

STUDIES ON THE MECHANISM OF THE SHWARTZMAN PHENOMENON*

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The intradermal injection of filtered bacterial toxin derived from certain Gram-negative microorganisms produces, in rabbits, little evidence of primary damage to the skin other than varying degrees of local inflammatory reaction. The skin may show mild erythema, usually with slight thickening of the injected area. After an interval of 12 to 18 hours, an intravenous injection of the same material causes the appearance of extensive hemorrhagic necrosis in the prepared skin site. This reaction, first described in 1928 (1), has become generally known as the Shwartzman phenomenon. No satisfactory explanation of its pathogenesis has yet been made.

Very little is known about the basic mechanisms which are implicated in cellular injury of any sort. In recent years, interest in the general problem of tissue damage has been stimulated by wartime investigations into the traumatic effects of arsenical poisons (2) and nitrogen mustards (3), which have demonstrated that certain types of tissue damage may be correlated with biochemical alterations involving cellular enzyme systems. It is probable that the various types of tissue injury caused by bacteria and their products, or by antigen-antibody reactions, will eventually become explainable at a biochemical level. As an approach to this problem, the Shwartzman phenomenon offers an experimental model in which one variety of injury can be studied from this point of view.

The phenomenon is, in a sense, non-specific. Preparation of the skin with culture filtrate from one bacterial species renders the skin susceptible to hemorrhage when filtrate from an entirely unrelated species is injected intravenously. Preparation of the skin can be accomplished by inducing local infection with certain living bacteria; *i.e.*, streptococci, staphylococci, and pneumococci, as well as by local virus infection (vaccinia) (4).

The capacity of antigen-antibody combinations to bring about the phenomenon has been studied in detail by Shwartzman (4). He showed that rabbits

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which had previously received horse serum exhibited typical hemorrhagic necrosis in skin sites prepared with bacterial filtrate, within 1 hour after the intravenous injection of horse serum. Other protein antigens produced similar and specific effects in appropriately sensitized rabbits. Moreover, the reaction could be induced in normal rabbits when mixtures of antigen and antibody, or the washed precipitates from such mixtures, were injected intravenously. The numerous investigations dealing with this and other features of the phenomenon have been amply reviewed by Shwartzman (4).

It is possible that in certain malignant neoplasms of animals a state of affairs may already exist which is similar to that created in rabbit skin by the local injection of bacterial toxin. It has been repeatedly demonstrated that hemorrhagic necrosis can be produced in such tumors by intravenous injection of those bacterial toxins capable of eliciting the Shwartzman phenomenon (4, 5). The mechanism by which hemorrhage is brought about in tumors has never been explained.

The hemorrhagic skin lesion which characterizes the Shwartzman phenomenon appears to be due to damage to the walls of small blood vessels. The events which precede the rupture of these vessels may be divided into two separate stages. The first stage is initiated by the intradermal injection of the bacterial toxin, and requires a period of 18 hours or so, during which time the skin becomes "prepared." There is no evidence of damage to blood vessels during this period, and the only indications of a disturbance in the tissue are edema and an infiltration by polymorphonuclear leucocytes. The second stage is initiated by the intravenous or "provocative" injection of bacterial filtrate, and consists of an injury to the blood vessels in the prepared area. One or 2 hours are required for the effects of this injury to become apparent. At this time, petechiae begin to appear in the skin, and during the ensuing 30 minutes a gross hemorrhage involving the entire prepared area occurs.

In attempting to investigate the mechanism of the Shwartzman phenomenon, it has been assumed as a working hypothesis that the preparatory injection brings about an alteration in local environmental conditions which does not of itself cause damage to the blood vessels. Under these new conditions, however, the vessels are rendered vulnerable to a second change in circumstances, which is brought about by the intravenous injection. On this basis, it appeared reasonable to approach the problem of the Shwartzman phenomenon along two separate lines, involving the mechanism of preparation and that of provocation. The first section of the present report is concerned with the results of a study of certain metabolic functions in rabbit skin following an intradermal injection of meningococcal toxin. In the second section, the effect of the intravenous injection of toxin is considered, and an attempt is made to formulate a theoretical explanation for the Shwartzman phenomenon.

Materials and Methods

Bacterial Toxins.—Most of the experiments described were performed with a meningococcal toxin which was supplied by Dr. Gregory Shwartzman. In some experiments a purified polysaccharide toxin derived from *Serratia marcescens* was employed; this material was supplied by Dr. Murray Shear. The methods used for the preparation of these materials are described in publications of these authors (4, 5).

Rabbits.—Male rabbits of hybrid white and grey stock, weighing approximately 2 kilos, were used in all experiments.

Intradermal Injection of Bacterial Toxin.—The hair over the entire abdomen was removed by shaving at least 24 hours in advance of all experiments. Meningococcal toxin was injected intradermally in a 1 to 4 dilution in physiological saline. A dose of 0.5 cc. was used in all experiments. *S. marcescens* toxin was given in an amount of 0.05 mg. contained in 0.5 cc. of saline. In the sections which follow, the term "prepared" will be used to designate skin into which bacterial toxin has been injected previously.

Intravenous Injection of Bacterial Toxin.—Meningococcal toxin was diluted 1 to 50 in physiological saline; and 2 cc. of this solution was injected intravenously into each rabbit. The dose of *S. marcescens* toxin was 0.05 mg. contained in 2 cc. of saline.

Manometric Measurements.—Eighteen hours after the intradermal injection of toxin, the animals were killed by a blow on the head. The abdominal skin was scrubbed with distilled water, dried with a cotton sponge, and strips of skin were quickly removed from the prepared site and from a normal skin area on the opposite side of the abdomen. Care was taken to include as little subcutaneous tissue as possible in the samples. The skin was then cut into small squares, each weighing approximately 50 mg., and several of these squares were accurately weighed and placed in Krebs-Ringer solution in Warburg flasks. Manometric determinations were carried out in duplicate, using the standard Barcroft-Warburg constant volume respirometer. The results are expressed in terms of microliters of gas per gram wet weight per hour. The dry weights of numerous samples of skin were measured and found to be from 19 to 24 per cent of the wet weight for the prepared skin samples, as compared with 25 to 30 per cent for normal skin. Lactic acid determinations were carried out according to the method of Barker and Summerson (6), and the results expressed as milligrams of lactic acid per gram dry weight of tissue.

Proteolytic Enzymes.—Crystalline trypsin was obtained from Armour Laboratories and employed as a 1 per cent solution in physiological saline. Partially purified, sterile papain was prepared from the commercial crude product by three successive precipitations with alcohol. The final precipitate was dissolved in saline to give a 1 per cent solution and filtered through a Seitz filter.

Sulphydryl Compounds.—Solutions of cysteine hydrochloride and BAL (2,3-dimercapto-propanol) were prepared in physiological saline and adjusted to pH 7.0 with NaOH. These solutions were freshly prepared before each experiment.

EXPERIMENTAL

1. Studies on the Metabolism of Prepared Rabbit Skin

Respiratory Exchange in Normal and Prepared Skin.—No significant differences were found between the oxygen uptake of normal and prepared skin, whether or not added glucose was supplied. The respiratory quotient of prepared skin, however, tended to be higher than that of normal skin, and this difference was more pronounced in the presence of added glucose. The results of a series of illustrative experiments are recorded in Table I.

TABLE I

Skin sample	Rabbit No.	Without glucose			With glucose		
		O ₂	CO ₂	R.Q.	O ₂	CO ₂	R.Q.
Prepared	1	288	204	0.71	270	248	0.90
	2	364	222	0.61	325	243	0.755
	3	402	294	0.73	345	301	0.87
	4	408	294	0.72	374	310	0.83
Normal	5	476	260	0.545	475	328	0.69
	6	286	154	0.54	295	197	0.67
	7	423	297	0.70	377	294	0.78
	8	384	219	0.57	362	254	0.71

The oxygen uptake and carbon dioxide output of rabbit skin tissue which had been prepared 18 hours previously, by an intradermal injection of meningococcal toxin, compared with normal rabbit skin. Tissue squares in Krebs-Ringer solution, without glucose and with 200 mg. per cent glucose. Oxygen in the gas phase and 0.2 cc. 10 per cent NaOH in the center well. Results are expressed as microliters of gas per gram wet weight per hour.

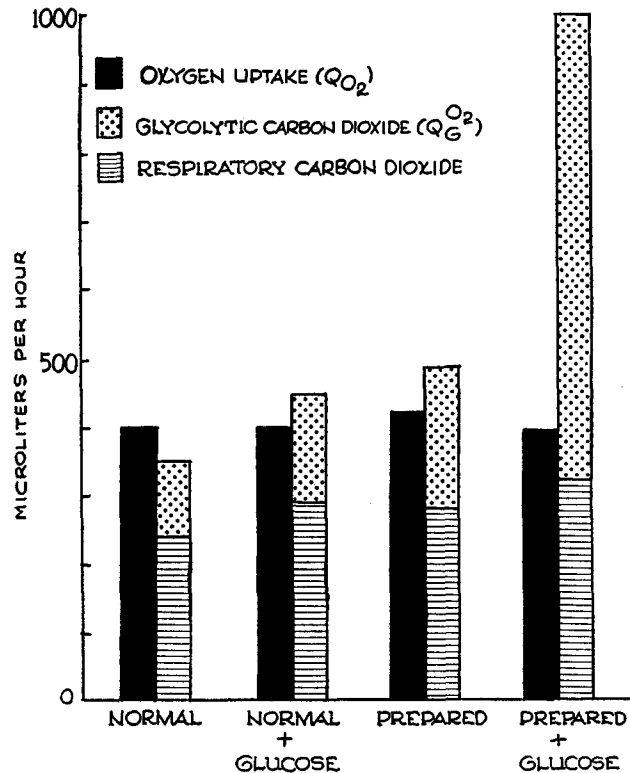
Aerobic Glycolysis of Normal and Prepared Skin.—

Samples of normal and prepared skin were placed in Krebs-Ringer solution containing added bicarbonate and glucose; and the ability of these tissues to produce lactic acid aerobically was measured by Dixon's (7) modification of Warburg's indirect method, based on the liberation of carbon dioxide from the bicarbonate buffer. Striking differences were observed between normal and prepared skin, the latter showing a twofold to fivefold increase over normal. In the absence of added glucose the differences were less pronounced but still evident. The results of a typical experiment, in which skin tissue was prepared with meningococcal toxin, are shown in Text-fig. 1, where a comparison is made between the degree of aerobic glycolysis of prepared and normal skin from a single rabbit, with and without the addition of glucose. In this figure, the tentative value for glycolytic carbon dioxide was obtained by subtraction of the respiratory carbon dioxide from the total carbon dioxide. Similar observations were made with skin tissue which had been prepared with *S. marcescens* toxin.

A summary of the determinations on prepared and normal skin samples from twenty-seven rabbits is presented in Text-fig. 2. Here the results are expressed as the ratio between the glycolytic carbon dioxide and oxygen uptake. It will be seen that this ratio was greater than unity for the majority of the prepared skin samples, and less than unity for all of the normal skin samples.

That the increased carbon dioxide output measured in these experiments was actually due to the aerobic formation of lactic acid was shown by directly determining the amount of lactic acid produced during a period of aerobic incubation in the Warburg flasks. The results are shown in Table II. It will be seen that the prepared skin samples produced four to five times as much lactic acid as did the normal tissue. In order to determine whether lactic acid

actually accumulated in prepared tissues *in vivo*, measurements of the lactic acid content were made directly on samples of prepared and normal skin which were removed from animals immediately after death and dropped at once into cold trichloroacetic acid. The results of these experiments are included in

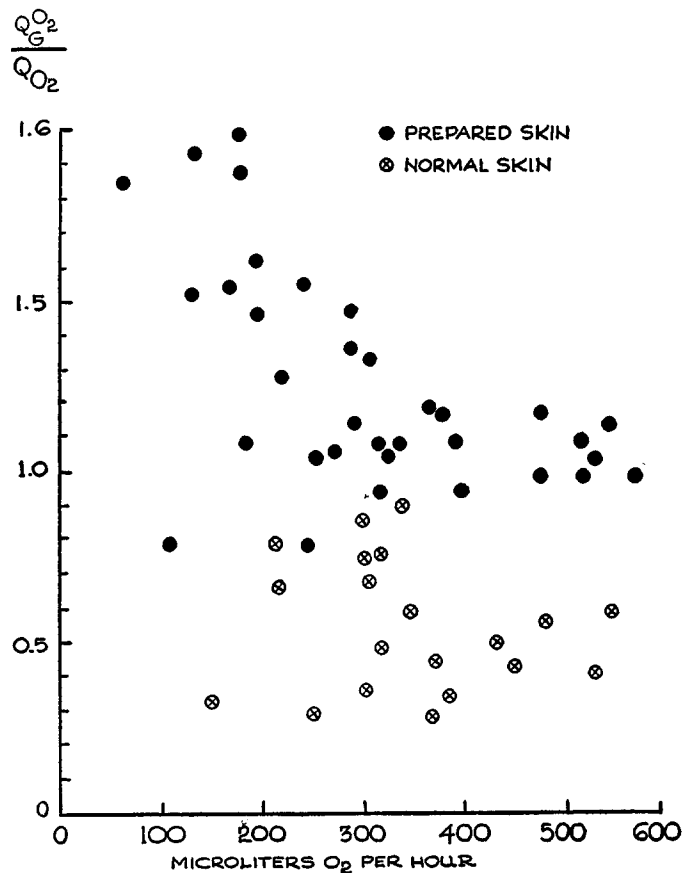


TEXT-FIG. 1. Aerobic glycolysis of normal and prepared rabbit skin, measured by Warburg's indirect method, in Krebs-Ringer solution with added 0.0014 M bicarbonate, and with 95 per cent O₂ per 5 per cent CO₂ as the gas phase. Glucose added to a final concentration of 200 mg. per cent where indicated. Results expressed as microliters per hour per gram wet weight of tissue.

Table II, in which it will be seen that the prepared skin sites contained up to five times as much lactic acid as was found in normal skin.

The increase in aerobic glycolysis was the most striking and consistent abnormality which appeared in the course of these experiments, and attempts were made to ascertain whether it was a result of some direct toxic action of the bacterial filtrate on the tissue, or a manifestation of some secondary effect. It was found that the phenomenon could not be produced *in vitro* by mixing various amounts of the bacterial toxin with normal skin samples in War-

burg vessels. No increase in the degree of aerobic glycolysis could be detected, even when such mixtures were incubated for 6 hours. This is to be contrasted with another series of experiments in which bacterial toxin was allowed to act



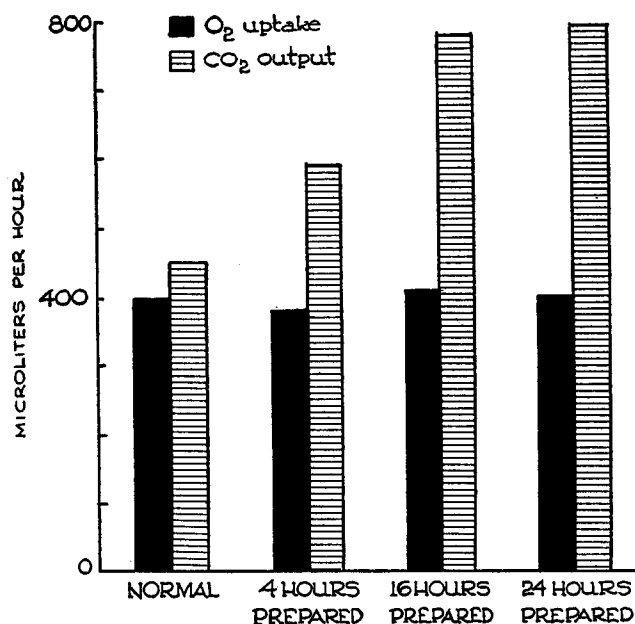
TEXT-FIG. 2. A comparison of the ratio of aerobic glycolysis to oxygen uptake in thirty-four samples of prepared rabbit skin and twenty samples of normal rabbit skin. Measurements made in Krebs-Ringer solution containing 200 mg. per cent glucose and 0.0014 M bicarbonate, in an atmosphere of 95 per cent O₂ and 5 per cent CO₂. Oxygen uptake of each sample of tissue indicated on abscissa.

in vivo for various periods of time before the animals were sacrificed, and the skin samples removed at these times were then compared. As is shown in Text-fig. 3, some increase in aerobic glycolysis appeared as early as 4 hours after the intradermal injection, but this was less marked than in skin samples taken 16 or more hours after the injection.

TABLE II

Rabbit No.	Initial content of lactic acid		Lactic acid formed in 2 hrs. at 37°C.	
	Normal	Prepared	Normal	Prepared
1	1.7	3.56	0.02	9.74
2	0.85	4.0	3.7	23.7
3	2.0	2.75	2.8	10.75
4	1.6	8.8	4.2	14.8
5	1.4	4.5	3.1	11.3

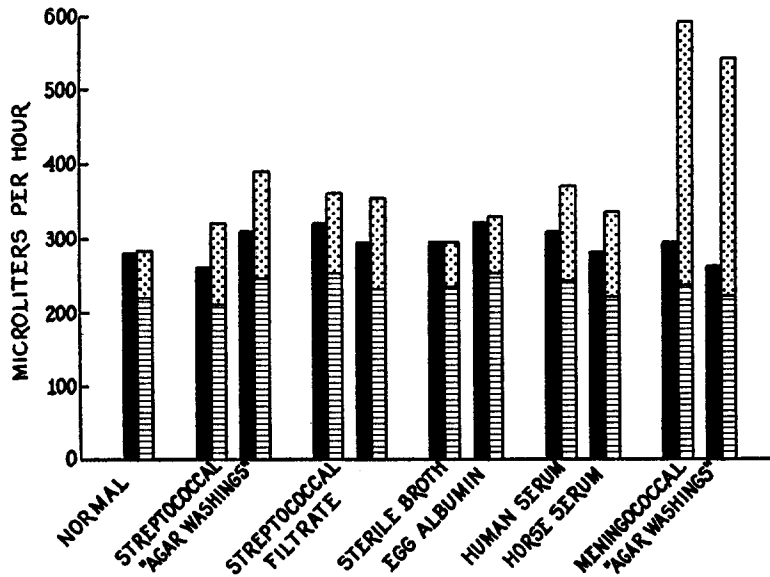
Lactic acid content of normal rabbit skin, and of prepared rabbit skin (injected intradermally with meningococcal toxin 18 hours previously), and the amount of lactic acid formed by normal and prepared skin during 2 hours at 37°C. in an atmosphere of 95 per cent O₂ and 5 per cent CO₂. Results expressed as milligrams lactic acid per gram dry weight of tissue.



TEXT-FIG. 3. Aerobic glycolysis of normal rabbit skin compared with that of skin prepared with meningococcal toxin 4, 16, and 24 hours prior to the experiment. Krebs-Ringer solution in flasks, with 200 mg. per cent glucose and 0.0014 M bicarbonate, in an atmosphere of 95 per cent O₂ and 5 per cent CO₂. Results expressed as microliters per gram wet weight of tissue per hour.

An attempt was made to determine whether the effect on glycolysis was confined to those bacterial filtrates which are capable of preparing the skin for the Schwartzman phenomenon. Several materials which produced visible local

inflammatory reactions in the skin but did not bring about a state of reactivity to the Shwartzman phenomenon were studied. These included fresh undiluted human and horse serum, 1 per cent egg albumin, sterile broth, filtered broth cultures of group C hemolytic streptococci, and concentrated "agar washings" of cultures of the latter organism which were prepared in the same manner as meningococcal toxin (4). The materials were injected intradermally in 0.5 cc. amounts, and 18 hours later the skin was removed and tested for aerobic glycolysis. The results of these experiments are illustrated in Text-fig. 4. It



TEXT-FIG. 4. Aerobic glycolysis of rabbit skin injected with various control substances compared with that of normal skin and of skin injected with meningococcal toxin. The experimental conditions were similar to those indicated in Text-fig. 1.

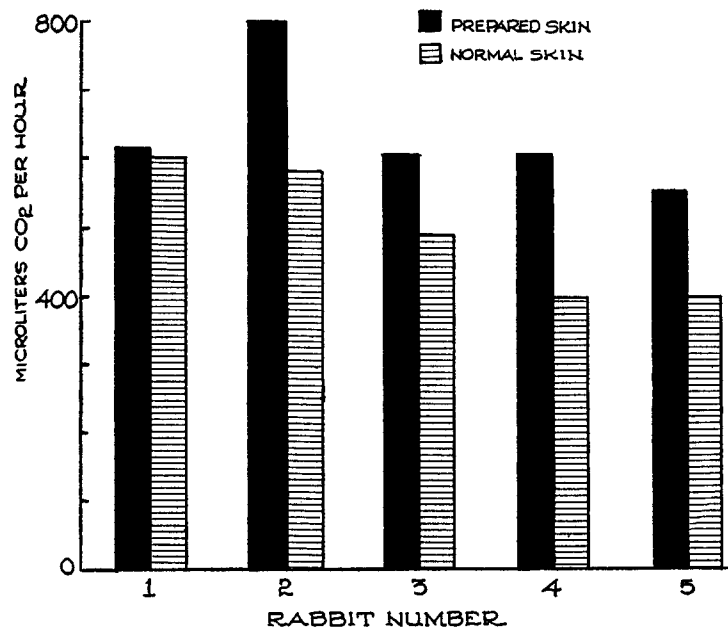
will be seen that none of these substances caused the appearance of aerobic glycolysis in significant degree. When the ratio $\frac{Q_G^{O_2}}{Q_{O_2}}$ was calculated for each skin sample, the results were found to lie well within the normal range shown in Text-fig. 2.

Anaerobic Glycolysis of Normal and Prepared Skin.—

Measurements of anaerobic glycolysis were carried out with samples of normal and prepared skin from twelve rabbits. The skin samples were placed in Krebs-Ringer solution with added bicarbonate and glucose, and incubated in an atmosphere of oxygen-free nitrogen and carbon dioxide. Simultaneous measurements of aerobic glycolysis were carried out with duplicate samples in each case.

It was found that the level of anaerobic glycolysis was consistently higher in prepared than in normal tissue, although the degree of difference was not as great as that encountered in aerobic glycolysis. The results of five illustrative experiments in which anaerobic glycolysis was determined in prepared and normal skin are shown in Text-fig. 5.

Respiration and Glycolysis Following Intravenous Injections of Bacterial Toxins.—Kun and Miller (8) have recently investigated some of the systemic



TEXT-FIG. 5. Anaerobic glycolysis of normal and prepared skin tissue in five rabbits. Measurements made in Krebs-Ringer solution containing 200 mg. per cent glucose and 0.0014 M bicarbonate. Oxygen-free nitrogen in the gas phase. Results expressed as microliters of carbon dioxide per hour per gram wet weight of tissue.

metabolic effects of intravenous injections of bacterial toxins similar to those used in the present study. They noted an inhibition of certain glycolytic enzymes in muscle and liver, which occurred within 90 minutes following the intravenous injection of meningococcal toxin. In order to determine whether similar effects might occur in either normal or prepared skin, the respiratory exchange and glycolysis were measured in skin samples taken from rabbits at various times after an intravenous injection of meningococcal toxin, up to and including the time when hemorrhage had actually begun in the prepared skin. The respiration and glycolysis of normal and prepared skin showed no significant changes following the intravenous injection. In a few

determinations made on prepared skin which had already begun to show hemorrhage, the oxygen uptake was somewhat below normal, but this finding was not constant and may have been related to the secondary tissue damage produced by hemorrhage.

2. *The Capacity of Proteolytic Enzymes to Produce Hemorrhage in Rabbit Skin, and the Effect of Intravenous Bacterial Toxin on Susceptibility to Such Hemorrhage*

It has been shown in the preceding section that rabbit skin, which has been prepared for the Shwartzman phenomenon by the intradermal injection of certain bacterial toxins, exhibits a striking degree of aerobic glycolysis, as compared with normal skin. Moreover, an accumulation of abnormal amounts of lactic acid in prepared skin was demonstrated. In attempting to relate these findings to the Shwartzman phenomenon, the known influence of glycolysis upon proteolysis in tissue was taken under consideration. Rubel (9), Menkin (10), and Maver, Johnson, and Voegtlin (11) have presented evidence which indicates that in tissues which undergo glycolysis there is a concomitant enhancement of the activity of tissue proteolytic enzymes. Stoner and Green (12) have shown that proteolysis occurs in ischemic muscle as the pH of the tissue becomes decreased, and may exceed the proteolysis of autolyzing muscle. The nature of the proteolytic enzymes involved is not clear, but they are presumed to belong to the class of tissue cathepsins.

It has been suggested that the increase in proteolytic activity may be designed for the removal of foreign and necrotic material from inflamed tissues (10). In view of the potentially destructive action of tissue protease, it is conceivable that an increase in enzyme activity would also result in damage to living tissue components if the latter were not protected by an inhibitory mechanism. It was therefore of interest to determine whether the intravenous injection of a bacterial toxin, capable of provoking the Shwartzman phenomenon, affected the susceptibility of normal skin tissue to the damaging effect of proteolytic enzyme. Since it was not feasible in this laboratory to undertake direct quantitative measurements of tissue protease activity in rabbit skin, an indirect approach to this problem was adopted. The response of normal rabbit skin to the intradermal injection of known proteolytic enzymes was compared with the response in rabbits which had been given an intravenous injection of bacterial toxin 1 hour previously; *i.e.*, at the time when hemorrhage would be beginning if these animals had been prepared for the Shwartzman phenomenon. The following section is concerned with the results of these and related experiments.

The Effect of Intradermal Injection of Proteolytic Enzymes.—Twelve normal rabbits were given an intradermal injection of 0.5 cc. of 1 per cent papain, in the abdominal skin. At the same time, twelve rabbits which had received

an intravenous injection of meningococcal toxin 1 hour previously were tested similarly.

Ten of the normal rabbits showed no reaction to papain other than local edema, while two developed small areas of circumscribed hemorrhage at the injected site, measuring approximately 0.5 cm. in diameter.

Eleven of the rabbits which had previously received meningococcal toxin exhibited extensive reactions of hemorrhagic necrosis following the injection of papain. These reactions began within 30 to 60 minutes after the intradermal injection and steadily increased in size and intensity during the next 2 hours, until they involved oval or circular areas measuring 5 cm. or more in diameter. The involved skin was dark blue or black, and usually showed some denudation of the superficial layers of the skin at the center. An example of a papain reaction is shown in Fig. 1. In general, the papain lesions differed in their gross appearance from the typical Shwartzman reaction in that the hemorrhage in the former involved the subcutaneous tissues more extensively and was followed, in some instances, by complete destruction of the skin with sloughing.

Similar reactions to papain were observed in each of five rabbits which had been given *S. marcescens* toxin 1 hour previously.

The optimal period for eliciting hemorrhagic reactions with papain was between 1 and 2 hours after the intravenous injection of toxin. When papain was injected at the same time as the toxin, the reactions were smaller and occurred less frequently. When the injection of papain was delayed until more than 4 hours after the bacterial toxin had been given, the hemorrhagic reaction did not occur.

It was evident from these observations that papain was capable of imitating the effect of prior skin preparation by bacterial toxin. Papain could not be used as a skin-preparing substance in the usual sense, however; when normal rabbits were given papain intradermally and then, after 18 hours, meningococcal toxin intravenously, no hemorrhage occurred.

The effect of crystalline trypsin on the skin of normal rabbits and animals receiving meningococcal toxin was tested in similar experiments. No differences were observed in the susceptibility of the two groups of rabbits. Small areas of hemorrhagic necrosis were produced in some animals of each group by 0.5 cc. of 1 per cent trypsin. None of the animals showed extensive or progressing lesions such as were seen with papain.

The existence of papain-like enzymes, having a relatively low pH optimum and requiring sulfhydryl or other reducing agents for their activation, has been demonstrated in a large number of tissues. The increased susceptibility of skin blood vessels to papain, but not to trypsin, suggested that the intravenous injection of bacterial toxin might have the capacity of interfering with a normal inhibitory mechanism for such a tissue protease. It was therefore of interest to determine whether sulfhydryl compounds were capable of activating

such a protease *in vivo* when injected into the skin. For this purpose, solutions of cysteine and BAL were injected intradermally in normal rabbits and in animals which had been given meningococcal toxin intravenously.

It was found that 0.5 M cysteine, and 0.1 M BAL, in volumes of 0.5 cc. produced little or no reaction in the skin of normal rabbits. In contrast, each of these compounds caused the appearance of extensive areas of hemorrhagic necrosis when injected 1 hour after the intravenous administration of meningococcal toxin. Similar results were obtained in rabbits given *S. marcescens* toxin intravenously. The reactions appeared at about 30 minutes after the intradermal injection, and slowly increased during the next hour. They consisted of oval or circular areas of deep blue hemorrhage, sometimes confluent and sometimes consisting of multiple petechiae. The lesions usually measured 3 to 5 cm. in diameter. In many instances they bore a striking resemblance to the gross appearance of the Shwartzman phenomenon. Typical reactions to cysteine are shown in Fig. 2.

The cysteine and BAL reactions could only be obtained when these substances were injected between 1 and 2 hours after the intravenous injection of bacterial toxin. When they were injected at the same time, or later than 2 hours after the intravenous injection, reactions did not occur.

Smaller concentrations of cysteine and BAL produced inconstant and smaller hemorrhagic lesions. The concentrations employed in the above experiments were considerably greater than would be required for the activation of papain or other sulfhydryl-activated proteolytic enzymes *in vitro*. If the hemorrhagic reactions observed following the intradermal injection of cysteine and BAL were due to the activation of a tissue protease, one might expect these substances to produce their effect in a relatively low concentration, but the following observation suggests that high concentrations may be necessary for activation *in vivo*. Five cc. of 1 per cent papain was injected intravenously into a number of rabbits, and at the same time 0.5 cc. of varying concentrations of cysteine and BAL was injected intradermally. In every instance, extensive hemorrhagic reactions occurred within 15 minutes in areas injected with 0.5 M cysteine and 0.1 M BAL. No reactions occurred, however, at sites injected with one-tenth of these concentrations of sulfhydryl compounds.

A number of other chemical solutions were injected into the skin of normal rabbits and animals which had received bacterial toxin intravenously, in an attempt to determine whether the reaction to sulfhydryl compounds and papain represented a specific event. These substances, none of which caused hemorrhagic reactions in either group of animals, included 10 per cent glutathione, 5 per cent sodium ascorbate, 5 per cent sodium lactate, 0.5 per cent potassium cyanide, 5 per cent sodium tetrathionate, 1 per cent hydroquinone, and 1 per cent gold thiosulfate. A solution of 5 per cent calcium chloride caused severe hemorrhagic reactions, but these were of the same intensity in both groups of animals.

DISCUSSION

It has been shown that rabbit skin which is prepared for the Shwartzman phenomenon by the intradermal injection of bacterial toxin exhibits a much increased degree of aerobic glycolysis and lactic acid formation *in vitro*. Evidence that this alteration occurs *in vivo* is obtained in the finding that there is a measurable increase in the concentration of lactic acid in prepared skin. The increase in aerobic glycolysis appears as early as 4 hours after the intradermal injection of toxin, but is not maximal until approximately 24 hours have elapsed.

The presence of increased aerobic glycolysis in adult mammalian tissues has generally been considered to be a manifestation of some form of damage. It has been reported, for example, in inflammatory exudates (10), in a tuberculous lymph node (21), in local vaccinia lesions in rabbit skin (14), and in various tissues damaged by mechanical means (13). It is also a characteristic feature of certain malignant tumors (15).

The concept that the release of aerobic glycolysis may be the result of damage to or interference with the so called "Pasteur mechanism" is supported by considerable evidence (13), and it is possible that some such mechanism is involved in the phenomena described in the present report. However, the failure of bacterial toxin to produce aerobic glycolysis when mixed with normal rabbit skin *in vitro* strongly suggests that the metabolic defect seen in the prepared skin samples may be due not to a direct toxic effect, but to some secondary reaction which can occur only *in vivo*. For example, it is possible that the polymorphonuclear leucocytes which appear in prepared skin tissue are the source of the increased aerobic glycolysis, and are responsible for the observed increase in total anaerobic glycolysis. This explanation was proposed by Crabtree (14) in the case of the vaccinia lesion. Available information is not sufficient to allow any definite conclusions as to whether the number of leucocytes present in the prepared skin samples could account for the increased aerobic glycolysis exhibited by the tissue as a whole.

Control experiments have been performed in which the intradermal injections consisted of various materials which are incapable of preparing the skin for the Shwartzman phenomenon. These substances included heterologous sera, broth, and culture filtrates of a group C hemolytic streptococcus. Each of these materials produced a visible local inflammatory reaction, but in no case did such tissue show the marked aerobic glycolysis characteristic of the samples prepared with meningococcal or *S. marcescens* toxin.

The studies of other workers (9-12) indicate that increased aerobic glycolysis with the accumulation of lactic acid may provide a stimulus for the action of tissue proteolytic enzymes, or cathepsins. The possibility that cathepsins may play a rôle in certain types of tissue damage has received little consideration. The function of these enzymes in tissues under normal conditions is not known, although it is generally believed that they are concerned with protein

synthesis. Their potentially destructive effect is illustrated by the familiar phenomenon of autolysis.

In the rabbit skin area prepared for the Shwartzman phenomenon, there is no evidence of physical damage to the components of this tissue until approximately 1 hour after the intravenous injection of bacterial toxin. At this time, necrobiosis involving the leucocytes and other cellular components of the tissue is observed (4), and rupture of the blood vessels occurs. If it is postulated that a proteolytic enzyme in prepared skin is responsible for this tissue damage, it is reasonable to assume that an inhibitory mechanism prevents the action of the enzyme until after the intravenous injection of toxin.

It has been observed that the intradermal injection of a 1 per cent solution of papain, which causes little or no hemorrhage in normal rabbits, results in extensive hemorrhagic and necrotic lesions in animals which have received an intravenous injection of meningococcal toxin 1 hour previously. These lesions bear a superficial resemblance to the Shwartzman phenomenon during the early period of their development, since they are characterized by profuse hemorrhage into the skin; later, when the papain lesions become wholly necrotic and sloughing occurs, the resemblance is less evident. No increase in susceptibility of skin to the hemorrhagic effect of trypsin was observed when rabbits were given intravenous toxin.

It has also been observed that the intradermal injection of neutralized solutions of cysteine and BAL causes a severe hemorrhagic reaction, 1 hour after an intravenous injection of bacterial toxin. The reactions elicited with these compounds were primarily hemorrhagic and bore a close resemblance to the Shwartzman reaction in their gross appearance.

In the absence of information regarding the actual status of protease inhibitor in skin tissue, the interpretation of these observations is a conjectural matter. The increased vulnerability of the skin to the action of papain may be due to the impairment of some inhibitory mechanism for this enzyme, but the nature of the inhibitor and the process involved in its impairment are not known. It is possible that the lesions produced by cysteine and BAL may be due to the direct chemical trauma of these reducing agents in tissue, with subsequent activation of tissue proteolytic enzyme and destruction of blood vessels. It is of interest that similar concentrations of cysteine and BAL produced hemorrhagic necrosis when injected into the skin of rabbits which were simultaneously given intravenous injections of papain solution.

In considering possible mechanisms by which susceptibility to intradermally injected protease may be affected by intravenously injected material, certain observations made by Jobling and Petersen (16-18) may be of significance. These workers reported that the inhibitor for crude trypsin could be removed from plasma by suspensions of bacteria, starch, agar, antigen-antibody mixtures, and kaolin, either when these substances were mixed with plasma *in*

vitro or injected intravenously into living animals. The authors considered that the inhibitor was probably adsorbed by the materials employed. It is known that each of these substances is capable of producing the Shwartzman phenomenon when injected intravenously into rabbits (4), including kaolin, which has been found in this laboratory to be highly effective in doses of 1 cc. of a 10 per cent suspension (19). It is not known whether the plasma inhibitor of trypsin is implicated in the control of tissue protease activity, but the possibility that a related mechanism may be involved in the Shwartzman phenomenon merits further investigation.

The mechanism by which intravenously injected bacterial toxin may affect the susceptibility of skin to proteolytic enzymes is unknown. It is possible that the systemic effects of toxin on carbohydrate metabolism may in some fashion be related to its action in the Shwartzman phenomenon. Kun and Miller (8) have reported that the injection of meningococcal toxin in rabbits causes a profound fall in the level of glycogen in liver and muscle tissue, during the hour following injection. They were unable to account for the disappearance of glycogen either on the basis of the hyperglycemia or the increased lactic acid formation exhibited by these animals. Bier and do Amaral (20) have recently shown that the intravenous injection of suspensions of glycogen will provoke the Shwartzman phenomenon, which they ascribed to the chemical similarity between glycogen and starch. We have confirmed this observation, using rabbit liver glycogen¹ in amounts of 100 mg. contained in 2 cc. of saline (19). It is conceivable that the disappearance of tissue glycogen described by Kun and Miller following the intravenous injection of meningococcal toxin and the capacity of glycogen to provoke the Shwartzman phenomenon may be related phenomena. Studies on this aspect of the problem are in progress.

In summary, the following theoretical explanation of the Shwartzman phenomenon is tentatively proposed. The effect of skin preparation with bacterial toxin is to bring about a change in the local metabolic activity of this tissue, with increased lactic acid formation. This new environment is favorable to the action of tissue protease, which is prevented from acting upon tissue components by a protease-inhibitory mechanism. The intravenous injection of bacterial toxin causes a decrease in the effectiveness of this inhibitory mechanism. In the prepared area, tissue protease is thus enabled to act on susceptible tissue components, the blood vessels undergo damage, and hemorrhage is the result.

¹ The glycogen used in these experiments was prepared from fresh rabbit livers by prolonged extraction with boiling water followed by precipitation with ethanol. Subsequent reprecipitation from aqueous solution with ethanol did not affect its hemorrhage-inducing activity. The conditions of preparation preclude the possibility of contamination of the material with bacterial products, which Shwartzman (4) has suggested as an explanation for the activity of suspensions of starch and agar.

The speculative nature of the above concept is apparent, and the evidence which is considered to support it is mainly indirect and circumstantial. It is presented because of its possible applicability to further investigation of the Shwartzman phenomenon, as well as its possible bearing on the problem of induced hemorrhage in tumors and the problem of tissue damage caused by antigen-antibody interaction.

SUMMARY

Rabbit skin which is prepared for the Shwartzman phenomenon by an intradermal injection of meningococcal toxin exhibits, *in vitro*, a high degree of aerobic glycolysis. This metabolic abnormality is reflected, *in vivo*, by a measurable increase in the concentration of lactic acid in the prepared skin. Some increase in anaerobic glycolysis also occurs in prepared skin; this is of less degree than the increase in aerobic glycolysis. The respiratory quotient of prepared skin tends to be somewhat higher than that of normal skin, although the oxygen uptake is not significantly altered.

Gross hemorrhagic lesions which resemble the Shwartzman phenomenon are produced by the intradermal injection of papain into rabbits which have received an intravenous injection of meningococcal toxin 1 hour previously. Such hemorrhagic reactions are not observed when papain is injected into normal rabbit skin.

Similarly, hemorrhagic lesions are produced by the intradermal injection of cysteine and BAL, following an intravenous injection of meningococcal toxin.

An hypothesis to explain the Shwartzman phenomenon, which implicates tissue protease in the damage to the blood vessels of the skin, is proposed.

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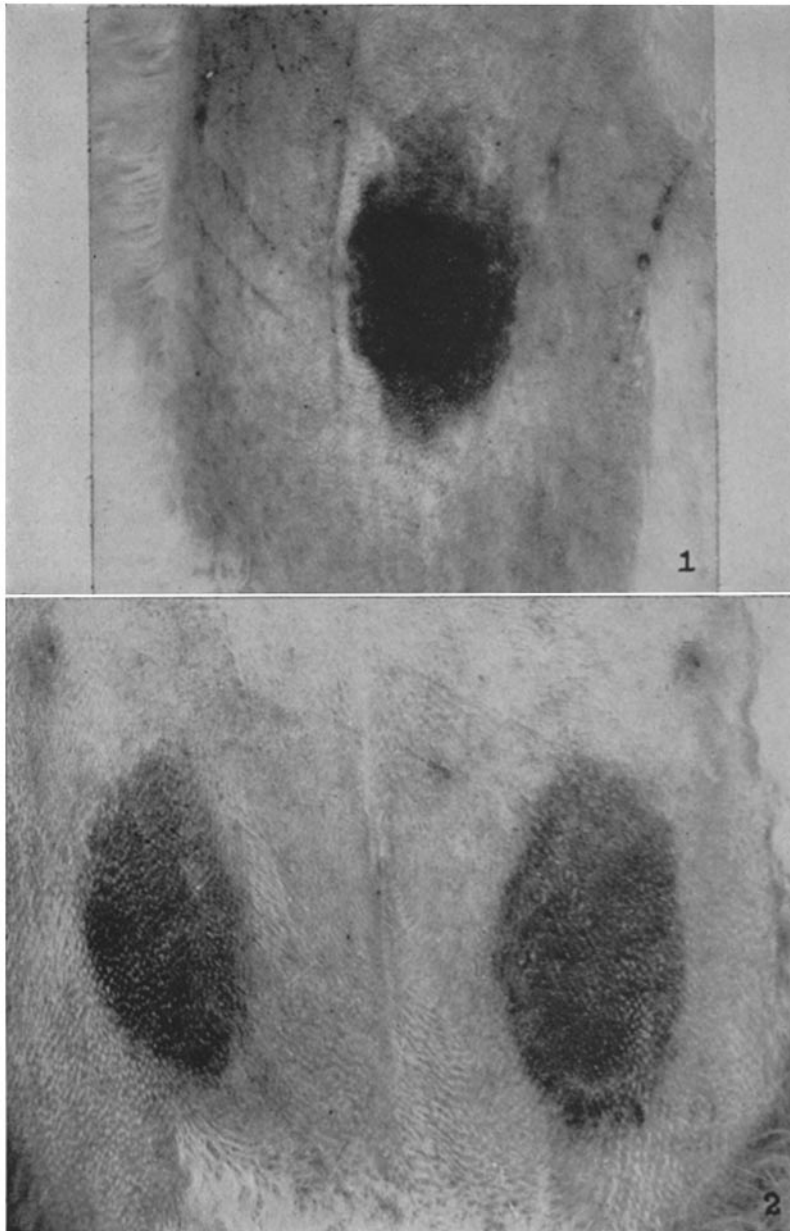
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EXPLANATION OF PLATE 21

FIG. 1. Hemorrhagic lesion produced in abdominal skin by 0.5 cc. of 1 per cent papain, in a rabbit which had received intravenous meningococcal toxin 1 hour previously.

FIG. 2. Two hemorrhagic lesions produced in abdominal skin by 0.5 cc. of 0.5 M cysteine, in a rabbit which had received intravenous meningococcal toxin 1 hour previously.



(Thomas and Stetson: Mechanism of Shwartzman phenomenon)