normal serum. Failure to include a mixture containing 10^1 dilution of the FE virus plus antiserum prohibits an exact calculation of the endpoint (LD_{50}) of neutralization for this virus. It is highly likely that more than 300 LD_{50} of the homologous virus, FE, were neutralized by the antiserum. In any case, it is apparent that, in the presence of a monovalent antiserum (prepared against strain FE) the FE and HF strains display an antigenic similarity, whereas, in the presence of the same antiserum, the LH strain differs significantly from both of them.

Strains FE and LH were isolated from primary herpetic infections. Convalescent serum of patient FE neutralized the homologous virus and the HF strain to about an equal degree. On the other hand, convalescent serum of patient LH, although it neutralized the homologous virus, showed decidedly less neutralizing capacity against the HF strain.[2] In other words, immune serum of human origin reveals the same antigenic dissimilarity between strain LH and strain HF as does the rabbit antiserum used in the present experiment. In like manner, human antiserum reveals an antigenic similarity between strains FE and HF. These findings lend weight to a supposition that, as judged by the method of comparison used in the present study, the antigenic constitution of strains FE and LH has not been greatly altered by artificial passage in laboratory animals. It remains to compare the strains by means of antiserums prepared against each of them.

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Allergic Sensitization to Penicillin: Experimental Results.

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Hypersensitivity reactions to penicillin have occurred frequently in patients treated with this antibiotic and also have been produced in healthy men and animals. Some have been attributed to impurities in the penicillin, and some to penicillin itself, although there is a paucity of reports on the pure crystalline preparation. This paper reports the successful production of allergic sensitization, particularly the Arthus phenomenon in rabbits, by the use of crystalline sodium penicillin G and of commercial calcium penicillin.

Previous reports have described clinical sensitization of several types. The most common have been urticaria, angioneurotic edema, vesicular, bullous or papular eruptions, contact dermatitis, the Arthus type of reaction, and a condition resembling serum sickness.[1-3] Reactions have been most frequent after topical application of the drug,[4-8] but have also occurred after parenteral administration following initial topical use,[9] and after parenteral administration only.[10,11] In some cases the symptoms appeared after the initial exposure, and in others at later intervals. Among the more comprehensive studies have been those of Keefer,[12] who

1 Price, D. E., McNairy, D. J., and White, E. L., *J. Am. Med. Assn.*, 1945, **128**, 183.

2 Editorial, *J. Allergy*, 1945, **16**, 302.

3 Gordon, E. J., *J. Am. Med. Assn.*, 1946, **131**, 727.

4 Vickers, H. R., *Lancet*, 1946, **1**, 307.

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7 Binkley, G. W., and Brockmole, A., *Arch. Dermat. and Syph.*, 1944, **50**, 326.

8 Seymour, H. S., *Arch. Dermat. and Syph.*, 1944, **50**, 328.

9 Haswell, R. E., and Wilkinson, J. F., *Lancet*, 1946, **1**, 308.

10 Macey, H. B., and Hays, T. G., *U. S. Nav. Med. Bull.*, 1945, **45**, 1143.

11 Flinn, L. B., *et al.*, *Delaware State Med. J.*, 1945, **17**, 133.

12 Keefer, C. S., Marshall, E. K., Lockwood, J. S., and Wood, W. B., *J. Am. Med. Assn.*, 1943, **122**, 1217.

found urticaria 14 times in 500 patients with infectious disease treated with penicillin, and Cormia, Jacobsen and Smith,[13] who reported reactions in 10 out of 200 patients.

Preexisting sensitivity has been studied by several reporters. Rostenberg and Welch[14,15] reported that among 144 normal persons injected intradermally with crystalline penicillin, 8 showed positive reactions, and in some, repeated injections produced an Arthus type of reaction. These authors assumed that persons showing the initial sensitivity had previous contact with penicillium mould in nature. Cohen and Pfaff[16] made patch tests with penicillin ointments on 524 human subjects, of whom 5 showed positive reactions.

In animals, McClosky and Smith[17] produced anaphylactic shock in 4 of 11 guinea pigs previously sensitized with commercial penicillin. With the isolated guinea pig uterus, a delayed response thought to indicate sensitivity was irregularly found in animals similarly sensitized.

The following is a summary of our results in animals and in one patient.

Results in Animals. Tests for Preexisting Sensitivity in Guinea Pigs and Rabbits. Eleven normal guinea pigs and 12 normal rabbits were injected intradermally with 100 u of crystalline penicillin G* in 0.1 cc of physiological salt solution. Two guinea pigs showed indurated wheals of 3 cm diameter and 0.5 cm thickness, without redness, lasting for one day. One rabbit showed a red wheal, 3 cm in diameter and 0.5 cm in height, lasting the same time. Simultaneous control injections of the same saline solution produced no lasting skin reaction. Three guinea pigs were tested symptomatically by intravenous injection of 10,000 u of crystalline penicillin G, and 3 for smooth muscle contractions in perfused isolated lungs (bronchi), and on isolated strips of intestine, bladder and uterus with 1,000 u in 50 cc of Tyrode's solution without demonstrable positive reactions.[18] All these smooth muscles reacted typically to applications of histamine. Thus infrequent urticarial reactions may occur in normal animals but typical general sensitivity could not be demonstrated.

Generalized Sensitization in Guinea Pigs. In an attempt to produce generalized, systemic sensitization, 40 guinea pigs were injected according to groups as follows: (1) 3 were given a single subcutaneous injection of 1000 u of crystalline penicillin G in 1 cc of physiological salt solution; (2) 6 were given 1000 u of crystalline penicillin G in 1 cc physiological salt solution subcutaneously and simultaneously 1 cc of a 1:10 dilution of normal horse serum in physiological salt solution at another site; (3) 6 were given 100 u of crystalline penicillin G in 1 cc of physiological salt solution subcutaneously every day for 5 days, and simultaneously 1 cc of a 1:10 dilution of normal horse serum on each day at another site; (4) 5 were sprayed in the nostrils with 1000 u of the penicillin in 1 cc of physiological salt solution on 5 successive days; (5) 3 had a similar amount of penicillin dropped on the conjunctivae on 5 successive days; (6) 7 had 100 units of the penicillin in 0.5 cc of physiological salt solution applied to scarified abdominal skin on 5 successive days; (7) 6 were given 100 u of penicillin in 0.1 cc of physiological salt solution subcutaneously daily for 5 days; (8) 5 were given a single subcutaneous injection of 500 u of penicillin which had been mixed with 0.5 cc of their own serum and incubated at 37°C for 2 hours; and (9) 4 were given a single subcutaneous injection of 500 u of penicillin which had been incubated at 37°C with 0.5 cc of normal horse serum for 2 hours. After intervals of from 18 to 31 days, tests

[13] Cormia, F. E., Jacobsen, L. Y., and Smith, E. L., *Bull. U. S. Army Med. Dept.*, 1945, **4**, 694.

[14] Welch, H., and Rostenberg, A., *J. Am. Med. Assn.*, 1944, **126**, 10.

[15] Rostenberg, A., and Welch, H., *Am. J. Med. Sci.*, 1945, **210**, 158.

[16] Cohen, T. N., and Pfaff, R. O., *Arch. Dermat. and Syph.*, 1945, **51**, 172.

[17] McClosky, W. T., and Smith, M. I., PROC. SOC. EXP. BIOL. AND MED., 1944, **57**, 270.

* Supplied by the Squibb Institute for Medical Research.

[18] Chow, Bacon F., and McKee, Clara, *Science*, 1945, **101**, 67.

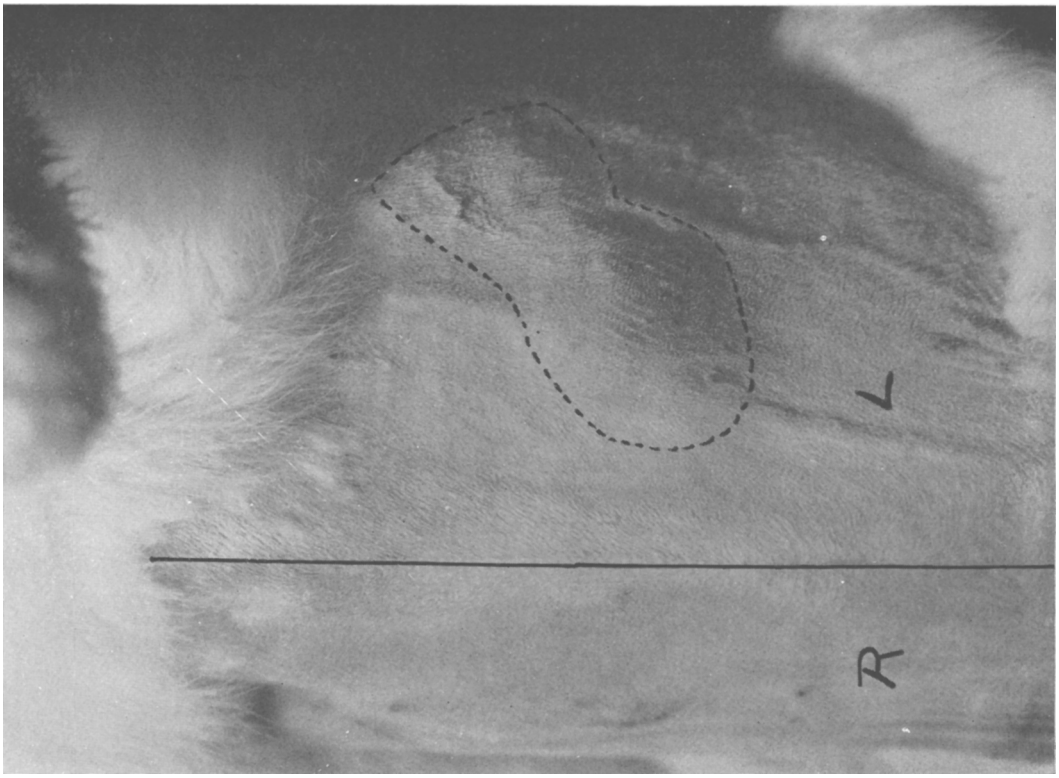

FIG. 1.
Typical Arthus Phenomenon in a Rabbit after Crystalline Sodium Penicillin G.
Shows a hard nodular swelling (broken line) in the left upper abdominal skin (L) after the fifth hypodermic injection, using 10,000 u in 5 cc physiological salt solution, on the thirtieth day after the first injection. Opposite right side (R) shows flat skin after the same injections of saline solution above (control).

for allergic sensitivity were made in a variety of ways.

Using doses of 1000 to 2000 u of crystalline penicillin G in 50 cc of Tyrode's solution, tests were made for smooth muscle contractions in isolated organs† in the following numbers of animals in the first 6 groups: 19, perfused lungs (bronchi); 17, intestinal strip (longitudinal muscle); 13, bladder strip; and 5, uterus strip. None showed a positive reaction, while 5 of the 7 animals simultaneously injected with horse serum showed bronchoconstriction, and one of the 2 tried showed uterine contraction, but none of 5 tested showed contractions of intestine or bladder. However, all of these smooth muscle organs reacted typically to histamine.

Eleven animals from groups 4, 6 and 7 were tested for local sensitivity by the intradermal injection of 1000 u of crystalline penicillin G in 0.1 cc of physiological salt solution, by the intravenous injection of 5,000 u of the penicillin in 0.5 cc of physiological salt solution, and by intravenous injection of 5000 u of the penicillin which had previously been incubated for 2 hours at 37°C with 0.5 cc of the guinea pig's own serum. Also, precipitation tests were made by incubating for 2 hours at 37°C one drop of each animal's serum, obtained before any other tests for sensitivity were performed, with one drop of a solution containing 20 u of penicillin in each cc, and with one drop of this penicillin solution previously incubated with normal guinea pig serum. Only

† The methods used for isolated organs throughout were those described in Sollmann, T., and Hanzlik, P. J., *Fundamentals of Experimental Pharmacology*, Second Ed., 1939.

one positive reaction appeared with any of these tests, namely, an urticarial flare with the intradermal injection of penicillin in an animal sensitized by spraying penicillin in the nose. Two animals, additionally tested by Prausnitz-Kuster's method showed no response. For this test, 1 cc of the animal's serum was injected into a normal animal, and again 24 hours later, another 1 cc was injected into the same area.

The 9 animals of Groups 8 and 9 were tested for sensitivity by the intradermal injection of 1000 u of crystalline penicillin G, in 0.1 cc of physiological salt solution, the intradermal injection of 0.1 cc of guinea pig or horse serum, and by mixtures of the penicillin solution and the guinea pig serum. Four dermal reactions to penicillin were obtained, 3 with penicillin-guinea pig's serum mixtures and one with penicillin alone. The intravenous injection of 1500 u of penicillin, and lung perfusion with penicillin gave no positive reactions in 3 animals each, although in one animal anaphylactic shock was produced by the intravenous injection of horse serum into the same animal sensitized by the penicillin-horse serum mixture.

Thus it appears that generalized sensitization of the guinea pig by penicillin administered by various routes, with or without incubation with homologous or heterologous serum, and with or without simultaneous sensitization to horse serum, is difficult or impossible to produce. As with preexisting sensitivity, occasional mild reactions may be demonstrated by the intradermal injection of penicillin or mixtures containing it.

Local Sensitization in Rabbits. Local skin sensitization of the Arthus type was studied in rabbits with pure crystalline penicillin G, and with commercial calcium penicillin.‡

Intradermal Injections. Two rabbits were injected 7 times intradermally in the same area of the abdomen at 5-day intervals with 100 u of penicillin G in 0.1 cc physiological salt solution. Both showed slightly red, edematous swellings about 3 cm in diameter and 0.5 cm in height, lasting 24 hours after the 5th injection, and somewhat smaller reactions after the 6th and 7th injections. One animal was simultaneously injected on each occasion on the opposite side of the abdomen with 0.1 cc of a 1:10 dilution of horse serum in physiological salt solution with the appearance of similar swellings following the 3rd to 6th injection, and a less intense swelling on the 7th. Similar repeated injections of physiological salt solution, as controls, produced no local reactions.

Intradermal Followed by Subcutaneous Injections Eight rabbits were injected intradermally twice at intervals of 7 days with 50 to 500 u of penicillin G in 0.1 cc of physiological salt solution. After a lapse of 15 days, injections were continued subcutaneously at 7-day intervals for 4 more injections, using 1000 u of penicillin G in 1 cc of physiological salt solution. Similar control injections with a 1:10 dilution of normal horse serum, or with physiological salt solution, were made on the opposite side of the abdomen. All but one showed dermal swellings at the site of the penicillin injections, similar to, but often larger than, those which received intradermal injections alone. These swellings started with the 1st to 4th injection, were maximal after the 3rd or 4th injection, and diminished thereafter. The serum controls gave similar, but more intense reactions, while the salt solution produced no effect.

Subcutaneous Injections. Nine rabbits were injected subcutaneously at intervals of 3 to 9 days for 5 to 7 injections with crystalline penicillin G, from 2 different lots of the drug. The dose varied from 500 to 10,000 u in volumes of 1 to 5 cc of physiological salt solution. Reactions appeared in 7 rabbits varying from 2 to 12 cm in diameter and from 0.5 to 6 cm in height. The more intense swellings occurred with the larger doses, and were maximal from the 3rd to 5th injection. The swellings were soft and boggy, usually faint to bright red in color, but sometimes without redness. They seldom lasted more than 24 hours, and never became necrotic. Fig. 1 shows a fairly typical reaction in the left upper abdominal skin of a rabbit, chiefly a large wheal, the opposite

‡ Supplied by the Squibb Institute for Medical Research.

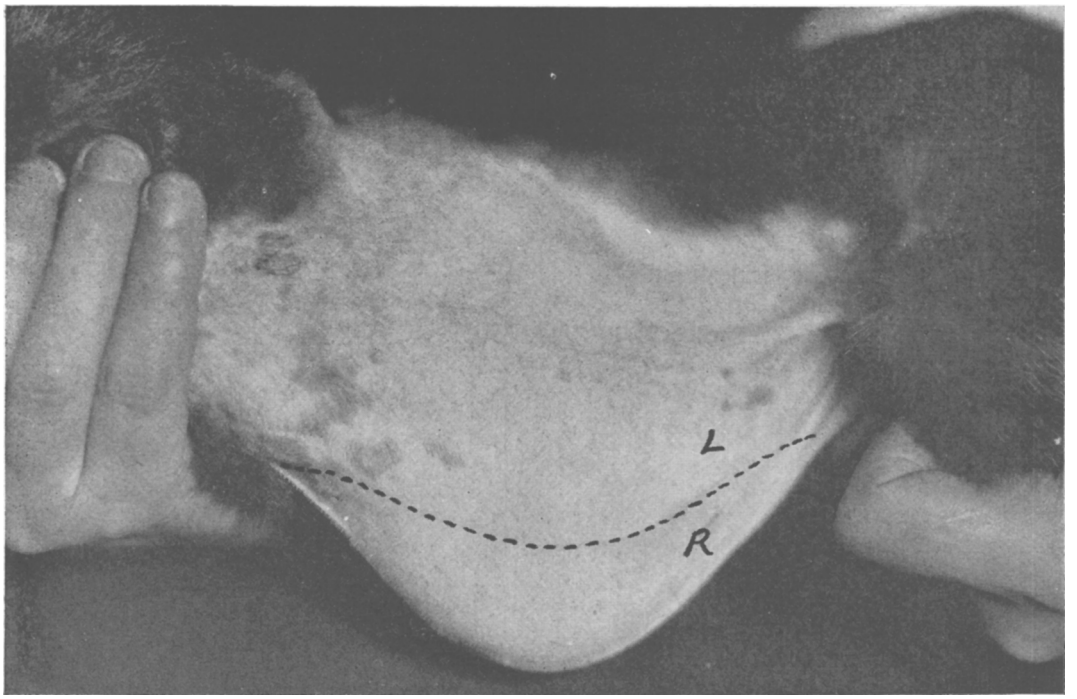

FIG. 2.
Marked Arthus Phenomenon in a Rabbit after Commercial Calcium Penicillin.
Shows a pendant, pouchy swelling in the right abdominal skin (R) after the fifth hypodermic injection of 5000 u in 1 cc of physiological salt solution, and on the twenty-third day after the first injection. The control area injected with saline solution alone in the same way is just above the linea alba (broken line), showing smooth, flat skin (L).

right side of the abdomen showing flat skin (control side).

Subcutaneous Injections with Commercial Calcium Penicillin. Four rabbits were injected subcutaneously with 1000 to 5000 u of commercial calcium penicillin in 1 cc of physiological salt solution at intervals of 4 to 6 days for 5 injections. Two animals were injected similarly on the opposite side with a 1:10 dilution of horse serum and 2 with the salt solution. Moderate to intense reactions to calcium penicillin occurred, similar to those described with pure sodium penicillin, but not more marked. Only one of the 2 control injections with horse serum produced swelling.

Thus, striking reactions like the Arthus type of phenomenon seen after injections of serum could be produced in rabbits by repeated intradermal or subcutaneous injections of either pure or commercial penicillin in saline solution. The route appeared to be unimportant, but larger doses gave greater reactions. Fig. 2 shows one of the more marked reactions to calcium penicillin in the right abdominal skin of a rabbit, the opposite skin above the linea alba being flat and smooth (saline control).

Clinical Case Report. A male physician, aged 40 years, injected himself subcutaneously in one anterior thigh with 300,000 u of commercial penicillin in oil and beeswax, and repeated the injection in the other thigh 2 days later. The injections were superficial and may have been intradermal, in part. Over the next 3 days he took 500,000 u of penicillin by mouth, and after an interval of 4 more days, was given 25,000 u of commercial penicillin intramuscularly in the buttocks every 3 hours for 3 days (25 doses). After the 20th dose the sites of the original penicillin injections in the anterior thigh itched intensely, urticaria appeared, and then tense red swellings. After 5 more in-

jections the lesions were orange-blue, slightly painful to the touch, and looked as though they would become necrotic. The intramuscular injections were then stopped, and the reactions subsided slowly over 2 weeks. A generalized macular eruption also appeared soon after the injections were stopped, most marked over the wrists and ankles, which cleared in 5 days. The local reactions in the thighs appear to have been typical Arthus phenomena, similar to those produced in the rabbits receiving penicillin alone. There is no good reason to believe that oil and beeswax would produce such typically localized reactions.

Discussion. Allergic sensitivity reactions were demonstrated in guinea pigs and rabbits with pure, white, crystalline penicillin G, and with commercial calcium penicillin. These took the form of mild local urticaria after intradermal injections, sometimes without previous known exposure to penicillin, and, more typically, of lesions characteristic of the Arthus phenomenon, some very intense. The reactions with commercial penicillin were no more intense than those with pure penicillin. It is, therefore, apparent that penicillin itself may act, presumably as a haptene in combination with body proteins, as a sensitizing agent, since Chow and McKee have shown that there could be a union between penicillin and serum albumin.[18]

General hypersensitiveness in guinea pigs was not demonstrable with crystalline penicillin, although McClosky and Smith[17] have reported this with less pure penicillin. This discrepancy may depend upon the purity of the preparation, reactivity of the guinea pigs used, or possibly upon differences in technic or dosage. However, it appears that with pure penicillin systemic sensitivity is definitely less important than the local, or contact sensitivity. The skin is an important organ through which penicillin sensitivity is mediated as with some other drugs.

Conclusions. 1. Mild preexisting sensitivity to pure crystalline sodium penicillin G, injected intradermally, was demonstrable occasionally in guinea pigs and rabbits. 2. Typical general sensitivity could not be demonstrated with pure penicillin G applied to guinea pigs by local or parenteral routes. 3. Local sensitization of the Arthus type phenomenon was produced consistently by repeated injections into rabbits of pure sodium penicillin G, or commercial calcium penicillin. These reactions were comparable to those produced by similar injections of horse serum, but somewhat less intense. 4. Similar local sensitization was observed in a patient injected with commercial penicillin in oil and beeswax. 5. Accordingly, the purest obtainable crystalline penicillin can act as a sensitizing agent with manifestations like those of native protein, and the skin is the outstanding organ for the seat of the allergic reaction.

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Comparative Numbers of Fungiform and Foliate Papillae on Tongues of Domestic and Wild Norway Rats.*

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Previous physiological and anatomical studies have revealed marked differences between domestic Norway rats which have lived for many years under laboratory conditions and wild Norway rats which have

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