larities may be caused by improper fixation since they may show up in only one or two amidst a large number of perfectly well stained consecutive serial sections. Nonspecific impregnations may also occur; they can be recognized as such by incubating inactivated slides (treated for 10 minutes with Lugol's solution or with any N mineral acid) as controls. The inactivated sections will show the same artifacts.

In successful slides, however, there is a typical localization of the reaction, varying somewhat with different species. The enzyme is present mainly in the cytoplasm (often in a granular form) but also in some of the nuclei. Moderate amounts are found in many tissues such as the liver, where its distribution often but not invariably follows that of glycogen; in the secretory portions of the renal tubules; in the adrenal cortex; in the small intestine where its localization is similar to that of alkaline phosphatase; in the epithelial lining of the bronchi; in the beta cells of the pancreatic islets of the mouse (not constantly); in the lachrymal gland of the hamster; and many others. However, a very intense reaction, much stronger than at the sites mentioned, is obtained in two tissues: the grey matter of the central nervous system (especially that of the cerebellum), and in malignant tumors. Twenty-four adenocarcinomas of the gastrointestinal tract, 4 squamous carcinomas, 2 hypernephromas and a number of other epithelial tumors such as embryonic carcinomas of the testis, cancers of the prostate, breast and bronchi, Walker rat carcinomas No. 256 and a butter yellow tumor stained very intensely and selectively. The reaction may start quite abruptly at the border of the malignant change, or the normal tissue may show a slight to moderate staining up to a distance of 1 or 2 mm from the edge of the neoplasm. In a few cases where the neoplasm showed various degrees of atypia in its different portions, the most atypical portions showed the most intense staining. So far not a single carcinoma has been found out of a total of 64 cases which did not show a sufficient reaction to set it off sharply against its environment. With sarcomas the results were much less constant. Some of them did not stain at all; others stained in an uneven, patchy way, and only a few stained as uniformly as carcinomas did. Two papillomas of the bladder, questionably malignant, stained moderately heavily. Granulomas such as sarcoidosis and human tuberculosis usually did not stain or showed a faint to moderate reaction in the giant cells and, rarely, even in the epitheloid cells. Tubercles in the guinea pig stained rather heavily.

Summary. A histochemical method for the demonstration of sites of phosphamidase activity is described. Small amounts of the enzyme are present in many normal tissues; large amounts are found in the grey matter of the central nervous system and in malignant epithelial tumors.

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Inhibition of the Shwartzman Phenomenon by Local Application of Bromobenzene and Other Solvents.*

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When an intradermal injection of toxin derived from certain Gram-negative microorganisms is followed, after a period of approximately 20 hours, by an intravenous injection of similar bacterial toxin, a gross hemorrhage involving the entire injected skin area occurs. This hemorrhagic reaction has become known as the Shwartzman phenom-

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enon, in acknowledgement of the investigator who originally described it.[1]

During the course of recent studies on the mechanism of the Shwartzman phenomenon, it was found that the hemorrhagic reaction could be completely inhibited by the application of certain organic solvents to the prepared skin site, prior to the intravenous injection. It was also observed that these substances caused a marked increase in the permeability of the vessels of normal skin to circulating dye.

Materials and methods. Male white rabbits weighing from 1.8 to 2.5 kg, were used in all experiments. The entire abdomen was freed of hair by clipping and shaving at least 24 hours before the experimental day.

The bacterial toxins employed were 1) Meningococcal toxin No. 44B,† 2) a purified polysaccharide derived from culture filtrates of *Serratia marcescens,*‡ and 3) an "agar washings" filtrate prepared from cultures of *B. coli* according to Shwartzman's technic.[2]

Each bacterial toxin was titrated in 3 or more rabbits to determine its skin preparing potency. Similar titrations were carried out to ascertain the optimal amount of each preparation for intravenous provocation of the phenomenon. The dose of each toxin to be used intradermally in the following experiments was arbitrarily chosen as that amount which, when injected intradermally in a volume of 0.5 cc, resulted in satisfactory skin preparation of at least 75% of the rabbits tested. The intravenous dose of each agent was chosen as that amount, which, when injected intravenously in a volume of 2.0 cc, elicited positive reactions in at least 75% of satisfactorily prepared rabbits.

In testing the inhibitory property of locally applied substances, two skin areas were prepared by the intradermal injection of toxin, one on each side of the abdomen. These injections were spaced as far apart as possible, and the side used for testing inhibition was alternated in the rabbits of each experimental group in order to avoid the effect of possible differences in the natural susceptibility of the two sides of the abdomen.[2]

Results. The effect of a single application of bromobenzene was tested by applying this substance with a cotton swab over one side of the abdomen, at various times before and after an intradermal injection of bacterial toxin had been made on both sides. The intravenous injection of bacterial toxin was given 20 hours after the intradermal injection.

The inhibitory effect of bromobenzene is illustrated in Table I, in which it will be seen that when the local application was made at the same time as the intradermal injection of meningococcal toxin, or at various times thereafter up until the time of the intravenous injection, the Shwartzman phenomenon did not occur. Similar results were obtained in experiments using *S. marcescens* and *B. coli* toxins.

Maximum inhibition was brought about when bromobenzene was applied between 16 and 20 hours after skin preparation, as is indicated in Table I. When the skin was painted before the intradermal injection, no inhibition occurred; indeed, in a limited number of observations it appeared that the lesions in such areas tended to be more extensive than in unpainted control sites. When painting was delayed until 21 hours, or one hour after the intravenous injection, inhibition was not observed.

Various amounts of bromobenzene were dissolved in ether and tested 18 hours after skin preparation, in order to determine the concentration necessary for the effect. It was found that inhibition occurred regularly with 25 and 50% solutions, while the effect was less uniformly obtained with 5% and not at all with 1% solutions.

In subsequent experiments, other organic solvents were tested for similar inhibiting properties. Chlorobenzene, iodobenzene and benzene caused inhibition of the Shwartzman phenomenon, although the degree of inhibition was not as complete as with bromobenzene.

[1] Shwartzman, G., PROC. SOC. EXP. BIOL. AND MED., 1928, **25**, 560.

† Obtained from Dr. Gregory Shwartzman, and prepared by the method of this author.[2]

‡ Obtained from Dr. Murray Shear.

[2] Shwartzman, G., Phenomenon of Local Tissue Reactivity. Paul B. Hoeber, Inc., New York. 1937.

TABLE I.
Inhibition of the Shwartzman Phenomenon by Local Application of Bromobenzene at Various Intervals Before and After the Intradermal Injection of Meningococcal Toxin.

Bromobenzene applied to skin	No. of rabbits	Shwartzman reaction* Bromobenzene treated side	Shwartzman reaction* Untreated side	% inhibition
24 hr before preparation	4	4*	4	0
4 " " "	7	7	7	0
At same time as preparation	6	3	6	50
4 hr after "	4	1	4	75
8 " " "	4	1	4	75
16 " " "	4	0	4	100
18 " " "	6	0	6	100
20 " " "	4	0	4	100
21† " " "	3	3	3	0

* Figures refer to number of rabbits showing hemorrhagic reaction at indicated skin area following intravenous injection of meningococcal toxin. Latter injection given at 20 hours after skin preparation.

† These rabbits received intravenous toxin one hour before the application of bromobenzene.

Chloroform and methyl salicylate, when applied 20 hours after the intradermal injection, caused inhibition in a majority of animals, but these agents yielded less constant results when applied at earlier intervals. Ether, acetone and alcohol had no effect on the Shwartzman phenomenon. In order to test the effect of a substance with primary skin irritative effects but without solvent action, 10% formalin was applied in a series of rabbits; this material had no effect on the phenomenon.

Observations were made on the local reaction of normal rabbit skin to single and repeated applications of bromobenzene. Within 30 to 60 minutes after a single painting, the skin exhibited a slight erythema. During the next few hours some edema occurred in the painted area in many of the animals. After 12 hours the skin usually appeared normal. However, after several days some superficial flaky desquamation of the skin commonly occurred. It was of incidental interest that the regrowth of hair on the shaven abdomen occurred more rapidly during the following weeks in areas which had been painted with bromobenzene. The effect of repeated painting with bromobenzene was similar to that following a single application, but the edema and desquamation were more extensive.

It is known that irritation of normal skin may cause an increase in local permeability to circulating dyes.[3] The effect of bromobenzene on permeability was tested by applying this substance to the skin of normal rabbits which were simultaneously given an intravenous injection of 5 cc of 5% Evans Blue Dye (T-1824). It was found that within 5 minutes after painting with bromobenzene, the painted area became deeply stained with dye. The local accumulation of dye was sharply confined to the painted area and outlined almost exactly the extent of application of the swab. The skin remained deeply stained for about 12 hours, after which fading occurred gradually.

Before testing the effect of bromobenzene on the permeability of the vessels in skin tissue which had been prepared for the Shwartzman phenomenon, it was of interest to determine whether such tissues showed any increase in permeability to Evans Blue dye. Accordingly, rabbits were given intradermal injections of meningococcal toxin and at various intervals thereafter were given an intravenous injection of 5 cc of 5% Evans Blue. It was observed that the prepared skin did not become stained at any time up until the actual precipitation of hemorrhage following the intravenous injection of toxin. These results were similar to those described by Bordet,[4] who used trypan blue dye in similar experiments.

[3] Menkin, V., Dynamics of Inflammation. The Macmillan Co., New York. 1940.

[4] Bordet, P., *Ann. Inst. Pasteur*, 1936, **56**, 325.

Having ascertained that skin areas with meningococcal toxin were not more permeable to circulating dye than normal rabbit skin, the effect of bromobenzene on prepared skin was determined. The results were quite different from those seen in normal skin. When bromobenzene was applied to an area which had been injected intradermally with toxin eighteen hours previously, and an intravenous injection of Evans Blue given simultaneously, no dye appeared in the prepared area. When a large surface of the abdomen of a prepared rabbit was painted, the contrast between the deep blue staining of the surrounding normal skin and the pink, unstained prepared area was striking.

These observations suggested that the vessels in a prepared skin area were actually less permeable to dye than normal vessels, or were less responsive to the permeability-enhancing property of bromobenzene. Similar results were obtained with surface applications of chlorobenzene, iodobenzene, and benzene, and to a less striking degree with chloroform and methyl salicylate; in each instance normal skin showed staining with the dye while prepared skin showed little or no staining.

Discussion. The inhibition of the Shwartzman phenomenon by the local application of bromobenzene and certain other organic solvents was a somewhat accidental finding. As a result of certain other experimental data,[5] an hypothesis was entertained which implicated sulfhydryl-activatable tissue proteolytic enzymes, or "cathepsins", in the mechanism of damage to blood vessel walls in the Shwartzman phenomenon. Crabtree[6] demonstrated that the application of bromobenzene caused prompt reduction in the amount of gluthathione in skin tissue, presumably as the result of local detoxification of bromobenzene. Accordingly, the possibility was considered that an application of bromobenzene to a skin area which had been prepared for the Shwartzman phenomenon might inhibit the hemorrhagic reaction by interfering with the activation of tissue protease. The finding that inhibition of the phenomenon was, in fact, produced by bromobenzene does not indicate that this is the mechanism by which the inhibition took place, in view of the observation that other unrelated substances such as chloroform and methyl salicylate caused a similar effect.

The capacity of bromobenzene, as well as the other solvents tested, to bring about a local increase in the permeability of normal skin to circulating Evans Blue dye offers a second possible explanation for the inhibitory action of these substances on the Shwartzman phenomenon. Although no increased permeability was demonstrable in prepared skin tissue by the dye method, it is reasonable to assume that a similar change, although less in degree, may have occurred. Under such a circumstance, inhibition of the Shwartzman phenomenon may have been caused by the advent of an inhibitory substance from the blood into the prepared area, or by the departure of a damaging substance from the prepared area.

The third possible explanation to be considered is that the inhibition is the result of a wholly non-specific damaging action of bromobenzene on the skin blood vessels, interfering with their reactivity to a variety of other stimuli.

The question of the permeability of skin capillaries is of importance in considering the pathogenesis of the Shwartzman phenomenon. If these vessels were more permeable than normal, as is reported to be the case in other varieties of inflammatory tissue,[3] the accumulation of toxic material from the circulating blood would require consideration as a possible mechanism for the tissue damage,[3,7] even though the likelihood of bacterial toxin having a direct hemorrhagic effect in such small concentrations seems remote. The studies of blood vessel permeability with intravenously injected Evans Blue dye indicate that the prepared skin is not more permeable to this material, and the results obtained with bromobenzene suggest that the vessels are less permeable, at least to this type of permeability-enhancing stimulus.

[5] Thomas, L., and Stetson, C. A., in press.

[6] Crabtree, H. G., *Cancer Research*, 1944, **4**, 688.

[7] Moritz, A. R., *J. Exp. Med.*, 1937, **66**, 603.

Other studies in this laboratory[5] have shown that skin tissue prepared for the Shwartzman phenomenon exhibits an abnormal degree of aerobic glycolysis and lactic acid accumulation. Whether this alteration is related to an impairment in the permeability of vessels in prepared skin is a subject for further investigation. It is of interest to note that the application of bromobenzene to prepared skin had no demonstrable effect on the degree of aerobic glycolysis exhibited by such tissue.[8]

Summary. Complete inhibition of the Shwartzman phenomenon was produced by a single application of bromobenzene to the surface of prepared skin areas at any time during the 20 hours after the intradermal injection of bacterial toxin. Similar results were obtained with other benzene derivatives, and, less constantly, with chloroform and methyl salicylate.

Areas of rabbit skin which were prepared by the intradermal injection of meningococcal toxin showed no increase in permeability to Evans Blue dye, when the dye was injected intravenously.

A single application of bromobenzene to the surface of normal rabbit skin resulted in the prompt appearance of intravenously injected Evans Blue dye in the painted area. In contrast, little or no dye appeared in painted areas which had previously been injected with meningococcal toxin. Similar results were obtained when skin was painted with other benzene derivatives, chloroform and methyl salicylate.

The possible bearing of these observations on the problem of the mechanism of the Shwartzman phenomenon is discussed.

8 Unpublished observation.

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Influence of Dinitrophenol on Body Temperature Threshold for Thermal Polypnea.*

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The capacity of dinitrophenol (DNP) to cause hyperthermia in animals through accelerating heat production is well known. In animals at low environmental temperatures, however, it reduces body temperature and total heat production,[1] apparently through reducing the activity of the cold defense mechanism, as shown by depression of shivering.[2] In discussing the mechanism of this inactivation of cold defense, Hall, Crismon and Chamberlin[2] suggested two possibilities: (a) DNP might depress the centers involved in cold defense by a nonspecific toxic action similar to that by which it depresses other nervous processes such as spontaneous running activity;[3] (b) DNP, which increases the oxygen consumption of cerebral cortical tissue in concentrations likely to be attained when ordinary doses are injected,[4] might accelerate the metabolic activity of the temperature regulating centers, so causing the centers to behave as if heated which would reduce or abolish shivering. If the latter were the case, one would expect from the known reciprocal relationship of the cold and heat defense mechanisms that, when the body temperature

* This work was done under a contract between the Air Materiel Command, Wright Field, and Stanford University.

1 Giaja, J., and Dimitrijevic, I. N., *Arch. internat. Pharm. Therap.*, 1933, **45**, 342; Tainter, M. L., *J. Pharm. Exp. Therap.*, 1934, **51**, 45.

2 Hall, V. E., Crismon, J. M., and Chamberlin, P. E., *J. Pharm. Exp. Therap.*, 1937, **59**, 193.

3 Hall, V. E., and Lindsay, M., *J. Pharm. Exp. Therap.*, 1934, **51**, 430.

4 Fuhrman, F. A., and Field, J., PROC. SOC. EXP. BIOL. AND MED., 1942, **49**, 504.