

## STUDIES IN PENICILLIN HYPERSENSITIVITY

### IV. ANTIGENIC PROPERTIES OF ALTERED PROCAINE PENICILLIN

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**R**EAGINS to penicillin have been found in the sera of many patients following allergic shock reactions produced by this antibiotic. We<sup>1</sup> found reagins in eleven successive cases of severe immediate allergic shock reactions resulting from penicillin administration. There are, however, occasional reports of failure to find antibodies to penicillin in similar cases. It is the purpose of this article to present experimental findings which may account for such negative reports.

This study began with the investigation of Patient M. F., a 60-year-old housewife, who on April 18, 1954, experienced an almost fatal allergic shock reaction following an intramuscular injection of 300,000 units of procaine penicillin G (Pfizer), which had been given for a sore throat. Within a few minutes, the patient developed severe generalized pruritus, dyspnea, asthmatic breathing, and loss of consciousness. Epinephrine was administered and the patient was hospitalized. After several hours, she began to improve. She eventually recovered with no untoward sequelae.

This patient, who gave a negative family history for atopic illnesses, had developed bronchial asthma at the age of 45. The attacks were severe, requiring injections of epinephrine for relief of symptoms. Skin tests revealed only a positive reaction to wool. The asthma improved when the patient stayed in a warm climate. At the age of 50, she underwent a tonsillectomy and adenoidectomy. Subsequently, the asthmatic attacks gradually subsided and eventually disappeared.

Since 1947, M. F. had received many injections of penicillin without untoward reactions. The last of these had been given approximately four months before the allergic shock reaction.

Direct intracutaneous tests with penicillin were not performed on this patient. It has been our<sup>1, 2, 3</sup> experience and that of others that patients who have recently had anaphylactic reactions to penicillin are prone to develop similar reactions following skin tests with this antibiotic. We have consistently demonstrated circulating reagins to penicillin in these cases, although at times the reagins have been found to be transient, disappearing from the circulation within several months after the last clinical reaction.<sup>1</sup> In such highly sensitive patients, with reaginic sensitivity to penicillin, a safe dilution for direct skin testing has not been established. Moreover, the presence of a positive skin reaction, when elicited by direct test, does not in itself establish the presence or absence of circulating reagins. Thus, it has

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been our practice to employ the passive transfer technique, not only because it is a nonhazardous procedure, but also because it permits the detection of reagins (circulating antibodies) to penicillin in these patients.

In preliminary passive transfer studies made with the patient's serum, tests with crystalline penicillin G in concentrations up to 50,000 units per milliliter yielded negative results. This was surprising in view of the fact that in all the sera of our previous cases, positive passive transfer reactions were demonstrated with this form of penicillin, often in higher dilutions than 50,000 units per milliliter. In the light of this apparent discrepancy, further studies seemed indicated in the search for the agent which was responsible for the shock reaction in this patient.

#### MATERIALS AND METHODS

Sera for passive transfer studies were obtained from bleedings taken six days and one month after the allergic shock reaction. According to the technique of Walzer<sup>4</sup> and Bowman, sites on the arms of normal recipients were prepared by the intracutaneous injection of 0.05 ml. of serum. After a four-day interval, the sensitized sites and adjacent untreated cutaneous areas were tested with approximately 0.01 ml. of various types of penicillin solutions or other materials. After fifteen minutes, the reactions on the test and control sites were read and compared. They were graded from negative to four plus, according to the size of the wheal. The two samples of serum showed similar responses to the test materials and all results were therefore combined.

The thick consistency and irritating properties of procaine penicillin suspension rendered it unsuitable for intracutaneous testing. This was also true of the undiluted supernate obtained by centrifuging the procaine penicillin suspension. A more satisfactory preparation for skin testing was obtained by diluting the supernate with nine parts of physiologic saline. Although the irritative properties of the test allergen were considerably reduced by this dilution, nonspecific plus-minus to one-plus reactions were still occasionally obtained on the control sites with the test material.

#### I. PRELIMINARY STUDIES WITH VARIOUS PENICILLIN PREPARATIONS

The initial passive transfer studies with serum M. F. in which sites were tested with aqueous potassium penicillin G, in concentrations up to 50,000 units per milliliter, produced consistently negative results.

Passive transfer sites were prepared with serum M. F. on four normal subjects and were tested with the following substances: (1) potassium penicillin G; (2) various brands and lots of procaine penicillin G suspension (Pfizer, Lilly, Sharpe & Dohme, and Commercial Solvents Corporation (C.S.C.)); (3) the diluent of procaine penicillin G (Pfizer); and (4) a 1 per cent procaine hydrochloride solution.

The results of the tests with the procaine penicillin G suspensions varied (Table I). Four of these suspensions gave positive passive transfer reactions

(Pfizer #JKL 538098 and Sharpe & Dohme #L 7972, #L 7920, and #1123 M). Three gave negative reactions (Pfizer #42083, Lilly, and C.S.C. #BAE 113). Potassium penicillin G, the diluent of procaine penicillin G, and the 1 per cent procaine hydrochloride consistently gave negative reactions (Table I).

TABLE I. PASSIVE TRANSFER REACTIONS ON SERUM M. F. SITES TO VARIOUS PENICILLIN PREPARATIONS

| SUBSTANCE TESTED                                                          | SUBJECT A |         | SUBJECT B |         | SUBJECT C |         | SUBJECT D |         |
|---------------------------------------------------------------------------|-----------|---------|-----------|---------|-----------|---------|-----------|---------|
|                                                                           | TEST      | CONTROL | TEST      | CONTROL | TEST      | CONTROL | TEST      | CONTROL |
| Potassium penicillin G solution—Squibb (50,000 units/ml.)                 | 0         | 0       | 0         | 0       | ±         | ±       | 0         | 0       |
| Procaine penicillin G suspension—Pfizer #42083                            | 0         | 0       | 0         | 0       |           |         |           |         |
| Procaine penicillin G suspension—Sharpe & Dohme #L 7972                   | ++        | +       | ++        | 0       | +±        | 0       | +         | 0       |
| #L 7920                                                                   |           |         |           |         | +++       | ±       |           |         |
| #1123 M                                                                   |           |         |           |         | +         | 0       |           |         |
| Procaine penicillin G suspension—Pfizer #JKL 538098                       | ++        | +       | ++        | 0       | +++       | ±       | ++++      | 0       |
| Procaine penicillin G suspension—Lilly Duracillin                         | 0         | 0       |           |         |           |         |           |         |
| Procaine penicillin G suspension—Commercial Solvents Corporation #BAE 113 | ±         | +       |           |         |           |         |           |         |
| 1% Procaine hydrochloride                                                 |           |         | 0         | 0       | 0         | 0       |           |         |
| Diluent of procaine penicillin G—Pfizer                                   | 0         | 0       | 0         | 0       | 0         | 0       | ±         | ±       |

Testing preparations of procaine penicillin suspensions were made by centrifugation of the suspension and dilution of the supernate with nine parts of isotonic saline.

## II. EFFECTS OF AGING AND HEAT ON ANTIGENICITY OF PROCAINE PENICILLIN G

In the search for an explanation for the inconsistent results obtained with the various procaine penicillin preparations, the effects of aging under various conditions were investigated. A suspension of procaine penicillin G (Pfizer #43870) and a supernate obtained from that specimen were incubated for ten days at 56° C. A portion of another lot of procaine penicillin G (Pfizer #42083) was treated in a similar manner and another portion was placed in a physician's bag which was carried on daily calls and rounds for one month. A solution of 1 per cent procaine hydrochloride solution and another of potassium penicillin G containing 300,000 units per milliliter were also incubated with the above specimens. Passive transfer tests were then made with the original preparations and with the treated samples of these same preparations.

The two untreated procaine penicillin suspensions produced negative reactions. These same preparations, after incubation at 56° C. for ten days, elicited three-plus (+++) to four-plus (+++++) positive passive transfer reactions (Table II).

As a result of incubation, the suspensions changed in color from white to amber, with a reduction in the amount of suspended material. The incubated solutions became more irritating to the skin. Control tests on normal

skin with these solutions originally produced negative reactions; after incubation, these solutions gave plus-minus ( $\pm$ ) or one-plus (+) positive reactions.

The incubated supernate of suspension Pfizer #43870 produced a one-plus (+) passive transfer reaction and a plus-minus ( $\pm$ ) reaction at the control site. This weak reaction with incubated supernate suggested that the change in the antigenicity of procaine penicillin suspension was not due primarily to an alteration in the small amount of dissolved procaine penicillin in the supernate. It seemed likely that the altered antigen found in solution in the supernate following incubation of the whole suspension was derived largely from the procaine penicillin crystals.

TABLE II. PASSIVE TRANSFER REACTIONS ON SERUM M. F. SITES TO PENICILLIN ALTERED BY AGING AND HEAT

| SUBSTANCE TESTED                                   | ALTERED BY |         |                                  |         |                                       |         |
|----------------------------------------------------|------------|---------|----------------------------------|---------|---------------------------------------|---------|
|                                                    | FRESH      |         | INCUBATION AT 56° C. FOR 10 DAYS |         | STORAGE IN DOCTOR'S BAG FOR ONE MONTH |         |
|                                                    | TEST       | CONTROL | TEST                             | CONTROL | TEST                                  | CONTROL |
| Procaine penicillin G suspension—<br>Pfizer #42083 | 0          | 0       | +++                              | +       | ++                                    | +       |
| Procaine penicillin G suspension—<br>Pfizer #43870 | 0          | 0       | ++++                             | +       |                                       |         |
| Procaine penicillin G supernate—<br>Pfizer* #43870 | 0          | 0       | +                                | $\pm$   |                                       |         |
| 1% Procaine hydrochloride                          | 0          | 0       | 0                                | 0       |                                       |         |
| Potassium penicillin G (300,000<br>units/ml.)      | 0          | 0       | 0                                | 0       |                                       |         |

Testing preparations of procaine penicillin suspensions were made by centrifugation of the suspension and dilution of the supernate with nine parts of isotonic saline.

Each suspension of procaine penicillin contained 300,000 units per milliliter.

\*The supernate of procaine penicillin suspension (Pfizer) is said to contain approximately 1.5 mg., or approximately 2500 units per milliliter of procaine penicillin dissolved in the diluent.

The suspension of procaine penicillin G (Pfizer #42083) which was carried in a physician's bag for one month and which originally had given negative transfer reactions produced a positive transfer reaction at the end of this period. It gave a two-plus (++) reaction on a sensitized site and a one-plus (+) reaction at the control site.

The incubated solutions of 1 per cent procaine hydrochloride and of the potassium penicillin G showed no changes in antigenic properties and continued to give negative passive transfer reactions.

### III. FURTHER STUDIES ON THE EFFECTS OF AGE AND TEMPERATURE

The effects of temperature on the antigenicity of penicillin were studied in greater detail. Samples of the most recently prepared stocks of penicillin were obtained directly from pharmaceutical companies. Included among these were procaine penicillin G suspension (Pfizer #40190 and Lederle #3865-R570), procaine penicillin G crystals (Pfizer #40748), potassium penicillin G crystals (Squibb #3M70102), potassium penicillin O (Cer-O-Cillin, Upjohn #CF585), and benzathine penicillin G suspension (Bicillin, Wyeth

TABLE III. PASSIVE TRANSFER REACTIONS ON SERUM M. F. SITES TO PENICILLIN PREPARATIONS KEPT AT VARIOUS TEMPERATURES FROM ONE TO FOUR WEEKS

| SUBSTANCE TESTED                                                                    | ALTERED BY |   |                           |   |                             |   |         |   |                      |   |         |   |                      |   |         |   |
|-------------------------------------------------------------------------------------|------------|---|---------------------------|---|-----------------------------|---|---------|---|----------------------|---|---------|---|----------------------|---|---------|---|
|                                                                                     | FRESH      |   | REFRIGERA-<br>TION, 6° C. |   | ROOM TEMPERATURE,<br>20° C. |   |         |   | INCUBATION AT 37° C. |   |         |   | INCUBATION AT 56° C. |   |         |   |
|                                                                                     |            |   |                           |   | 2 WEEKS                     |   | 4 WEEKS |   | 2 WEEKS              |   | 4 WEEKS |   | 1 WEEK               |   | 3 WEEKS |   |
|                                                                                     |            |   |                           |   |                             |   |         |   |                      |   |         |   |                      |   |         |   |
| Procaine penicillin G suspen-<br>sion—Pfizer #40190                                 | ±          | ± | 0                         | 0 | 0                           | 0 | +       | 0 | ±                    | ± | ++      | ± | ++++                 | + | ++++*   | + |
| Procaine penicillin G suspen-<br>sion—Lederle #3865-B570                            | ±          | 0 | +                         | 0 | 0                           | 0 | ±       | 0 | ±                    | 0 | +++     | 0 | ++++                 | + |         |   |
| Procaine penicillin G crys-<br>tals—Pfizer #40748 (sus-<br>pended in saline)        | 0          | 0 | 0                         | 0 | 0                           | 0 | +       | 0 | +                    | ± | +++±    | 0 | ++                   | 0 | ++++    | ± |
| Potassium penicillin G—<br>Squibb #3M70102 (dis-<br>solved in saline)               | 0          | 0 | 0                         | 0 | 0                           | 0 | 0       | 0 | 0                    | 0 | 0       | 0 | 0                    | 0 | 0       | 0 |
| Potassium penicillin O<br>(Cer-O-Cillin)—Upjohn<br>#CF 585 (dissolved in<br>saline) | 0          | 0 |                           |   |                             |   |         |   |                      |   |         |   |                      | 0 | 0       |   |
| Benzathine penicillin G<br>(Bicillin suspension)—<br>Wyeth #2540838                 | 0          | 0 |                           |   |                             |   |         |   |                      |   |         |   |                      |   | +       | 0 |

Testing preparations of procaine penicillin suspensions were made by centrifugation of the suspension and dilution of the supernate with nine parts of isotonic saline.  
All suspensions and solutions of penicillin contained 300,000 units per milliliter, except potassium penicillin 0 which contained 200,000 units per milliliter.  
T = Test site.  
C = Control site.  
\*Positive passive transfer reaction obtained with 1:1,000,000 dilution.

#2540838). Sufficient physiologic saline was added to the crystalline preparations to make their concentrations comparable to those of the suspensions, that is, 300,000 units per milliliter. The penicillin preparations were tested on passively sensitized sites and then divided into four parts which were stored at 6°, 20°, 37°, and 56° C. Samples of each specimen were tested at various intervals during the processing period. Sterility tests were performed on all specimens to rule out bacterial or fungal growths as a factor in the experiments.

It was found that procaine penicillin G crystals (Pfizer #40748), freshly suspended in saline, gave no positive passive transfer reactions when refrigerated for one month or when kept at room temperature for two weeks (Table III). When samples of this suspension were kept at room temperature for four weeks, a one-plus (+) transfer reaction was obtained. After incubation at 37° C., a one-plus reaction (+) was obtained at two weeks and a much stronger one at four weeks (+++±). After incubation at 56° C., a two-plus (++) reaction was obtained at one week and a four-plus (+++++) reaction at three weeks. Similar results were obtained under the same experimental conditions with two suspensions of procaine penicillin G (Lederle #3865-R570 and Pfizer #40190) (Table III). After incubation at 56° C. for three weeks, procaine penicillin G (Pfizer #40190) produced a positive passive transfer reaction in saline dilutions as high as 1:1,000,000.

Potassium penicillin G and potassium penicillin O showed no changes in their antigenic properties following incubation. Benzathine penicillin G gave a one-plus (+) reaction after incubation for one week at 56° C. In its original state it had produced a negative reaction.

#### DISCUSSION

Considerable evidence has now been accumulated to indicate that the immediate allergic shock reaction to penicillin is mediated by a reaginic mechanism. We have found reagins to penicillin or its derivatives in every one of eleven cases which we have had the opportunity to study thoroughly. Some of the reported failures to find reagins in similar cases may now be explained. One possibility previously demonstrated by us<sup>1</sup> is that reagins can disappear so rapidly after the occurrence of a shock reaction that passive transfer tests performed after an interval of several weeks give negative reactions.

Another possible explanation is presented in this study. A nonallergenic suspension of fresh procaine penicillin G can become an extremely potent antigen when aged or incubated for several weeks. In tests with serum M. F., a 1:1,000,000 dilution of incubated procaine penicillin G gave positive passive transfer reactions while the concentrated fresh penicillin was inactive. There is little doubt that the allergic shock reaction experienced by Patient M. F. was caused by such an altered procaine penicillin.

The nature of the change which occurs in the procaine penicillin molecule as a result of aging is not known at this time. It is of interest that the separate components of this compound—namely, procaine and potassium penicillin G—failed to develop antigenic activity with aging. Incubated benzathine penicillin G cross-reacted slightly with altered procaine penicillin G, while potassium penicillin O did not. It may be significant that both benzathine and procaine penicillin contain two amine radicals, while the other types of penicillin tested contain only one such radical.

In view of these experiments, there is reason to question the advisability of storing procaine penicillin suspension at room temperature, as is now recommended by the manufacturers, or of keeping it in the physician's bag for prolonged periods of time. During the summer months, the rear compartment of an automobile, where the bag is usually kept, may act as an incubator. Since the experimental evidence suggests that refrigeration of procaine penicillin does not promote alteration of its allergenic properties, this type of storage would appear to be preferable.

The above experiments also cast considerable light on the limitations of the present skin-testing techniques for determining the presence of an allergy to penicillin. A negative reaction to potassium penicillin G or to fresh procaine penicillin G does not rule out the possibility of a sensitivity to altered procaine penicillin G. Under these circumstances, if tests for penicillin sensitivity are performed, the material to be injected is the test substance that should be employed. This subject will be discussed at greater length in a subsequent communication.

#### SUMMARY

1. The serum of a patient who developed an immediate allergic shock reaction following an injection of procaine penicillin G contained no reagins for potassium penicillin G or fresh procaine penicillin G. In this serum, reagins for procaine penicillin G which had been altered by aging and heating were demonstrated.

2. Fresh nonallergenic suspensions of procaine penicillin G became actively allergenic after standing for four weeks at room temperature, for two weeks at 37° C., and for one week at 56° C.

3. Refrigeration retarded the alteration of procaine penicillin. This method of storage is to be preferred to storage at room temperature or in the physician's bag, as is a common practice.

4. All allergic shock reactions to penicillin, in our experience, have been based upon a reaginic mechanism.

5. There are two possible explanations for failure to demonstrate reagins to penicillin in such cases. Since reagins to penicillin may disappear from the circulation within a few weeks after a shock reaction, improper timing of the test for reagins may explain some of the failures. Furthermore, reagins may be present to *altered* procaine penicillin G and not to crystalline penicillin G, as was the case in the studies herein presented.

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