and hyperplasia of cells responsible for ACTH elaboration. Nevertheless, the difference in relative amounts of DNA between control and operated rats does not account entirely for larger nucleic acid ratio in the 2-day post-adrenalectomy group. The absolute increase in cytoplasmic RNA at that time is sufficient to overcome this relative DNA differential and indicate accelerated protein synthesis.

Summary. Adenohypophyses of adrenalectomized male rats were analyzed for RNA and DNA at various post-operative intervals. The ratio of RNA/DNA at 2 days after adrenalectomy was more than twice that observed in control animals. Rats sacrificed at 3, 5, and 7 days post-adrenalectomy revealed nucleic acid ratios comparable to those of controls. The increased RNA level in the pituitary 2 days after adrenalectomy correlates well with the time when glandular ACTH content is rapidly increasing. The increased elaboration of protein hormone ACTH induced by removal of adrenals can be correlated with alterations in nucleic acid levels reflecting increased protein synthesis.

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Zymosan as a Possible Reagent in Local Shwartzman Reaction. (24772)

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Recently, one of us (WTB) reported the tumor-necrotizing effect of yeast polysaccharide zymosan when administered to mice bearing implants of Sarcoma 180(1). Phenomena similar to this in which polysaccharide extracts of certain Gram-negative bacteria (endotoxin) produce hemorrhagic necrosis of tumors have been well documented in the literature(2,3,4). This action of bacterial endotoxins is thought by some to be a special form of localized Shwartzman reaction in which the tumor tissue is "naturally" prepared. Indeed, evidence supporting this view has been advanced by Shear and co-workers (5). Inasmuch as zymosan is a polysaccharide of microbial derivation(6), the possibility exists that its anti-tumor activity is due to endotoxin-like properties rather than to host-mediated immune phenomena postulated by Bradner *et al.*(7). It was our purpose to explore this possibility with tests designed to determine competence of zymosan in a system in which bacterial endotoxin plays a known part, namely, the localized Shwartzman reaction. It would appear that if tumor-necrotizing action of zymosan is due to "endotoxic" properties, its effectiveness as a preparing and/or provoking agent in the Shwartzman phenomenon should be readily demonstrable at concentrations active for tumor-necrosis. The dermal Shwartzman reaction, which was long undetected in mice has recently been demonstrated in selected mouse stocks(8,9). Inasmuch as the original observations of zymosan-induced tumor necrosis were made with mice, it seemed appropriate in so far as possible, to test the effects of this material in mice as well as in the classical system with rabbits.

Materials and methods. Mice were adult (20-24 g) females from either brother-sister inbred BSVS strain(10), or the Webster-Swiss strain (O'Grady Farms). Zymosan was

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Fleischmann Lot #5B-171† suspended to proper concentration in physiologic saline and sterilized by boiling 1 hour. The endotoxin employed was commercially prepared (Difco) *E. coli* lipopolysaccharide from Lot #0127B:8. Rabbits were New Zealand does from the Rockefeller Institute stock, weighing about 1.5 kg. All intradermal (I.D.) injections in mice were 0.05 ml delivered through a sharp, short-bevel #27 needle. Intraperitoneal (I.P.) injections were uniformly 0.5 ml. Intravenous (I.V.) injections in rabbits were made in marginal vein of ear with a #26 needle.

Results. It might be expected that if the tumor-necrotizing action of zymosan is mediated through mechanisms of the Shwartzman reaction, the mice in which the zymosan effect is seen would be capable of demonstrating this reaction when suitably prepared and challenged with known Shwartzman-active reagents. Previous tests for the phenomenon in other representatives of the Webster-Swiss strain had consistently yielded negative results regardless of age of mice or dose-levels of endotoxin used for preparation or provocation(11). This was retested, however, in the identical Webster-Swiss strain employed by Bradner *et al.*(1), using an optimum preparatory dose of endotoxin (based on results with susceptible strains) and 5 different provocative doses. In confirmation of prior results, no evidence of skin hemorrhage was seen in any of the test animals. It was concluded, therefore, that this strain is unreactive to endotoxin, at least in amounts capable of eliciting the dermal Shwartzman reaction in 7 other mouse strains(unpublished). Further tests of zymosan's potency in this regard then, were made with the Shwartzman-competent BSVS strain in which the phenomenon has been more extensively studied.

The general plan of all such tests involved injecting the test substance (zymosan) I.D. on one side of shaved abdomen with a control site of endotoxin (30 μg) injected on the other side. (Experience has indicated that up to 3 sites can be placed on the abdomen of an adult mouse without interaction.) Twenty-four hours following the I.D. preparation, all mice were challenged with the test provoking material, delivered I.P. Immediately prior to challenge injection, the mice were scored for "single-injection reactions" which occur irregularly in this strain(8,9). This phenomenon can, in most cases, be distinguished from the "induced" reaction by an increase in severity of hemorrhage and necrosis at the prepared site following I.P. challenge. The pooled results of several such experiments are represented in Table I. From these data it can be seen that while the endotoxin provoked reactions at all endotoxin-prepared sites, no hemorrhages were noted at any of the zymosan-prepared areas. Zymosan challenge provoked reactions at neither endotoxin nor zymosan-prepared areas. Although no hemorrhagic lesions were observed, intradermal deposition of zymosan resulted in formation of inflammatory lesions which, on histological examination (H-E stain), proved to be sterile abscesses.

TABLE I. Zymosan Compared with *E. coli* Endotoxin in Localized Shwartzman Reaction in BSVS Mice.

Preparing (I.D.) dose	Provoking (I.P.) dose	No. tested	R/T*
Zymosan .4 mg	Zymosan .4 mg	26	0/26
" "	Endotoxin 30 μg	20	0/20
Endotoxin 30 μg	Zymosan .4 mg	26	6/26†
" "	Endotoxin 30 μg	20	20/20
Zymosan .4 mg	Saline	5	0/ 5
Endotoxin 30 μg	"	5	3/ 5†
Saline	Zymosan .4 mg	5	0/ 5
"	Endotoxin 30 μg	5	0/ 5

* No. reactors/total No. tested.
† "Single inj. reactions"—see text(8).

Rabbit experiments. Although the hypothetical Shwartzman activity of zymosan in mice is probably a closer parallel of its effectiveness in a tumor-necrotizing system in the same species than in some other, it seemed desirable to further test the material in the classical system using rabbits. Again zymosan was tested for both its preparatory and provoking capabilities. In these experiments

† Received from Special Products Division, Standard Brands, Stamford, Conn.

TABLE II. Zymosan Compared with *E. coli* Endotoxin in Localized Shwartzman Reaction in Rabbits.

Provoking (I.V.) dose (24 hr post-prep'n)	No. rabbits	No. positive reactions	
		Zymosan site*	Endotoxin site†
E. coli endotoxin (120 μg/kg)	2	0	2
Zymosan (20 mg/kg)	4	0	0
" (200 ")	2	0	0

* Zymosan dose: 20 mg/kg, delivered in 0.3 ml, I.D.

† Endotoxin dose: 30 μg/kg, delivered in 0.3 ml, I.D.

preparation was effected by I.D. injections with the provocative injections (I.V.) being given 24 hours later. As with mice, control sites of endotoxin were placed on one shaved flank to establish the basic reactivity of the animals being tested. Table II summarizes results of several experiments using 2 different doses of zymosan for provocation. These results clearly demonstrate that zymosan is incapable of preparing for, or provoking the dermal Shwartzman reaction in rabbits at concentrations tested, and under conditions in which bacterial endotoxin is active.

Discussion. The literature regarding the effects of bacterial extracts on neoplasms has been reviewed by others(4,11,12). Nauts *et al.*(11) indicated their belief that certain of these substances, *e.g.*, Coley's Toxin, exert their effects through indirect stimulation of host defense mechanisms. Another category of substances, namely certain Gram-negative bacterial endotoxins have been postulated to involve the mechanisms of the Shwartzman reaction as the basis for their activity(3), and indeed such materials are competent reagents for this phenomenon. Clearly, the foregoing data indicate that zymosan lacks this competence when used in concentrations active in promoting tumor necrosis. Mediation of its activity through mechanisms of the Shwartzman reaction is thus rendered unlikely.

Summary. To investigate the possibility of zymosan's tumor-necrotizing activity in mice being mediated through a modification of the Shwartzman reaction, the mouse strain in which the phenomenon has been studied, was examined for its capacity to display the Shwartzman reaction when tested with competent bacterial endotoxin. No evidence of reaction was seen under conditions appropriate for induction of skin hemorrhages in other mouse strains. Zymosan was also tested for its ability to prepare for and/or provoke the Shwartzman reaction in an inbred, Shwartzman-responsive strain of mice (BSVS), and in rabbits. Consistently negative results were obtained in both systems under conditions in which bacterial endotoxin was uniformly effective. It is concluded, therefore, that zymosan is ineffective as a reagent for the Shwartzman reaction when used at concentrations effective in causing tumor-necrosis.

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