taneous administration had elevated blood glucose concentrations and weight loss. On discontinuation of 2-DG administration blood glucose concentrations returned to control values. 3. Rabbits following intravenous or oral administration of small doses of 2-DG had a rapid rise in blood glucose concentrations. The glucose concentrations remained elevated for at least 5 hours. In glucose tolerance tests done shortly after 2-DG administration glucose concentrations decreased more slowly than in control tolerance tests, but the animals did respond to insulin administrations.

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Two Local Hemorrhagic Skin Responses to Bacterial Endotoxin in an Inbred Mouse Strain. (24269)

WILLIAM F. ARNDT AND HOWARD A. SCHNEIDER
Rockefeller Institute for Medical Research, New York City

Although the localized Shwartzman reaction was long undetected in mice and rats, Homma(1), and more recently Kelly *et al.* (2), have demonstrated that this phenomenon, or an analagous one, does indeed occur with adequate "preparation" or in properly selected mouse strains. In addition to the normal Shwartzman reaction, the latter workers have described a "Shwartzman-like reaction" which differs from the classical phenomenon in that it is produced by but a single injection of bacterial polysaccharide. In other respects, however, this reaction is indistinguishable from the normal 2-injection phenomenon.

It was the purpose of the experiments reported here to confirm and extend the abovementioned observations in an effort to elucidate the mechanisms underlying the single-injection reaction, which, because of its morphological characteristics, was thought to be related to the local Shwartzman reaction.

The following experiments were performed with a mouse strain bearing a genetic relationship to one of the "positive" strains (Rockefeller "pen-bred") described by Kelly *et al.,* but differing in that it now reflects some 44 generations of brother-sister inbreeding. Such a strain offers the advantage of genetic homogeneity, thereby defining an important variable of this system.

Materials and methods. The mice used were from a recently established pen-bred subline of the brother-sister, 44 generations inbred BSVS strain, originally extracted by Webster(3) from the Rockefeller Institute Stock. They are maintained under conditions of constant temperature (80° F.) and relative humidity (50%) with a uniform 12 hour artificial (fluorescent lamp) light day.

TABLE I. Skin Reactions in BSVS Mice following Injection with *E. coli* Endotoxin.*

Exp. No.	No. & sex		No. mice with skin hemorrhage I.D. only R/T†	% reactors	I.D. & I.P. R/T†	% reactors
1	12	♂	12/12	100	—	—
	18	♀	18/18	100	—	—
2	10	♂	1/10	10	9/9	100
	10	♀	2/10	20	8/8	100
3	20	♂	20/20	100	—	—
	8	♀	8/8	100	—	—
4	10	♂	8/10	80	1/2	50
5	10	♀	0/10	0	9/10	90
6	13	♂	13/13	100	—	—
	13	♀	13/13	100	—	—
Totals	124		95/124	76.6	27/29	93.1

* Prep. No. 0127 A, B from Difco Labs., Detroit, Mich.
† R/T = reactors/total No. tested.

The standard diet was Purina Laboratory Chow and tap water supplied *ad lib.*

Except where indicated, the bacterial endotoxin employed was commercially available (Difco) *E. coli* purified lipopolysaccharide (Lots No. 0127 A. and B), which, as tested by us according to the method of Reed and Muench(4), has an LD_{50} of 150 μg for 20 g adult BSVS mice when injected intraperitoneally.

For intradermal (I.D.) and intraperitoneal (I.P.) injections, the endotoxins were dissolved in normal saline. The standard volume for all I.D. injections was 0.05 ml delivered through a No. 27 needle. The volume for all I.P. injections was 0.5 ml.

Experimental results. In a series of experiments with these mice, confirming Kelly's observations, we have observed both the normal Shwartzman reaction and the single-injection "Shwartzman-like reaction." The latter occurred after as little as 25 μg of *E. coli* endotoxin or 50 μg of *S. marcescens* endotoxin* was injected into the skin, appearing after 18 hours, and reaching maximum intensity in from 24-48 hours. The intensity of this reaction was apparently dependent, to a degree, on the dosage since in some mice a slight difference could be noted between the responses to 30 μg and 50 μg of the *E. coli* material. This difference was not marked however, and the 30 μg dose was chosen as a standard for these experiments.

Maximum lesions produced averaged 15 x 20 mm, and were composed of an intense area of hemorrhagic necrosis surrounded by a margin of erythema. Lesser lesions varied from scattered petechiae to that just described with all gradations between.

The results of 6 separate experiments are summarized in Table I. In these experiments, the mice received 30 μg of endotoxin I.D. All animals were scored for number and intensity of reactions after 24 hours. At this time, those mice showing no reactions at injection site were challenged I.P. with 30 μg of the same material. Twenty-four hours following the second injection, all previously negative skin sites were again scored for hemorrhagic necrosis. Table I shows that 93% of the mice not responding to a single injection of endotoxin exhibited lesions when challenged I.P. after 24 hours. The other fact clearly demonstrated was that response to a single injection was quite variable among experiments, ranging from 0% to 100%. In view of the uniform response of these mice to 2 injections, such marked variability to the single injection in a system of presumed genetic homogeneity presented an interesting problem. Our attention was next focused on those environmental variables which might explain these events. A previous history of transient bacterial epizootics in the colony, coupled with the fact that the inciting substance for these lesions was a bacterial product, led to the hypothesis

* Lot P-25, obtained from Dr. M. Shear, Nat. Inst. of Health, Bethesda, Md.

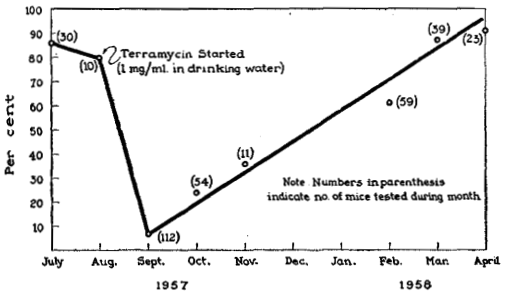

FIG. 1. Frequency of hemorrhagic skin response of BSVS mice to single intradermal inj. of *E. coli* endotoxin.

that the "natural" flora in the animals were related, in some way, to the observed variability. To test this proposal, a large portion of the colony was placed on oral oxytetracycline therapy by dissolving the antibiotic (Pfizer Animal Terramycin) in drinking water in a concentration of 1 mg active drug per ml. The effect of this therapy over a course of 8 months is shown in Fig. 1. This curve represents the percentage of mice that gave a positive reaction when tested with a single 30 μg I.D. injection of endotoxin. Each point is the average percent response of all experiments conducted during that month. Therapy was begun where indicated and continued, at the same level, throughout the following months. In Sept. 1957, the period during which the greatest effect was seen, a corresponding control group of 57 mice reared on tap water showed an average reactivity of 65% as opposed to the 7.1% observed for the experimental group. A chi-square test for the difference between these groups showed a $P<<0.001$. A similar test for random deviation from the mean of all the experimental groups during the course of the therapy showed χ^2 to be 142 ($P<<0.001$).

Preliminary results indicate that chloramphenicol is also effective in this regard, although its use has not been sufficiently long to permit the rise of any resistance effect such as was noted with Terramycin.

Discussion. The preceding data obtained with BSVS mice confirm the observations of Kelly *et al.* on the related Rockefeller strain. In contrast with the latter report however, BSVS mice have consistently displayed a higher frequency in response (93% in BSVS vs. 57% in Rockefeller "pen-bred") to 2 injections (I.D. + I.P.) of endotoxin. Further, in BSVS mice, doses of endotoxin one-tenth that employed by Kelly and co-workers were sufficient to incite lesions in the single-injection system. From these findings, it would seem that the role of the host genotype is important not only in a qualitative sense, but in a quantitative way as well.

Perhaps the most interesting aspect of these observations was the single injection phenomenon. It would appear that this reaction is a special form of the Shwartzman reaction inasmuch as both are so similar in their gross histological characteristics. It seems clear that Terramycin had an effect on the responsiveness of the mice in this regard, but that this effect diminished on continued therapy. Such data suggest the role of a microbial agent, initially sensitive to the antibiotic, but gradually developing some resistance on continuing exposure. Investigations currently in progress deal with the problems of isolating the microorganisms presumably responsible for increased "sensitivity" of these mice toward bacterial endotoxin.

Summary. The observations previously made by Kelly *et al.* on production of hemorrhagic skin lesions by dermal injection of bacterial endotoxin in Rockefeller mice have been repeated with the genetically related, highly inbred, BSVS strain. BSVS mice displayed all of the reactions reported by the above authors, but with a significantly increased frequency, and at much lower doses of endotoxins. Pretreatment with Terramycin caused a marked decrease in frequency of reactors to a single injection of endotoxin. This effect gradually diminished over a period of 8 months of continued treatment. It is suggested that the single-injection reaction is a special form of the dermal Shwartzman reaction in which some microbial agent(s) plays an as yet unknown part.

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