Hemorrhagic and Necrotic Reactions in Guinea Pigs During Protracted Anaphylactic Shock. (24341)

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During experiments on protracted anaphylactic shock(1) a massive local hemorrhagic reaction was found by Stone to occur at the site of an eliciting subcutaneous injection of large amounts of egg albumin in guinea pigs sensitized to this antigen, using paraffin oil-in-water adjuvants containing mycobacteria. This observation recalled experiments reported by Dienes(2) in which hemorrhagic reactions were observed in egg white sensitive guinea pigs infected with a slightly virulent strain of *M. tuberculosis*. A severe protracted anaphylactic shock was elicited by an intraperitoneal injection of large amounts of egg white. In the peritoneum and tuberculous lesions of the omentum and mesenterium large hemorrhagic areas were observed extending sometimes over the entire peritoneum. In some of the guinea pigs, hemorrhagic reactions occurred at skin-test sites of intracutaneous injection of egg white. From the standpoint of effect of mycobacteria on sensitization to unrelated antigens, it is of interest that killed mycobacteria suspended in a paraffin oil emulsion with other antigens alter the immune response to the antigens. Freund and McDermott found that necrosis occurred at the site of intracutaneous injection of horse serum in guinea pigs sensitized with the adjuvants but was not observed in guinea pigs sensitized with horse serum alone(3) and only rarely was seen in animals immunized using horse serum, paraffin oil, and Falba, omitting the mycobacteria(4). Bordet(5) and Freund(6) observed that the intravascular injection of bacterial filtrates from cultures of gram negative organisms caused hemorrhages in tuberculous guinea pigs at the site of skin reactions to tuberculin(5,6) and horse serum(7). It was suggested(8) that this effect of endotoxin was due to increased vulnerability of blood vessels of the tissues containing tubercles, or of the skin sites reacting to tuberculin. The pathologic findings of protracted anaphylactic shock in guinea pigs are similar to those of endotoxin shock and systemic tuberculin shock(1). This similarity became more significant when a massive local hemorrhagic reaction was observed at the site of subcutaneous injection of large amounts of egg albumin in animals previously sensitized by a single dose of this antigen incorporated in paraffin oil adjuvants containing killed mycobacteria. This report describes the characteristics of this reaction.

Methods and materials. Adult male Hartley guinea pigs were sensitized by a single dose of 2.5 mg of egg albumin (Ea) with or without paraffin oil adjuvants. The sensitizing dose was divided into five parts and injected intracutaneously into five sites of the nuchal region. For details on the preparation of adjuvant mixtures see(9). Serum antibody titers were determined by the passive cutaneous anaphylaxis (PCA) technic. The ear vein of the guinea pigs was split with a scalpel. Blood was taken by and diluted in a white blood cell pipette. Two guinea pigs were used to test each sample of plasma; 8 to 10 mm of blueing was taken as the threshold reaction.

Results. Table I shows that guinea pigs sensitized by the intracutaneous route with egg albumin incorporated in paraffin oil adjuvants containing mycobacteria react with massive hemorrhagic reactions at the site of subcutaneous injection of large amounts of the homologous antigen. Fig. 1 shows the gross appearance of the lesion at 24 hours. It did not occur in guinea pigs sensitized with incomplete adjuvants, *i.e.* those not containing mycobacteria. The hemorrhagic area became edematous; there was extensive necrosis and finally a sloughing of large areas of skin over a period of several days. At necropsy, the hemorrhage was found to extend through the subcutaneous tissues to include the peritoneal wall. Table II describes the systemic reactions in these guinea pigs. It may be seen

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TABLE I. Local Hemorrhagic Reaction in Sensitized Guinea Pigs.

No. of guinea pigs	Method of sensitization*	Amt of Ea inj. S.C. (mg)	Reaction at 24 hr†	Avg size of hemorrhagic area, mm²
6	Ea + Arlacel/Bayol + *M. tuberculosis* I.D.	50	5/5	2880
2	*Idem*	20	2/2	1440
2	"	5	1/2§	600§
6	Ea + Arlacel/Bayol + *M. butyricum* I.D.	50	4/4	1475
6	Ea + Arlacel/Bayol I.D.	50	0/6	
2	*Idem*	20	0/2	
2	"	5	0/2	
4	Ea in saline only I.D.	50	‡	

* Ea, 2.5 mg; Arlacel/Bayol, 0.25 ml; mycobacteria, 1 mg. Single dose in 5 sites I.D.

† No. of surviving animals with hemorrhage/No. of surviving animals.

‡ All guinea pigs dead of anaphylactic shock by 1 hr. At death no local reactions were apparent.

§ Necrosis central to erythematous area.

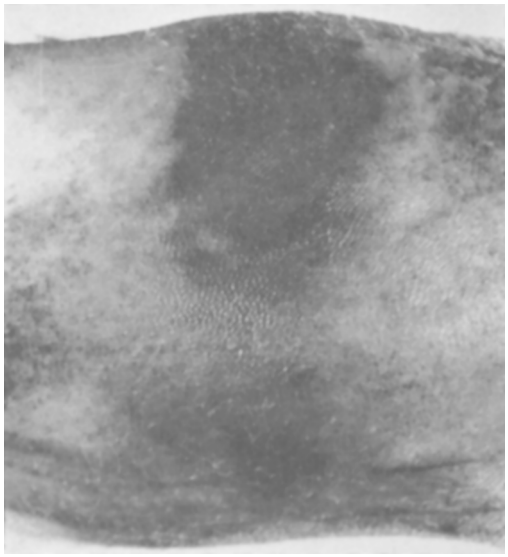

FIG. 1. Hemorrhagic reaction 24 hours after subcutaneous eliciting injection of 50 mg of egg albumin in a guinea pig sensitized with egg albumin in complete paraffin oil adjuvants containing *M. butyricum*.

that the difference in local response described above occurred although antibody titers and systemic reactions were of the same order in guinea pigs immunized intradermally with or without mycobacteria in the oil emulsion. In both groups, the number of animals which died of the protracted anaphylactic shock was small compared to the percentage of fatalities seen in guinea pigs immunized with egg albumin in incomplete adjuvants by subcutaneous injection(1). The results of the systemic reaction were in sharp contrast to the effects in guinea pigs sensitized with egg albumin in saline alone. Such guinea pigs died of anaphylactic shock in from 39 to 66 minutes and necropsy revealed emphysematous lungs as well as stasis or hemorrhages in the intestines, *i.e.* a combination of manifestations was observed indicating a certain degree of pro-

TABLE II. Systemic Reactions and Antibody Titer in Guinea Pigs Sensitized to Egg Albumin.

No. of guinea pigs	Method of sensitization*	Amt of Ea inj. S.C., mg	Anaph. reaction: Surv.	Anaph. reaction: Deaths within 24 hr	Anaph. reaction: Time of death (avg, max)	PCA antibody titers† $\times 10^{-3}$
6	Ea + Arlacel/Bayol + *M. tuberculosis*	50	5	1	Between 8-21 hr	4, 8, 8, 8, 16, ND
2	*Idem*	20	2	0		8, 8
2	"	5	2	0		4, 16
6	Ea + Arlacel/Bayol + *M. butyricum*	50	4	2	Between 8-23 hr, 23 hr	8, 8, 8, 8, 8, 8
6	Ea + Arlacel/Bayol	50	6	0		1, 2, 8, 8, 8, 8
2	*Idem*	20	2	0		4, 8
2	"	5	2	0		2, 4
4	Ea in saline only	50	0	4	49 min., 66 min.	<1, $<.4$, $<.4$, $<.4$

* Ea, 2.5 mg; Arlacel/Bayol, 0.25 ml; mycobacteria, 1.0 mg.

† Dilution used 0.1 ml in the skin. A PCA unit causes blueing 8-10 mm; thus a serum with titer 1:8000 contains 80,000 PCA units/ml.

longation of the anaphylactic shock, although the time necessary for lethal outcome was relatively short.

Since tuberculous guinea pigs show hemorrhagic reactions at intracutaneous sites of injection of tuberculin or horse serum after intravascular injections of endotoxin (5,7), it was decided to test the similarity of endotoxin shock and the protracted anaphylactic shock by noting the reactions to purified protein derivative (PPD) (tuberculin reactions) in guinea pigs sensitized with various proteins incorporated in adjuvants containing *M. tuberculosis*. Gross hemorrhages at the site of tuberculin reactions were not observed in such animals during or after the protracted systemic reaction, although tuberculin test sites became grossly hemorrhagic upon intravenous injection of 100 μg of *E. coli* or *S. typhosa* endotoxin (7 out of 11 guinea pigs).

Discussion. Local hemorrhagic reaction. The massive hemorrhagic reaction observed in guinea pigs sensitized with egg albumin in adjuvants containing mycobacteria is due to specific hypersensitivity. It is noteworthy that the hemorrhage does not occur in animals sensitized with egg albumin in adjuvants without mycobacteria even though these guinea pigs have circulating antibody in amounts comparable to that in guinea pigs sensitized with complete adjuvants.

The association of killed mycobacteria and the hemorrhagic reaction suggests sensitivity of the delayed type; however, the mechanism of the entire reaction leading to the hemorrhage remains obscure. It may be related to a "higher" degree of delayed hypersensitivity, for it is possible that animals immunized by the cutaneous route acquired some "delayed" hypersensitivity even when mycobacteria are omitted from the paraffin oil emulsion(10). It has been shown that anaphylactic shock (11) or local antigen-antibody reactions(11, 12) elicit hemorrhages in sites previously injected with endotoxins or in tumors in mice not previously given lipopolysaccharides(13). The latter observation, indicating the presence of potentially toxic material in mammalian tissue, has been recently studied by Landy and Shear(14). The role of anaphylaxis in the mechanism of hemorrhagic reactions does not appear to be a major one in our guinea pigs since similar amounts of circulating antibody were found in the two groups of animals. Shock of the "delayed" type may be involved; however, the suggestion that delayed shock (or anaphylactic shock) might elicit hemorrhages in tissues whose endotoxin-like lipopolysaccharides have been "activated" by mycobacterial products is not supported by experimental evidence.

The alternative considerations revolve around the possible endotoxin activity of mycobacteria themselves. An increased vulnerability of blood vessels in tuberculous animals would be the outcome of the presence of large amounts of endotoxin-like material in the circulation. Such a concept does not exclude the role of factors outside the blood vessels discussed above, and both elements may take part, *i.e.* (1) injury capable of damaging fundamentally normal blood vessels, (2) lesser injury capable of damaging "vulnerable" blood vessels.

Protracted anaphylaxis. It has been shown that circulating antibody prolongs anaphylactic shock in passively sensitized guinea pigs given an eliciting injection of large amounts of antigen subcutaneously(15). The syndrome was very prolonged in guinea pigs sensitized by intracutaneous injection of egg albumin in complete or incomplete adjuvants (Table II). In this case, prolongation led to protection from a lethal outcome for the majority of guinea pigs. The death rate from protracted anaphylaxis in these animals is in sharp contrast with that observed in guinea pigs immunized by the subcutaneous route with incomplete adjuvants(1). Such guinea pigs regularly succumb after an eliciting injection of 50 mg of Ea given subcutaneously. Since prolongation of the anaphylactic syndrome is associated with the presence of circulating antibodies, these results confirm direct evidence(16) of the efficacy of the intracutaneous route in immunization.

In the guinea pigs which died of the extremely protracted anaphylactic shock (Table II) hemorrhages were observed in the lungs and stomach wall. The role of a "delayed" hypersensitivity component in this protracted,

usually non-lethal syndrome is not known and is under investigation.

The guinea pigs which were sensitized with Ea in saline only and which had small amounts of circulating antibody died within a relatively short period of time. At necropsy, there was both emphysema of the lungs and stasis in the gastrointestinal tract.

Summary. 1. A massive hemorrhagic reaction was observed at site of subcutaneous injection of large amounts of egg albumin in guinea pigs sensitized with egg albumin incorporated in water-in-oil emulsions containing killed *M. tuberculosis* or killed *M. butyricum.* No hemorrhages were seen in guinea pigs sensitized with paraffin oil adjuvants without mycobacteria, although these animals had circulating antibodies in amounts comparable to those in guinea pigs sensitized using mycobacteria. 2. Guinea pigs sensitized by intracutaneous injection of egg albumin in paraffin oil adjuvants with or without mycobacteria underwent a protracted but usually non-lethal anaphylactic shock after a subcutaneous eliciting injection of egg albumin. On the contrary, in guinea pigs sensitized by intracutaneous injection of egg albumin in saline alone, death occurred in 39 to 66 minutes and at necropsy there were manifestations of a combination of symptoms of acute (pulmonary emphysema) and protracted shock. In accordance with previous work, prolongation of the anaphylactic syndrome was associated with the presence of antibody in the circulation. 3. Protracted anaphylaxis did not cause grossly apparent hemorrhages at sites of tuberculin reactions to PPD. Intravenous injection of *S. typhosa* or *E. coli* endotoxin 24 or 48 hours after the intracutaneous injection of PPD caused hemorrhages at the sites of tuberculin reactions.

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Biological Activity of 9α-Fluoro-11β-Hydroxy-Δ⁴-Androstene-3,17-Dione.*† (24342)

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Considerable interest in 9α-halogenated C_{21}-steroids has resulted from the findings of Fried and co-workers(1) that substitution of the 9α-hydrogen by fluorine results in compounds having intensified adrenocortical activity. Thus the 9α-fluoro derivative of cortisol has a glycogenic activity approximately 5 times greater than cortisol in the adrenalectomized rat. The 9α-fluoro derivative of corticosterone has a sodium retaining activity 15 to 30 times that of desoxycorticosterone, and actually of the same order as aldosterone. The synthesis of 9α-fluoro-11β-hydroxy-Δ⁴-andro-

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