig's fixative(3), pH 7.3-7.5, for 4 hours, and washed overnight in buffered sucrose, pH 7.3-7.5. Frozen sections (6 μ) were prepared with a cryostat. Acid phosphatase activity of the sections was determined at pH 5 by the Barka-Anderson technique(4), using naphthol AS-BI phosphate, and by the Gomori technique(5).

Adjacent conceptuses were fixed in alcoholic formalin, embedded in paraplast, and sectioned at 6 μ. Non-glycogen carbohydrate was determined by periodic acid-Schiff reagent (PAS) following treatment of the sections with 0.1% diastase of malt in phosphate buffer. For morphological study, representative sections were stained with a polychrome stain.

Results. Of the rats injected with 200 mg Triton WR-1339 on day 10 (48 hours), 50% had dead and resorbing litters. Of the rats injected on day 11 (24 hours), 21% were in stages of resorption. These exceed the resorption observed in the controls (5%).

The morphological changes following the injection of Triton WR-1339 were similar with all injection regimens. They included a

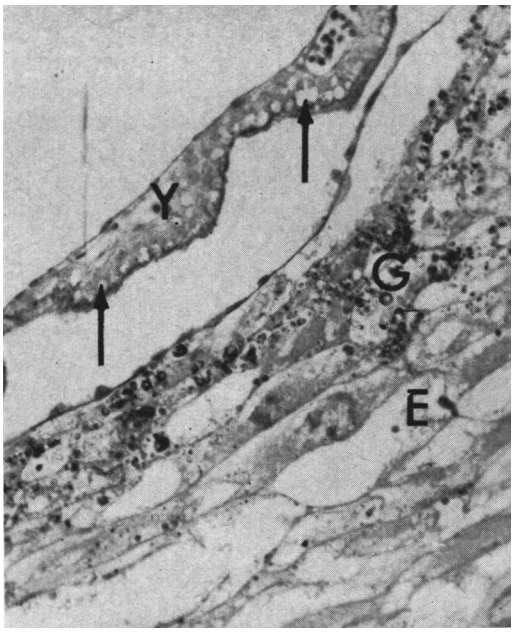

FIG. 1. Triton-injected rat placenta. Periodic acid-Schiff reaction following pretreatment with diastase. Note: yolk sac (Y) with vacuoles (arrows) ringed with diastase-resistant PAS reaction and the giant cells (G) containing PAS-positive erythrocytic debris. The maternal erythrocytes in the capillaries (E) do not react with PAS. × 165.

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marked vacuolation of the apical region of the visceral yolk sac epithelial cells (Fig. 1), a pronounced increase in the amount of debris, primarily fragments of erythrocytes, present within the cytoplasm of the giant cells (Fig. 1), and a loss of cell cohesion in the fetus,

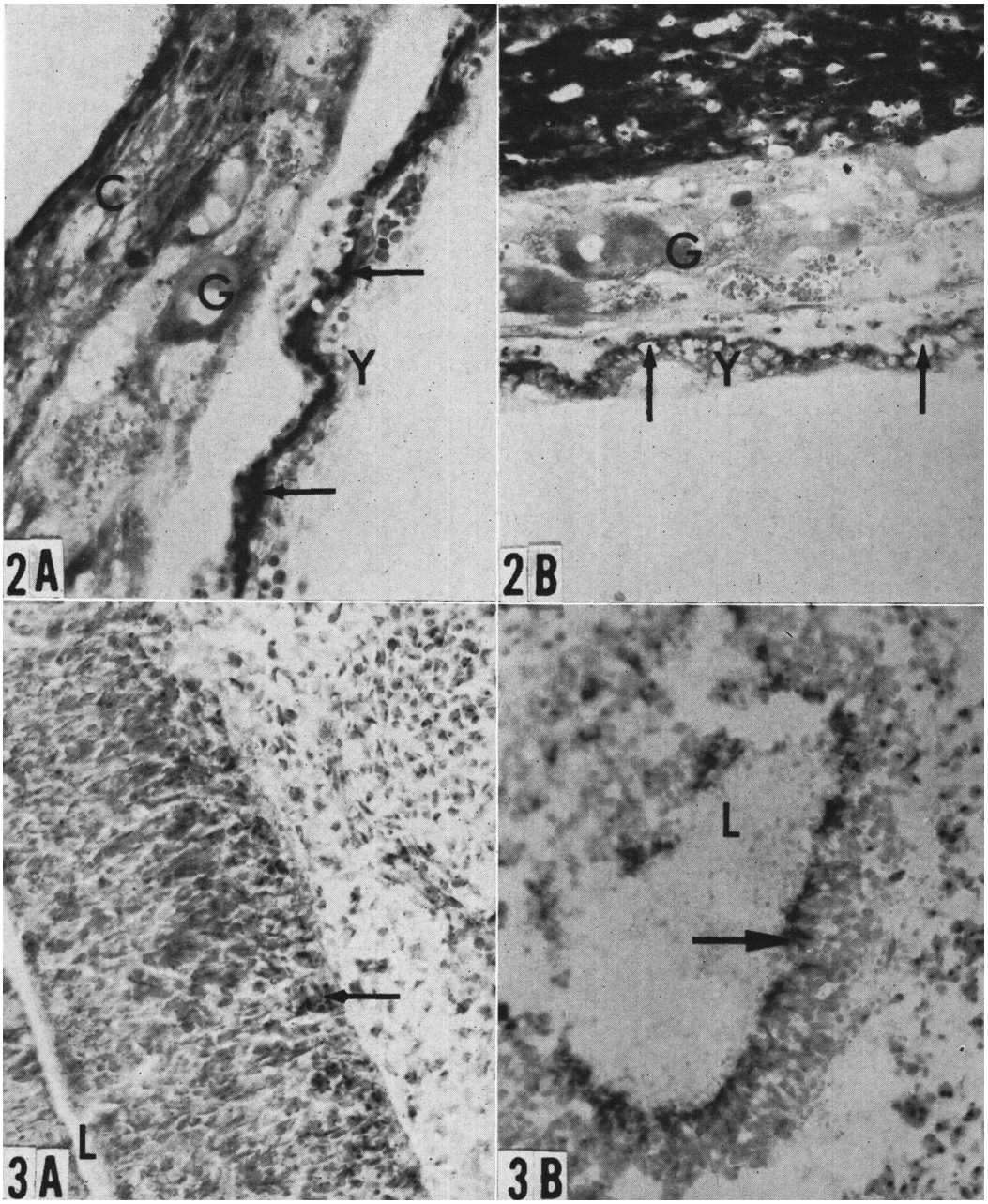

FIG. 2. Decidua capsularis (C) region, containing visceral yolk sac (Y) and giant cells (G). A. Control. Arrows indicate lysosomes. B. Triton-injected. Arrows indicate vacuoles. Barka-Anderson reaction for acid phosphatase. × 165.

FIG. 3. Section of neural tube of 12-day rat fetus with lumen (L). A. Control. Note concentration of lysosomes in basal layer of neural tube (arrows). B. Triton-injected, resorbing fetus. Note concentration of lysosomes in cells adjacent to lumen of neural tube (arrow). Barka-Anderson reaction for acid phosphatase. × 165.

placental labyrinth, and decidua basalis. The debris within the cytoplasm of the giant cells was strongly PAS-positive and the Triton-induced apical vacuoles of the visceral yolk sac epithelial cells were ringed with granular PAS-positive material (Fig. 1).

After reaction for acid phosphatase, sections of the conceptus showed the following patterns of activity: a pronounced ring of activity on the perimeter of the vacuoles at the apical end of the visceral yolk sac cells corresponding to the non-vacuolated lysosomal area of normal yolk sac cells (Fig. 2), a decrease in activity in the debris-filled giant cells (Fig. 2), and some vacuolation and increase in size of the lysosomes of the cells of the placental labyrinth. These changes were accompanied by a migration of macrophages from the basal region of the endometrium into the decidua basalis. In some of the treated conceptuses, there was a concentration of macrophages in the region of the decidua basalis adjacent to the placental labyrinth.

In non-resorbing conceptuses, Triton itself produced no changes in the appearance of fetal lysosomes; however, when resorption occurred, either spontaneously or Triton-induced, a general increase in the size of the lysosomes of all fetal tissues occurred as well as a change in the localization of lysosomes within the neural tube (Fig. 3).

Discussion. The uptake of Triton WR-1339 by the lysosomes of the placental tissues, similar to that shown by the lysosomes of the liver(2), is indicated in this study by the vacuolated appearance of the lysosomes of the yolk sac epithelial cells and, to a lesser extent, of the lysosomes of the placental labyrinth. Two other studies, one biochemical and one cytochemical, have provided additional support for this observation. The cytochemical study(6) has shown that Triton appears in the yolk sac within 15 minutes after injection and is retained within the lysosomal bodies for at least 4 days. This can be correlated with other studies which have shown the accumulation of foreign materials within the lysosomal region of the yolk sac epithelial cell(7,8) suggesting that the yolk sac may be acting as a barrier to the movement of foreign materials to the fetus. A fractionation and biochemical study (Schultz, Thines-Sempoux and Jacques, in preparation) has shown that the lysosomes of the labyrinth of the chorio-allantoic placenta take up Triton within 18 hours after injection. None of these studies prove conclusively that there is a preferential uptake of the detergent by any one part of the conceptus, but the lack of cellular change in the basalis and fetus in contrast to the yolk sac and labyrinth lead us to believe that some tissues have a stronger sensitivity to Triton treatment.

The uptake of erythrocytic fragments by the giant cells is a demonstration of the phagocytic nature of these cells which has been previously shown(7,9). The strong PAS reaction of the debris, which has been related to lysosomal structure(10), and the diffuse nature of the acid phosphatase reaction indicate a fusion of the lysosomes with the erythrocytic fragments following phagocytosis. This phenomenon of fusion of lysosomes with phagosomes containing engulfed foreign material has been well established(11).

Summary. Histochemical studies of the conceptus were made using the Barka-Anderson and Gomori techniques for acid phosphatase and periodic acid-Schiff reagent. Triton injection caused a marked vacuolation in the lysosomes of the visceral yolk sac cells, a pronounced increase in the amount of PAS-positive debris in the giant cell cytoplasm, and a loss of cell cohesion in general. A significant increase in resorption occurred with all dosage levels of Triton.

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Photosensitization of an Actinophage by Heteroanthracenes.* (31277)

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Several heteroanthracenes have been reported to photosensitize bacteriophages; for example, methylene blue(1), toluidine blue (2), and acridine(3). This is not surprising inasmuch as heteroanthracenes are known to participate in photo-oxidation of both proteins(4,5) and nucleic acids(6,7). However, the heteroanthracenes eosin and neutral red, and triphenyl methanes such as fuchsin and crystal violet have been reported as having no photodynamic action on bacteriophage(8). The present report is concerned with dyes which photoinactivate an actinophage for *Streptomyces venezuelae*. Of the 8 dyes found to be effective, 7 were heteroanthracenes. The conditions for photodynamic killing by different dyes were not the same.

Materials and methods. Actinophage MSP2 was propagated and enumerated on *Streptomyces venezuelae*(9); actinophage MSP2 was purified and concentrated by a combination of chromatography and differential centrifugation(10). The phage and dyes were prepared and diluted in 0.15 M NaCl, pH 7, unless otherwise indicated. Mixtures of phage and dye, 1 mm deep, were illuminated by 2 photoflood bulbs (General Electric BBA) placed 12 inches above the samples.

Results. Of the 27 diverse substances tested for power to inactivate actinophage MSP2, only crystal violet, hydroxylamine and methylene blue were markedly viricidal at the tested concentrations (Table I). Because acriflavine and methylene blue were known to kill bacteriophage photodynamically, 16 of

TABLE I. Effect of Diverse Chemicals on Actinophage MSP2.

Treatment	Conc (μg/ml)	Surviving phage
Acriflavine	75	1.5×10^9
2-amino,3-phenyl butanoic acid	500	1.7×10^9
Anthrone	75	1.7×10^9
Benzopurpurin	50	1.6×10^9
Brilliant cresyl blue	50	6.0×10^8
Brilliant vital red	50	1.3×10^9
Bromthymol blue	75	2.8×10^9
Carmine	75	2.0×10^9
Chlorophyll	75	2.8×10^9
Cholic acid	500	3.5×10^9
Colchicine	500	1.8×10^9
Crystal violet	500	9.5×10^6
Dianil blue 2R	50	1.4×10^9
Diphenylamine	500	2.9×10^9
Eosin Y	250	7.3×10^8
Fumaric acid	500	2.7×10^9
Hydroxylamine	500	1.2×10^6
Janus green B	250	6.5×10^8
Linoleic acid	500	2.1×10^9
2-mercaptobenzthiazole	500	2.6×10^9
Methylene blue	50	$<10^5$
Orcinol	500	2.7×10^9
Pronase	500	1.7×10^9
Quinine	500	2.9×10^9
Riboflavin	500	2.6×10^9
Safranin O	50	1.2×10^9
Thionin	50	8.0×10^8
None (control)	—	2.7×10^9

Chemicals were suspended or dissolved in 0.15 M NaCl and adjusted to pH 7 with 1 N NaOH or 1 N HCl. Phage was added and the mixture was incubated at 30°C for 30 min.

Acriflavine, Janus green B, Safranin O: Allied Chemical Corp., Nat. Analine Div., New York. Bromthymol blue: J. T. Baker Co., Phillipsburg, N. J. Cholic acid, fumaric acid, linoleic acid, pronase, riboflavin: Calbiochem, Los Angeles, Cal. Diphenylamine: Eastman Organic Chemicals, Rochester, N. Y. Anthrone, crystal violet, hydroxylamine hydrochloride, orcinol, quinine: Fisher Co., Minneapolis, Minn. Benzopurpurin 4B, brilliant cresyl blue, brilliant vital red, carmine, dianil blue 2R, methylene blue, thionin: Hartman-Leddon Corp., Philadelphia, Pa. Eosin Y: Matheson, Coleman and Bell, Cincinnati, Ohio. 2-amino,3-phenyl butanoic acid, chlorophyll, colchicine: Nutritional Biochemicals Corp., Cleveland, Ohio. 2-mercaptobenzthiazole: Sharp & Dohme, Rahway, N. J.

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