tion but it is curious that the few remaining cells prior to initiation of the cycle of regeneration have the cytological appearance of infection.

Summary. It has been shown with a modification of the usual tissue culture technic that the Minnesota strain of vaccinia virus has been adapted to Earle's L strain fibroblasts. Following establishment of the infection, several cycles of cell growth and degeneration occurred. The adapted strain was identified by conventional cytological and serological criteria. The method described might lend itself to adaptation of other viruses.

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Potentiation of the Local Shwartzman Phenomenon by Trypsin.* (25650)

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It has been reported that extracts of bacteria, tumors and normal mammalian tissues produced the Shwartzman Phenomenon in rabbits and hemorrhage into sarcoma 180(1, 2,3) and sarcoma 37(4,5) in mice. It was subsequently found that a number of other preparations of animal, plant and bacterial origin could effect many host responses including the Shwartzman Phenomenon and tumor damaging ability(6,7,8). In these preparations trypsin was employed in the extraction process(7), therefore the final products could contain residual trypsin which was not removed by dialysis. On the other hand we were able to obtain potent extracts, without use of trypsin, from pancreas and testes(2), organs which normally contain proteolytic enzymes(9). Accordingly, it did not seem unreasonable to assume that trypsin *per se* might play a role in production of lesions by bacteria or tissue extracts.

Material and methods. Stock Albino female rabbits were maintained on Rockland rabbit ration pellets which did not contain antibiotics. The abdominal skin was shaved with an electric clipper. Various concentrations of *E. coli* endotoxin (Lipopolysaccharide extract prepared by Difco Laboratories) were injected at different sites in the right flank. The same concentrations in combination with 0.070 mg crystalline trypsin (Takamine Laboratories, Clifton, N. J.) were injected into corresponding sites of the left flank. Other rabbits were treated similarly with *P. vulgaris* polysaccharide(1) instead of *E. coli* endotoxin. A provocative intravenous injection of 0.5 mg *E. coli* endotoxin was administered 24 hours later and the skin reaction was read as in the classical Shwartzman Phenomenon. All solutions were made with pyrogen free saline; the final volume injected in each site was 1 cc. The trypsin solutions were cultured and were not used if found to be contaminated by bacteria.

Results. In a characteristic experiment, in 16 out of 20 rabbits, *E. coli* endotoxin or *P. vulgaris* polysaccharide in combination with trypsin evoked a stronger skin reaction than was produced without use of trypsin. In 10 animals at least one concentration of the bacterial extract was positive in combination with trypsin but did not produce any reaction at the site without added trypsin (Fig. 1). Various concentrations of bacterial extracts in the remaining 6 animals produced skin reaction

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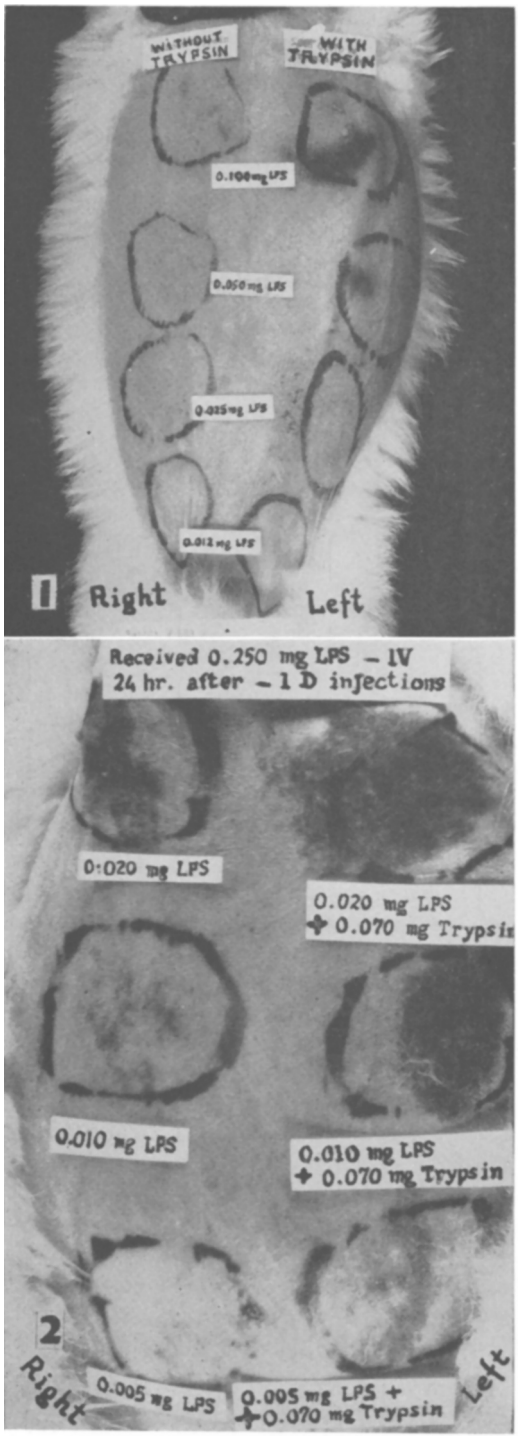

FIG. 1. Left of figure (right flank). Concentrations of *E. coli* endotoxin without trypsin showing negative reaction. Right of figure (left flank). Two corresponding concentrations (.100 mg and .050 mg) in combination with trypsin show positive reaction.

both with and without trypsin, but in combination with trypsin the reactions were considerably stronger (Fig. 2). In no instance did the site injected with endotoxin alone show a stronger reaction than the corresponding site where endotoxin was administered in combination with trypsin. Various amounts of sterile trypsin up to 1 mg in 1 cc saline solution injected intradermally alone in 15 rabbits did not cause a positive Shwartzman reaction.

Table I indicates that both *E. coli* endotoxin and *P. vulgaris* polysaccharide in concentrations varying from 6 to 25 γ, in combination with trypsin, produced a greater number of positive reactions than the corresponding injection of the endotoxin without added trypsin. From a total of 50 sites injected with endotoxin in combination with trypsin, 36 produced positive reactions and 14 remained negative. From the 50 corresponding sites injected with endotoxin alone, only 24 showed a positive reaction, and 26 remained negative. (A Chi-square test applied to these frequencies reveals that the differences between the compared groups are significant at the 0.02 level of confidence. The same significance is obtained if the percentage of positives is compared — 72% in the group with trypsin *vs.* 48% in the group without trypsin.) In the overall comparative evaluation, *P. vulgaris* extract in combination with trypsin produced positive reactions at 2 concentrations lower than when injected alone; with *E. coli* endotoxin this usually occurred at one concentration lower.

Discussion. It is apparent that trypsin potentiates the effect of skin preparatory substances in elicitation of the local Shwartzman Phenomenon. This indicates that in interpreting the results obtained with trypsin treated substances, it is necessary to equate the effect of the basic substance *per se* with that of the substance in combination with trypsin so that potentiating or activating roles

FIG. 2. Left of figure (right flank). Concentrations of *E. coli* endotoxin without trypsin showing weakly positive reaction. Right of figure (left flank). Corresponding concentrations of *E. coli* endotoxin in combination with trypsin showing stronger positive reaction. Potentiation by trypsin is more apparent with .010 mg of endotoxin (center sites).

TABLE I. Potentiation of Local Shwartzman Reaction by Trypsin.

	.025 mg intraderm.			.012 mg intraderm.			.006 mg intraderm.		
		Positives			Positives			Positives	
	No. of cases	Without trypsin	With trypsin	No. of cases	Without trypsin	With trypsin	No. of cases	Without trypsin	With trypsin
E. coli endotoxin	9	5	8	8	3	4	2	0	0
P. vulgaris polysaccharide	10	9	10	11	6	9	10	1	5
Total	19	14	18	19	9	13	12	1	5

might be differentiated and evaluated. The importance of this is exemplified in other reactions which are potentiated or activated by trypsin(10-15).

It is also worthy of note that heparin, which inhibits the Shwartzman Phenomenon(16) also inhibits action of trypsin and serum tryptase(17).

Summary. 1) Trypsin potentiates the effect of skin preparatory substances in elicitation of the Shwartzman Phenomenon. 2) In combination with trypsin, the skin reacts to a greater degree and with lower concentrations of the preparatory factor.

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Tissue Culture Bioautographic System.* (25651)

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A bioautographic system under agar has been developed for paper chromatographic analysis of cytotoxic agents *in vitro*. Applications of paper chromatography and bioassay methods in fermentation beer analyses are described.

Materials and methods. Eagle's KB line of human epidermoid carcinoma cells was maintained and harvested as reported previously (1). Cells grown in suspension culture provide a more convenient inoculum than bottle cultures and studies of submerged growth will be reported elsewhere. Cell content of bottle cultures was determined as protein concentration(2); suspension culture populations were determined by hemacytometer count.

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