# THE PHENOMENON OF LOCAL SKIN REACTIVITY TO BACTERIAL FILTRATES: ELICITATION OF LOCAL REACTIVITY BY WAY OF THE VASCULAR SYSTEM

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In the phenomenon of local skin reactivity to bacterial filtrates the state of reactivity is obtained by means of an intradermal injection of a potent bacterial filtrate. It seemed of interest to determine whether local reactivity could be elicited by way of the vascular system. This report deals with experiments on rabbit ears in which the question was investigated.

## *Methods*

Twenty-four to 48 hours prior to the experiments the skin of the rabbit's ear was epilated by means of barium sulfide. When deemed necessary, the circulation was stopped by applying a clamp to the base of the ear for periods of time indicated in the respective protocols. The efficiency of the clamp was checked by injection of a dye of slow diffusibility recently used extensively by Rous and his coworkers (1), namely Niagara sky blue, which was prepared according to the method described by Rous (1).

*Rabbit 5-38.*—1 cc. of a 17 per cent solution of the dye was injected into the marginal vein of the left ear. A few seconds later, left and right ears and the conjunctiva of the eyes stained blue. A little later the entire body became blue, showing, however, during the following hour a more intense color in both ears and eyes than in the rest of the body.

*Rabbit 5-39.*—A clamp was applied to the base of the left ear. 5 minutes later, 1 cc. of 17 per cent solution of Niagara sky blue was injected into the marginal vein of the same ear. The left ear became blue a few seconds later but the rest of the body did not stain. The clamp was removed 13 minutes after the injection of the dye. At this time, the color spread instantaneously to the right ear and then to the conjunctiva of the eyes. Approximately 2 minutes after the removal of the clamp, the body began to stain blue. For the following period of 1 hour

the staining of the left ear remained more intense than of the right ear and eyes.

As is seen from these experiments, the application of the clamp used was efficient in stopping entirely the spread of the substance injected from the clamped ear into the general circulation.

### *Phenomenon of Local Skin Reactivity to Bacterial Filtrates in Rabbit's Ear*

Attempts to reproduce the phenomenon of local skin reactivity to bacterial filtrates in the rabbit's ear have been made previously by Gratia and Linz (2), Klein in these laboratories (3), and recently by Alechinsky (4).

When appropriate doses of the toxic material are used for the preparatory and provocative injections, strong reactions can be obtained in the rabbit's ear. The reactions may be well circumscribed and intensely hemorrhagic. Very frequently, however, there is a tendency towards generalization of the reaction through the entire ear and sometimes, as also reported by Gratia and Linz (2), there may be an extension of the reaction to the opposite ear. Frequently there is observed blue discoloration through the entire ear. Fig. 1 illustrates the typical reaction of the ear. Fig. 2 presents the histological appearance of the tissues at the prepared site. As is seen, the reaction is accompanied by hemorrhage, pronounced capillary thrombosis, subsequent severe necrosis, and inflammation. Fig. 3 demonstrates the microscopic appearance of discolored parts of the ear well removed from the local reaction. The conspicuous feature of the discolored tissues is extensive thrombosis in the venules.

Before attempting the series of studies planned in this investigation, it seemed important to compare the susceptibility of the rabbit's ear to the phenomenon with that of the abdominal skin on which all the previous studies were carried out.

*Comparative Titrations.*—All titrations described were done within 1 month, using the same preparation; *i.e.*, *B. typhosus* "agar washings" filtrate (T.1986). The preparatory intradermal injections consisted each of 0.25 cc. of undiluted filtrate. The doses used in the intravenous injections ranged from 5 to 80 reacting units. Each intravenous dose was tested in a group of three rabbits. The results are summarized in Table I.

As is seen from Table I, severe reactions were consistently obtained in the skin of the abdominal wall beginning with the intravenous

dose of 2 reacting units. The incidence of reactions was the same in groups receiving the provocative injection into the right and into the left marginal ear veins.

When the intradermal injections were given into the skin of the ear and the provocative injection into the marginal vein of the same ear, at least 20 reacting units were necessary for the elicitation of the

TABLE I

*Comparative Titrations in the Skin of the Ear and of the Abdominal Wall*

| Group No. | Prepared skin site | Toxin and dose used for preparatory injection* | Vein used for provocative injection | Dose used for provocative injection | Reactions following provocative injection |
|---|---|---|---|---|---|
| 1 | Upper right quadrant of abdomen | 0.25 cc. B. $TyT_L$† T. 1986 | Right marginal ear vein | 2 units | 1/2‡ |
| 2 | " " | 0.25 " " " | " " | 5 " | 2/1 |
| 3 | " " | 0.25 " " " | " " | 20 " | 2/0 |
| 4 | " " | 0.25 " " " | Left marginal ear vein | 2 " | 1±/2 |
| 5 | " " | 0.25 " " " | " " | 5 " | 1/2 |
| 6 | " " | 0.25 " " " | " " | 20 " | 2, 1±/0 |
| 7 | Left ear | 0.25 " " " | " " | 5 " | 0/2 |
| 8 | " " | 0.25 " " " | " " | 20 " | 2±/1 |
| 9 | " " | 0.25 " " " | " " | 40 " | 1/2 |
| 10 | " " | 0.25 " " " | " " | 60 " | 2/1—1 died |
| 11 | " " | 0.25 " " " | Right marginal ear vein | 5 " | 0/3 |
| 12 | " " | 0.25 " " " | " " | 20 " | 0/3 |
| 13 | " " | 0.25 " " " | " " | 40 " | 0/3 |
| 14 | " " | 0.25 " " " | " " | 60 " | 2/1 |

* 0.25 cc. was injected intradermally.

† Abbreviation B. $TyT_L$ designates "agar washings" filtrates of *B. typhosus*, Strain $T_L$ cultures.

‡ The numerator indicates the number of positive rabbits. The denominator indicates the number of negative rabbits. The sum of both indicates the total number of rabbits in each group.

reactions. Rabbits with prepared ears receiving provocative injections into the marginal veins of the non-prepared ears, gave reactions only when at least 60 reacting units were used. As is seen from these titrations, the skin of the ear is considerably more resistant to the phenomenon than the skin of the abdomen. It is of interest that ten times the provocative dose is required if it is given into the vein of the prepared ear and thirty times the dose if it is given into

the vein of the non-prepared ear. The obvious interpretation of this observation is that a certain amount of toxin injected into the vein of the non-prepared ear is lost in the general circulation before reaching the skin of the prepared ear.

### *Elicitation of Local Reactivity in the Rabbit's Ear by Way of the Vascular System*

Various attempts were made to prepare the tissue of the ear by an intravenous injection instead of intradermal. In planning these experiments the above information was taken into consideration.

*Group 1.*—1 cc. of meningococcus Group III (44B.) "agar washings" filtrate (T.1968) diluted 1:50 (80 reacting doses) was injected into the marginal vein of the left ear of each of three rabbits tested. 24 hours later, the filtrate was injected into the right marginal ear vein in a dose of 50 reacting units, per kilo of body weight. No reactions were observed.

*Group 2.*—The ears of three rabbits were clamped off at the base. 1 cc. containing 50 reacting units of meningococcus Group III (44B.) "agar washings" filtrate (T.1968) was injected into the left marginal ear vein of each rabbit. The clamps were removed 5 minutes after the intravenous injections were completed. 24 hours later, 50 reacting units (per kilo of body weight) of the same filtrate were injected into the left marginal vein. No reactions followed.

*Group 3.*—Clamps were applied to the base of the left ear of three rabbits. 1 cc. containing 100 reacting units of *B. typhosus* "agar washings" filtrate (T.1968) was injected into the left marginal vein of each rabbit. Clamps were removed 5 minutes after the intravenous injection. 24 hours later, 100 reacting units (per kilo of body weight) were injected into the left marginal ear veins. No reactions followed.

As is seen from these experiments, preparatory intravenous injections of potent filtrates fail to elicit the state of reactivity of the phenomenon under discussion. The same doses given intradermally elicited this state in most rabbits tested. Unfortunately, larger doses could not be used because of the lethal effect of these preparations. It was assumed, then, that the perivascular preparation was necessary for elicitation of the state of reactivity. In experiments, such as the above, under conditions of normal resistance, the capillaries may not allow the diffusion of the preparatory factors into the tissues when given intravenously. In view of this assumption, it was decided to accompany the preparatory intravenous injections of the toxin by various agents known to modify capillary permeability, as follows:

*Preparatory Intravenous Injections of Toxins Accompanied by Applicaton of Heat*

In these experiments sausage shaped rubber bags filled with water of the desired temperature were firmly applied to both sides of the ear for 5 minutes and the preparatory injections were given into the marginal vein. In some experiments the ear was clamped off at the base simultaneously with the application of heat and the clamps were removed 5 minutes after the intravenous injections were completed. The provocative injections were given into the vein of the same or of the opposite ear. The results of these experiments are summarized in Table II.

In the experiments recorded in Table II, clamped and non-clamped ears exposed to 45°, 50°, and 55°C. for 5 minutes and injected with various amounts of active bacterial filtrates intravenously, showed immediate intense hyperemia with subsequent swelling and moderate hyperemia 24 hours after the treatment.

Provocative injections of toxin in the various amounts recorded in Table II, elicited no secondary reactions in non-clamped ears.

The preparatory intravenous injections of potent preparations given into the veins of ears exposed to 45°, 50°, and 55°C. and clamped off at the base were capable of eliciting the state of reactivity of the phenomenon under consideration. Reactions following the provocative injections were intense. Diffuse hemorrhages throughout the entire ear were accompanied frequently by deep cyanosis. Microscopically there was pronounced thrombosis in the venules (Fig. 3). There were also observed petechial hemorrhages in various parts of the ear and sometimes in portions far removed from the site of the injected vein. In the gross, these petechiae closely resembled purpuric spots seen in the skin of human cases of meningococcemia.

As is seen from Table II, ears exposed to 45°C. for 5 minutes gave definite reactions following the provocative injection, provided at least 50 reacting units were used for the preparatory intravenous injection. When exposure to 50°C. was combined with the preparatory injection of toxin, distinct reactions were seen with as little as 5 units, provided the provocative injection was given into the same vein. It is curious that a preparatory dose of 25 reacting units in ears exposed to 50°C. failed to elicit the state of reactivity. This observation is difficult to explain and the experiment should be repeated. Provocative injections of toxins into the ears prepared by larger doses

TABLE II

*Preparatory Intravenous Injections of Toxins Accompanied by Application of Heat*

| Group No. | Time during which ear was clamped | Dose of preparatory injection of toxin into left marginal ear vein | Application of heat to the left ear | Reactions following preparatory intravenous injection | Vein used for provocative intravenous injection | Dose of provocative injection | Reactions following provocative injection |
|---|---|---|---|---|---|---|---|
| 1 | None | 50 units Mg. 44B.* T. 1968 | Bags 45°C. 5 min. | 3†—negative | Right marginal ear vein | 25 units per kilo | 2†—negative; 1—died |
| 2 | 5 min. | 25 " " " | " 45°C. 5 " | 3 " | Left marginal ear vein | " " | 3—negative |
| 3 | 5 " | 50 " " " | " 45°C. 5 " | 3 " | Right marginal ear vein | " " | 2—hemorrhages and thrombosis of marginal veins; 1—negative |
| 4 | None | 80 " " " | " 50°C. 5 " | 3 " | " " | " " | 3—negative |
| 5 | 5 min. | 1 " " " | " 50°C. 5 " | 3—ears swollen and hyperemic; 3—negative | Left marginal ear vein | " " | 1—hemorrhage along marginal vein; 5—negative |
| 6 | 5 " | 5 " " " | " 50°C. 5 " | 6—negative | " " | " " | 2—diffuse hemorrhagic reaction over entire right ear; 2—doubtful; 2—negative |
| 7 | 5 " | 5 " " " | " 50°C. 5 " | 3—swellings | Right marginal ear vein | " " | 3—negative |
| 8 | 5 " | 25 " " " | " 50°C. 5 " | 3—negative | Left marginal ear vein | " " | 3 " |
| 9 | 5 " | 25 " " " | " 50°C. 5 " | 3—swellings | Right marginal ear vein | " " | 2 " ; 1—died |
| 10 | 5 " | 50 " " " | " 50°C. 5 " | 1—swelling; 2—negative | Left marginal ear vein | " " | 2—diffuse cyanosis; 1—negative |
| 11 | 5 " | 80 " " " | Water bath 50°C. 5 min. | 3—swellings | " " | " " | 2—intense diffuse hemorrhagic reaction over entire ear; 1—negative |
| 12 | 5 " | 50 " " " | Bags 55°C. 5 min. | 3 " | Right marginal ear vein | " " | 1—tip cyanosis (6 x 5 cm.) intense and sharply demarcated; 1—small cyanotic area; 1—negative |

* Abbreviation Mg. 44B. designates "agar washings" filtrates of meningococcus Group III cultures.

† Number of rabbits.

(*i.e.*, 50 and 80 reacting units in ears exposed to 50° and 55°C.) gave intense and diffuse reactions.

It may be concluded from these experiments that preparatory intravenous injections of toxins are capable of eliciting the state of reactivity in the skin of the ear provided they are combined with temporary stasis (clamping) and thermal hyperemia.

### *Preparatory Intravenous Injections of Toxins Accompanied by Chilling*

*Protocol 1.*—The left ears of three rabbits were chilled by means of ice bags applied to both sides for a period of 10 minutes. About 2 minutes after completion of chilling, when the ears became flushed, they were clamped off at the root and 80 reacting units of meningococcus Group III (44B.) "agar washings" filtrate (T.1968) were injected into the marginal veins. The clamps were kept on for 2 minutes following the intravenous injection. 24 hours later the rabbits were injected into the marginal veins of the same ears with 25 reacting units of the above toxic filtrate. No reactions were observed.

*Protocol 2.*—In this experiment the left ears were clamped off at the base and 80 reacting units of meningococcus, Group III (44B.) "agar washings" filtrate (T.1968) were injected into the left marginal veins. Immediately after completion of the intravenous injection, ice bags were applied to the left ears. 5 minutes later the bags were removed and the clamps released. The ears appeared distinctly hyperemic shortly afterwards. 24 hours later the left marginal veins were injected intravenously with 25 reacting units, per kilo of body weight, of the same toxin. One rabbit died and the two surviving rabbits showed no reactions.

As is seen from these experiments, preparatory intravenous injections were given into the clamped ears in adequate doses. The ears were chilled prior to and following the intravenous injection for periods of 5 and 10 minutes. Distinct hyperemia followed the treatment. The injections failed, however, to elicit the state of reactivity.

### *Preparatory Intravenous Injections of Toxins in Xylol Treated Ears*

*Protocol 1.*—The left ears of six rabbits were clamped off at the base and rubbed with xylol until the veins became very prominent. Immediately afterwards, rabbits received 50 reacting units of meningococcus, Group III (44B.) "agar washings" filtrate (T.1968) into the marginal veins of treated ears. The clamps were removed 5 minutes after the intravenous injections were completed. 24 hours later the ears appeared swollen and one was slightly hemorrhagic along the marginal vein. All the rabbits received 50 reacting units of the same toxin, per kilo of body weight, into the right marginal ear veins 24 hours later. In the rabbit

in which slight hemorrhage along the marginal vein was observed before the provocative injection, there was diffuse cyanosis with petechial hemorrhages in various parts of the ear. In another rabbit an extensive thrombosis of the marginal ear vein and its tributaries was observed. All the remaining rabbits showed no reactions.

*Protocol 2.*—The left ears of two rabbits treated with xylol until the veins became prominent were clamped off at the base. Immediately afterwards, 80 reacting units of meningococcus, Group III (44B.) "agar washings" filtrate (T.1968) were injected into the left marginal veins and the clamps removed 5 minutes later. 24 hours later, 25 reacting units, per kilo of body weight, were injected into the left marginal ear veins of these rabbits. No reactions followed.

As is seen, the provocative injection of active filtrate elicited reactions in two out of eight rabbits prepared by intravenous injection of toxin and application of xylol. In one of these, the reaction was doubtful and in another, it represented an enhancement of a primary hemorrhagic lesion following the preparatory treatment. The results of this experiment, therefore, remain inconclusive.

### *Preparatory Intravenous Injections of Toxins Combined with Ethyl Urethane, Acetylcholine, Pilocarpine Hydrochloride, Atropine, Calcium Gluconate, and Guinea Pig Liver Extract*

In this series of experiments preparatory injections were made intravenously in combination with the following substances: 10 per cent solution of ethyl urethane; 1 per cent solution of calcium gluconate; 3 per cent solution of atropine; 2 per cent solution of pilocarpine hydrochloride; acetylcholine diluted 1:500; and guinea pig liver extract in an amount of 0.85 per cent NaCl solution equal to the moist weight of liver.

The ears of the rabbits tested were clamped off at the base and the left marginal veins injected with 1 cc. each of a mixture of equal parts of 50 reacting units of meningococcus, Group III (44B.) "agar washings" filtrate (T.1968) with each of the above substances in dilutions indicated. Each mixture was tested for its preparatory effect in three rabbits. 24 hours later no reactions were observed. At this time, each of the rabbits received into the right marginal ear vein meningococcus, Group III (44B.) "agar washings" filtrate (T.1968) in a dose of 50 reacting units, per kilo of body weight. No reactions followed the provocative injections.

As is seen, preparatory intravenous injection of toxins in combination with ethyl urethane, acetylcholine, pilocarpine hydrochloride, atropine, calcium gluconate, and guinea pig liver extract failed to elicit the state of reactivity to the phenomenon under discussion.

TABLE III

*Preparatory Intravenous Injections of Toxins in Combination with Histamine*

| Group No. | Histamine | | Time of clamp application | Dose of preparatory injection of toxin into left marginal ear vein | Reactions following preparatory intravenous injection | Vein used for provocative intravenous injection | Dose of provocative injection | Reactions following provocative injection |
|---|---|---|---|---|---|---|---|---|
| | Dose | Site | | | | | | |
| 1 | 0.25 cc. dil. 1:1000 | Dermis in vicinity of left marginal vein | None | 40 units Mg. 44B.* T. 1968 | 3†—negative | Right marginal ear vein | 50 units Mg. 44B. T. 1968 | 3†—negative |
| 2 | " " | " " | " | 28 " B. TyT$_L$‡ T. 1986 | 3 " | " " | 50 " B. TyT$_L$ T. 1986 | 3 " |
| 3 | " " | " " | 5 min. | 25 " Mg. 44B. T. 1968 | 3 " | " " | 50 " Mg. 44B. T. 1968 | 3 " |
| 4 | " " | " " | None | 50 " " " | 3—erythema and swelling | " " | 25 " " " | 3 " |
| 5 | " " | " " | 5 min. | 50 " " " | 3—erythema | " " | 50 " " " | 3 " |
| 6 | " " | " " | 5 " | 50 " " " | 3—negative | " " | 50 " " " | 3 " |
| 7 | " " | " " | 5 " | 28 " B. TyT$_L$ T. 1986 | 3 " | " " | 50 " B. TyT$_L$ T. 1986 | 3 " |
| 8 | " " | " " | 5 " | 40 " " " | 2—swelling; 1—negative | " " | 50 " " " | 3 " |
| 9 | 1 cc. dil. 1:1000 | i. v. in mixture with toxin | None | 80 " Mg. 44B. T. 1968 | 1—tip of ear slightly hemorrhagic; 2—negative | " " | 25 " Mg. 44B. T. 1968 | 1—definite reaction at tip ($2\frac{1}{2}$ x $1\frac{1}{2}$ cm).; 2—negative |
| 10 | " " | " " | 5 min. | 20 " " " | 3—negative | " " | 25 " " " | 3—negative |
| 11 | " " | " " | 5 " | 5 " " " | 3 " | Left marginal ear vein | 25 " " " | 3 " |
| 12 | " " | " " | 5 " | 80 " " " | 2—hemorrhagic reactions; 1—negative | " " | 25 " " " | 2—slight accentuation of primary hemorrhage; 1—negative |
| 13 | " " | " " | 5 " | None | 3—negative | " " | 25 " " " | 3—negative |

i. v. = intravenously. Dil. = diluted.

* Abbreviation Mg. 44B. designates "agar washings" filtrates of meningococcus Group III cultures.

† Number of rabbits.

‡ Abbreviation B. TyT$_L$ designates "agar washings" filtrates of *B. typhosus*, Strain T$_L$ cultures.

### *Preparatory Intravenous Injections of Toxins in Combination with Histamine*

In these experiments histamine dihydrochloride in dilution 1:1000 was used. In Groups 1 to 8, histamine dihydrochloride was injected into the dermis of clamped and non-clamped ears in the vicinity of marginal veins. The preparatory injection of varying amounts of toxin was given intravenously immediately afterwards. The clamps were released 5 minutes after the intravenous injections were completed. In Groups 9 to 13, histamine dihydrochloride mixed with toxin in various proportions was injected intravenously into clamped and non-clamped ears. The clamps were also removed 5 minutes after completion of the intravenous injection. Provocative injections of toxins in various amounts were given into the same or other ears 24 hours later.

As is seen from Table III, the preparatory intravenous injection of toxin in mixture with histamine dihydrochloride or combined with intradermal injections of histamine dihydrochloride failed to elicit the state of reactivity. Large doses of toxin mixed with histamine dihydrochloride elicited some primary injury in clamped ears. These reactions only occasionally were augmented by the subsequent provocative injection of toxin.

### *Preparatory Intravenous Injections of Toxins Combined with Adrenalin*

*Protocol 1.*—The left ears of three rabbits were clamped off at the base. The marginal veins were injected each with 1 cc. of a mixture consisting of equal parts of 50 reacting units of meningococcus, Group III (44B.) "agar washings" filtrate (T.1968) and adrenalin chloride in dilution 1:1000. The clamps were removed 5 minutes after the intravenous injections were completed. 24 hours later, Rabbit 1 showed a pronounced hemorrhage along the injected marginal vein, Rabbit 2 showed a well marked hemorrhage at the tip of the ear and Rabbit 3, an intense hemorrhagic lesion over the entire ear. At this time the above filtrate was injected into the marginal vein of the right ear in a dose of 50 reacting units, per kilo of body weight. No enhancement of primary hemorrhagic reactions was observed.

As is seen from these experiments, intravenous injection of mixtures of adrenalin chloride with meningococcus, Group III (44B.) "agar washings" filtrate (T.1968) into clamped ears was capable of eliciting severe hemorrhagic lesions. The primary reactions were not augmented by the provocative injection of toxin, 24 hours later. It is obvious, therefore, that the state of reactivity is not induced by the intravenous injection of toxin in combination with adrenalin chloride.

## TABLE IV

*Preparatory Intravenous Injections of Toxins in Combination with Pituitrin*

| Group No. | Pituitrin | | Time of clamp application | Dose of preparatory injection of toxin into left marginal ear vein | Reactions following preparatory intravenous injection | Vein used for provocative intravenous injection | Dose of provocative injection | Reactions following provocative injection |
|---|---|---|---|---|---|---|---|---|
| | Dose | Site | | | | | | |
| 1 | 0.5 cc. dil. 1:200 | Dermis in vicinity of marginal vein | None | None | 3*—erythema and swellings | Right marginal ear vein | 25 units Mg. 44B.† T. 1968 | 3*—negative |
| 2 | " " | " " | " | " | 3—negative | Left marginal ear vein | 25 " " " | 3 " |
| 3 | " " | " " | 5 min. | " | 3—slight s. c. hemorrhages | " " | 25 " " " | 3—no accentuation |
| 4 | " " | " " | 5 " | " | 3—negative | Right marginal ear vein | 25 " " " | 3—negative |
| 5 | " " | " " | 5 " | 5 units Mg. 44B.† T. 1968 | 3—swellings, hemorrhages along marginal vein | " " | 25 " " " | 1—negative; 1—hemorrhage along marginal vein; 1—slight accentuation of primary hemorrhage |
| 6 | 1 cc. dil. 1:200 | Into left marginal vein | 5 " | None | 3—swellings and small hemorrhages along marginal vein | Left marginal ear vein | 25 " " " | 3—no accentuation |
| 7 | " " | i. v. in mixture with toxin | 5 " | 5 units Mg. 44B. T. 1968 | 3—swellings and slight hemorrhages along marginal vein | Right marginal ear vein | 25 " " " | 1—no accentuation; 2—venous stasis and slight accentuation of hemorrhage |
| 8 | " " | " " | 5 " | 80 " " " | 3—swellings and diffuse hemorrhages | Left marginal ear vein | 25 " " " | 3—no accentuation |

s. c. = subcutaneous. i. v. = intravenously. Dil. = diluted.

* Number of rabbits.

† Abbreviation Mg. 44B. designates "agar washings" filtrates of meningococcus Group III cultures.

### *Preparatory Intravenous Injections of Toxins in Combination with Pituitrin*

Pituitrin in dilution 1:200 was used. In these experiments there were made preparatory intravenous injections of toxin in mixture with pituitrin or simultaneously with intradermal injections of pituitrin in the vicinity of the marginal vein. In some groups, the ears were clamped before the preparatory injection and the clamps removed 5 minutes after the injection was completed. 24 hours later the rabbits received 25 reacting units of the same toxin into the vein of clamped or non-clamped ears.

As is seen from Table IV, intradermal or intravenous injections of certain doses of pituitrin into clamped ears are prone to elicit hemorrhages. 1 hour later they may already appear along the injected vein. Approximately 24 hours later there develops cyanosis and pronounced hemorrhage which extends along the tributaries of the injected vein. These reactions become more pronounced if accompanied by intravenous injections of meningococcus, Group III (44B.) "agar washings" filtrate (T.1968). The provocative injection of toxin into veins of ears thus prepared brings about insignificant accentuation of primary reactions. It is obvious that the reactions may be interpreted as primary damage to blood vessels which are not related to the state of reactivity under discussion.

### *Preparatory Intravenous Injections of Toxins Combined with Testicular Extract*

In these experiments, there was employed rabbit testicular extract prepared according to the method described by Duran-Reynals (5).

As is seen from Table V, in Groups 1 to 4, the extract was injected into the dermis in the vicinity of the marginal veins of clamped and non-clamped ears. The preparatory injection of toxin in various doses was given intravenously immediately afterwards. The clamps were released 5 minutes after the intravenous injection was completed. The provocative injection of toxin also in various doses was given into the vein of the same or other ear 24 hours later.

The provocative injection of 50 reacting units of meningococcus, Group III (44B.) "agar washings" filtrate (T.1968) elicited definite reactions in clamped ears in two out of three rabbits prepared 24 hours previously by combined intradermal injections of testicular extracts and intravenous injections of 50 reacting units of the same filtrate (Group III). The same provocative injection failed to elicit

reactions in clamped ears prepared by combined intradermal injection of testicular extract and 25 and 80 reacting units of the filtrate (Group III), respectively.

In Groups 5 to 23, the testicular extract was mixed with the toxin in various proportions. The mixture was injected intravenously into clamped and non-clamped ears. The clamps were released 5 minutes after the intravenous injection. Provocative injections of toxins in various amounts were given into the same or other ears after different intervals of time.

In experiments of Groups 1 to 8, the interval of time between the preparatory and provocative injections was 24 hours.

In Group 7, provocative injection of 50 reacting units of *B. typhosus* $T_L$ "agar washings" filtrate (T.1968) elicited an intense reaction in one out of three rabbits prepared by intravenous injection of a mixture of undiluted testicular extract with 10 reacting units of the above filtrate. Rabbits of Group 8 prepared with 20 reacting units of the filtrate in mixture with the same amount of testicular extract showed no reactions following the provocative injection of 25 reacting units of the same filtrate. The latter experiment was carried out only with two rabbits and, therefore, remains inconclusive.

As is also seen from Table V, intense and diffuse hemorrhagic reactions followed the provocative injection of the filtrate in rabbits of Groups 9, 10, 11, 12, 14, 17, and 18. In these experiments the incubation period was short, *i.e.*, ½, 1, and 2 hours; the amount of testicular extract was not less than 0.9 cc. diluted 1:4; 3 reacting units of the filtrate were used for the preparatory injection and the provocative dose varied from 2 to 25 reacting units.

Doses of three and five reacting units for preparatory and provocative injections, respectively, effective with a short incubation period, failed to elicit reactions when longer incubation periods (*i.e.*, 4, 6, and 24 hours) were allowed (Groups 19, 20, and 21).

It may be concluded from these observations that mixtures of bacterial filtrate and testicular extract are capable of inducing the state of reactivity in the rabbit's ear by way of the vascular system. The reactivity is elicited provided the circulation is interrupted for a few minutes and the amount of the filtrate is quite small. It is suggestive that the state may disappear within 4 hours following the preparation. Although the possibility of inducing the state of re-

TABLE V

*Preparatory Intravenous Injections of Toxins Combined with Testicular Extract*

| Group No. | Rabbit testicular extract | | Time of clamp application | Dose of preparatory injection of toxin into left marginal ear vein | Reactions following preparatory intravenous injections | Vein used for provocative intravenous injections | Dose of provocative injection | Time interval between preparatory and provocative injection | Reactions following provocative injection |
|---|---|---|---|---|---|---|---|---|---|
| | Dose | Site | | | | | | | |
| | | | | | | | | *hrs.* | |
| 1 | 0.25 cc. | Dermis in vicinity of left marginal vein | 5 min. | 25 units Mg. 44B.* T. 1968 | 1†—swelling; 2—negative | Left marginal ear vein | 50 units Mg. 44B. T. 1968 | 24 | 2†—negative; 1—deep cyanosis |
| 2 | 0.25 " | " " | None | 25 " " " | 3—negative | " " | 50 " " " | 24 | 3—negative |
| 3 | 0.5 " | " " | 5 min. | 50 " " " | 2 " | Right marginal ear vein | 50 " " " | 24 | 1—pronounced venous stasis; 1—hemorrhage 4 + (1 x 1 cm.) |
| 4 | 0.5 " | " " | 5 " | 80 " " " | 3 " | " " | 50 " " " | 24 | 2—no reactions; 1—died |
| 5 | 1 cc. dil. 1:5 | Left marginal ear vein | 5 " | None | 3 " | " " | 50 " B. $TyT_L$‡ T. 1986 | 24 | 3—negative |
| 6 | 1 cc. | " " | 5 " | " | 2—negative; 2—doubtful hemorrhage | " " | 50 " Mg. 44B. T. 1968 | 24 | 3 " |
| 7 | 0.9 " | " " | 5 " | 10 units B. $TyT_L$ T. 1986 | 3—erythema | Left marginal ear vein | 50 " B. $TyT_L$ T. 1986 | 24 | 2—negative; 1—diffuse hemorrhage 3+ |
| 8 | 0.9 cc. dil. 1:4 | " " | 5 " | 20 " " " | 2—slight erythema | " " | 25 " " " | 24 | 2—slight erythema |
| 9 | " " | " " | 5 " | 3 " " " | 2—negative; 1—slight hemorrhage at tip | " " | 5 " " " | $1/_2$ | 1—negative; 1—hemorrhage 4 + (7 x $1\frac{1}{2}$). Petechial hemorrhage over entire ear; 1—diffuse petechial hemorrhage over ear |

| | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 10 | 0.9 cc. | " | " | 5 | " | 3 | " | " | " | 2—negative | " | " | 5 | " | " | " | 1 | 1—hemorrhage 2+ tip of ear; 1—slight erythema |
| 11 | 0.9 cc. dil. 1:4 | " | " | 5 | " | 3 | " | " | " | 3 " | " | " | 25 | " | " | " | 1 | 1—negative; 2—extensive hemorrhage 2+ |
| 12 | " " | " | " | 5 | " | 3 | " | " | " | 1—erythema 4+; 2—slight erythema | " | " | 5 | " | " | " | 2 | 2—negative; 1—hemorrhage 4 + (8½ x 6 cm.) |
| 13 | 0.9 cc. | " | " | 5 | " | 3 | " | " | " | 3—negative | " | " | 15 | " | " | " | 2 | 3—negative |
| 14 | 0.9 cc. dil. 1:4 | " | " | 5 | " | 3 | " | " | " | 1—negative; 1—doubtful hemorrhage at tip | " | " | 2 | " | " | " | 2 | 1—negative; 1—hemorrhage 4+ (4 x 4 cm.) |
| 15 | " " | " | " | 5 | " | 1 | " | " | " | 3—negative | " | " | 5 | " | " | " | 2 | 3—negative |
| 16 | 0.9 cc. dil. 1:10 | " | " | 5 | " | 3 | " | " | " | 3 " | " | " | 5 | " | " | " | 2 | 3 " |
| 17 | 0.9 cc. dil. 1:4 | " | " | 5 | " | 3 | " | " | " | 3 " | " | " | 25 | " | " | " | 2 | 2—died; 1—hemorrhage 4 + (2½ x 2 cm.) and diffuse petechial hemorrhage in both ears |
| 18 | " " | " | " | 5 | " | 3 | " | " | T. 2002 | 3 " | Right marginal ear vein | | 15 | " | " | T. 2002 | 2 | 1—diffuse hemorrhagic reaction along inner border of ear |
| 19 | " " | " | " | 5 | " | 3 | " | " | T. 1986 | 3 " | Left marginal ear vein | | 5 | " | " | T. 1986 | 4 | 3—negative |
| 20 | " " | " | " | 5 | " | 3 | " | " | " | 3 " | " | " | 5 | " | " | " | 6 | 3 " |
| 21 | " " | " | " | 5 | " | 3 | " | " | " | 3 " | " | " | 5 | " | " | " | 24 | 3 " |

Dil. = diluted.

* Abbreviation Mg. 44B. designates "agar washings" filtrates of meningococcus, Group III cultures.

† Number of rabbits.

‡ Abbreviation B. $TyT_L$ designates "agar washings" filtrates of *B. typhosus*, Strain $T_L$ cultures.

activity is obvious from the experiments cited, the exact conditions of its reproduction should be considered with a great deal of reserve. Apparently, there exist individual fluctuations in susceptibility of rabbits which may serve as a source of error. These fluctuations are illustrated by the unexpectedly negative results of Group 13. The negative results of Group 16 were possibly due to a high dilution of testicular extract (*i.e.*, 0.9 cc. diluted 1:10), and those of Group 15 to the use of only 1 reacting unit for preparation.

If the assumption be granted that the state of reactivity induced by way of the vascular system with the aid of testicular extract is of short duration, it becomes clear why larger provocative doses are necessary when the interval of time is longer. Thus, positive results were obtained in Group 7 where as many as 50 reacting units were used for the provocative injection 24 hours after preparation. Rabbits of Group 21, yielding negative results, received only 5 reacting units for the provocative injection after the same interval of time.

## RÉSUMÉ AND COMMENTS

In the experiments recorded in this paper, attempts were made to determine whether local reactivity to bacterial filtrates could be elicited by way of the vascular system. Preparatory injections of bacterial filtrates of ascertained skin-preparatory potency were given into the marginal ear vein. In the greater portion of the experiments the circulation of the ear was interrupted by application of a clamp for short periods of time prior to and following the preparatory injection of filtrates. In most of the experiments injections of filtrates were given intravenously in mixture with agents intended to modify capillary and tissue permeability. In the remaining experiments, the preparatory intravenous injections of filtrates were accompanied by simultaneous intradermal injections of some of these agents. Various intervals of time between preparatory and intravenous injections were allowed in the experiments with testicular extract. The following was observed.

The state of reactivity could not be elicited by preparatory intravenous injections of adequate doses of bacterial filtrates alone into clamped and non-clamped ears. The state also failed to appear in combination with cold, xylol, ethyl urethane, acetylcholine, pilocar-

pine hydrochloride, atropine, calcium gluconate, guinea pig liver extract, histamine dihydrochloride, adrenalin chloride, and pituitrin. However, preparatory intravenous injections of toxins were capable of eliciting the state of reactivity in the rabbit's ear when they were accompanied by a thermal hyperemia produced by exposure to 45°, 50°, and 55°C. It was also possible to induce a state of reactivity of short duration when a mixture of the preparatory factors with testicular extract was given into the veins of clamped ears. In some experiments provocative injections of small doses of a filtrate given ½, 1, and 2 hours following the preparatory injections with such a mixture, elicited severe and diffuse reactions. It is noteworthy that in these experiments the incubation period and the duration of the reactivity was considerably shorter than that following the intradermal preparatory injection. In the latter, at least 8 hours of incubation period are required and it may last as long as 96 hours (6).

It is difficult to interpret the observation that heat and testicular extract were the only agents which allowed the preparation of the rabbit's ear by way of the vascular system whilst numerous substances employed which are capable of influencing profoundly the capillary permeability failed to do so. According to Duran-Reynals (7), McClean (8), and Favilli (9), the Reynals factors are capable of producing striking and immediate increase in dermal permeability which lasts for 24 hours. The well known rapid spreading of testicular extract in the injected site is due to an extreme dilatation of the capillaries and lymph spaces. It is obvious that when an intradermal injection of a bacterial filtrate is made for the purpose of elicitation of the state of reactivity of the phenomenon under discussion, a perivascular depot of the injected material is formed which comes into continuous contact with the cells and blood vessels of the site injected. The fact that an incubation period of at least 8 hours is required for the elicitation of the state of reactivity may be due to relative impermeability of the cells which does not permit a rapid entrance of the preparatory factors into them. The rôle of the testicular extract and heat accompanying the intravascular preparation may, then, be twofold; *i.e.*, to allow the passage of the injected substances into the surrounding tissues through a rapid increase in the capillary permeability; and to enhance the cell permeability. The latter assumption is based

on the fact that a considerably shorter incubation period is necessary for preparation with mixtures of the preparatory factors with testicular extract injected intravenously than when the preparatory factors alone are injected intradermally.[1]

In 1924, Sanarelli (10) described experiments in which rabbits received an intravenous injection of a sublethal dose of a live culture of cholera vibrio followed by an intravenous injection of *B. coli* or *B. proteus* culture filtrate, 24 hours later. The second injection elicited hemorrhagic lesions in the small intestines, mesentery, and kidneys, and killed a large percentage of rabbits. Invariably it was possible to demonstrate cholera vibrio in the intestinal wall of animals injected. Combined injections of heat killed or autolyzed cholera vibrio with *B. coli* or *B. proteus* culture filtrates produced no effect. The reverse order of injections, *i.e.*, *B. coli* culture filtrate followed by injection of live cholera vibrio culture, also gave little effect. Sanarelli concluded from his experiments that there exists a definite selective affinity of cholera vibrio for the intestines; that the reactions described are anaphylactic in nature (*epithalaxie*) requiring sensitization with live cholera vibrio; and that the experimental picture obtained is pathognomonic of human cholera.

After the author of this paper described the phenomenon of local skin reactivity to bacterial filtrates in 1928, Gratia and Linz interpreted Sanarelli's observation in the light of the latter phenomenon. They assumed that the first intravenous injection of the live cholera vibrio induced in the intestines a state of reactivity by means of the preparatory factors operative in the phenomenon of local skin reactivity to bacterial filtrates. The provocative injection of the filtrate of *B. coli* or *B. proteus* elicited, then, hemorrhagic lesions in the intestines. To prove this contention, Gratia and Linz (2) gave to guinea pigs two intravenous injections of bacterial filtrates potent in the elicitation of the phenomenon of local skin reactivity (cholera vibrio), 24 hours apart and obtained hemorrhages in the peritoneal cavity and, in one instance, hemorrhagic lesions in the large intestines subsequently to the second injection.

Phenomenon of general reactivity to *B. coli* culture filtrates was later studied

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[1] The effect of testicular extract upon the phenomenon under discussion was previously studied by Duran-Reynals by means of experiments somewhat different from those described in this paper. Rabbits were either prepared by intradermal injection of a mixture of testicular extract with bacterial filtrate, or received an intravenous injection of testicular extract 24 hours after the intradermal preparatory injection of bacterial filtrate. Following the intravenous injection of bacterial filtrate there was observed spreading of the lesion accompanied by a definite reduction in intensity. The author concluded that testicular extract did not enhance the susceptibility to the phenomenon of local skin reactivity to bacterial filtrates.

again by Gratia and Linz (2) and Apitz (11). Gerber (12) in these laboratories, recently studied this phenomenon with *B. typhosus* and meningococcus "agar washings" filtrates. These authors gave repeated intravenous injections of filtrates potent in the elicitation of the phenomenon of local skin reactivity to bacterial filtrates. In most of the experiments there were given two intravenous injections 24 hours apart. Diffuse vascular lesions were observed in the liver, spleen, kidneys, adrenals, pancreas, bone marrow, and lungs. Apitz and Gerber made extensive gross and histologic studies. No intestinal lesions were noted by them.

Gratia and Linz (2), Dienes (13), Bordet (14), Apitz (11), Freund (15), and Koplik (16) in these laboratories, observed hemorrhagic lesions at the sites of tuberculous and other bacterial and virus infections following an intravenous injection of certain potent heterologous bacterial filtrates.

Thus, there is in the literature a group of observations apparently related to each other. In the light of experiments described in this paper, an attempt will be made to point out in a form of a working hypothesis possible differences in essential mechanisms of the above observations.

In the phenomenon of local skin reactivity to bacterial filtrates the state of reactivity is elicited through the influence of certain soluble bacterial factors introduced into the perivascular tissue. The provocative intravenous injection of suitable material produces lesions in the capillary network, especially in the small veins and venules, and subsequently in the tissues themselves. No arterial damage is observed.

In the phenomenon of general reactivity to bacterial filtrates (*i.e.*, two intravenous injections, 24 hours apart, of bacterial filtrates potent in the elicitation of the phenomenon of local skin reactivity) diffuse vascular lesions occur in organs above mentioned. As in the phenomenon of local skin reactivity, there is observed damage in the capillary network, small veins, and venules. It was shown in the work on the rabbit's ear that preparation by way of the vascular system takes place provided agents capable of enhancing capillary permeability and possibly cell permeability are employed. In experiments on general reactivity, when the state of reactivity is elicited by way of the vascular system in the absence of any auxiliary agents increasing the vascular permeability, it is reasonable to expect that the physiologic differences of capillary permeability of organs and especially varia-

tions in the venous supply, may condition the occurrence of lesions in different organs. Rous (17) in collaboration with F. Smith, Hudack, and McMaster, showed clearly that there exist essential differences in the permeability of capillaries and venules based on structural features. It is of interest in this connection that the lesions of the phenomenon of general reactivity occur in organs in which a high degree of capillary permeability exists under physiologic conditions (kidney, liver, spleen, bone marrow, etc.); and also that they take place mostly in the venules. Further proof is also brought by the finding of Stolyghwo (18) that the factors operative in the phenomenon of local skin reactivity are excreted by the kidneys of rabbits injected intravenously with bacterial filtrates and also of typhoid fever patients. The phenomenon of general reactivity to bacterial filtrates may, then, be identical with the phenomenon of local skin reactivity, the only differences being in the intravascular route of elicitation of reactivity, and in the fact that the distribution of lesions in normal animals is conditioned by the vascular permeability of various organs.

Next, then, one should consider the additional rôle of various agents capable of modifying the vascular and tissue permeability, and thus being indirectly responsible for the elicitation of the state of general reactivity. Sanarelli's observation belongs to this group in which, in addition to soluble bacterial factors operative in the phenomenon of local skin reactivity to bacterial filtrates, the effects of the live organisms used for the preparatory injection are to be considered. These effects are possibly as follows:

Local inflammatory reactions to live bacteria which may change the capillary permeability; formation of bacterial foci which may act as provocative agents upon distant reactive areas; and the inherent selective affinity of the organisms employed for various organs, etc. With our present knowledge, an attempt is made merely to outline roughly the possible complex rôle that the combined effect of live bacteria with the soluble bacterial factors may have in the elicitation of the lesions described by Sanarelli. It is noteworthy in this connection that in his experiments, reactions were obtained in the small intestines whilst no intestinal lesions were observed by Apitz and Gerber in the phenomenon of general reactivity to bacterial factors alone; and that live cholera vibrio was invariably isolated from the intestinal wall.

As noted before, sites of spontaneous or induced infections may possess the state of reactivity of the phenomenon under discussion. For this reason the possible effect of bacterial filtrates upon pre-existing foci in the experimental animals is to be considered in the experiments on the phenomenon of general reactivity to bacterial filtrates.

Freund (19) recently observed that hemorrhagic reactions may appear at sites injected with silver nitrate in tuberculous guinea pigs following intravenous injection of potent heterologous bacterial filtrates. In such experiments the inflammatory reaction set up by silver nitrate may be able to localize bacteria and bacterial toxic substances circulating in the vascular system of the infected tuberculous animal. This possibility is clearly postulated by the work of Opie (20), Menkin (21), Cannon and Pacheco (22), and others. Returning to Freund's observations, the localization from the blood stream of certain factors secreted by the bacteria during the course of the active infection may now induce a state of reactivity at the site of fixation. Bacterial factors capable of eliciting the phenomenon of local skin reactivity were recently shown to exist in tuberculous cultures (Shwartzman (23)). Obviously, subsequent provocative injection of bacterial filtrates could produce a reaction at the site of the silver nitrate injection. It is noteworthy in this connection that silver nitrate alone (*i.e.*, without the presence of active infection in the animal) has no skin-preparatory potency (Shwartzman (6)). This mechanism is not implied necessarily as one operative in the experiments by Freund. An attempt is made, as in other instances, to outline the complex interference of additional agents in studies on the phenomena of general and local skin reactivity to bacterial filtrates. Data reported in this paper concerning the elicitation of the state of reactivity by way of the vascular system make these considerations necessary in the evaluation of the various facts reported in the literature.

Following intravenous injections into clamped ears of mixtures of pituitrin, adrenalin chloride, and histamine dihydrochloride with bacterial filtrates, primary hemorrhagic and thrombotic lesions were observed. These lesions were rarely enhanced by the provocative injections of potent filtrates. Possibly, similar effects were obtained by Marcus and Schmidt-Weyland. These authors, quoted by Rössle

(24),[2] gave repeated injections of adrenalin to rabbits previously treated with bacterial toxins and, in some instances, they obtained gangrene and thrombosis.

It is of interest that in spite of the primary hemorrhagic lesions obtained with mixtures above described, the state of reactivity did not take place. This fact may serve as an additional argument in favor of the contention that the ability of bacterial factors to induce the state of reactivity to the phenomenon under discussion is entirely independent of primary inflammatory and hemorrhagic effects in otherwise normal animals.

Experiments are under way to determine the effect of bacterial invaders and various agents capable of changing the capillary permeability, upon the phenomenon of general reactivity to bacterial filtrates.

## SUMMARY

The skin of the rabbit's ear is considerably more resistant than the abdominal skin to the phenomenon of local skin reactivity to bacterial filtrates. Ten times the provocative dose is required if it is given into the vein of the prepared ear and thirty times the provocative dose if it is given into the vein of the non-prepared ear.

The state of reactivity cannot be elicited by a preparatory intravenous injection of bacterial filtrates alone into clamped and non-clamped ears. The state also fails to appear in combination with cold, xylol, ethyl urethane, pilocarpine hydrochloride, atropine, calcium gluconate, guinea pig liver extract, histamine dihydrochloride, adrenalin chloride, and pituitrin.

Preparatory intravenous injections of toxins are capable of eliciting the state of reactivity in the rabbit's ear when they are accompanied by thermal hyperemia (*i.e.*, exposure to 45°, 50°, and 55°C.). It is also possible to induce the state of reactivity when a mixture of the preparatory factors with testicular extract is given into the veins of clamped ears. The incubation period required may be less than 2 hours.

In the light of the above experiments, there are discussed various

[2] Unfortunately, the reference to the original paper describing these experiments was not available.

observations concerning the elicitation of the phenomena of organ reactivity by means of live bacterial cultures and the filtrates thereof.

## BIBLIOGRAPHY


1. Rous, P., Gilding, H. P., and Smith, F., *J. Exp. Med.*, 1930, **51,** 807.
2. Gratia, A., and Linz, R., *Ann. Inst. Pasteur.*, 1932, **49,** 131.
3. Klein, H. M., unpublished observations.
4. Alechinsky, A., *Compt. rend. Soc. biol.*, 1935, **118,** 1496.
5. Duran-Reynals, F., *J. Exp. Med.*, 1929, **50,** 327.
6. Shwartzman, G., *J. Exp. Med.*, 1935, **61,** 383.
7. Duran-Reynals, F., *Compt. rend. Soc. biol.*, 1928, **99,** 6, 1908; *J. Exp. Med.*, 1929, **50,** 327; 1933, **58,** 161, 451; 1932, **55,** 703; *Proc. Soc. Exp. Biol. and Med.*, 1933–34, **31,** 341; *Am. J. Cancer*, 1931, **15,** 2790.
8. McClean, D., *J. Path. and Bact.*, 1930, **33,** 1045.
9. Favilli, G., *J. Exp. Med.*, 1931, **54,** 197.
10. Sanarelli, G., *Ann. Inst. Pasteur.*, 1924, **38,** 11.
11. Apitz, K., *Virchows Arch. path. Anat.*, 1934, **293,** 1; *Z. ges. exp. Med.*, 1934, **94,** 222.
12. Gerber, I. E., *Am. J. Path.*, 1935, **11,** 843.
13. Dienes, L., *Proc. Soc. Exp. Biol. and Med.*, 1929–30, **27,** 690.
14. Bordet, P., *Compt. rend. Soc. biol.*, 1933, **114,** 572, 574.
15. Freund, J., *Proc. Soc. Exp. Biol. and Med.*, 1932–33, **301,** 535.
16. Koplik, L. H., *Am. J. Path.*, 1935, **11,** 842.
17. Rous, P., and Smith, F., *J. Exp. Med.*, 1930, **51,** 807. Smith, F., and Rous, P., *J. Exp. Med.*, 1931, **53,** 195. Rous, P., and Smith, F., *J. Exp. Med.*, 1931, **53,** 219. Smith, F., and Rous, P., *J. Exp. Med.*, 1931, **54,** 499. McMaster, P. D., Hudack, S., and Rous, P., *J. Exp. Med.*, 1932, **55,** 203. McMaster, P. D., and Hudack, S., *J. Exp. Med.*, 1932, **55,** 417. Hudack, S., and McMaster, P. D., *J. Exp. Med.*, **55,** 431.
18. Stolyghwo, N., *Bull. Soc. biol. Lettonie*, 1931, **2,** 129.
19. Freund, J., *J. Exp. Med.*, 1934, **60,** 669.
20. Opie, E. L., *J. Immunol.*, 1929, **17,** 329.
21. Menkin, V., *J. Exp. Med.*, 1930, **51,** 285; *Arch. Path.*, 1931, **12,** 802.
22. Cannon, P. R., and Pacheco, G. A., *Am. J. Path.*, 1930, **6,** 749.
23. Shwartzman, G., *J. Exp. Med.*, 1935, **61,** 369.
24. Rössle, R., *Klin. Woch.*, 1933, **12,** 574.


## EXPLANATION OF PLATES

### PLATE 26

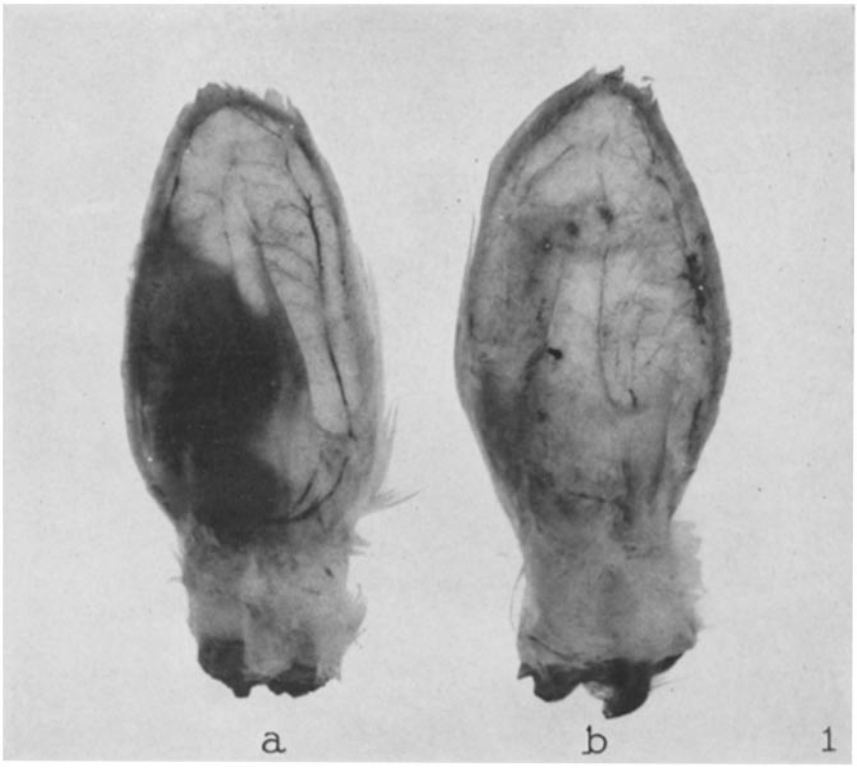
FIG. 1. Phenomenon of local skin reactivity to meningococcus filtrate in the rabbit ear. (Meningococcus, Group III (44B.) "agar washings" filtrate (T.1968).) 0.25 cc. diluted 1:2 was injected intradermally and 100 reacting units were in-

jected intravenously 24 hours later. (*a*) Typical lesion 4 hours after the intravenous injection. (*b*) Control ear which received no preparatory injection.

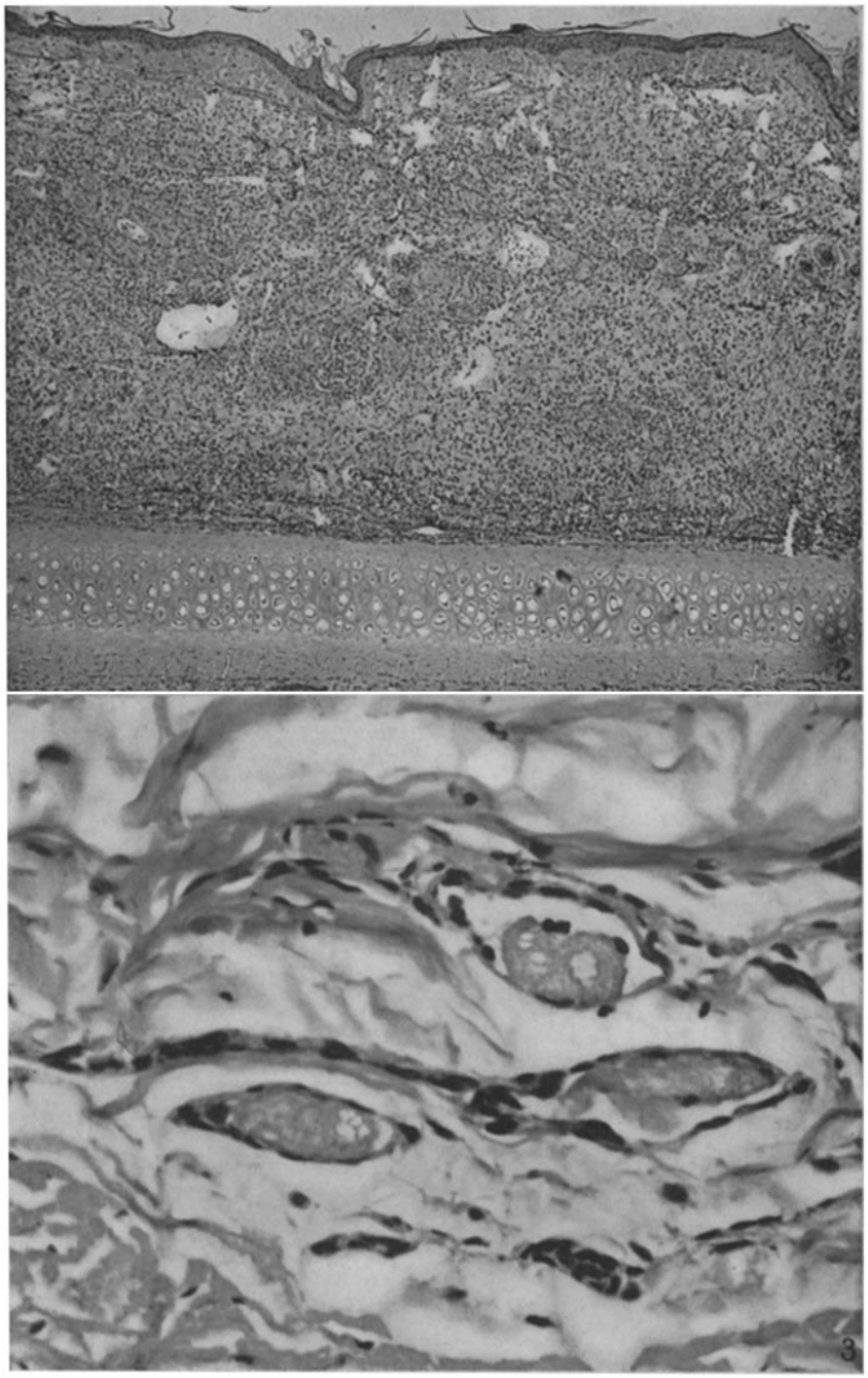
PLATE 27

FIG. 2. Treatment the same as in Ear *a* of Fig. 1. Note severe hemorrhage, edema, inflammatory reaction, and vascular thrombosis. ×50.

FIG. 3. Treatment the same as in Ear *a* of Fig. 1. Section taken of an area 3 cm. away from the site of the reaction and showing in the gross cyanosis and edema. Note thrombi in the venules. ×400.




(Shwartzman: Local skin reactivity to bacterial filtrates)



(Shwartzman: Local skin reactivity to bacterial filtrates)