STUDIES ON EXPERIMENTAL SHOCK: PRODUCTION OF ISCHEMIC NECROSIS OF THE SKIN BY AN INTRADERMAL INJECTION OF ENDOTOXIN OR VASOPRESSOR AMINE

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In 1928 Shwartzman[1] reported the production of cutaneous hemorrhagic necrosis in rabbits by an intradermal and an intravenous injection of bacterial filtrates. Since that time, it has been shown that a number of substances, when injected intradermally, are capable of inducing similar lesions if given in association with an intravenous injection of gram-negative endotoxin.[2,3] It has been demonstrated also that the administration of substances such as heparin[4] or nitrogen mustard[5,6] inhibit the development of the dermal lesion.

In 1956 Thomas[7] reported that dermal hemorrhagic lesions which resembled the cutaneous Shwartzman lesion could be produced in rabbits by an intravenous injection of endotoxin followed by an intradermal injection of epinephrine or levarterenol or by an intradermal injection of a mixture of endotoxin and epinephrine. Zweifach, Nagler, and Thomas[8] described also an altered state of reactivity to epinephrine in the vessels of the rat meso-appendix when this material was applied topically after an intravenous injection of endotoxin. The results of these studies led these investigators to postulate that endotoxin altered the reactions of blood vessels to epinephrine in such a manner that the hormone became a capable necrotizing agent.

In 1958 Gatling[9] reported the induction of hemorrhagic skin lesions in rabbits by an intradermal injection of epinephrine or levarterenol following the intravenous administration of horse serum to which the animals had been sensitized previously. This investigator postulated that circulating antigen and its antibody were necessary for the production of the alteration, and he presented data identifying the lesion with that of the Arthus phenomenon. In 1959 Anderson and Brunson[10] described certain lesions produced in rabbits subjected to acute rotational shock. They reported that the lethal outcome and the incidence and severity of the

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lesions were increased greatly if the shock procedure was carried out in conjunction with an intravenous injection of endotoxin. It was suggested that these effects were mediated through adrenal medullary hormones.

The present paper reports the results of a series of experiments in which cutaneous necrosis developed in rabbits given an intradermal injection of endotoxin or a vasopressor amine in conjunction with rotational shock. These lesions, while similar in some respects to those described by Thomas [7] and Gatling,[9] exhibited striking differences in other respects.

Material and Methods

Hybrid albino rabbits of both sexes, weighing approximately 1.5 kg., were used in the study. They were fed Purina Rabbit Pellets and given free access to water.

The animals were subjected to a 15-minute period of rotation in a Noble-Collip drum [11] (450 revolutions), modified to the extent that it had no baffles and was padded with foam rubber to reduce trauma. This duration of rotation, as described previously,[10] produced a state of marked prostration and shock in the animals. In conjunction with rotation in the drum, the animals were given an intradermal injection of endotoxin or a sympathomimetic amine. For this purpose the abdominal hair was depilated, using the method described by Pitesky and Last.[12] No significant differences were observed in the dermal lesions in those depilated before and those depilated after rotation.

In initial studies, endotoxin, epinephrine, and levarterenol were used. The endotoxin consisted of the lipopolysaccharide fraction derived from *Escherichia coli* (0:111 B4) obtained from Difco Laboratories, Detroit, Michigan. Dilutions were made in sterile, isotonic, pyrogen-free saline solution and given in an amount of 100 μg. in a volume of 0.2 ml. The epinephrine and levarterenol were given in dosages of 100 μg. in a volume of 0.1 ml. In later studies a variety of other substances was used. These included phenylephrine (Neo-Synephrine ®), ephedrine sulfate, isopropylarterenol (Isuprel ®), heparin, nitrogen mustard, and certain phenothiazine derivatives. The ephedrine was injected in a dosage of 100 μg. in 0.5 ml., phenylephrine in a dosage of 100 μg. in 0.1 ml., and isopropylarterenol in a dosage of 10 μg. in 1.0 ml.

Heparin sodium, in a concentration of 10 mg. per ml., was given intravenously in a dosage of 20 mg. immediately prior to drum rotation and intradermal injections of 100 μg. of endotoxin and epinephrine. In other animals an intravenous injection of nitrogen mustard was given in a dosage of 1.5 mg. per kg., 3 days prior to intradermal injections of endotoxin and epinephrine in conjunction with drum rotation. An additional group of rabbits was given an intravenous injection of 2.5 mg. each of promethazine (Phenergan ®) and promazine (Sparine ®) mixture. This mixture was used because it had been shown to modify the pressor responses to epinephrine and levarterenol.[13] Following administration of the mixture, the animals were given an intradermal injection of endotoxin and levarterenol, and subjected to drum shock.

In a majority of the animals, two test substances were injected at separate sites on the abdomen. Two groups of control animals were used: one group was given intradermal injections of the test substances without rotation, and the other was given an intradermal injection of 0.2 ml. of sterile isotonic saline solution in conjunction with drum rotation. Details concerning the numbers of animals used in the various experiments are summarized in the text.

Results

The results obtained by an intradermal injection of gram-negative endotoxin, epinephrine, or levarterenol are summarized in Table I. The

figures in the table include only those animals that survived longer than 4 hours after rotation. A high percentage of the animals given the test substances simultaneously with the onset of rotation developed cutaneous necrosis. No lesions developed in control animals given only intradermal endotoxin, epinephrine, or levarterenol, or in those given intradermal saline simultaneously with drum rotation.

TABLE I

INCIDENCE OF CUTANEOUS NECROSIS IN RABBITS GIVEN INTRADERMAL INJECTIONS OF ENDOTOXIN, EPINEPHRINE, OR LEVARTERENOL SIMULTANEOUSLY WITH ROTATIONAL SHOCK

Material injected	No. of animals	Dermal necrosis
Endotoxin	30	21 (70%)
Epinephrine	14	10 (71%)
Levarterenol	15	9 (60%)
Controls *	12	0 (0%)

* See text for details.

The lesions were similar in all groups of animals, and were evident on inspection within 4 hours after rotation. They were characterized by a localized area of ischemia about the injection site in which the skin was a pearly white color. Over the next few hours the skin became dry, brown, and parchment-like. The area of involvement extended from the injection site for varied distances, and invariably was greater in those given epinephrine (Figs. 1, 3, 4 and 7) of levarternol (Fig. 5) than in those given endotoxin (Fig. 2). Dilatation of superficial blood vessels over the area was noted occasionally, but frank hemorrhage in or around the lesion was never observed. In the following 18 to 24 hours the skin became progressively drier and more brittle, and cracks or fissures appeared (Figs. 1 and 2). There was little evidence of edema, however. Within 72 hours, areas of eschar formation appeared and fragments of the dried skin began to drop off (Fig. 3). In 96 hours, extensive eschar formation was present and desquamation occurred (Figs. 4 and 5).

To determine the role of ischemia in causation of the lesions, the animals were given an intravenous injection of fluorescein (2 ml. of a 2 per cent solution) at varied intervals after the intradermal injections, and the abdomens were examined under ultraviolet light (Wood's lamp) for evidence of fluorescence. In those animals which had been given epinephrine or levarterenol, nonfluorescence, frequently over as much as 50 per cent of the abdominal skin area, was noted for as long as 6 hours after intradermal injection. On the contrary, in those given endotoxin, the period of ischemia and nonfluorescence diminished by the end of 2 to 3 hours, and at 6 hours the injection site was marked by high intensity fluorescence.

Microscopic examination of the skin in animals that died or were sacrificed at varied periods after intradermal injection and rotation showed similar alterations in all groups, with one exception. Early changes were characterized by complete ischemia of the skin and subcutaneous tissues, with necrosis of the superficial portions of the epi-

TABLE II

EFFECT ON DERMAL NECROSIS OF VARIATIONS IN THE INTERVAL BETWEEN INTRADERMAL INJECTION AND ROTATION

Procedure	Material injected	No. of animals	Dermal necrosis
Injection 4 hours prior to rotation	Endotoxin	8	1 (13%)
	Epinephrine	9	2 (22%)
	Levarterenol	3	0 (0%)
Rotation 4 hours prior to injection	Endotoxin	7	5 (71%)
	Epinephrine	4	3 (75%)
	Levarterenol	3	2 (66%)

dermis. At 18 to 24 hours this was marked by a heavy crust composed of necrotic squamous cells, nuclear debris, and minimal amounts of fibrin and red cells (Figs. 9 to 11). These lesions persisted for as long as 5 days. Rarely were occluded blood vessels observed, and the arterial walls displayed no evidence of necrosis. In all instances there was a striking absence of hemorrhage.

In the lesions produced by endotoxin, varying degrees of inflammatory reaction composed of heterophils and mononuclear cells were observed. In some, the reaction was quite sparse (Fig. 9), but in others it was severe and extended into the underlying connective tissue (Figs. 6 and 7). In those lesions produced by the vasopressor substances, a paucity of inflammatory cellular reaction was noted in lesions examined as long as 3 to 5 days after rotation (Figs. 8 and 10). Sections of skin from animals of both groups at this time showed extensive deposits of subepidermal hyaline material and varying degrees of connective tissue proliferation (Figs. 10 and 12). One other change, common to both groups, consisted of extensive foci of muscle calcification unaccompanied by any cellular reaction.

It has been reported that the systemic effects produced by drum rotation and intravenous injections of endotoxin are dependent on the interval between rotation and the administration of endotoxin.[10] To investigate the effect of time variation, endotoxin, epinephrine, and levarterenol, in the same dosages and volumes as described previously, were administered to one series of animals 4 hours before and to another series 4 hours after drum rotation. As may be seen in Table II, there was a low incidence

of lesions when the test substances were injected 4 hours before rotation. Only 1 of 8 animals given endotoxin, and only 2 of 9 animals given epinephrine developed cutaneous ischemic necrosis; the size and extent of these lesions was considerably less than that described in the previous groups of animals. When the injections were carried out 4 hours after rotation, however, a high percentage of the animals developed cutaneous necrosis. These lesions were similar in incidence and severity to those which occurred following intradermal injections of these substances simultaneously with rotation (Table I).

In the studies described by Thomas[7] and Gatling,[9] dermal necrosis failed to develop in rabbits given ephedrine, suggesting that vasoconstriction *per se* was not an important factor in the production of the hemorrhagic lesions. The effects of other vasopressor amines on the development of dermal lesions in rabbits subjected to rotational shock, are summarized in Table III. As may be seen, a high percentage of the animals in each group developed dermal lesions. These were similar in appearance and severity to those produced by endotoxin, epinephrine, or levarterenol.

It has been shown that the dermal Shwartzman phenomenon is inhibited by heparin[4] or nitrogen mustard,[5,6] but that the administration of chlorpromazine, a phenothiazine derivative, does not influence its development.[7] Thomas[7] reported that the epinephrine-endotoxin dermal lesions were prevented by the administration of chlorpromazine, but were enhanced by heparin or nitrogen mustard. Gatling,[9] on the other hand, reported that heparin "ameliorated" but did not prevent hemorrhagic dermal necrosis in his experiments. To test the effects of these substances on the drum-induced dermal lesions described previously, experiments were carried out in which heparin, nitrogen mustard, or a mixture of

TABLE III

INCIDENCE OF CUTANEOUS NECROSIS IN RABBITS GIVEN INTRADERMAL INJECTIONS OF VARIOUS SYMPATHOMIMETIC AGENTS SIMULTANEOUSLY WITH ROTATIONAL SHOCK

Material injected	No. of animals	Dermal necrosis
Phenylephrine	6	5 (83%)
Ephedrine	8	4 (50%)
Isopropylarterenol	4	4 (100%)

promazine-promethazine was given to rabbits subjected to drum shock simultaneously with an intradermal injection of endotoxin, epinephrine, or levarterenol.

The results, summarized in Table IV, show that neither heparin nor nitrogen mustard, in the dosages used, prevented the development of the

dermal lesions, which occurred in all of the animals given either endotoxin or epinephrine. On the contrary, the lesions were either prevented or markedly attenuated by prior administration of the mixture of promethazine-promazine. Only one of the animals in each group developed ischemic dermal necrosis (Table IV), and this was considerably

TABLE IV

EFFECT OF HEPARIN, NITROGEN MUSTARD, OR PHENOTHIAZINES ON THE PRODUCTION OF DERMAL LESIONS

Intravenous injection *	Intradermal injection	No. of animals	Dermal necrosis
Heparin	Endotoxin	4	4 (100%)
	Epinephrine	4	4 (100%)
Nitrogen mustard	Endotoxin	4	4 (100%)
	Epinephrine	4	4 (100%)
Phenothiazine	Endotoxin	5	1 (20%)
	Levarterenol	4	1 (25%)

* Details of administration in text.

less severe and more localized than the lesions in animals given endotoxin or levarterenol alone in conjunction with drum rotation.

DISCUSSION

A previous report [10] described the systemic lesions induced in rabbits by acute rotational shock alone and in conjunction with an intravenous injection of gram-negative endotoxin. The effects in those subjected to rotation alone were similar to those in rabbits following a single intravenous injection of endotoxin, suggesting that the rotational procedure acted in a manner basically similar to that of endotoxin. The acute stress procedure, undoubtedly accompanied by an increased secretion from the adrenal gland, led the authors to postulate that adrenal medullary hormones in some manner participated in the actions of endotoxin, or that the actions of endotoxin were mediated through adrenal medullary hormones.

The occurrence of ischemic and hemorrhagic intestinal lesions in rabbits subjected to drum rotation suggested also that there might be absorption into the blood stream of either gram-negative micro-organisms, or products thereof (lipopolysaccharides) which could contribute to the development of systemic lesions. Thus, it seemed plausible to believe that drum-shocked animals might have not only increased quantities of adrenal hormones in their circulatory system, but also, perhaps simultaneously, endotoxin or endotoxin-like substances derived from the intestinal flora. The experiments described by Thomas,[7] in which dermal

hemorrhagic necrosis was produced by an intradermal injection of epinephrine in rabbits given intravenous endotoxin, or by an intradermal injection of a mixture of endotoxin and epinephrine, suggested an ideal method for testing the hypotheses mentioned above. Consequently, the series of experiments described here were performed.

The results of these studies show that cutaneous necrosis may, indeed, be induced in shocked rabbits by the intradermal injection of epinephrine (or levarterenol) or endotoxin. Although the method used is similar to that used by Thomas [7] and Gatling,[9] the resulting lesions bear little gross or microscopic similarity to the lesions reported by these investigators. The differences warrant consideration. The lesions described in these experiments seem to be caused primarily by a prolonged interval of ischemia. Evidence for this fact is their gross appearance, the absence of fluorescein penetration into the injected sites for extended periods of time, and absence of dilated vessels, petechiae, or hemorrhage. Histologically also, the lesions are characteristic of ischemic necrosis. Secondly, the lesions resolve by desquamation of the skin in and about the sites of injection; this is associated with subepidermal accumulations of hyaline material and connective tissue proliferation. They do not, in contrast to the lesions described by Thomas, "gradually fade in color." [7] Thirdly, it should be emphasized that similar dermal lesions were produced by injections of other vasopressor amines such as ephedrine, isopropylarterenol, or phenylephrine. In this respect they differ sharply from those reported previously, since neither Gatling [9] nor Thomas [7] was successful in producing dermal hemorrhagic necrosis by the administration of such substances as ephedrine.

The epinephrine (or other vasopressor) lesion observed in this investigation is similar to the lesions described by Thomas in its striking absence of inflammatory reaction. On the other hand, the lesion induced by endotoxin resembles that induced by the intradermal injections of mixtures of epinephrine-endotoxin,[7] in that there was often a heavy infiltrate of inflammatory cells. The lesions were similar also to those of Thomas in that they exhibited no evidence of vascular occlusion or vascular fibrinoid deposition. A comparison of the various features is shown in Table V.

The influence of several modifying agents on the production of dermal necrosis is summarized in Table VI. The lesions observed in the present series of experiments resembled more closely those of Thomas [7] than of other investigators, in that they were prevented by the administration of phenothiazine derivatives (chlorpromazine or promazine-promethazine), and were either unaltered or enhanced by administration of heparin or nitrogen mustard. In an attempt to study the effects of cortisone on the

TABLE V

COMPARISON OF THE FEATURES OF CUTANEOUS LESIONS REPORTED BY VARIOUS AUTHORS

Author or reaction	Gross features					Microscopic features			Fate of lesion
	Ischemia or pallor	Petechiae	Hemorrhage	Edema	Induration	Vascular lesions	Hemorrhage	Cellular infiltrate	
Shwartzman [1]	Transient	+	+	±	−	+	+	+	Fades
Thomas [7]	Transient *	+	+	−	−	±	+	−	Fades
	Transient †	+	+	+	+	±	+	+	Fades
Gatling [9]	Transient	+	+	+	+	+	+	+	Fades
Evers & Brunson	Persistent	−	−	−	−	±	−	+ (endotoxin)	Desquamates
								− (vasopressor)	

* Reaction produced by intradermal epinephrine or endotoxin after an intravenous injection of endotoxin.

† Reaction produced by intradermal injection of mixture of endotoxin and epinephrine.

shock-induced dermal lesions, rabbits were pretreated for 3 days with cortisone acetate, following which they were subjected to rotation in conjunction with intradermal injections of epinephrine or endotoxin. Unfortunately, it was not possible to ascertain definitive results, since all animals so treated died during the period of rotation.[14]

TABLE VI

COMPARISON OF MODIFYING ACTION OF VARIOUS SUBSTANCES ON CUTANEOUS LESIONS

Author or reaction	Modification of lesion			
	Heparin	Cortisone	Phenothiazine	Nitrogen mustard
Shwartzman[1]	Prevents	Enhances	No effect	Prevents
Thomas[7]	Enhances	Prevents	Prevents	Enhances
Gatling[9]	Ameliorates	?	No effect	?
Evers & Brunson	No effect	?*	Prevents	Enhances

* All animals died within 4 hours after pretreatment with cortisone and rotation in drum.

From these observations and other data now extant, it appears reasonable to conclude that vasospasm, with prolonged dermal ischemia, plays a major role in the development of the lesions in this series of experiments. This period of ischemia appears to be particularly severe in the vasopressor induced lesions, to such an extent that an inflammatory reaction is inhibited, but of less severity and shorter duration in the endotoxin-induced lesions. Further, inhibition of vasospasm by agents known to exert adrenolytic effects, such as the phenothiazine derivatives, prevented the development of the lesions. On the contrary, agents such as heparin had no effect on their development, indicating that vascular thrombosis and hemorrhage were relatively unimportant in this regard. It is difficult, however, to assess the role of nitrogen mustard, since its administration seemed to enhance the development of the lesions.

That vasospasm may not be the only factor, however, is shown by other studies in which rabbits were given repeated intradermal injections of epinephrine alone, without developing cutaneous necrosis.[7] Similarly, certain other substances such as normal rabbit plasma or old tuberculin, given intradermally just prior to drum rotation, have been shown to induce lesions comparable in all respects to those described here.[14] Furthermore, it has not been possible to induce cutaneous lesions in rabbits given intradermal epinephrine or endotoxin during hemorrhagic shock,[15] a state which is usually accompanied (at least transiently) by peripheral vasoconstriction.

These observations and the recorded differences in the dermal lesions, suggest two hypotheses: (1) The shock state induced by drum rotation differs in its pathogenesis from that induced by endotoxin or hemorrhage.

(2) Certain other, endogenous agents or factors, in addition to those injected intradermally, participate in the production of dermal lesions in rabbits subjected to drum shock. The nature and origin of these factors, if such exist, and the mechanisms involved in the production of rotational shock remain obscure, and their elucidation requires further investigation.

Summary and Conclusions

Ischemic cutaneous necrosis was produced in rabbits subjected to rotational shock in conjunction with an intradermal injection of epinephrine, levarterenol, or endotoxin. The incidence of the lesions was approximately 70 per cent when the test substances were given simultaneously with or 4 hours after drum rotation, but was less than 25 per cent when they were administered 4 hours prior to rotation.

The lesions were evident grossly within 4 hours after rotation. They were characterized by extensive pale, flat ischemic areas which progressed rapidly to desiccation and, later, to desquamation. The histologic features consisted of necrosis of the superficial epidermis with a paucity of inflammatory reaction except in the endotoxin lesions. Vascular occlusion was significantly absent. Lesions observed 3 to 5 days after rotation consisted of extensive accumulations of subepidermal hyaline substance and connective tissue proliferation. Similar dermal lesions were induced by rotational shock in association with the intradermal injection of ephedrine, phenylephrine, or isopropylarterenol.

The lesions were prevented by the intravenous administration of a mixture of promazine and promethazine, but were unaffected by the prior administration of heparin. Pretreatment with nitrogen mustard appeared to increase the severity of the lesions.

The pathogenesis of the dermal lesions, and their similarity to those described by other authors, are discussed. It is concluded that vasospasm, although playing a major role in the causation, is probably not the only factor involved.

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[*Illustrations follow*]

Legends for Figures

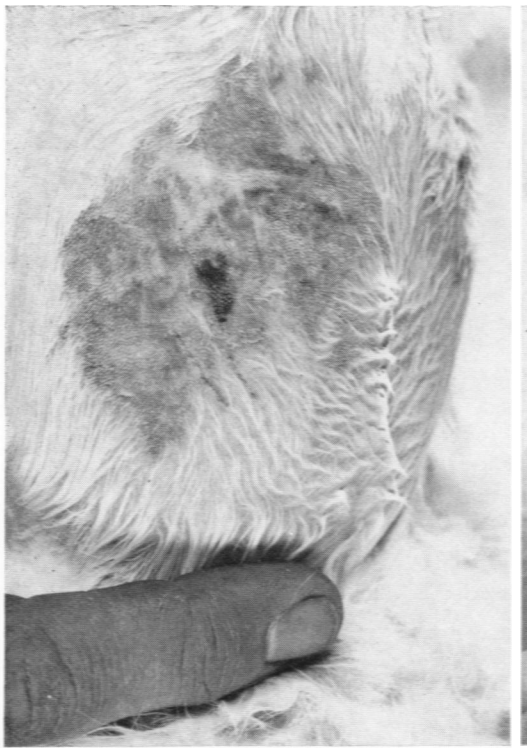
All microscopic sections were stained with hematoxylin and eosin.

Fig. 1. Abdomen of a rabbit given epinephrine intradermally immediately prior to rotation; photographed 24 hours after rotation. Note the eschar in the central portion of the lesion.

Fig. 2. Abdominal lesion at the site of intradermally injected endotoxin 24 hours after rotation. The lesion is similar to that in Figure 1, but is much less extensive.

Fig. 3. Abdominal lesion at the site of intradermally injected epinephrine 72 hours after rotation. Note progression of the lesion as compared to that in Figure 1.

Fig. 4. Abdominal lesion at the site of intradermally injected epinephrine 96 hours after rotation. Note extensive eschar formation and beginning desquamation.



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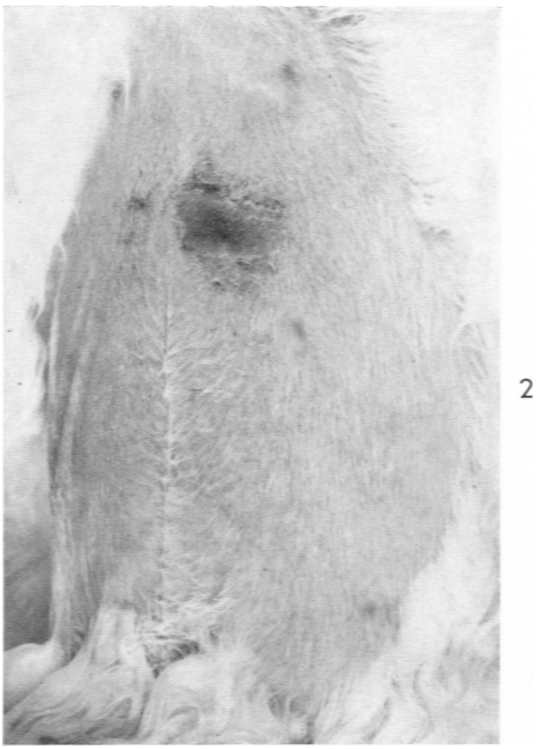

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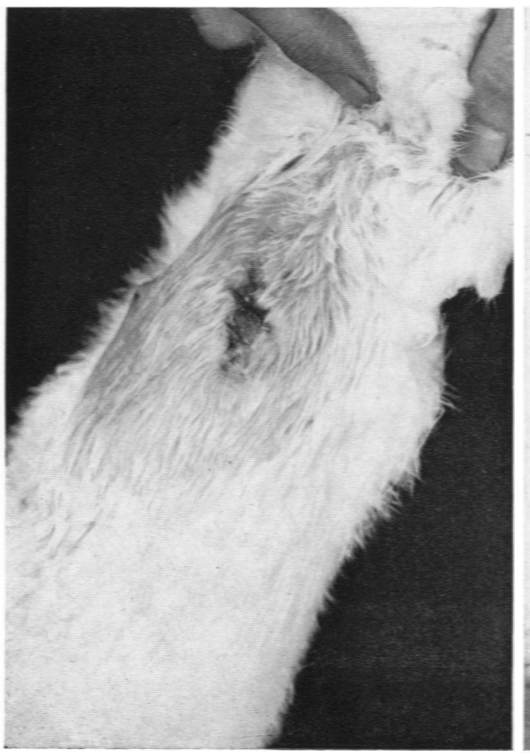

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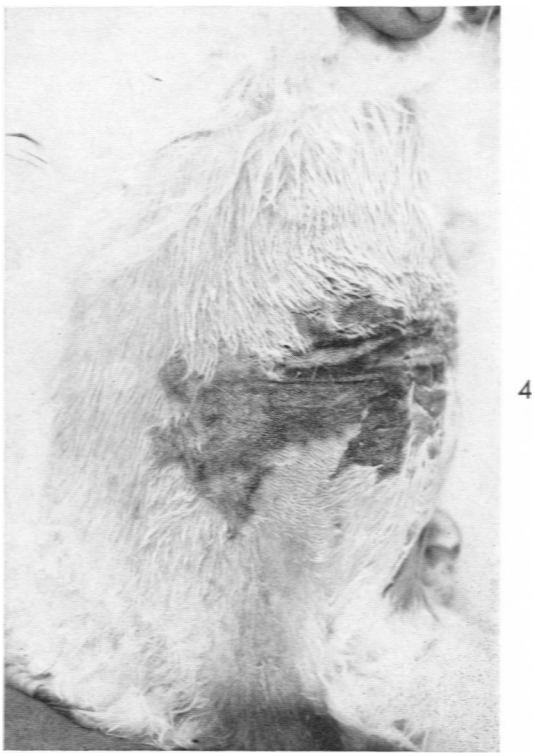

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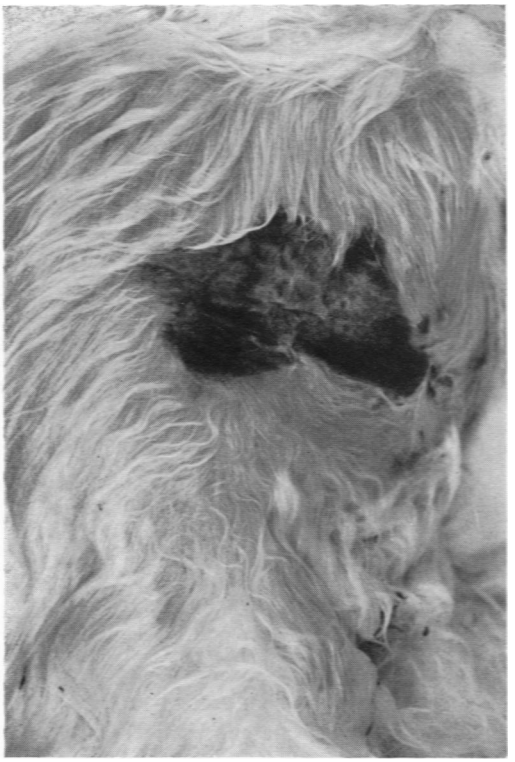
FIG. 5. Abdominal lesion at the site of intradermally injected levarterenol 96 hours after rotation. The changes are similar to those shown in Figure 4.

FIG. 6. A dermal lesion in an animal receiving an injection of endotoxin immediately prior to rotation and sacrificed 3 days later. Note the epidermal and dermal necrosis with extensive cellular reaction composed predominantly of heterophils and a few mononuclear cells. These extend through all layers of the skin into the muscle. There is beginning subepidermal hyalinization. × 40.

FIG. 7. Another animal treated as indicated in Figure 6; section taken at 3 days. Ischemic necrosis is evident in all layers of the skin, with infiltration by heterophils into underlying tissue and muscle. There is also muscle necrosis. × 40.

FIG. 8. An intradermal epinephrine injection site; section taken 3 days after rotation. Note the absence of cellular reaction or hemorrhage and the extensive ischemic necrosis. × 40.



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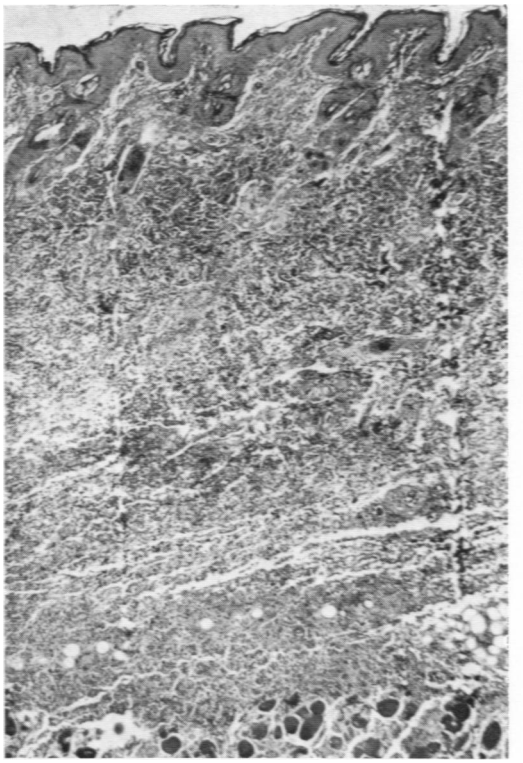

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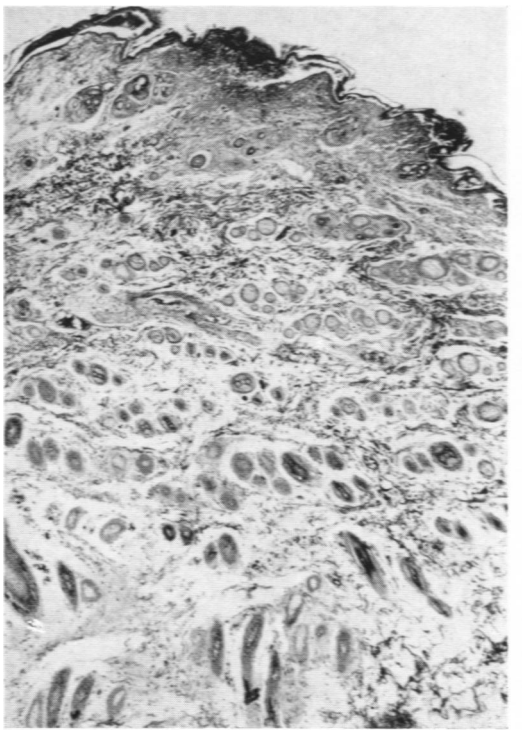

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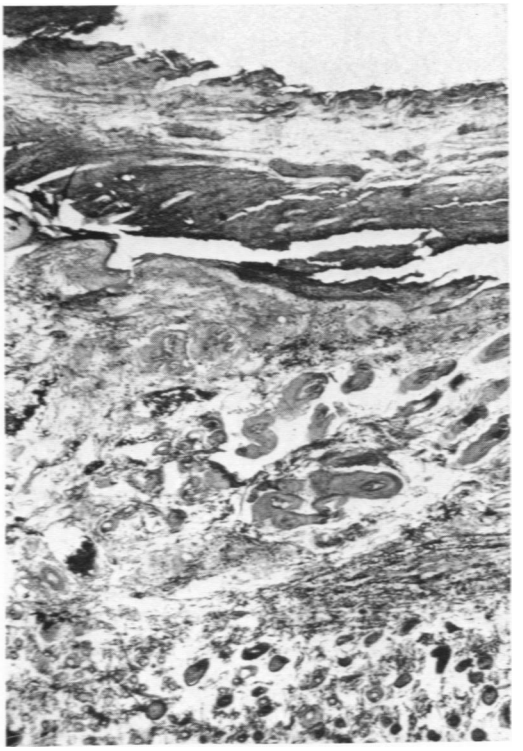
FIG. 9. An intradermal endotoxin site; section taken 4 days after rotation. The exudate is composed of nuclear debris and some red cells. There is extensive superficial necrosis and a mild underlying cellular infiltrate. × 40.

FIG. 10. An animal given intradermal levarterenol; section taken 4 days after rotation. Epidermal necrosis shows absence of cellular reaction, extensive hyalin deposition and beginning connective tissue proliferation. × 40.

FIG. 11. An intradermal phenylephrine site 4 days after rotation. Note ischemic necrosis, paucity of inflammatory cells, and absence of edema and hemorrhage. × 40.

FIG. 12. An intradermal endotoxin injection site 7 days after rotation. Note the remnant of the crust, regenerated epithelium, subepidermal hyalin deposition and extensive connective tissue proliferation. × 40.



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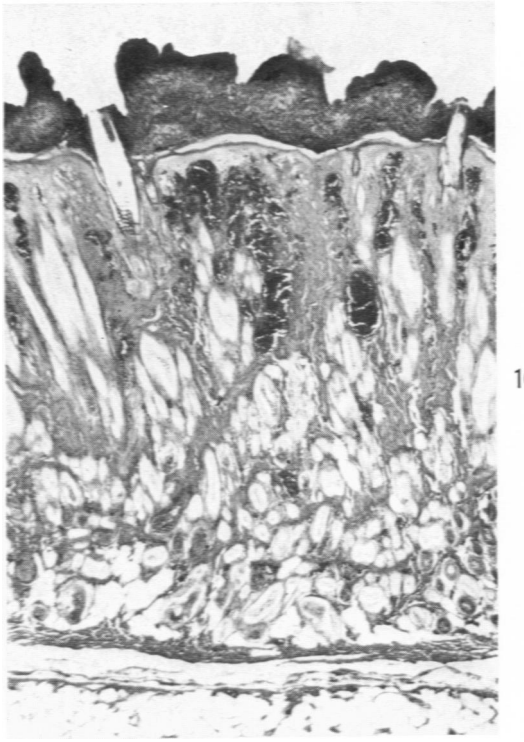

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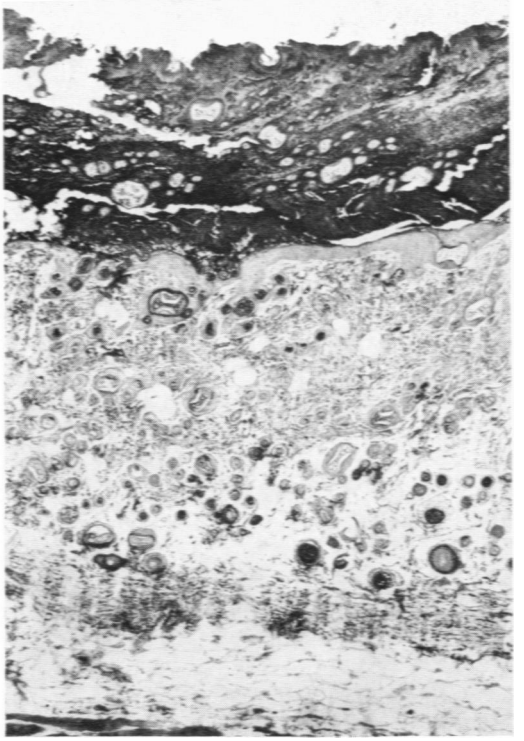

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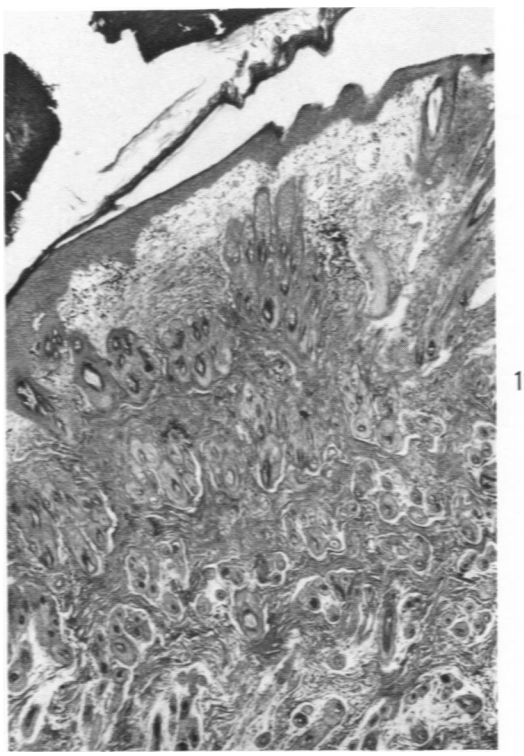

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