

## PASSIVE CUTANEOUS ANAPHYLAXIS (PCA) IN THE GUINEA PIG AN IMMUNOLOGIC AND PATHOLOGIC STUDY

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BIOZZI, Mene, and Ovary<sup>1</sup> studied the quantitative aspects of endothelial granulopexy induced by intradermal injection of histamine. Guinea pigs were given intrajugular injections of India ink and, after two hours, intradermal injections of histamine dilutions. Due to an increase in the permeability of the vessels, an effusion of plasma colored by India ink accumulated at the histamine-injected skin sites. The microscopic examination of the test sites revealed phagocytosis of the India ink particles by endothelial cells (granulopexy).

The authors included in their investigation an observation of granulopexy induced at the site of antibody-antigen reaction. A skin site passively sensitized with guinea pig antihorse-serum serum was challenged by intravenous injection of antigen (horse serum) and India ink. There was an effusion of India ink at the antigen-antibody reaction site. The microscopic examination revealed minute hemorrhages, vascular lesions, and granulopexy activity of cells in connective tissue, rather than in vascular endothelium only. Pretreatment with an antihistamine prevented both the effusion and the granulopexy. The authors concluded that granulopexy induced in guinea pig skin at the site of an antigen-antibody reaction is somewhat different from that induced by intradermal injection of histamine.

The antibody-antigen reaction in the skin of guinea pigs was termed passive cutaneous anaphylaxis (PCA) and tentatively attributed to the action of histamine liberated in the course of the reaction.<sup>2</sup>

Ovary and Briot<sup>3</sup> reported that an intracutaneous injection of the most minute amounts of rabbit immune serum (such as a 1:30,000 dilution of rabbit antiovalbumin serum) can still passively sensitize a site of guinea pig skin and induce a seepage of plasma. Determinations of precipitable antibody N (determined by the method of Heidelberger and Kendall<sup>4</sup>) indicated that about 0.003 mcg. of precipitable antibody N was present in 0.1 ml. of 1:30,000 dilution of antiserum used for passive sensitization.

Various studies by Ovary,<sup>5, 7</sup> Ovary, Biozzi, and Mene,<sup>6</sup> Ovary and Bier,<sup>8</sup> and Biozzi, Halpern, and Benacerraf<sup>9</sup> established the basic observations on passive cutaneous anaphylaxis in the guinea pig as follows:

A skin site is passively sensitized with intracutaneously injected sensitive serum. The challenge with a mixture of antigen and dye is administered by an intravenous or intracardial route. An interaction of antibody and specific antigen is signified by a rapid accumulation of the dye in the subepidermal tissues surrounding the sensitized skin site.<sup>7</sup>

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The induction of passive sensitivity after intracutaneous injection of immune serum requires a latent period which was found to be inversely proportional to the amount of antibody used for passive transfer. The more potent the antiserum, the shorter the latent period before onset of passive sensitization.<sup>9</sup> This latent period cannot be reduced to less than thirty minutes between intradermal sensitization and subsequent challenge with antigen.<sup>5</sup> It has been noted that antihistaminics do not abolish the PCA reaction, but only modify it, preventing reactions at weakly sensitized sites and lessening the degree of reactions where more potent antisera induced the local sensitization.<sup>6</sup> The distinction between reversed Arthus phenomenon and PCA was postulated from experiments with antihistaminics which do not have any influence on reversed Arthus phenomenon but modify the PCA reaction. As another evidence aiding the differentiation of the two, it is stated that horse antityphoid polysaccharide immune serum is able to induce a severe reversed Arthus phenomenon in guinea pig, but is not effective in inducing local sensitization for passive cutaneous anaphylaxis.<sup>8</sup>

The passive sensitivity of guinea pig skin can be induced by the injection of certain human sensitive sera. Successful transfer of human diphtheria precipitating and nonprecipitating antisera and antityphoid and antibrucella convalescent sera has been reported by Ovary and Biozzi.<sup>10</sup> In our previous investigation,<sup>11</sup> using antisera of human origin, we have established that human sera of the *spontaneous* immediate (edema-erythema) type of sensitivity (against pollen antigens, horse epithelium, and horse serum) were not capable of sensitizing guinea pig skin. However, cutaneous reactivity was obtained with human sera of the *induced* immediate (edema-erythema) type of sensitivity against horse serum and with rabbit sera immunized with ragweed pollen extract. *Human blocking antibody*<sup>14</sup> against ragweed pollen failed to transfer and induce PCA in guinea pig.

The present study is an examination of the relationship of passive cutaneous anaphylaxis to immunological properties of various antibodies, and aims at the elucidation of the pathologic physiology of this cutaneous phenomenon.

#### EXPERIMENTAL TECHNIQUES

*Passive Cutaneous Anaphylaxis (PCA).*—Male albino guinea pigs, each weighing 350 to 400 grams, were used throughout the study. Abdominal skin, shaved with an electric clipper, was employed. Three to six intradermal sites were prepared, each with 0.1 ml. of sensitive serum or serum dilutions. Six hours later an intracardial injection of corresponding antigen-dye mixture was made. After thirty and sixty minutes, the reactions were read and considered positive if an accumulation of dye 7 mm. or greater in diameter appeared. Absence of dye seepage or discoloration of less than 7 mm. in diameter was considered negative.

For quantitative estimation of sensitizing activity, dilutions of sensitive sera were prepared with 0.85 per cent saline solution. The serum-saline dilution responsible for the minimal (7 mm. diameter) effusion of dye established the titration end point, that is, the maximal dilution of antibody capable of inducing reaction. The titration by dilution permitted fairly accurate estimation of sensitizing potency of investigated sera and facilitated quantitative comparisons.

In each test animal, a known positive reacting system in maximal dilution and normal serum were included as controls. Each serum was examined for PCA at least three times.

*Schultz-Dale Phenomenon.*—Isolated guinea pig ileum was used. Fresh segments of ileum were immersed in the sensitive serum for five hours at 37° C.; then they were washed and suspended in 0.85 per cent saline (37° C.). The reaction was considered positive if the challenge with specific antigen caused immediate contraction, followed by a refractoriness of the muscle to subsequent addition of the same antigen. Addition of histamine confirmed the remaining ability to respond, while a challenge with nonspecific antigen after a change of bath served as a control. Parallel experiments with ileum strips immersed in normal human or animal sera failed to produce reactivity upon addition of antigens.

*Systemic Anaphylaxis.*—Systemic sensitization of the guinea pigs was established by an intraperitoneal injection of 5 to 18 ml. of sensitive serum. The animals were challenged intracardially twice: with nonspecific antigen eighteen hours after sensitizing injection and six hours later with specific antigen. They were observed for signs and symptoms of anaphylaxis. The reaction was considered positive if dyspnea ended in death and the autopsy revealed emphysematous lungs.

*Precipitation.*—The technique described as agar gel diffusion Oudin,<sup>12</sup> as well as the classical test tube method of precipitation, was used.

*Antigens.*—

*Passively sensitized animals:* These were challenged intracardially with the proper antigens. The following amounts were used: 1 ml. of concentrated antigen extracts containing 100,000 to 200,000 protein nitrogen units\* per milliliter; 0.2 ml. of horse serum; and 0.2 ml. of 5 per cent solution of the offending plasma protein in 0.85 per cent saline.

*Passive cutaneous anaphylaxis:* The challenging dose of antigen, as stated above, was mixed with 0.25 ml. per 100 grams of body weight of 1 per cent Evans blue solution just prior to injection.

*Schultz-Dale experiments:* Two-tenths milliliter of antigen extracts, horse serum, or plasma protein antigen solutions were used for each 10 ml. of saline constituting the suspension bath for tested intestinal segments.

*Precipitation:* Several dilutions of the antigens were employed.

*Sensitizing Serums.*—The various investigated sensitive sera, the sensitized species, and the offending antigens (respective test antigens) are listed in Table I. In the *spontaneous* human allergies of the immediate type (edema-erythema group) the antisera had skin-sensitizing property in at least 1:800 dilution in the Prausnitz-Küstner tests. The skin-sensitizing ability of human sera of the *induced* human immediate (edema-erythema) type of sensitivity was not demonstrable in dilutions exceeding 1:300 dilution.

Titration of *sensitive sera of animal origin* indicated only a limited ability to sensitize human skin. For the purpose of the present investigation, only

\*One protein unit contains 0.00001 mg. phosphotungstic precipitable nitrogen.

undiluted antiserum and 1:10 dilutions were employed to demonstrate human-skin-sensitizing property of animal antisera. The capacity to induce guinea pig skin sensitivity, reflected by PCA titers, did not relate proportionally to the human-skin-sensitizing activity.

Sera capable of transferring sensitivity to guinea pig skin induced positive PCA reactions in dilutions as high as 1:24,000 (rabbit antiovalbumin serum), 1:12,000 (human antihorse-serum serum), 1:6000 (guinea pig antibovine-albumin serum). Only very few ranged as low as 1:800 (human anti-Friedlander and antipneumococcus S.S.S. III-convalescent sera, rabbit anti-ragweed serum, and guinea pig antitubercleulopolysaccharide serum).

*Serum Fractions.*—These were derived from induced human antihorse-serum serum\* and normal human serum and were employed for investigation of PCA phenomenon. Fractions designated as euglobulin, intermediate globulin, pseudoglobulin, and albumin were separated from serum on the basis of their solubility properties in ammonium sulfate solutions and then resolved by dialysis into water-soluble and water-insoluble constituents.<sup>17</sup> Electrophoretic pattern and mobility indicated that the fraction designated as *water-soluble euglobulin* is essentially gamma globulin.<sup>13</sup> This euglobulin dissolved in saline in about the same proportion as found in the original serum, demonstrated approximately 28 per cent of human-skin-sensitizing activity of the whole serum.<sup>13</sup> Its ability to sensitize guinea pig skin and induce a PCA reaction was reduced by about 50 per cent. The *water-soluble albumin* derived from the same horse-serum-sensitive serum failed to reveal activity in any of the above mentioned immunological tests.

Fractions designated as *normal human euglobulin* and *normal human albumin* were water-soluble constituents of fractions derived from normal non-allergic human serum (Krs.). They were isolated by ammonium sulfate fractionation and dialysis, as described above, for use as controls and as diluents as specified in the text.

#### EXPERIMENTAL FINDINGS

Table I shows the results of PCA in guinea pig and the contrasting immunological properties† of investigated sensitive serum as determined by testing their activities in terms of:

1. Passive transfer of sensitivity to human skin (skin-sensitizing property—term based on passive sensitizing activity upon human skin as demonstrable in Prausnitz-Küstner tests).
2. Passive transfer of sensitivity to guinea pig (muscle-sensitizing property—term based on passive sensitizing activity upon guinea pig smooth muscle as demonstrated by means of passive systemic anaphylaxis and Schultz-Dale phenomenon).

\*Serum obtained from a patient who had suffered serum sickness following administration of tetanus antitoxin (horse serum).

†The nomenclature defining antibodies by their properties or functions is that suggested by Cook, R. A., and Spain, W. C.: Studies in Hypersensitiveness: A Comparative Study of Antibodies Occurring in Anaphylaxis, Serum Disease and Naturally Sensitive Man, J. Immunol. 17: 295, 1929.

TABLE I. COMPARATIVE STUDY OF IMMUNOLOGICAL PROPERTIES

| SENSITIVE SPECIES | ANTIGEN                                | TYPE OF IMMEDIATE SENSITIVITY | DIRECT SKIN TEST, IMMEDIATE WHEAL, AND ERYTHEMA REACTION |
|-------------------|----------------------------------------|-------------------------------|----------------------------------------------------------|
| Human             | Ragweed pollen                         | Spontaneous                   | Pos.                                                     |
|                   | Timothy pollen                         |                               | Pos.                                                     |
|                   | Horse epithelium                       |                               | Pos.                                                     |
|                   | Horse serum                            |                               | Pos.                                                     |
|                   | Pork                                   |                               | Pos.                                                     |
|                   | Peanuts                                |                               | Pos.                                                     |
|                   | Egg white (hen)                        |                               | Pos.                                                     |
|                   | Penicillin (e)                         | Induced                       | Pos.                                                     |
|                   | Tetanus antitoxin                      |                               | Pos.                                                     |
|                   | (test antigen: horse serum)            |                               |                                                          |
|                   | Pneumococcus T.III                     |                               | Pos.                                                     |
|                   | (test antigen: S.S.S. III)             |                               |                                                          |
|                   | <i>Klebsiella friedländeri</i> (f)     |                               | Neg.                                                     |
|                   | <i>Eberthella typhi</i> (g)            |                               | n.r. (a)                                                 |
|                   | <i>Brucella melitensis</i> (g)         |                               | n.r.                                                     |
|                   | <i>Diphtheria toxoid</i> (i)           |                               | Pos.                                                     |
|                   | <i>Diphtheria toxoid</i> (i)           |                               | Pos.                                                     |
|                   | Ragweed pollen extract (j)             | Induced blocking              | Neg.                                                     |
| Rabbit            | Ragweed pollen extract                 | Induced                       | Arthus (k)                                               |
|                   | Human albumin                          |                               | Arthus                                                   |
|                   | Bovine albumin                         |                               | Arthus                                                   |
|                   | Ovalbumin                              |                               | Arthus                                                   |
|                   | Pneumococcus T.III                     |                               | Arthus                                                   |
| Guinea pig        | (test antigen: S.S.S. III)             |                               |                                                          |
|                   | Bovine albumin                         | Induced (m)                   | Arthus                                                   |
|                   | Horse serum                            |                               | Arthus                                                   |
|                   | Tubercle bacilli (n)                   |                               | Pos. (p)                                                 |
|                   | (test antigen: TBB-polysaccharide) (o) |                               |                                                          |

**Key to symbols:**

- (a) *Abbreviations*: n.r. = not reported; n.d. = not done.  
 (b) *Precipitation determined by agar gel diffusion* (Oudin).<sup>12</sup>  
 (c) Published and discussed in our previous study.<sup>11</sup>  
 (d) Sensitivity to horse epithelium and horse serum.  
 (e) Four investigated specimens of this group were selected out of nineteen penicillin-sensitive sera because in these four instances only the patients from whom the bleedings were obtained suffered an anaphylactoid shock after administration of the drug, demonstrated positive immediate skin tests, and the sensitivity was passively transferrable to normal human skin. (Prausnitz-Küstner reaction in at least 1:200 dilution.)  
 (f) A filtrate of bacterial vaccine (derivative of *Klebsiella friedländeri* culture growth) was used as the test antigen.  
 (g) Published by Ovary and Blozzi.<sup>10</sup>  
 (h) Agglutination.  
 (i) Published by Kuhns and Pappenheimer.<sup>16</sup>  
 (j) Two nonallergic volunteers were given injections of low ragweed pollen extract; after repeated series of injections neither of the two demonstrated positive skin tests to the extract. The sera were obtained for use in testing of their blocking effect; 1 ml. of such immune serum "blocked" the activity of low ragweed pollen extract in the order of 1,600 protein nitrogen units.<sup>17</sup>  
 (k) Direct skin testing of the sensitized animals with antigen resulted in Arthus phenomenon characterized by hemorrhagic necrosis, verified by histologic examination.

- Precipitation (precipitating property—term based on formation of specific precipitate upon interaction of antigen and antibody in vitro).
- Agglutination (agglutinating property—term based on formation of specific agglutinate upon interaction of antigenic particles, that is, bacteria, and antibody in vitro).

## OF VARIOUS ANTIBODIES IN SERA OF MAN AND ANIMALS

| NO. OF SERA<br>EXAMINED | IMMUNOLOGICAL PROPERTIES OF ANTIBODIES<br>DETERMINED BY APPROPRIATE TESTS |                                                                |                                                                  |                      |                                                              |
|-------------------------|---------------------------------------------------------------------------|----------------------------------------------------------------|------------------------------------------------------------------|----------------------|--------------------------------------------------------------|
|                         | PASSIVE<br>TRANSFER IN<br>HUMAN<br>SKIN                                   | PASSIVE<br>SYSTEMIC<br>ANAPHYLAXIS IN<br>GUINEA PIG<br>(SHOCK) | SCHULTZ-DALE<br>PHENOMENON<br>ON ISOLATED<br>GUINEA PIG<br>ILEUM | PRECIPITATION<br>(b) | PASSIVE<br>CUTANEOUS<br>ANAPHYLAXIS<br>IN GUINEA PIG<br>SKIN |
| 3                       | Pos.                                                                      | Neg.                                                           | Neg.                                                             | Neg.                 | Neg. (c)                                                     |
| 1                       | Pos.                                                                      | Neg.                                                           | Neg.                                                             | Neg.                 | Neg. (c)                                                     |
| 4 (d)                   | Pos.                                                                      | Neg.                                                           | Neg.                                                             | Neg.                 | Neg. (c)                                                     |
|                         | Pos.                                                                      | Neg.                                                           | Neg.                                                             | Neg.                 | Neg. (c)                                                     |
| 1                       | Pos.                                                                      | Neg.                                                           | Neg.                                                             | Neg.                 | Neg.                                                         |
| 1                       | Pos.                                                                      | Neg.                                                           | Neg.                                                             | Neg.                 | Neg.                                                         |
| 2                       | Pos.                                                                      | Neg.                                                           | Neg.                                                             | Neg.                 | Neg.                                                         |
| 4                       | Pos.                                                                      | Neg.                                                           | Neg.                                                             | Neg.                 | Neg.                                                         |
| 4                       | Pos.                                                                      | Pos.                                                           | Pos.                                                             | Pos.                 | Pos.                                                         |
| 1                       | Pos.                                                                      | Pos.                                                           | Pos.                                                             | Pos.                 | Pos.                                                         |
| 1                       | Neg.                                                                      | Pos.                                                           | Pos.                                                             | Pos.                 | Pos.                                                         |
| 1                       | n.r.                                                                      | n.r.                                                           | n.r.                                                             | Pos. (h)             | Pos.                                                         |
| 1                       | n.r.                                                                      | n.r.                                                           | n.r.                                                             | Pos. (h)             | Pos.                                                         |
| 1                       | Pos.                                                                      | Pos.                                                           | n.r.                                                             | Neg.                 | Pos. (g)                                                     |
| 1                       | Pos.                                                                      | Pos.                                                           | n.r.                                                             | Pos.                 | Pos. (g)                                                     |
| 2                       | Neg.                                                                      | Neg.                                                           | Neg.                                                             | Neg.                 | Neg. (c)                                                     |
| 3                       | Pos.                                                                      | Pos.                                                           | Pos.                                                             | Pos.                 | Pos. (c)                                                     |
| 1                       | Neg. (1)                                                                  | Pos.                                                           | Pos.                                                             | Pos.                 | Pos.                                                         |
| 2                       | Pos.                                                                      | Pos.                                                           | Pos.                                                             | Pos.                 | Pos.                                                         |
| 2                       | Pos.                                                                      | Pos.                                                           | Pos.                                                             | Pos.                 | Pos.                                                         |
| 1                       | Pos.                                                                      | Pos.                                                           | Pos.                                                             | Pos.                 | Pos.                                                         |
| 2                       | Neg.                                                                      | Pos.                                                           | Pos.                                                             | Pos.                 | Pos.                                                         |
| 2                       | Neg.                                                                      | Pos.                                                           | Pos.                                                             | Pos.                 | Pos.                                                         |
| pooled                  | n.d. (a)                                                                  | Pos. (r)                                                       | Pos. (r)                                                         | Pos. (r)             | Pos. (r)                                                     |

- (l) Rabbit antihuman albumin immune serum, when injected in human skin, did not produce a typical wheal and erythma reaction. After five to six hours the injected site became indurated. The infiltration was found maximal after twenty-four hours. This might be regarded as reversed Arthus phenomenon, although a confirming histological diagnosis was not made.
- (m) Guinea pigs were immunized by series of subcutaneous injections of antigen with Freund's adjuvant.<sup>28</sup>
- (n) Male guinea pigs were immunized by series of subcutaneous injections of autoclaved, washed, and lyophilized tubercle bacilli of human type (10 mg. of TBB in 1 ml. of paraffin oil biweekly for four to six weeks.
- (o) Tuberculopolysaccharide S-4 for our investigation of the immediate type of reactivity of TBB-sensitized guinea pigs (direct skin tests and immunologic examinations of pooled serum) was kindly supplied to us by Dr. Dennis W. Watson, professor of bacteriology and immunology, University of Minnesota.
- (p) Out of eighteen TBB-sensitized guinea pigs tested with TBB-polysaccharide S-4 six guinea pigs exhibited immediate erythma at least 15 mm. in diameter which became edematous within one to two hours. (Skin test doses of 10 mcg. of TBB-polysaccharide S-4 were used. Skin tests on normal guinea pigs failed to produce reactivity.) Twenty-four hours after skin testing with polysaccharide S-4 an induration was found at all sites irrespective of presence or absence of immediate response. Minute amounts of tuberculo-protein present in the polysaccharide may be regarded as responsible for this delayed type of reaction. All eighteen TBB-immunized animals, when tested with 7 mcg. of P.P.D. (prepared from Parke, Davis & Company commercial tablets) demonstrated intensive delayed tuberculin reaction with central necrosis.
- (r) P.P.D. used in identical control experiment failed to produce reactivity.

5. Blocking effect (blocking property—term based on behavior of these antibodies in skin tests<sup>14</sup> and must not be confused with the term “blocking” in serologic tests for Rh sensitization<sup>15</sup>).

*Relation of Muscle-Sensitizing Property to PCA.—From the present experiments, it can be seen that the ability to induce PCA reaction is always parallel*

*to the ability of antibodies to sensitize guinea pig smooth muscle (compare passive systemic anaphylaxis and Schultz-Dale phenomenon with PCA reactions in Table I).*

*Relation of Skin-Sensitizing Property to PCA.*—The human-skin-sensitizing sera of spontaneous immediate (edema-erythema) type and of the induced immediate (edema-erythema) type of sensitivity to penicillin, highly active in human skin by direct and indirect tests, were not capable of inducing guinea pig smooth muscle sensitivity; nor were they able to transfer to guinea pig skin (PCA).

Four hours' heating (at 56° C.) of human sera (induced antihorse-serum serum, antipneumococcus S.S.S. III-convalescent serum) as well as animal antisera, all capable of inducing human skin sensitivity, smooth muscle sensitivity, and PCA reaction, destroyed the human-skin-sensitizing property but did not alter the other two. Schultz-Dale experiments repeated with heated sera demonstrated the remaining muscle-sensitizing property, and repeated PCA titrations confirmed the transfer of sensitivity to guinea pig skin with no more than slight change of titration end points.

*There is no indication that the human-skin-sensitizing property of antibodies, both spontaneous and induced, is responsible for or identical with that inducing guinea pig smooth muscle or guinea pig skin sensitivity (PCA).*

*Relation of Precipitins to PCA.*—In our previous study,<sup>11</sup> using induced human antihorse-serum serum, we have reported that precipitins demonstrable by the Oudin method withstand heating at 56° C. for sixty hours. A marked decrease in precipitin content was evident after four hours' heating, as estimated by classical test tube method. Using a variety of precipitating sera, this observation may be confirmed. Most precipitins decrease markedly after heating for four hours; however, Schultz-Dale phenomenon remains positive and PCA reactions remain essentially unchanged as estimated by end point titration.

Human nonprecipitating antidiphtheria serum (included in Table I) has been reported to transmit smooth muscle sensitivity to guinea pig<sup>16</sup> as well as passive cutaneous reactivity.<sup>10</sup> This we have confirmed.\*

Our experience with nonprecipitating guinea pig antbovine gamma globulin serum duplicates the latter report.

Eighteen guinea pigs were sensitized by a single intracardial injection of 1 ml. of 10 per cent bovine gamma globulin solution in saline. On the fifth, ninth, twelfth, fifteenth, and eighteenth days after injection of the offending antigen, 2 to 4 ml. of blood was withdrawn by cardiac puncture. Three guinea pig antbovine gamma globulin sera (one obtained on the fifth, two on the ninth day after sensitizing injection) were able to induce passive cutaneous reactions

\*Subsequent to the first draft of this report, we have examined a human antidiphtheria serum supplied through the courtesy of Dr. A. M. Pappenheimer of New York University and another human antidiphtheria serum from our service at the Roosevelt Hospital. Both sera were nonprecipitating but gave a positive Prausnitz-Küstner reaction and a positive Schultz-Dale phenomenon, as well as positive PCA reactions, in dilutions as high as 1:256. The diphtheria toxoid concentrate (1950 Lf per milliliter) used in several dilutions as test antigen for precipitation, Schultz-Dale and PCA, was supplied through the courtesy of Dr. J. Freund by the Department of Health, City of New York. The purified diphtheria toxoid (50 Lf per milliliter) for skin testing as well as booster injections was donated by the Department of Public Health, Commonwealth of Massachusetts, Division of Biologic Laboratories.

(with end point titrations of 1:16, 1:64, and 1:128) and, when pooled, transferred smooth muscle sensitivity to normal guinea pig (positive passive systemic anaphylaxis) as well as to isolated intestinal segments (positive Schultz-Dale phenomenon). Precipitins in these sera were not detectable by classical test tube precipitation or by Oudin's agar gel diffusion.<sup>12</sup> Of these three animals, one died and the remaining two developed precipitins. Antisera of the other animals in the group either simultaneously transferred passive cutaneous reactivity to guinea pig skin and contained precipitins or simultaneously failed in both.

Furthermore, we were unable to elicit any anaphylactic phenomenon (passive systemic anaphylaxis, Schultz-Dale, or PCA), using rabbit anti-ragweed sera\* to transfer sensitization and fraction 2 of low ragweed pollen extract<sup>19</sup> as the challenge. Precipitins in these sera, against fraction 2 of the low ragweed extract (determined by Oudin method), were repeatedly positive. Precipitation of normal rabbit serum by fraction 2, as controls, failed.

*From the above-cited results, it seems that neither the passive cutaneous reactivity (PCA) nor passive muscle-sensitizing activity in guinea pigs is quantitatively or qualitatively related precipitin content of sensitive sera.*

**Fixation of PCA Reactivity.**—Presence of normal serum (if used for preparation of sensitive serum dilutions) strongly interferes with fixation of antibody responsible for sensitization of guinea pig skin (PCA). Ovary and Bier,<sup>20</sup> using two rabbit antisera (antiovalbumin and antipneumococcus S.S.S. III), have demonstrated, by PCA end point titrations, that rabbit sensitive serum diluted in saline is capable of inducing passive cutaneous reactivity in about 50 to 100 times greater dilution than when diluted in normal rabbit serum.

Results of an extension and several variations of the quoted study are tabulated in Table II. Section A confirms the results reported by Ovary and Bier.<sup>20</sup> The variations of this model experiment (Sections B and C) ascertain that the intensity of passive sensitivity in guinea pig skin (PCA) following transfer of rabbit or human antisera (*heterologous serum transfers*), as well as guinea pig antisera (*homologous serum transfer*), is dependent upon quantitative relationship of antibody and serum proteins.

Experiments with sensitive euglobulin (essentially pure gamma globulin) isolated from induced human horse-serum-sensitive serum and fractions (euglobulin and albumin) of normal human serum (Krs.) are listed in Table II, Section D. In this section tabulated results reveal that the normal euglobulin, which is a fraction of normal human serum (Krs.), when in the same concentration as in the whole serum (1.17 per cent), inhibits the PCA reactions as much as the whole normal serum (Krs.). In contrast, the normal albumin, also a fraction of the same normal human serum (Krs.), in the same concentration as in the whole serum (4.28 per cent) does not inhibit the passive cutaneous sensitivity (PCA). In other experiments, to be reported later, the normal euglobulin solutions (Krs.) of double (2.34 per cent) and greater

\*Rabbits were sensitized with alum-precipitated low ragweed pollen extract, containing fraction 1, fraction 2 and fraction 3.<sup>10</sup>

TABLE II. THE EFFECT OF NORMAL SERUM AND OF FRACTIONS OBTAINED FROM NORMAL SERUM UPON PASSIVE CUTANEOUS ANAPHYLAXIS

| SEC-<br>TIONS | ANTIBODY<br>0.1 ML. OF SENSITIVE<br>SERUM INTRA-<br>DERMALLY                                                         | ANTIGEN<br>SIX HOURS AFTER<br>PASSIVE TRANSFER<br>OF ANTIBODY<br>INTRACARDIALLY | DILUTIONS OF SENSITIVE SERUM<br>PREPARED WITH                                                                                        | EVALUATIONS OF PASSIVE CUTANEOUS ANAPHYLAXIS<br>IN GUINEA PIG SKIN (a) |   |    |    |    |     |     |     |     |      |
|---------------|----------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------|---|----|----|----|-----|-----|-----|-----|------|
|               |                                                                                                                      |                                                                                 |                                                                                                                                      | DILUTIONS 1:                                                           |   |    |    |    |     |     |     |     |      |
|               |                                                                                                                      |                                                                                 |                                                                                                                                      | 4                                                                      | 8 | 16 | 32 | 64 | 128 | 256 | 512 | 800 | 1600 |
| A             | Rabbit antiragweed                                                                                                   | Ragweed pollen<br>extract                                                       | 0.85% saline<br>Normal rabbit serum                                                                                                  | +                                                                      | + | +  | +  | +  | +   | +   | +   | +   | -    |
| B             | Human antihorse se-<br>rum                                                                                           | Horse serum                                                                     | 0.85% saline<br>Normal human serum (Krs.)                                                                                            | +                                                                      | + | +  | +  | +  | +   | +   | +   | +   | +    |
| C             | Guinea pig antihorse<br>serum                                                                                        | Horse serum                                                                     | 0.85% saline<br>Normal guinea pig serum                                                                                              | +                                                                      | + | +  | +  | +  | +   | +   | +   | -   | -    |
| D             | Euglobulin derived<br>from a human anti-<br>horse serum sensi-<br>tive serum 0.5%<br>solution in 0.85%<br>saline (e) | Horse serum                                                                     | 0.85% saline<br>Normal human serum (Krs.)<br>Normal human euglobulin<br>1.17% (Krs.) (d)<br>Normal human albumin 4.27%<br>(Krs.) (d) | +                                                                      | + | +  | +  | +  | +   | +   | +   | +   | -    |

(a) Each PCA evaluation represents an average of three experiments.  
(b) Similar results were obtained in experiments using rabbit antiovine-albumin immune system.  
(c) Similar results were obtained in experiments using guinea pig antiovine-albumin immune system.  
(d) The water-soluble normal human euglobin and normal human albumin isolated from normal human serum (Krs.) were dissolved in 0.85% saline in about the same proportion as found in the whole serum (euglobin 1.17%, albumin 4.27%).  
(e) Serum was obtained from a patient who had suffered serum sickness following administration of horse tetanus antitoxin (induced type of sensitivity).

(4.68 per cent) amounts of the concentration used in the experiment tabulated in Section D (normal euglobulin, 1.17 per cent), showed that the inhibition of PCA increased proportionately to the increase of concentration of normal euglobulin (Krs.) solutions used for preparation of sensitive euglobulin dilutions.

From these experiments, it is apparent that the fixation of antibody gamma globulin to guinea pig skin is interfered with and the PCA reactions are inhibited when the content of normal serum gamma globulin is increased.

*The passive sensitization of guinea pig skin (PCA) seems to be dependent upon competitive fixation of reactive antibody gamma globulin and serum gamma globulin. The ratio of antibody to serum globulin influences the degree of saturation of the skin receptors with reactive and nonreactive gamma globulins; the intensity of the PCA reaction varies accordingly.*

*Effect of Drugs on the PCA Reaction.*—In an attempt to relate the mechanism of passive cutaneous anaphylaxis of the guinea pig to a mediatory action of substances possibly liberated in the course of antibody-antigen interaction, drugs known to influence allergic mechanisms were tested for their effectiveness upon the PCA reaction. Sensitive sites in guinea pigs were prepared with dilutions of 0.5 per cent induced human horse-serum-sensitive euglobulin, and six hours later the challenging doses of a mixture of horse serum, Evans blue, and either antihistamine, heparin,\* hydrocortisone,† or epinephrine were injected intracardially.

The results are compiled in Table III and show that antihistamine, heparin, or hydrocortisone is able only to reduce the intensity of passive cutaneous anaphylaxis as evidenced by decrease of end point titers and reduction of size of positive reactions. The effectiveness of these three drugs seems to be limited by the role and/or the degree of importance of the partial mechanism in PCA antagonized by their pharmacodynamic activity. Administration of increased doses did not promote an increase of inhibition. The results recorded in experiments with epinephrine, a potent constrictor of the skin vessels, seem to emphasize the importance of vasoconstriction, a pharmacomechanic interference, with the development of PCA.

*Gross Pathology and Histopathology of PCA Reaction.*—Preliminary experiments and observations indicated a need for several minor modifications of the experimental procedure for macroscopic and microscopic observations of the development of passive cutaneous anaphylaxis in guinea pigs.

One per cent and 3 per cent saline dilutions of water-soluble euglobulin isolated from induced human horse-serum-sensitive serum were employed for transfer of sensitivity. Dilutions (1 per cent and 3 per cent) of normal human euglobulin derived from normal nonallergic serum served as nonreactive controls. The 1 per cent solution of sensitive gamma globulin, in amounts of

\*Heparin, an inhibitor of Arthus<sup>21</sup> and Schwartzman phenomena,<sup>22</sup> prevents thrombus formation. Being an ingredient of hyaluronidase inhibitor,<sup>22</sup> it increases the viscosity of blood and diminishes the permeability of intracellular substances.<sup>23</sup>

†The water-soluble hydrocortisone has, as heparin, an antihyaluronidase activity and also a relaxing effect on guinea pig smooth muscle, antagonizing histamine.<sup>24</sup>

TABLE III. THE INHIBITORY EFFECT OF SOME DRUGS UPON PASSIVE CUTANEOUS ANAPHYLAXIS IN GUINEA PIGS

| 0.1 ML. OF SENSITIVE SERUM<br>INTRADERMALLY                                                                | ANTIGEN<br>SIX HOURS AFTER<br>PASSIVE TRANSFER OF<br>ANTIBODY | SIMULTANEOUSLY WITH<br>ANTIGEN INTRACARDIALLY<br>INJECTED: | EVALUATION OF PASSIVE CUTANEOUS ANAPHYLAXIS IN<br>GUINEA PIG 60 MINUTES AFTER CHALLENGE (a) |    |     |     |     |     |      |  |  |  |
|------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------|------------------------------------------------------------|---------------------------------------------------------------------------------------------|----|-----|-----|-----|-----|------|--|--|--|
|                                                                                                            |                                                               |                                                            | DILUTIONS 1:                                                                                |    |     |     |     |     |      |  |  |  |
|                                                                                                            |                                                               |                                                            | 0                                                                                           | 10 | 100 | 200 | 400 | 800 | 1000 |  |  |  |
| Euglobulin derived from a hu-<br>man horse-serum-sensi-<br>tive serum 0.5% solution in<br>0.85% saline (f) | 0.2 ml. horse serum                                           | Control without drug                                       | +                                                                                           | +  | +   | +   | +   | +   | +    |  |  |  |
|                                                                                                            |                                                               | 3 mg. antihistamine (b)                                    | +                                                                                           | +  | +   | +   | +   | +   | +    |  |  |  |
|                                                                                                            |                                                               | 5 mg.                                                      | +                                                                                           | +  | +   | +   | +   | +   | +    |  |  |  |
|                                                                                                            |                                                               | 7 mg.                                                      | +                                                                                           | +  | +   | +   | +   | +   | +    |  |  |  |
|                                                                                                            |                                                               | 11 mg.                                                     | +                                                                                           | +  | +   | +   | +   | +   | +    |  |  |  |
|                                                                                                            |                                                               | 3 mg. heparin (c)                                          | +                                                                                           | +  | +   | +   | +   | +   | +    |  |  |  |
|                                                                                                            |                                                               | 5 mg.                                                      | +                                                                                           | +  | +   | +   | +   | +   | +    |  |  |  |
|                                                                                                            |                                                               | 7 mg.                                                      | +                                                                                           | +  | +   | +   | +   | +   | +    |  |  |  |
|                                                                                                            |                                                               | 9 mg.                                                      | +                                                                                           | +  | +   | +   | +   | +   | +    |  |  |  |
|                                                                                                            |                                                               | 10 mg. hydrocortisone (d)                                  | +                                                                                           | +  | +   | +   | +   | +   | +    |  |  |  |
|                                                                                                            |                                                               | 15 mg.                                                     | +                                                                                           | +  | +   | +   | +   | +   | +    |  |  |  |
|                                                                                                            |                                                               | 20 mg.                                                     | +                                                                                           | +  | +   | +   | +   | +   | +    |  |  |  |
|                                                                                                            |                                                               | 25 mg.                                                     | +                                                                                           | +  | +   | +   | +   | +   | +    |  |  |  |
|                                                                                                            |                                                               | 0.01 mg. adrenalin (e)                                     | +                                                                                           | +  | +   | +   | +   | +   | +    |  |  |  |
|                                                                                                            |                                                               | 0.1 mg.                                                    | +                                                                                           | +  | +   | +   | +   | +   | +    |  |  |  |

(a) Each PCA evaluation represents an average of three to five experiments.

(b) Chlor-Trimeton (chlorpheniridine) maleate, Schering Corporation.

(c) Isotonic solution of Liqueair: sodium (brand of heparin), Organon, Inc.

(d) Intravenous Solu-Cortef (hydrocortisone sodium succinate), The Upjohn Company.

(e) Epinephrinehydrochloride (also containing chlorbutanol and sodium bisulfite), Armour Laboratories.

(f) Serum was obtained from a patient who had suffered serum sickness following administration of horse tetanus antitoxin (induced type of sensitivity).

0.05 ml. per site, was able to induce minimal PCA (7 mm. in diameter) in 1:800 dilution; the 3 per cent solution, 0.05 ml. per site, titrated as far as 1:2,400.

Two skin sites were prepared with 0.05 ml. on each side of the abdominal wall, one with the sensitive gamma globulin and the other with the corresponding dilution of normal gamma globulin. The initial sensitizing intradermal injection bleb was 2 to 3 mm. in diameter. It rapidly flattened and spread out. In one hour the margins became diffuse. By the second hour it was difficult to identify the site of the injection. After three or four hours the skin had a normal appearance. Whenever, due to individual intolerance of the guinea pig skin, an irritant effect of the injected material occurred, such as slight swelling or mild erythema, the animal was eliminated from further experimentation.

The histology of the passively sensitized site before challenge with antigen was that of a very mild, nonspecific, inflammatory reaction with its maximum at the third hour after the injection. It consisted mainly of polymorphonuclear leukocytes, both diffuse and perivascular. This very mild infiltration was confined solely to the initially injected site and the adjacent area, totaling about 5 to 6 mm. in diameter. Toward the sixth hour, the infiltration had undergone major involvement, and it was of minimal density eight hours after the initial injection. On the basis of the microscopic findings, it was decided to begin the further observation eight hours after the initial intradermal injection of sensitizing material on both *the quiescent (before challenge) sensitized sites and the reacting (after antigen-dye challenge) sensitized sites*.

The skin on the belly, including the subcutaneous connective tissue and subdermal layer containing cutaneous blood-supply vessels, was cut along the entire midline, carefully separated from the abdominal musculature, and reflected for about 3 to 5 cm. The parallel venules and arterioles, as well as capillaries, of the *quiescent sensitized site* (that is, before challenge), viewed by means of thirtyfold surface magnification, were of unchanged size, the venules being slightly larger than the corresponding arterioles. There was no obstruction of the circulation due to contraction of the vessels or obliteration of the lumina by leukocytes or erythrocytes. At this point, the animal was sacrificed and speedily drained of blood. The subdermal layer was stripped away and the skin and subcutaneous connective tissue were exposed. The blood-free vessels in a quiescent sensitized site collapsed and became grossly invisible in the connective tissue. Only a faint yellowish discoloration, about 3 to 4 mm. in diameter, indicated the initial intradermal injection bleb.

The same technique was employed for observation of changes in the *reacting sensitized areas* (that is, after challenge with antigen-dye mixture). For the photographs of reacting areas on the reflected skin, the animals were sacrificed and bled out at times specified in the legends for the respective illustrations, and the subdermal layer was removed from the skin and subcutaneous connective tissue as described above.

The major changes in the *reacting sensitized area* occur within ten minutes after the intracardial challenge with antigen-dye mixture. Fifty to ninety seconds after the challenge, an elliptical area of the skin (about 40 by 20 mm.), surrounding the site of initial intradermal injection of sensitized material, flares up. The skin becomes vividly erythematous for several seconds (Plate I, *A*). The hyperemic flare is caused by an instant distention of capillaries and venules (Plate I, *B*). Arterioles seem not to take part in the reaction, and neither dilate nor contract. The flow of blood into the reacting area continues, and the dilation of venules and capillaries increases as more

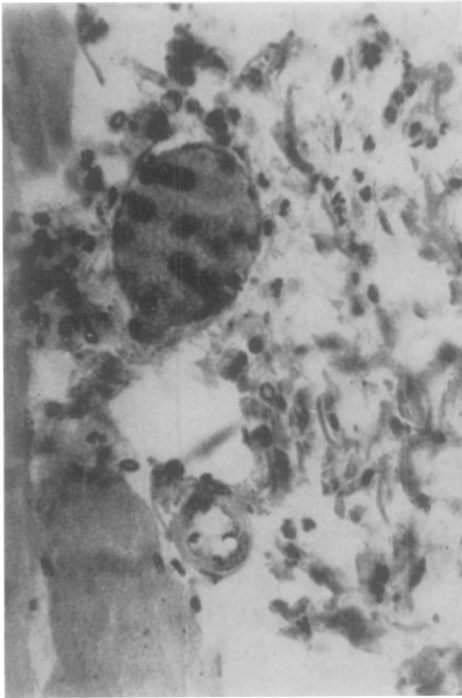


Fig. 1.

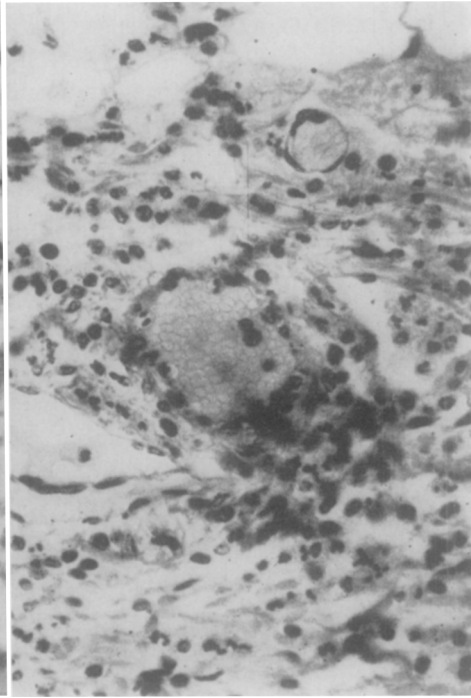


Fig. 2.

Fig. 1.—A venule, congested with erythrocytes and a patent, not contracted arteriole. The perivascular infiltration by degenerate leukocytes is a residue of a moderate, nonspecific inflammatory reaction caused by the intradermal injection of sensitizing gamma globulin eight hours before the challenge and the onset of the PCA reaction.

Fig. 2.—A capillary and a venule, both maximally congested with erythrocytes, shortly after the onset of the PCA. The perivascular infiltration rapidly increases by diapedesis of leukocytes.

blood cells enter (Figs. 1 and 2). The movement of erythrocytes in the engorged veins and capillaries is almost completely halted at the point of highest congestion. White blood cells accumulate at the walls of the venules, at times in layers several cells thick. Some leave the lumen and enter the surrounding tissues by rapid diapedesis, some adhere to the endothelial lining of the venules, and some form clusters among the congested erythrocytes (Figs. 3 and 4). The appearance of leukocytic clusters coincides with a short but

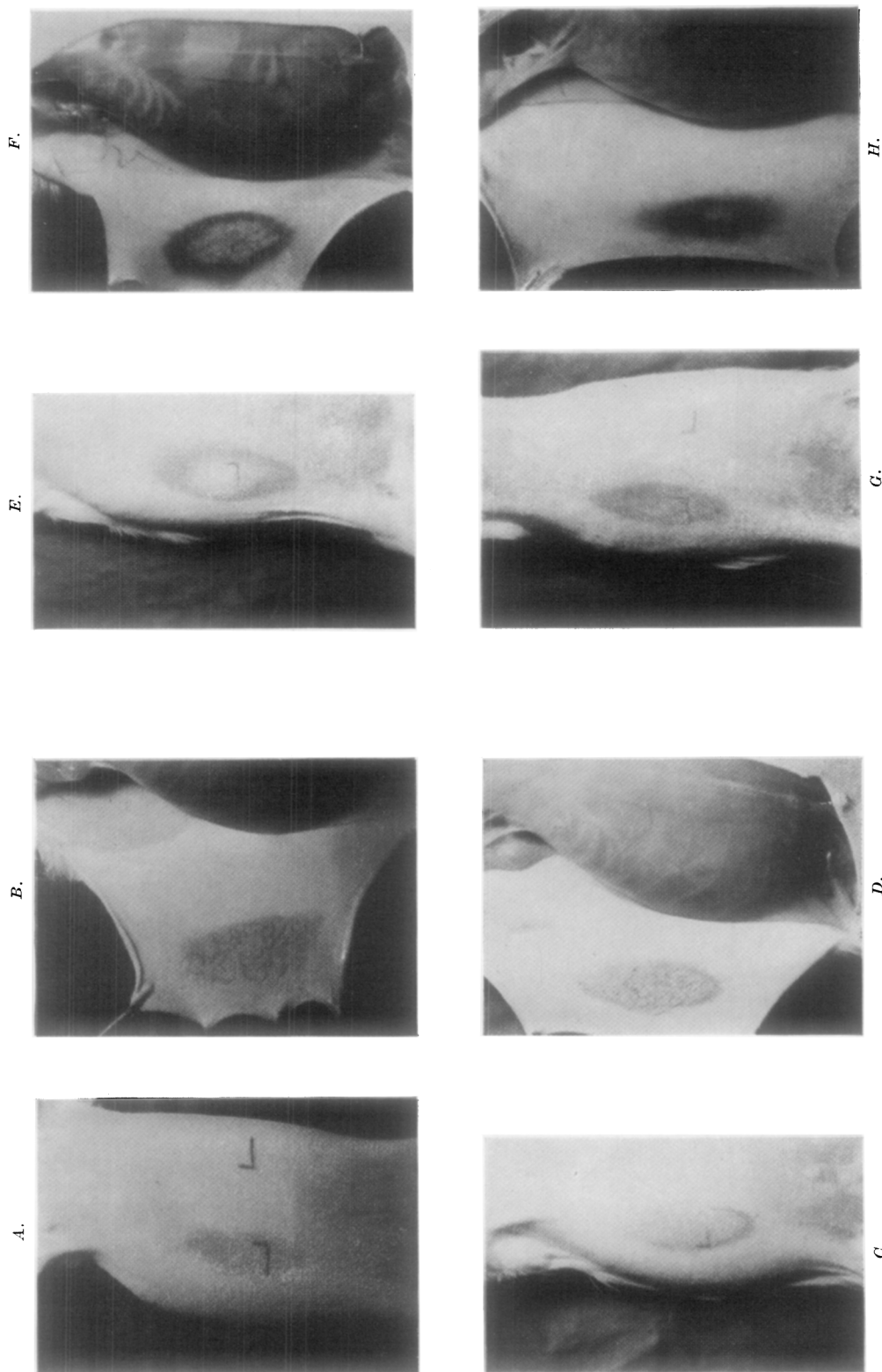


Plate I.—Illustration of chronological development of PCA reactions in guinea pigs. Gamma globulin, derived from human induced horse-serum-sensitive serum, was used for passive transfer of PCA sensitivity; 0.05 ml. of 3 per cent dilution was injected intradermally into guinea pigs A and B; and 0.05 ml. of 1 per cent dilution was injected in guinea pigs C through H. (Nonreactive gamma globulin derived from human nonallergic serum served as control. See guinea pigs A and G.) Eight hours later the antigen (horse serum) with Evans blue was administered intracardially and the development of PCA reactions photographed at intervals as follows:

A, PCA on the skin surface 50 to 90 seconds after challenge.  
 B, PCA on the reflected skin 50 to 90 seconds after challenge.  
 C, PCA on the skin surface 70 to 120 seconds after challenge.  
 D, PCA on the reflected skin 10 to 12 minutes after challenge.  
 E, PCA on the skin surface 3 to 5 minutes after challenge.  
 F, PCA on the reflected skin 3 to 5 minutes after challenge.  
 G, PCA on the skin surface 10 to 12 minutes after challenge.  
 H, PCA on the reflected skin 10 to 12 minutes after challenge.

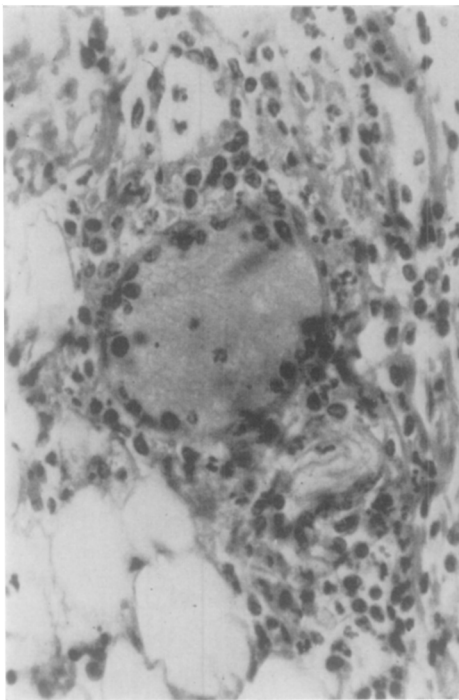


Fig. 3.

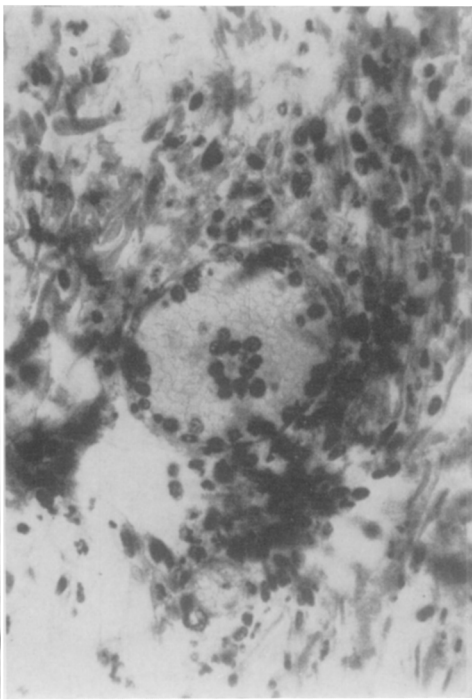


Fig. 4.

Fig. 3.—A maximally dilated venule, congested with erythrocytes. Leukocytes adhere to the endothelial lining. Note the interstitial edema with infiltration.

Fig. 4.—A venule with changes as in Fig. 3. In addition to a number of white blood cells adherent to the wall, there is a cluster of leukocytes in the center of the congestion.

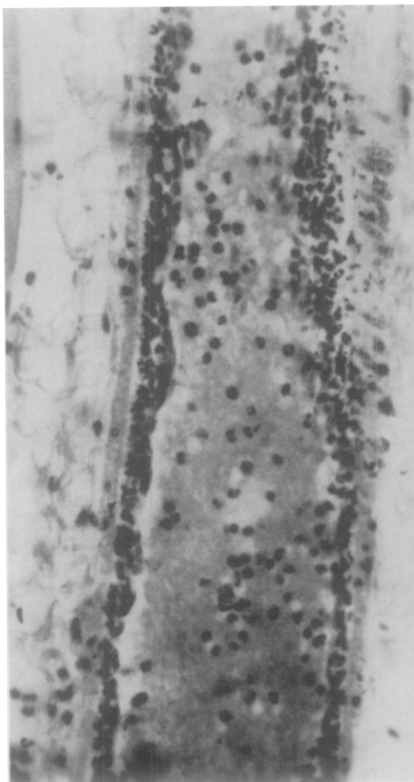


Fig. 5.

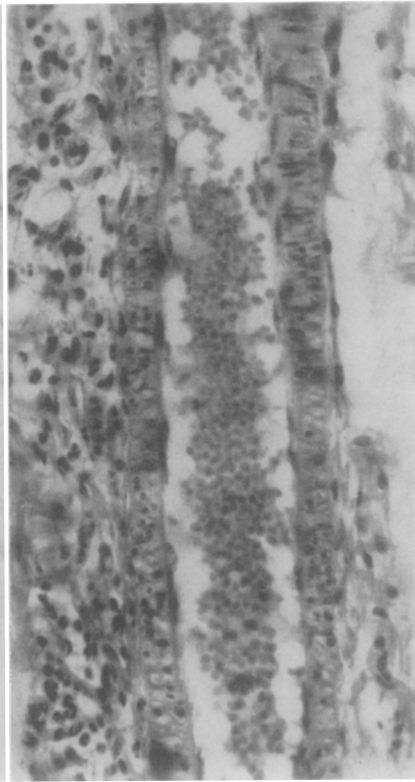


Fig. 6.

Fig. 5.—A venule with an aggregate of erythrocytes. The whole endothelial lining is lifted away from the musculature of the venule wall by intruded leukocytes.

Fig. 6.—No such changes are found in the concomitant arteriole. The lumen is patent to blood flow. There is no adherence or clustering of leukocytes. The endothelial lining is free of changes.

complete arrest of flow movement in the affected venules. This aggregation and compression of erythrocytes reaches its maximum four to six minutes after the challenge.

The endothelial cells of the venules are gravely damaged. The cellular protoplasm is swollen and the nuclei are pyknotic and achromic. In some venules, the leukocytes creep under the damaged endothelium and separate the endothelial lining from the musculature of the vessel wall (Fig. 5). No such changes can be observed in the concomitant arterioles (Fig. 6).

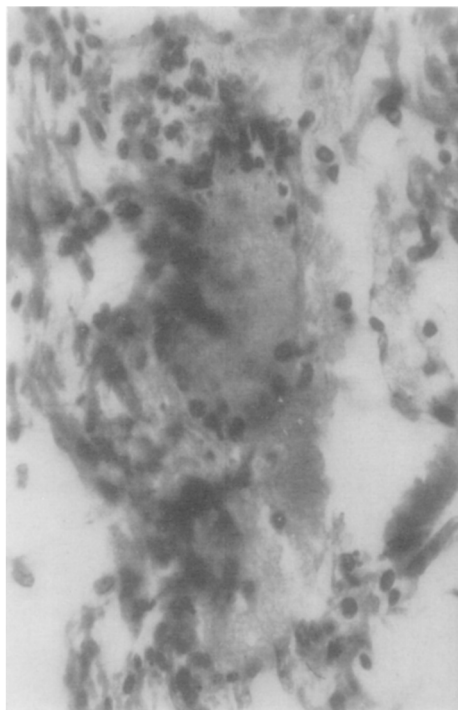


Fig. 7.

Fig. 7.—An engorged venule surrounded by a mass of extravasated erythrocytes caught in adjacent connective tissue.

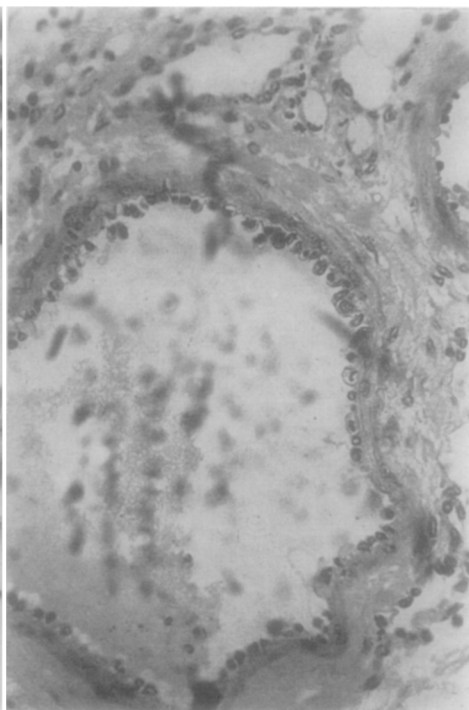


Fig. 8.

Fig. 8.—A venule freed of erythrocytic congestion. The endothelial lining is covered by adherent white cells.

At the time of the initial flare-up (fifty to ninety seconds after challenge) and throughout the above-described process to maximal congestion, extravasations of erythrocytes occur alongside the venules. The intensity and number of these extravasations are proportionally related to the sensitizing dose of the material used for passive sensitization of the skin site. The extravasations are markedly more numerous in the reacting areas sensitized with 3 per cent solution of sensitive gamma globulin than in those sensitized with 1 per cent solution (Plate I, compare *B* and *D*). The circulation in the venules interrupted by extravasation of erythrocytes ceases and the blood cells are caught permanently in the connective tissue surrounding the venule (Fig. 7).

Simultaneously with (or a few seconds after) the initial hyperemic flare, an exudation of the blue-colored plasma begins on the periphery of the reaction, demarcating the outer areas of sensitization (Plate I, *C* and *D*). Five minutes later, the blue effusion has spread centripetally and forms a band around the reacting site (Plate I, *E* and *F*).

A difference in intensity and speed of changes, within the outer and central portions of the reacting area, affecting the flow rate of blood, may be responsible for the early peripheral and delayed central exudation. It would seem that the flow of blood in the marginal areas is slowed down by dilatation of the venules and capillaries, but not brought to a complete stop by clustering and adherence of leukocytes and peeling of the endothelial lining, as noted in the venules of the central portion of the reacting area (the outer exudation starts immediately after the onset of the reaction). The flow of blood in the central areas is almost completely halted and, for a short period of time, is arrested; thus, the central exudation is delayed during the time of circulation stoppage.



Fig. 9.—Six hours after the initial flare, the subcutaneous edema is of maximal thickness (about 3 to 4 mm.). The cellular infiltration is now homogeneously distributed throughout the edematous connective tissue. There are a venule and concomitant arteriole, both free to flow of blood.

Approximately four to six minutes after the beginning of the reaction, the masses of red blood cells in the venules of the central area break apart and begin to move. The erythrocytic aggregates, as well as the clusters of white blood cells occluding the lumina and thickly covering the endothelium, disappear in the circulation. With the blood flowing through the dilated capillaries and veins, many of them stripped of their endothelial lining, voluminous exudation begins instantly. Within several minutes, the entire reacting area is covered and the tissues are soaked with blue-colored plasma (Plate I, *G* and *H*).

The preparation of the skin for the photographs is described above. The dilated veins, freed of obstructing masses of erythrocytes and leukocytes, are

emptied of circulating blood and are collapsed in the subcutaneous connective tissue (Fig. 8). Veins permanently blocked by extravasation of red cells and those, at the time of exposition after the animal has been sacrificed, still plugged and engorged by erythrocytic and leukocytic thrombi, show in the Kodachromes of PCA reactions (Plate I, *B, D, F*, and *H*).

For six hours after the initial flare-up, the subcutaneous interstitial edema gradually increases in thickness to 3 to 4 mm. (Fig. 9) and centrifugally in size to 65 by 35 mm. Thereafter, no apparent increase of edema occurs. During the time of actual reaction (first ten minutes) and thereafter, a great number of polymorphonuclear and mononuclear white blood cells leave the circulation by rapid diapedesis. The thick, essentially perivascular infiltration (Fig. 10) spreads homogeneously throughout the reacting site simultaneously with increase of the interstitial edema fluid, entering into spaces between the dissociated connective tissue fibrils (Fig. 9). In the six- to eight-hour lesions, many leukocytes are found to be already degenerate. No further major quantitative increase or qualitative change (such as eosinophilic or

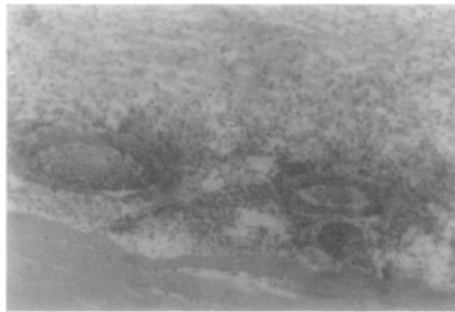


Fig. 10.—A venule with a mass of leukocytes adhering to the endothelium, obstructed by an erythrocytic aggregate, and a concomitant arteriole free of such changes. The vessels are surrounded by a dense perivascular infiltration. The infiltrative cells begin to spread into spaces between the dissociated connective tissue fibrils.

mononuclear reactions) of the infiltration becomes evident. In twelve to thirty-six hours the edema undergoes a progressive and total resorption without any tissue damage, such as necrosis of connective tissue or epithelium of the skin. In thirty-six-hour lesions there are still some pyknotic polymorphonuclear leukocytes. There is also a fresh, diffuse, but sparse, infiltration by mesenchymal cells, histiocytes, and local proliferative-reparatory reaction around veins obliterated by extravasation of erythrocytes. However, within three days after the reaction, all remnants of edema and cellular elements completely disappear, leaving no trace of damage.

*Comment on the Pathology of PCA Reaction.*—Passive cutaneous anaphylaxis in the guinea pig is a vascular phenomenon with dominant venous and capillary manifestations. It is the result of an interaction (*occurring simultaneously at all sensitive points*) between passively fixed antibody and corresponding antigen brought in via circulation. The fixation of antibody occurs during the resorption of sensitizing material in the cutaneous network of

capillaries and venules, and is conditioned by binding affinity of globulins to guinea pig skin. (There seem to be globulins which are not bound, such as human blocking antibody,<sup>11</sup> and horse,<sup>8</sup> bovine, and goat gamma globulins.<sup>25</sup>) During the PCA reaction, a hyperemic flare develops as a result of dilatation of venules and capillaries. Arterioles do not seem to contract or dilate. Possibly the hydrodynamic pressure in the venules and capillaries increases due to dilatation of the lumina,\* and as a result the damaged vessel walls become maximally pervious to plasma.

In contrast to our findings stands the work of Abell and Schenck,<sup>27</sup> who observed microscopically a reaction in the ear of an actively sensitive rabbit after introduction of antigen (Arthus phenomenon). Arteriolar contraction with obliteration of the lumina and shutting-off of the circulation in the reacting area was noted. Their observation is extended by Benacerraf and Kabat,<sup>28</sup> who noted in passive Arthus phenomenon in guinea pig skin a contraction and fragmentation of arterioles after intradermal injection of antigen. In the PCA there is no evidence of a protracted spastic constriction of arterioles and, also contrary to the observations by Abell and Schenck,<sup>27</sup> there is a maximal dilatation of venules and capillaries. The intravenous changes in the PCA (adherence and clustering of leukocytes, endothelial damage, congestion, and extravasation of erythrocytes) are similar to: (1) those seen by Lecomte and Hugues<sup>29</sup> in mesenteric vessels of sensitive rabbits and guinea pigs after contact with antigen, (2) those reported by Irwin, Weille, and Burrage<sup>30</sup> during systemic anaphylaxis in the guinea pig, and (3) those reported by Abell and Schenck<sup>27</sup> in the ear venules of sensitive rabbits after antigen has been repeatedly instilled.

To dispel doubts and ascertain a distinction between PCA (an interaction of intradermally *fixed* antibody and circulating antigen) and reversed Arthus phenomenon (an interaction of circulating antigen and intradermally *instilled* antibody), both reactions were demonstrated with identical antisera in the same guinea pig.

On one side of the belly a skin site was injected intradermally with anti-serum (0.1 ml. of 0.5 per cent or 1.5 dilutions of induced horse-serum-sensitive gamma globulin). Eight hours later, antigen (0.5 ml. of horse serum) and dye were injected in the circulation. PCA reaction developed. Next, the identical antiserum was injected intradermally on the other side of the belly. At this skin site, a reversed Arthus phenomenon (severe with 1.5 per cent, moderate with 0.5 dilution) developed within six to eight hours. The changes at the reversed Arthus phenomenon site were similar to the changes described for Arthus phenomenon† resulting in a necrosis of connective tissue and epithelium within eight to thirty-six hours<sup>31, 32</sup> contrary to the evanescent changes at the site of PCA reaction.

\*Bernoulli's theorem<sup>26</sup> states that along a stream line where the velocity of a fluid is high (narrow lumen) the hydrodynamic pressure on the wall is low or negative, where the velocity is low (wide lumen) the pressure is high.

†That is, blanching of the reactive site for three to four hours' contraction and fragmentation of the arterioles<sup>27, 28</sup> centrifugally developing edematous infiltration,<sup>31</sup> quantitative and qualitative changes of cellular infiltration.<sup>31, 32</sup>

## SUMMARY

1. In view of present experiments, the immunologic property of antisera to induce passive cutaneous sensitization to guinea pig skin is attributed to the guinea pig smooth-muscle-sensitizing antibody, not conditioned by presence of precipitins. The human-skin-sensitizing antisera property (of human or animal origin) is neither related to the muscle-sensitizing property nor responsible for the induction of passive sensitization of guinea pig skin (PCA).

2. The fixation of antibody responsible for passive cutaneous reactivity is dependent upon competitive fixation of reactive antibody gamma globulin and serum gamma globulin. The ratio of antibody to serum globulin seems to determine the degree of saturation of the skin receptors with reactive and nonreactive gamma globulin.

3. The efficacy of antihistamine, heparin, and hydrocortisone to inhibit passive cutaneous anaphylaxis in guinea pig is limited. Administration of increased doses does not promote increased inhibition. Nontoxic doses of adrenaline are the most effective. The vasoconstricting effect of adrenaline appears to emphasize the vasodilation as an important partial mechanism in passive cutaneous anaphylaxis.

4. Passive cutaneous anaphylaxis in the guinea pig is a manifestation of an interaction between a passively fixed antibody and circulating antigen simultaneously at all points of sensitivity. It is a vascular phenomenon with dominant venous and capillary manifestations, rather than an arteriolar response as in Arthus phenomena. This is evidenced by the macroscopic observations and the microscopic findings in passive cutaneous anaphylaxis.

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