

The Association of Viral Activation with Penicillin Toxicity in Guinea Pigs and Hamsters¹

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Penicillin toxicity in the guinea pig may be manifested in several different ways, and it is proposed that these toxic effects be categorized into three syndromes: (1) toxic syndrome, characterized by acute fatal illness; (2) hemorrhagic syndrome, characterized by delayed illness with leukopenia and thrombocytopenia, and culminating in massive visceral hemorrhages; (3) chronic syndrome, characterized by retardation of growth and alopecia, a condition somewhat resembling "runt disease." A virus having some of the properties of a parvovirus has been isolated repeatedly from animals ill or dying of penicillin-induced disease. This finding has been construed as being activation of a latent virus by this antibiotic, but the relationship, if any, of the phenomenon of viral activation to the syndromes produced by penicillin and its frequent lethal toxicity is unknown. That a strong association exists, however, has been established. Of some 60 guinea pigs which received injections of penicillin three developed tumors and four others were found to have gallstones. A virus similar or identical to the guinea pig virus also has been isolated from hamsters dying of penicillin-induced disease. It is hypothesized that the absorption of endotoxin, resulting from the well known change in intestinal flora caused by penicillin, produces a state of immunodeficiency which regularly gives rise to activation of a latent virus, and perhaps, rarely, to the development of malignant neoplasms.

Although penicillin shows a remarkable lack of toxicity for most animals, its lethal effect on the guinea pig, first reported by Hamre *et al.* in 1943 (1), is well known (1-8). This toxic effect is rather unpredictable, but most guinea pigs given a single injection of 5000 units or more rapidly become ill and die; somewhat larger amounts administered orally also kill most guinea pigs (2, 3, 6, 8). Within a day or two they display listlessness, ruffled fur, anorexia, and hypothermia, and usually die by the fifth day. Very large doses cause an acute fatal neurologic syndrome, comparable to that seen in other animals, and very small doses, or intermittent injections in "penicillin-resistant" animals may cause a chronic syndrome in which the animals display weight loss or retardation of growth, loss of hair, and neurologic aberrations (2, 3). However, within rather broad limits, the delayed lethal toxic

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effect, and the survival time are not dose related (4, 5). At autopsy, an almost constant finding is a greatly dilated cecum containing liquid feces; some investigators also have reported generalized vasodilatation and petechiae in the intestinal walls, while others have found severe cecitis and ileitis with regional lymphadenitis (2, 6, 7).

The unique toxicity of penicillin for the guinea pig has been the subject of a number of studies, but its mode of action has not been clearly elucidated. The intestinal flora of the guinea pig, which anomalously comprises mostly gram-positive organisms, predominantly rods and anaerobic cocci, is rapidly and drastically altered by penicillin; within 2 days these organisms are largely replaced by gram-negative bacteria, especially coliform bacilli and anaerobic rods (2, 6). Some investigators have found that the illness and death, which usually ensue, often are accompanied by enteric bacillary bacteremia, and they have concluded that death results from an overwhelming infection with these organisms (6). DeSomer *et al.* found no consistent evidence of bacteremia, and initially attributed the lethal effect of penicillin to toxemia caused by the newly acquired gram-negative intestinal flora; in a later report, however, they expressed doubt that toxemia could be the cause, and advanced the view that death might result from the destruction of a microorganism that normally produced an essential growth factor, or from the eventual utilization of such a factor by the newly developed flora (2, 8). Others have suggested that the toxic effect of penicillin is due to a direct neurotoxic action (9–11), a toxic substance produced in the animal itself (12–14), an anaphylactic reaction (15), lack of nutritive factors (16), a deficiency state (17), and acute necrosis of the adrenal glands (18).

Subsequent to the discovery of this unusual toxicity of penicillin, it was found that certain other antibiotics have a similar effect upon the guinea pig. Ambrus *et al.* (15), and Roine and Ettal (19), first reported that chlortetracycline is toxic for the guinea pig. Certain other antibiotics active against gram-positive bacteria, including bacitracin (8, 20), erythromycin (21), and spiramycin (22), also are highly toxic. Streptomycin given in a single oral dose to guinea pigs resulted in a mortality rate ranging from 10% to 100%, while, on the other hand, chloramphenicol administered orally for 2 wks resulted in no evidence of toxicity (2). Although all of these antibiotics are relatively nontoxic for mice, rats, rabbits, and other laboratory animals, small doses of erythromycin (21), and penicillin (23) are lethal for hamsters, and produce a syndrome similar to the disease induced in guinea pigs by penicillin.

A recent preliminary report from this laboratory has shown that the lethal effect produced by penicillin in the guinea pig is associated with activation of a latent virus (24). Further investigation of this phenomenon and of its possible etiologic relationship to penicillin toxicity, as well as attempts to define and elucidate the pathogenesis of penicillin-induced disease in the guinea pig and the hamster constitute this report.

MATERIALS AND METHODS

Weaning female guinea pigs of the Hartley strain, weighing about 200 g each, and weanling Syrian hamsters of both sexes were used exclusively. Animals were housed in clear plastic, wire-covered cages containing wood shavings as bedding. They were provided with water *ad lib.*, and with Purina "guinea pig chow" as the sole source of food.

Potassium penicillin G, dissolved in distilled water, was injected in 0.1-ml amounts into the thigh muscles of a hind extremity, using a tuberculin-type syringe and a 25-gauge 5/8-in. needle. Initially, various regimens including daily and thrice daily injections of relatively small amounts (1000–5000 units) for 7–10 days were employed. Later, a standard regimen consisting of a single injection of 50,000 units was used. Some animals were given a single intramuscular injection of 60,000 units of dibenzyl ethylenediamine dipenicillin G (Bicillin).

Animals were observed once or twice daily for evidence of illness, and, in some instances, daily temperatures and periodic determinations of weight were recorded. Many animals receiving penicillin were found dead; others were sacrificed when moribund, or died under observation. Autopsies usually were performed immediately thereafter. In a few instances, dead animals were frozen at -70°C , and autopsied later after thawing. Organs were removed aseptically and selected specimens were placed in 10% formaldehyde and in 2% glutaraldehyde. The remaining tissues were ground individually in a mortar and pestle with sterile alundum, and with the addition of sufficient phosphate-buffered saline (PBS), pH 7.0, to make approximately 10% suspensions. Portions of the specimens were frozen and stored at -70°C for future reference. For bacterial cultures, aliquots of such organ suspensions, as well as of blood and, in some instances, of bile and urine were inoculated onto duplicate sheep blood agar and MacConkey's agar plates. Both sets of plates were then incubated at 37°C , one set aerobically and the other in an anaerobic jar. Bacterial isolates were identified by Gram stain, sugar fermentations, and other conventional tests. Many specimens also were cultured on Sabouraud's agar plates and incubated at room temperature.

For mycoplasmal cultures, similar aliquots were inoculated onto special agar plates consisting of Difco PPLO agar plus 20% gamma globulin-free horse serum, 10% fresh yeast extract, 0.5% glucose, penicillin 1000 U/ml, amphotericin 2.5 $\mu\text{g}/\text{ml}$, and thallium acetate 1–50, 2.5 ml/100 ml. Incubation of these plates was carried out at 35°C in a humid atmosphere containing about 5% CO_2 in a candle jar, and in an anaerobic jar.

For viral isolations, specimens prepared as described above were treated with gentamicin 50 $\mu\text{g}/\text{ml}$ and amphotericin 2.5 $\mu\text{g}/\text{ml}$ and allowed to stand at 4°C for 30 min. Two-tenth milliliter aliquots were then inoculated into tubes containing monolayers of various tissue cultures maintained in Medium 199 as modified by Hallauer *et al.* (25), and with the substitution of gentamicin 50 $\mu\text{g}/\text{ml}$ and amphotericin 2.5 $\mu\text{g}/\text{ml}$ for penicillin, streptomycin, and mycostatin. The nonspecific cytopathic effect (CPE), which often is produced by tissue suspensions and by some body fluids, was avoided by adsorbing the inocula to the tissue culture monolayers for 1 hr at 35°C , decanting, and washing once with maintenance medium. Cultures subsequently were incubated at 35°C and examined daily for CPE. Those showing CPE were immediately passed by inoculating 0.2-ml amounts of the fluid component onto fresh tissue culture monolayers. It was concluded, arbitrarily, that virus had been isolated if destructive CPE occurred, in the absence of bacterial contamination, in at least two consecutive passages in WI-38 cells. Neutralization tests were carried out in tubes of WI-38 cell monolayers, using serial 2-fold dilutions of serum and approximately 10 TCID_{50} of virus.

In some instances, duplicate monolayer cultures also were tested for hemadsorption at various intervals after inoculation. In many others, body fluids, organ suspensions, and tissue culture supernatant fluids were tested for hemagglutination.

Both of these tests were carried out with the use of 0.4% guinea pig RBC in PBS. Hemagglutination-inhibition tests were done in the standard fashion using serial 2-fold dilutions of serum in PBS and 8 units of hemagglutinin.

Monolayer tube cultures of WI-38 cells, primary human embryonic kidney and monkey kidney cells were obtained from commercial sources. Other cell cultures were prepared in our laboratory by passage of HeLa and other cell line stocks, and by trypsinization of various organs of freshly killed guinea pigs, rabbits, hamsters, and other small laboratory animals.

For light microscopy the tissues which had been fixed in 10% formaldehyde were embedded in paraffin, sectioned, and stained with hematoxylin and eosin, and with Lendrum's inclusion body stain. WI-38 cells grow on cover slips in Leighton tubes were inoculated with virus, fixed, and stained with acridine orange, when the monolayer showed 1+ to 2+ CPE. For electron microscopy, those portions of tissues fixed in 2% glutaraldehyde were embedded in Epon, sectioned, and stained with uranyl acetate and lead citrate, and examined for fine detail, and for virus particles with a Philips EM 300 electron microscope.

RESULTS

Penicillin-Induced Disease

Guinea pigs given various amounts and regimens of penicillin displayed a variety of toxic manifestations. These toxic effects were categorized into three different syndromes, as follows: (a) toxic syndrome, (b) hemorrhagic syndrome, and (c) chronic syndrome.

(a) *Toxic syndrome.* Although not so-named in previous literature on the subject, this syndrome is the one originally described (1), and was the one most frequently encountered. Animals afflicted with it usually displayed ruffled fur, listlessness, and anorexia within a day or two; subsequently, they lost weight and became hypothermic. A few animals recovered, but most died within 3–5 days. Although the incidence of this syndrome varied markedly from one experiment to another, with a mortality rate ranging from 20% to 100%, the vast majority of animals receiving a single injection of 50,000 units of potassium penicillin G were affected. It occurred infrequently among animals receiving repeated daily injections of 1000–3000 units.

The only consistent finding at autopsy has been a greatly distended cecum which is filled with gas and liquid feces. In some instances, the adrenal glands have appeared to be enlarged. This is of interest because it has been reported that the adrenal glands of guinea pigs dying of penicillin increase in weight by some 31% (3), and that injections of penicillin in guinea pigs produce a sustained rise of plasma 17-hydroxycorticoid levels (26). The histopathology has not yet been studied systematically but, as previously reported by others (2, 6, 7), there is a severe enteritis which, in our experience, involves not only the cecum and ileum but also the entire small intestine and colon. This enteritis is characterized by edema, vascular congestion, at times with hemorrhages in the submucosa, sloughing of mucosa, and a diffuse submucosal exudate comprising mononuclear cells and some pigment-containing macrophages. Occasionally, eosinophilic bodies, thought to be viral inclusion bodies, have been seen in cells of the intestinal mucosa and the adrenal glands.

(b) *Hemorrhagic syndrome*. This syndrome, described briefly in a preliminary report (24), appears to be similar, if not identical, to that described by Anderson and Nowell, and attributed by them to a graft-versus-host reaction (27). It also is reminiscent of the panleukopenic disease of cats which is said to be caused by a feline parvovirus (38, 29).

These animals were almost invariably found dead, after appearing to be well when observed earlier in the day or on the previous day. This syndrome occurred sporadically, and often involved groups of animals which were caged either together or in close proximity. Frequently, the first animal to die in a particular group was one which had survived an injection of penicillin given some weeks earlier. Subsequently, other animals, including some controls which had received saline or no injections, housed in the same or adjacent cages, died in similar fashion.

There often was fresh blood around the mouth and nostrils and, in most instances, death appeared to have resulted from massive visceral hemorrhages. The peritoneal and/or thoracic cavities contained large amounts of liquid blood which usually did not clot. The spleen often was enlarged, and many organs showed evidence of recent hemorrhage. The liver was enlarged, congested, and occasionally contained sharply demarcated pale-yellow, necrotic areas. Wright-stained smears of peritoneal blood showed some fragmentation of red blood cells and a number of large mononuclear cells resembling monocytes, and in some instances, small lymphocytes and eosinophils, but no polymorphonuclear neutrophils, and very few, if any platelets. Stains of blood obtained by cardiac puncture showed a similar picture, and blood counts revealed a marked leukopenia and thrombocytopenia.

(c) *Chronic syndrome*. This syndrome, so named and first described by DeSomers *et al.* (8), and Eyssen, DeSomers, and Van Dijck (2), was found by them to occur occasionally after the intermittent injection of penicillin (30,000–100,000 units every 3 days) in "resistant" guinea pigs. They reported that such animals showed loss of weight, depilation, and neurologic aberrations, generally a spastic paraplegia of the hind legs. In some guinea pigs there also was erection and necrosis of the penis, and pseudoincontinence, while others only developed areas of alopecia. Many animals so affected became progressively worse and died, but a number recovered.

We have independently observed a similar, if not identical, syndrome in some animals which had received 1000 U of potassium penicillin G three times daily for 10 days, as well as in some which had received a single intramuscular injection of 60,000 units of Bicillin. Some of these animals also showed erythema and scaldiness of ears and foot pads, and their general appearance was similar to that of animals suffering from runtling or a graft-versus-host-like reaction. Preliminary experiments suggest that this syndrome also may be induced in guinea pigs receiving a single injection of 50,000 units of penicillin G, by maintaining them at an environmental temperature of 75° to 80°F.

Experience with hamsters has been somewhat limited but these animals have displayed an even higher and more consistent mortality from penicillin than guinea pigs. The vast majority have died 2–3 days after a single injection of 50,000 units, with manifestations similar to those seen in the toxic syndrome of the guinea pig.

Results of a fairly typical experiment illustrating the toxic effect of penicillin for guinea pigs and hamsters, and its lack of toxicity for some other rodents, are shown in Table 1. After a single injection of 50,000 units of penicillin G most guinea pigs and hamsters died, but mice and rats suffered no ill effects. The day

TABLE 1
TOXICITY OF PENICILLIN^a FOR GUINEA PIGS AND HAMSTERS

Animal	Mortality		
	No. died	No. injected	Day of peak mortality
Guinea pigs	5	7	4
Hamsters	7	8	3
Mice	0	8	—
Rats	0	8	—

^a Single im dose of 50,000 U of potassium penicillin G.

of peak mortality occurred earlier by one, and the mortality rate was slightly higher among hamsters than among guinea pigs.

VIRUS ISOLATES

Similar viruses were isolated from guinea pigs ill or dying of the three penicillin-induced syndromes described above. Virus isolates were made from organs of most animals which had received penicillin, as well as from untreated animals dying of the hemorrhagic syndrome that had been in close contact with penicillin-treated animals. As shown in Table 2 virus was isolated from every one of the 35 guinea pigs sacrificed when ill, or dying after receiving penicillin, but from none of the 19 animals receiving saline or no injections. However, virus was isolated from only 2 of 11 sacrificed penicillin-treated animals which did not die or become ill.

Virus was isolated, at least once, from blood, bile, feces, urine, and every organ tested, including adrenal, brain, cecum, colon, gallbladder, ileum, kidney, liver, lung, spleen, thymus, and stomach. Virus was not invariably isolated from any organ, but those most often found to be virus positive were cecum, colon, stomach, kidney, adrenal, liver, and spleen. Contents of both small and large intestine as well as homogenates of virtually all of the organs of guinea pigs ill or dying of penicillin have been found, on one or more occasions, to agglutinate guinea pig RBC, often to high titer. Intestinal contents and homogenates of intestine were almost invariably positive, and usually had the highest titers of hemagglutinin, often as high as 640 or 1280. The percentage of other organs containing hemagglutinin

TABLE 2
PENICILLIN-TREATED AND CONTROL GUINEA PIGS TESTED FOR
HEMAGGLUTINATION AND VIRUS ISOLATION

	Hemagglutination ^a			Virus isolation ^b		
	No. positive	No. tested	% Positive	No. positive	No. tested	% Positive
Penicillin-treated ^c (35)	35	35	100	35	35	100
Controls ^d (19)	14	19	74	0	19	0

^a Hemagglutination, positive if body fluid or homogenates of one or more organs, in a dilution $\geq 1-10$, agglutinated RBC (guinea pig).

^b Virus isolation, positive if body fluid or homogenates of one or more organs produced CPE for at least two tissue culture passages (WI-38 cells).

^c Died or sacrificed when moribund.

^d No treatment or saline; sacrificed while healthy.

varied from 15% for lung to about 60% for spleen, kidney, and liver, and 90% for adrenal. With few exceptions, those specimens from penicillin-treated animals, which produced hemagglutination, as well as a small percentage of those which did not hemagglutinate, also produced CPE in WI-38 fibroblasts. In contrast to these findings intestinal contents and organ suspensions of uninoculated controls, and of animals which had received saline injections, and subsequently were sacrificed, failed, in every instance, to produce CPE in WI-38 cells. In spite of the fact that these specimens were not infectious (i.e., did not produce CPE) many of them did agglutinate guinea pig RBC. The percentage which did so, however, was somewhat lower than that of specimens from animals which had received penicillin. Moreover, when hemagglutination did occur, the titers generally were lower among the specimens obtained from control animals. Examples of the results of these studies are presented in Table 3. Examination of other specimens not shown in Table 3 but including blood, gallbladder, ileum, stomach, and thymus yielded similar results. Table 4 provides a summary, showing the total numbers of specimens tested, as well as the numbers and percentages showing hemagglutination and CPE in penicillin-treated animals as compared to controls.

As shown in Table 2, one or more organs of every one of the 35 animals given penicillin produced hemagglutination of guinea pig RBC and CPE in WI-38 cells.

TABLE 3
SELECTED ORGAN HOMOGENATES AND BODY FLUIDS SHOWING HA AND/OR CPE
35 PENICILLIN-TREATED AND 19 CONTROL GUINEA PIGS^a

Organ or fluid tested	Adrenal		Bile		Brain		Colon		Kidney		Liver		Lung		Spleen	
	HA	CPE	HA	CPE	HA	CPE	HA	CPE	HA	CPE	HA	CPE	HA	CPE	HA	CPE
Penicillin ^b (35)	18/20 ^c	17/20	4/4	2/4	3/9	5/9	30/35	31/35	12/17	14/17	25/32	25/32	2/12	6/12	16/26	16/26
Controls ^d (19)	6/17	0/17	5/11	0/11	1/4	0/4	6/6	0/6	9/16	0/16	11/19	0/19	2/8	0/8	12/19	0/19

^a HA = hemagglutination in a dilution $\geq 1-10$ (guinea pig RBC); CPE = cytopathic effect for at least two passages (WI-38 cells).

^b Died or sacrificed when moribund.

^c Denominator = total number of specimens tested; numerator = number of specimens showing HA or CPE.

^d No treatment or saline; sacrificed while healthy.

TABLE 4
TOTAL ORGANS AND BODY FLUIDS SHOWING HA AND/OR CPE
35 PENICILLIN-TREATED AND 19 CONTROL GUINEA PIGS

	HA ^a			CPE ^a		
	No. positive	No. tested	% Positive	No. positive	No. tested	% Positive
Penicillin ^b (35)	116	169	69	122	169	72
Controls ^c (19)	65	135	48	0	135	0

^a HA = hemagglutination in a dilution $\geq 1-10$ (guinea pig RBC); CPE = cytopathic effect for at least two passages (WI-38 cells).

^b Died or sacrificed when moribund.

^c No treatment or saline; sacrificed while healthy.

On the other hand, organs of only 14 of 19 control animals produced hemagglutination, and not a single one of the 135 specimens tested produced CPE (Tables 2 and 4). In sharp contrast to the control group, some 122 of 169 or 72% of the specimens from penicillin-treated animals produced CPE in WI-38 cells (Table 4).

CHARACTERIZATION OF THE VIRUS

The agent or agents isolated appear to infect and produce CPE in tissue cultures of various organs of a number of vertebrates. These cultures include WI-38 diploid cells, primary rhesus monkey, African green monkey, guinea pig, rabbit, and hamster kidney cells, but not human embryonic kidney cells. In order to avoid endogenous contaminating agents, WI-38 cells, which generally are thought to be free of viruses, usually were used for isolation and subsequent passage. The CPE which occurs in these cells generally begins with cellular swelling and granulation, followed by separation and rounding, resulting in round cells of small to medium size, and quite frequently, crescents and ring-shaped cells; cells then begin to fall away from the glass surface and eventually destruction of the monolayer is complete. CPE is not always progressive and, on occasion, it regresses after reaching the 1+ or 2+ stage, and the cell monolayer regains a normal appearance. Moreover, the production of CPE is erratic and unpredictable, and, not infrequently, ceases to occur after several passages. There is some indication that this is associated with a relatively static state or slow rate of growth of the host cells. For this reason, it has not been possible to prepare virus pools of predictable titer, and consequently further characterization of the virus has not been possible. However, the approximate size of this virus has been determined by filtration through Millipore filters. Ten percent suspensions of organs of animals dying of penicillin retained both infectivity and hemagglutinating capacity after successive passage through 0.45, 0.2 and 0.05- μ m filters. Similar suspensions, passed through such filters and pelleted by centrifugation at 30,000 rpm for 2 hr were stained with uranyl acetate and lead citrate, and examined in a Phillips 300 electron microscope. Many particles of fairly uniform size measuring about 20 nm in diameter were seen and were thought to represent virus particles. Smaller numbers of similar particles, however, also were seen in preparations made of organs of normal guinea pigs which had not received penicillin. Thin sections of various organs of animals dying of penicillin-induced disease, similarly stained and examined by electron microscopy, in a few instances have revealed particles of fairly uniform size and shape, measuring about 22 nm in diameter. Acridine orange stains of virus-infected WI-38 cells have shown occasional cells containing one or more, usually two, bright-red intranuclear bodies, which suggests that this agent may be a single-stranded DNA virus. Infectivity of virus-containing organ homogenates was not significantly reduced after treatment with dilute HCL at pH 3.0, and after treatment with ether. Neither hemagglutinating capacity nor infectivity titer was appreciably diminished after storage for several months at -70°C .

The possibility that endotoxin or some other product elaborated by the intestinal flora, rather than a virus, produces the CPE in WI-38 cells, seems remote, because many of the isolates were successively passed in tissue culture, in some instances for 10 or more passages.

An agent similar to that recovered from guinea pigs was isolated repeatedly from hamsters dying of penicillin, but not from control animals which had received saline. Moreover, hemagglutinins were found in tissues of both penicillin-treated

and control hamsters. On the other hand, no virus could be isolated in WI-38 cells from the tissues and intestinal contents of mice and rats, which were killed several days after having received penicillin. However, a hemagglutinin for guinea pigs RBC was found in some specimens from these animals, as well as in those of some animals which had not received penicillin.

Hemagglutination, Hemolysis, and Hemadsorption

The hemagglutinin in organ homogenates of penicillin-treated guinea pigs could be detected with a variety of RBC. Those of the following species were agglutinated, and are listed in decreasing order of magnitude of hemagglutination titers: guinea pig, mouse, rabbit, rat, sheep, man, hamster, and baby chick. Hemagglutination occurred equally well at 22°C and at 4°C. A hemolysin for guinea pig and other RBC also was found in virus-containing organ homogenates. Hemolysis occurred in virus-treated suspensions of RBC which had been allowed to stand at 22°C for 24 hr; the hemolysin usually had a titer 2- to 4-fold greater than that of the hemagglutinin. Great difficulty has been encountered in demonstrating hemagglutination by the virus grown in tissue culture. Tissue culture fluids, as well as frozen and thawed suspensions of infected WI-38 cells have uniformly failed to hemagglutinate. However, similar tissue culture materials concentrated several fold by centrifugation and resuspended in PBS have agglutinated guinea pig RBC, although only to low titer. Hemadsorption has been demonstrated with some virus-infected WI-38 cells, but the specificity of this has not been established.

Bacterial and Other Isolates

Repeated efforts were made to isolate mycoplasmas from both penicillin-treated and control animals, as well as from tissue cultures inoculated with body fluids and organ homogenates of such animals, but none was found. Moreover, candida and other apparently saprophytic fungi were only occasionally recovered from such material. However, coliform bacteria have been found in large numbers in intestinal contents of penicillin-treated animals. These organisms also have been found not infrequently, but not invariably, in various tissues of animals dying after the administration of penicillin. They most commonly were recovered from animals which had been dead for some hours. Tissues of animals which were sacrificed usually were bacteriologically sterile, and virus was isolated on numerous occasions from penicillin-treated animals whose tissues contained no demonstrable bacteria.

Serological and Transmission Studies

Sera of guinea pigs resistant to penicillin-induced disease and of those immunized with virus-rich organs or tissue cultures inhibited hemagglutination by virus-containing organ homogenates, usually to titers of 256 or higher. However "normal" guinea pig sera also inhibited hemagglutination, although to somewhat lower titers. The apparent nonspecific nature of, at least, part of this inhibition has made it difficult to assess the validity of the hemagglutination-inhibition test for antibodies to this agent.

Attempts to transmit penicillin-induced disease to normal guinea pigs by intraperitoneal inoculation of bacteria-free, virus-containing organ homogenates and by the virus grown in tissue culture have met with no success, although some animals so treated developed neutralizing antibodies to the virus and were resistant to the toxic effects of penicillin.

Resistance to Penicillin Toxicity

We have found, as previously reported by other investigators (2, 8), that some guinea pigs are resistant to the toxic effects of penicillin. As previously pointed out, the over-all mortality usually averages about 75%. However, occasionally it has been as low as 10% or less. There was some indication that the mortality rate might be related to ambient temperature, and preliminary experiments suggested that an ambient temperature of 75°–80°F as compared to that of 65°–70°F, protected guinea pigs but not hamsters against the toxic effects of penicillin. However, it has become apparent that other, as yet unidentified factors play a role in this resistance. At times almost all guinea pigs obtained from a given breeder have been resistant, irrespective of ambient temperature.

Development of Tumors and Gallstones

Three of 60 guinea pigs given penicillin G developed tumors. These animals were among those which appeared to be resistant to penicillin and, except for a temporary retardation of growth, had not manifested any evidence of toxicity. One to two months later however, they died, and at autopsy yellowish-white masses involving the peritoneal wall, mesentery, omentum, intestine, and other organs were found. In one instance, a large portion of the intestine was affected, giving it a pipstem-like appearance. On microscopic examination this proved to be a spindle cell sarcoma which had invaded the intestinal wall and lumen. Tumors found in the other two animals have not yet been characterized microscopically. However, one of these when ground up and injected intraperitoneally into guinea pigs resulted in the appearance of similar tumors and death in some of the animals. Most of these tumors contained large amounts of hemagglutinin and infectious virus. One or more gallstones have been found in the gallbladders of 4 of 18 guinea pigs dying of penicillin-induced disease; none has been found in 17 control animals, in which a careful search was made for stones.

DISCUSSION

The data presented here, as well as observations previously made by other investigators, establish beyond any doubt the fact that penicillin exerts unusual toxic effects upon the guinea pig (1–19). Although not generally recognized, the manifestations of this toxicity may assume various forms; that most commonly encountered and the only one described in most reports has been designated in our studies as the toxic syndrome. It is characterized by anorexia, listlessness, hypothermia, and death within 2–5 days. A second form, the chronic syndrome, previously described by others as following intermittent injections of penicillin in “resistant” animals, was observed in this laboratory to occur after frequently repeated injections of very small amounts of penicillin. A third form, designated here as the hemorrhagic syndrome, is characterized by leukopenia and thrombocytopenia, followed by death from massive hemorrhages (24).

There has been no agreement concerning the mechanism of the toxicity of penicillin. Many theories have been advanced but none is really convincing. Perhaps the most popular hypothesis is that the toxic effects are due to toxemia resulting from the intestinal overgrowth of enteric bacilli, or to bacteremia with these organisms (2, 5, 6). Indeed, a bacteremia frequently does occur but this may well be an agonal event because some recently dead and moribund animals do not have

bacteremia. Moreover, cultures of various organs often are sterile, and little if any evidence of bacterial infection is found on microscopic examination (2, 5, 6, 24). Furthermore, germ-free guinea pigs are reported to be resistant to penicillin toxicity, and when such animals are fed enteric bacilli they suffer no ill effects (5, 30).

The isolation of a virus from virtually all guinea pigs ill or dying of penicillin-induced disease, but from none of the control animals, has been interpreted as activation of a latent virus by this antibiotic (24). This finding also suggests, but by no means proves, that this agent bears a causal relationship to penicillin toxicity. The virus or its precursor appears to be present in the gastrointestinal tract of most if not all conventionally reared guinea pigs and hamsters. The failure to find infectious virus in intestinal contents, in the intestine itself, as well as in other organs and body fluids of normal guinea pigs and hamsters suggests that complete virus is not present in such animals. However, the appearance of infectious virus in intestinal contents and in many organs and body fluids very soon after the administration of penicillin suggests that the virus had been present in some latent or otherwise noninfectious form, and somehow had been converted into infectious virus. The frequent presence of a hemagglutinin, usually in low titer in intestinal contents, and less often in some organs, in the absence of any demonstrable infectious activity, in "normal" animals and the apparent increase in hemagglutinating activity accompanying the appearance of infectious virus after the administration of penicillin, also suggests that the virus is present in defective or incomplete form, and that it may be demonstrable by its capacity to hemagglutinate. The possibility exists, of course, that the presence of the hemagglutinin is a coincidental phenomenon, unrelated to the infectious agent, but this seems unlikely. The same or a similar hemagglutinin has been found in rats and mice; however, in these animals, no infectious agent appears after the administration of penicillin. If the hemagglutinin actually is part of a defective virus or virus precursor, it would seem to be widespread among rodents and perhaps other animals. One might speculate that it is activated by antibiotics only in the guinea pig and hamster because of the anomalous nature of the intestinal flora in these two species.

The mechanism of viral activation and that whereby penicillin (and various other antibiotics active against gram-positive organisms) exerts its toxic effect in the guinea pig (and the hamster) appear to be as elusive as ever. However, the material presented in this report does provide sufficient data for formulating some speculative hypotheses.

The rapid and drastic alteration of the guinea pig's intestinal flora from predominantly gram-positive bacteria to mostly gram-negative anaerobic and enteric rods appears to be such a constant accompaniment of penicillin toxicity that one cannot escape the impression that this phenomenon plays an important role. That the changes in intestinal flora are coincidental rather than causally related to the toxic syndromes induced by penicillin seems unlikely because several other antibiotics, unrelated to penicillin, also produce changes in flora and toxic manifestations similar to those caused by penicillin (2, 8, 15, 19-22). This finding, as well as the fact that, within rather broad limits, penicillin toxicity is not dose related, also makes it seem unlikely that penicillin-induced toxicity is due to some unusual specific toxic effect of this antibiotic for the guinea pig, although our studies indicate that penicillin is, at least, 10 times more toxic *in vitro* for guinea pig kidney tissue cultures than for similar human and rhesus monkey kidney cultures. Moreover, the resistance of some guinea pigs to the toxic effect of penicillin is well known, although it occurs sporadically and unpredictably, and is sometimes of a transient

nature (2). Interestingly enough, coliform organisms seldom are found among the flora of penicillin-resistant animals (2). Perhaps this resistance to penicillin-induced disease is associated with a gram-positive intestinal flora which is of the usual variety, but which is not sensitive to penicillin.

While it is quite possible that penicillin may eliminate gram-positive organisms which normally suppress or inhibit the emergence of a latent virus, it seems more reasonable to assume that the newly acquired flora somehow is responsible for its activation. The enteritis accompanying penicillin toxicity might enhance the absorption of endotoxin and perhaps other toxic products elaborated by the coliform organisms. This, in turn, could lead to a state of endotoxemia, either acute and overwhelming or chronic and protracted, depending upon the new bacteria acquired, degree of damage to the intestinal mucosa, amount and rate of toxin absorption, and possibly such environmental factors as ambient temperature. The question then arises as to how endotoxemia might activate a latent virus and whether the virus, the endotoxin, or both could account for all of the toxic manifestations of penicillin, or, more specifically, for the three syndromes described above. The toxic syndrome, with its rapid onset, hypothermia, extensive vascular damage to the intestinal wall, and fulminating course, is compatible not only with an acute endotoxemia, but also with an acute viral disease comparable to infectious enteritis of cats or feline panleukopenia (28, 29).

It is conceivable that the administration of penicillin leads to an autoimmune-like state or a graft-versus-host-like reaction (GVHLR); and, indeed the chronic syndrome bears some resemblance to this entity. The enteritis which is such a constant feature of penicillin-induced disease resembles that seen in runt animals, although it has been assumed to be of bacterial or viral etiology. Moreover, guinea pigs dying of penicillin show a disproportionate loss in weight of the thymus; such animals, as compared to controls, showed a mean percentage loss of weight of this gland of 56, which represented a much greater loss than that of any other organ (3). This is reminiscent of the GVHR, in which the thymus undergoes rapid involution, progressing to almost complete disappearance (31). It is well known that *Salmonella typhimurium*, a potent producer of endotoxin, as well as purified endotoxin can produce runting, a type of wasting disease, in infant mice (31-33). The analogy with penicillin-induced disease which could be considered here, is that the absorption of endotoxin might give rise to a GVHLR which impairs the animal's immune defenses and results in activation of the latent virus. Although the proliferative phase of the GVHR is characterized by hyperphagocytosis, and some increased resistance to bacterial infection, especially pneumococcal bacteremia (34), immunological competence may be impaired, rendering the animal particularly susceptible to infection (31, 33, 35). On the other hand, there are close similarities between runt disease and chronic endotoxicity, and some have concluded that the severe wasting induced by endotoxin may be due to an infectious process (33, 35). That some viruses produce a graft-versus-host-like reaction (which resembles the chronic syndrome) is well known. This occurs in certain mice inoculated with polyoma virus (36), the virus of lymphocytic choriomeningitis (37), and reovirus (38). The parvoviruses also are known to cause runting, as well as tooth and bone deformities (39, 40). Moreover, it is suspected that wasting disease and the GVHR may enhance the susceptibility of some animals to infection with oncogenic viruses and may lead to the development of tumors (35, 41). Another possibility is that the large antigenic mass imposed upon the animal by the sudden appearance of an over-whelming number of "foreign" gram-negative bacilli might result in a gen-

eralized immune paralysis, or a sort of vaccine-induced wasting disease such as that described by Ekstedt and Nishimura (42), which is very similar to immunological runt disease and the GVHR. Thus, it seems entirely possible that the chronic syndrome may be a type of GVHLR resulting either from a chronic endotoxemia itself, or from infection with a latent virus activated by endotoxin.

It also appears possible that the third syndrome associated with penicillin toxicity, the hemorrhagic syndrome, may be due either to an autoimmune response such as a GVHLR or to a viral infection resulting from activation of a latent virus. The occurrence of a fatal hemorrhagic disease characterized by leukopenia and thrombocytopenia in F₁ hybrid guinea pigs was reported by Anderson and Nowell and ascribed by them to a graft-versus-host reaction, induced by inoculation of parental strain lymphoid cells (27). This disease appears to be similar, if not identical, to the hemorrhagic syndrome, and may have been caused by a virus which was activated as a result of a state of immunodeficiency associated with the GVHR. Furthermore, both it and the hemorrhagic syndrome closely resemble feline panleukopenia which is thought to be caused by a parvovirus (28, 29). In contrast to the toxic and chronic syndromes which appear not to be transmissible, the hemorrhagic syndrome occasionally appears to be transmitted by contact. How this is accomplished is unknown but, since most guinea pigs harbor the latent virus, it may be that transfer is mediated through colonization of the contact's gastrointestinal tract with gram-negative enteric bacilli which, in turn, result in activation of the latent virus, rather than by transmission of the virus itself.

Whether the activation of a virus by penicillin is a coincidental phenomenon or is casually related to penicillin-induced disease remains a moot question. If it is causally related, the resistance to penicillin displayed by some animals could be due to the fact that the latent agent is not present in the resistant host. The resistance of germ-free guinea pigs to the toxic effects of penicillin, and the absence of untoward effects after the introduction of coliform bacteria into such animals would lend support to this possibility (5, 30, 43). That the virus was recovered from every one of 35 guinea pigs ill or dead of penicillin-induced disease, from not one of 19 sacrificed controls, and from only 2 of 11 animals showing no evidence of disease after the administration of penicillin also supports the theory that a causal relationship exists. On the other hand, failure to reproduce the disease by injection of large amounts of virus, both in the form of organ homogenates of animals dying of penicillin and of tissue cultures, militates somewhat against such a relationship. Preliminary data, however, suggest that such injections may provide some protection against penicillin-induced disease. Conceivably, the newly acquired bacteria or their toxins, in some way, condition the host so as to make it highly susceptible to infection with the activated virus, or perhaps the virus enhances susceptibility to the bacterial toxins. Regardless of their modes of action, presently available evidence strongly suggests that both the virus and an abnormal (for the guinea pig and probably the hamster) intestinal flora, comprising coliforms and possibly other gram-negative bacilli, play essential parts in the pathogenesis of penicillin-induced disease.

Although penicillin is known to be lethal for the hamster as well as the guinea pig, this facet of the problem appears to have received little attention, and only scant and occasional references to it have been found. In preliminary studies in this laboratory penicillin has been found to be just as lethal, if not more so, for the hamster. The mechanism of penicillin toxicity in the hamster would seem to be similar to that in the guinea pig since the hamster's intestinal flora also appears

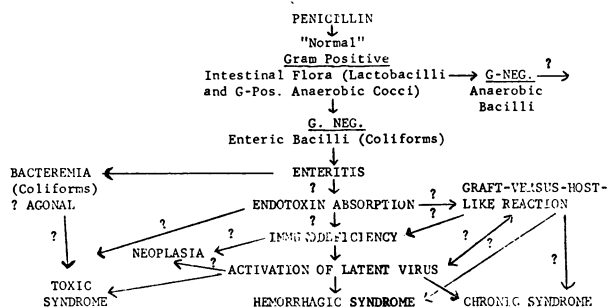


FIG. 1. Hypothetical mechanism of penicillin toxicity in the guinea pig. Penicillin is known to alter the intestinal flora and to produce an enteritis which sometimes is accompanied by bacteremia. Activation of a latent virus, associated with one of the three syndromes, usually follows. As indicated by question marks, the other steps depicted in the diagram are speculative. The enteritis may be of viral rather than bacterial etiology or, alternatively, it may be part of a graft-versus-host-like reaction.

to be composed mostly of gram-positive bacteria. Moreover, a virus, similar or identical to that isolated from guinea pigs, also has been recovered from hamsters dying of penicillin.

It is concluded that penicillin toxicity in both the guinea pig and the hamster is associated with activation of a latent virus which probably belongs to the parvovirus group. Theories concerning the mechanism of viral activation remain speculative, and evidence for a causal relationship of the virus to the toxic effects is inconclusive. However, as graphically depicted in Fig. 1, it is hypothesized that the suprainfection with gram-negative enteric rods and the enteritis which are induced by penicillin lead to the elaboration and absorption of endotoxin. The resulting endotoxemia produces a state of immunodeficiency which is responsible for activation of a latent virus. The viral infection or endotoxemia either directly, or indirectly, through a graft-versus-host-like reaction, might induce each of the syndromes associated with penicillin toxicity. The appearance of gallstones in a significant number of penicillin-treated guinea pigs remains unexplained, but, conceivably, cholesterol stones may form because of an interference with the intestinal absorption of bile salts brought about by the ileitis which accompanies penicillin-induced disease. Finally, the development of intestinal tumors in some guinea pigs which had survived injections of penicillin suggests that this form of neoplasia may be related to a state of immunodeficiency associated with penicillin toxicity.

Any implications concerning human disease which one might draw from these studies would be highly speculative, to say the least. Nonetheless, one is tempted to consider whether some instances of idiopathic thrombocytopenia and granulocytopenia, granulomatous and antibiotic-associated enteritis, and perhaps even colonic neoplasia may be human counterparts. Regardless of the validity of any such associations, the demonstration that an antibiotic can activate a latent virus would seem to add a new dimension to the toxic potential of these widely used drugs.

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