ENTERITIS AND COLIFORM BACTEREMIA IN GUINEA PIGS GIVEN PENICILLIN

W. Edmund Farrar, Jr., M.D., and Thomas H. Kent, M.D.

From the Departments of Applied Immunology and Experimental Pathology, Walter Reed Army Institute of Research, Walter Reed Army Medical Center, Washington, D.C.

The observation that small doses of penicillin may be lethal for the guinea pig was first made by Hamre, Rake, McKee and MacPhillamy in 1943,[1] and the mechanism of this surprising phenomenon has intrigued investigators ever since. Very large doses of penicillin produce an acute lethal neurologic syndrome in most experimental animals, including the guinea pig.[2] Much smaller doses, however, produce a delayed type of illness in the guinea pig and hamster[3] which has not been described in other species. Guinea pigs appear well for 2 or 3 days after penicillin injection, and then rapidly sicken and die on the third to the sixth day.[4]

Early theories of the nature of the toxicity of penicillin for the guinea pig invoked a direct chemical effect of penicillin on the animal.[5] But in 1955 de Somer, Van de Voorde, Eyssen and Van Dijck[4] demonstrated that the administration of penicillin is followed by a massive proliferation of gram-negative coliform organisms in the intestine of the guinea pig.[4] The intestinal flora of the guinea pig, unlike that of most other animals, is predominantly gram-positive, and coliform organisms are not usually present in significant numbers.[6,7] De Somer postulated that penicillin suppresses the normal gram-positive flora, and allows the overgrowth of gram-negative coliform organisms, which are somehow responsible for the fatal illness. If this hypothesis is correct, then this disease would represent a reproducible and relatively well defined experimental model for the study of bacterial superinfection associated with the administration of an antibiotic agent. Problems of this nature are commonly encountered in clinical medicine, but under clinical circumstances are very difficult to study in a systematic manner. The present study was therefore undertaken to quantitate the changes in the intestinal microbial flora of the guinea pig associated with penicillin administration, and to evaluate the relationship of these changes to the clinical and morphologic manifestations of disease in these animals.

Material and Methods

Hartley strain guinea pigs of either sex, weighing between 300 and 400 gm, were used in all experiments. They were housed in groups of 6 to 8 in a room in which no other species of experimental animals were kept. Diet consisted of Purina ®

Accepted for publication, May 6, 1965.

Rabbit Chow, supplemented with lettuce and other greens, and water *ad libitum*. The principles of laboratory animal care as promulgated by the National Society for Medical Research were observed.

Aqueous penicillin was administered as a single intramuscular dose of 50,000 units. Other antimicrobial agents were used as described under Results.

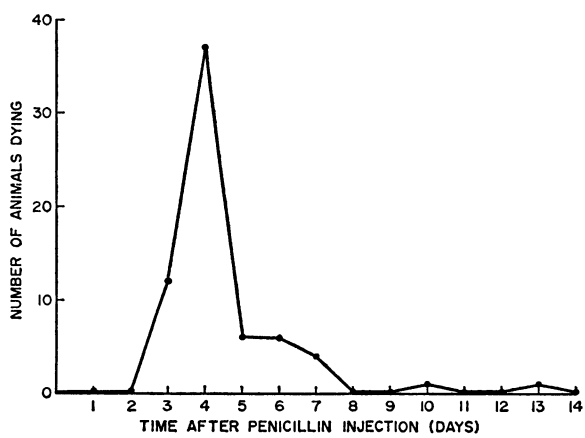
Intestinal microorganisms were enumerated by preparing serial 10-fold dilutions of cecal contents in Penassay broth (Difco Laboratories, Detroit, Mich.), and spreading 0.1 ml of appropriate dilutions on each of the media employed by means of a motor-driven turntable and a bent glass rod. The media used and the conditions under which the cultures were incubated are given in Table I. Emphasis was placed upon accurate enumeration and generic grouping of organisms, rather than exact species identification in every case. Lactobacilli were identified by morphologic characteristics and ability to grow on LBS agar. *Escherichia coli* and *Aerobacter aerogenes* (all Klebsiella-Aerobacter organisms were included under this term) were identified by typical colonial appearance on MacConkey agar and reactions on Kligler's iron agar. Atypical coliform bacteria were classified as "paracolon" organisms. Strictly anaerobic gram-negative rods were considered to be Bacteroides. Streptococci which grew on SF agar at 45.5° C were considered to be enterococci.

Blood and lymph node cultures were made in thioglycollate broth, and were held for 2 weeks before being discarded as negative.

Tissues taken from sacrificed animals for histologic study were fixed in 10 per cent neutral formalin and stained with hematoxylin and eosin.

Results

Clinical Effects of Penicillin. For 24 to 48 hours after a single injection of 50,000 units of penicillin the animals appeared normal. During the second and third days many showed inactivity and loss of appetite, and stopped defecating. By 72 hours some were more seriously ill, with ruffling of the fur, unsteady gait and rapid respiration. Most of these obviously sick animals died within the next 6 hours, with increasing prostration, cyanosis and gasping respiration. Throughout the illness obstipation, rather than diarrhea, was a characteristic feature. Text-figure 1 shows the number of animals dying each day after penicillin among a group of 91 animals. More deaths occurred between 72 and 96 hours



TEXT-FIG. 1. Number of guinea pigs dying each day following a single intramuscular injection of 50,000 units of penicillin G. Total number of animals given injections was 91.

TABLE I

CULTURAL METHODS USED AND BACTERIA ISOLATED FROM CECAL CONTENTS OF NORMAL GUINEA PIGS

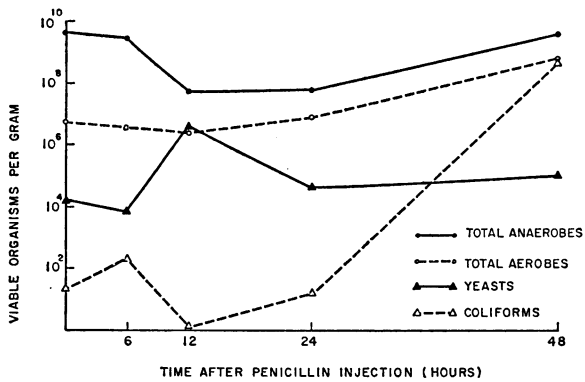
Medium	Atmospheric environment	Incubation time (days)	Temperature (° C)	Organisms enumerated	Mean $\log_{10}$ organisms per gram (range)
Sheep blood agar	H_2 + 10% CO_2	4	37	total count (predominantly streptococci)	9.6(9.0–9.9)
LBS agar *	H_2 + 10% CO_2	4	37	lactobacilli	3.8(< 2–6.6)
Sheep blood agar	air	2	37	total aerobes (predominantly Bacillus)	6.7(6.0–7.3)
Sabouraud dextrose agar + chloramphenicol (50 μg/ml)	air	7	37	yeasts (Candida)	4.2(< 2–5.9)
MacConkey agar	air	1	37	coliform bacteria	< 2(< 2)
SF agar †	air	4	45.5	enterococci	2.6(< 2–3.8)

* Baltimore Biological Laboratories, Inc.

† Difco Laboratories.

than during the remainder of the 14-day experimental period. The mortality in this experiment was 74 per cent, and was approximately the same in many additional experiments.

Microbiologic Effects of Penicillin. The microorganisms cultured from



TEXT-FIG. 2. Changes in the numbers of various microorganisms in the cecum following a single injection of penicillin. The line labeled "total anaerobes" includes two separate effects: a fall in gram-positive cocci occurring between 0 and 12 hours and a rise in gram-negative rods occurring between 24 and 48 hours. The rise in "total aerobes" occurs because coliform organisms are included in this group. The values for each time period represent the geometric means of the counts obtained from 6 animals.

the cecal contents of 6 normal guinea pigs are shown in Table I. The findings in 25 additional normal animals examined at various times during these experiments did not differ significantly from the data given in the table. Anaerobic organisms outnumbered aerobic bacteria by at least 100-fold. Almost all of the organisms isolated on blood agar plates maintained anaerobically were gram-positive cocci. Most appeared to be streptococci, although a minority had the morphologic characteristics of micrococci. A few exhibited alpha hemolysis, but most were nonhemolytic. Further identification of these organisms was not carried out. Contrary to the findings of others,[6,7] gram-positive cocci were considerably more abundant than lactobacilli. The lactobacillus counts varied widely among normal animals. Bacteroides organisms were present in moderate numbers (up to 10^7) in a few animals but were always outnumbered by anaerobic gram-positive cocci.

Most of the organisms which appeared on blood agar plates incubated aerobically were gram-positive rods of the genus Bacillus. Coliform bacteria were the only members of the family Enterobacteriaceae encountered and were very rarely found in numbers considered to be significant; only 3 of 31 normal animals whose intestine was cultured had more than 100 of these organisms per gram of cecal content, and none had more than 1,000. Yeasts (Candida) were usually present but the numbers varied widely. Enterococci constituted only a small proportion of the streptococci present.

The principal changes occurring in this flora during the first 48 hours

after penicillin administration are shown in Text-figure 2. The earliest definite change was seen at 12 hours, when the total anaerobe count (mainly gram-positive cocci) had fallen by approximately 100-fold to less than 10^8 organisms per gram. Coincident with this was a 600-fold increase in the yeast count, which rapidly subsided toward normal levels. The population of aerobic gram-positive rods (predominantly Bacillus) did not change significantly. Between 24 and 48 hours after penicillin administration a 10 million-fold increase in the numbers of gram-negative coliform bacteria occurred. The increase in the total anaerobe count during this period was due to the appearance in many animals of large numbers of gram-negative anaerobic bacteria, rather than to a resurgence of gram-positive organisms. Precise enumeration of streptococci at this time was not possible because of the overgrowth of gram-negative organisms. A few of the anaerobic gram-negative organisms were coccoid in form, but most were pleomorphic rods (Bacteroides). In about half the animals Bacteroides outnumbered the coliform organisms. The flora established by this time did not change significantly during the next 48 hours, when most of the deaths occurred.

No single type of coliform organism was clearly predominant in the cecal flora following the administration of penicillin. Among 23 animals examined 48 hours after penicillin, paracolon organisms were found in 14 (predominant in 10), *Aerobacter aerogenes* in 13 (predominant in 7), and *Escherichia coli* in 12 (predominant in 6). In most animals at least 2 different types of coliforms were present.

Culture of heart blood from 36 randomly-selected animals 72 to 96 hours after penicillin revealed that 10 (27 per cent) had bacteremia due to coliform organisms (Table II). Among the 10 positive cultures, paracolon organisms were found in 4, *E. coli* in 3, *A. aerogenes* in 1, and both

TABLE II

BLOOD CULTURES IN GUINEA PIGS GIVEN PENICILLIN

	Number cultured	Number positive	Percent positive
Controls	23	0	0
24–48 hours	40	1	2.5
72–96 hours	36	10	27
72–96 hours (sick animals)	9	8	89

E. coli and *A. aerogenes* in 2. With 1 exception positive blood cultures were never obtained earlier than 3 days or later than 5 days after penicillin, even though many of these animals had large numbers of coliform bacteria in the cecum. When animals that were obviously sick (though not necessarily moribund) were selected for blood culture, 8 of 9 (89 per cent) were found to have bacteremia at 72 to 96 hours. One of these cultures contained Bacteroides; the others contained coliforms.

Ileocecal lymph nodes were cultured in an attempt to determine the route by which organisms reached the blood stream. In control animals

these nodes were always sterile, but coliform organisms were found in 2 of 5 nodes removed 48 hours after penicillin, and in 9 of 10 nodes removed at 72 to 96 hours.

Morphologic Changes after Penicillin. Guinea pigs were sacrificed for histologic study at 6 and 12 hours and 1, 2, 3, 4, 5 and 7 days after penicillin administration. All viscera including brain were studied in at least 6 animals from each time interval and the intestines were examined in many other animals sacrificed at various times. Untreated controls were examined in each experiment.

As early as 6 hours after penicillin, the cecal contents were softer than normal, and by 24 hours they were liquid. By 48 hours the cecum was distended with liquid and gas, and the content had a distinctly feculent odor not detected in normal animals. In sick animals the right colon and ileum showed intense vascular congestion, and in some cases appeared deeply cyanotic. In contrast to the cecum, the content of the distal large intestine was inspissated. Lymph nodes in the ileocecal region were enlarged and congested. Other viscera were grossly normal.

At 12 and 24 hours after penicillin administration a definite but mild infiltration of the lamina propria of the distal ileum by neutrophils was present (Figs. 1 and 2). In a few animals a slight focal acute inflammatory reaction was seen in the cecum at this time, but in most animals the cecum was uninvolved.

At 48 hours the inflammatory reaction in the ileum was more variable, being slight in some animals and somewhat more severe in others. At 72 to 96 hours in animals that appeared sick the distal ileum and cecum exhibited a more severe lesion. The ileal mucosa contained chronic inflammatory cells and the epithelium appeared hyperplastic with lengthened crypts and short irregular villi (Fig. 3). The cecal mucosa was severely congested and the epithelium was altered with shortened crypts lined by more irregular and hyperchromic cells. Many degenerate cells were seen in the process of sloughing into the lumen. The lamina propria contained variable numbers of acute and chronic inflammatory cells (Figs. 5 and 6). Focal erosions were also seen. Inflammatory cells were present in the submucosa of the cecum in some areas. No lesions were seen in the upper small intestine.

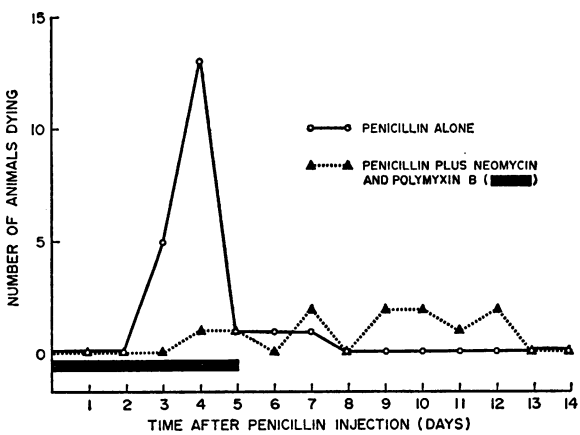
The lymphoid tissue of the ileum and cecum contained foci of purulent exudate with focal ulceration of the surface. In animals that did not appear sick at 72 hours the lesions were much less severe. An acute lymphadenitis of regional lymph nodes was seen in sick animals sacrificed at 72 hours (Fig. 4) but not in animals sacrificed at earlier times.

Other viscera exhibited no significant change except for mild fatty metamorphosis of the liver in some animals. Surviving animals exhibited no lesions at 7 days.

Protection against the Lethal Effect of Penicillin. A group of 25 ani-

mals was given neomycin, 5 mg, and polymyxin B, 3 mg, twice daily by gastric tube for 5 days after penicillin injection. Administration of these non-absorbable antibiotic agents protected these animals almost completely from the lethal effect of penicillin for the duration of treatment (Text-fig. 3). The animals did not develop a coliform flora in the cecum, although many showed small numbers of proteus organisms. At necropsy only minimal inflammatory changes were seen in the gut.

Nature of the Early Inflammatory Changes. As described above, mild inflammatory changes were seen in the distal ileum before the appear-



TEXT-FIG. 3. Number of animals dying each day among 25 animals given a single injection of 50,000 units of penicillin G compared with the number dying in a similar group given a single injection of penicillin and then treated for 5 days with oral neomycin and polymyxin B.

ance of coliform organisms in the cecal content. Experiments were designed to elucidate, if possible, the pathogenesis of these early changes. Two possible mechanisms were studied: (1) release of some toxic substance from intestinal bacteria killed by the penicillin, and (2) irritation of the mucosa in association with the early, transient proliferation of yeasts.

To investigate the first possibility, normal guinea pigs were given, either by intragastric or intracolonic instillation, 20 ml of a 25 per cent suspension of normal guinea pig cecal content which had been frozen and thawed twice using a dry ice-alcohol bath. Control animals received a suspension which had not been frozen and thawed. None of the 36 animals sacrificed at various intervals up to 24 hours after administration of the suspension showed any inflammatory changes in the alimentary tract.

To assess the role that yeasts might play in the early inflammatory changes, a group of animals was given amphotericin B, 2 mg, by gastric tube at the time of penicillin injection, followed by similar doses of amphotericin B, 6 and 12 hours later. Administration of amphotericin B neither prevented nor delayed the development of the early inflamma-

tory lesions, even though proliferation of yeasts was entirely suppressed; in fact, yeasts were completely eradicated from the bowel within 24 hours. The mortality was greater among animals given penicillin plus amphotericin B (13 of 15) than among animals given penicillin alone (7 of 15). None of 9 animals given amphotericin B alone died.

Resistance to the Lethal Effect of Penicillin. During the period when most of these studies were being performed, approximately 25 per cent of the animals given 50,000 units of penicillin survived. In an effort to elucidate the reasons why some animals were apparently resistant to the lethal effect of penicillin, serum was collected from animals still living 3 weeks after the administration of penicillin. When 2 ml of this pooled serum was given subcutaneously at the time of penicillin injection, the mortality (35 of 45 animals) was not significantly different from that observed when either pooled normal serum or no serum was employed. Thus, it was not possible by these experiments to demonstrate the presence of a protective factor, such as antibody, in the serum of "resistant" animals.

After several months of experimentation, during which the mortality following this dose of penicillin (50,000 units) had remained in the range of 70 to 75 per cent, the mortality suddenly fell to less than 25 per cent, and remained low for at least 2 months. Two possible mechanisms for this change in response were considered: (1) the animals might have acquired a flora of penicillin-resistant organisms from the environment of the animal room, where large amounts of this antibiotic agent had been used during the preceding months, and (2) sufficient numbers of coliform organisms might not have been present in the environment to initiate superinfection after penicillin administration. Using anaerobic cultures on blood agar plates containing penicillin (10 units per ml) it was not possible to demonstrate any more penicillin-resistant organisms in the cecal contents of animals which had been quartered in the animal room for 2 weeks than in animals just received from the supplier. The possibility that insufficient numbers of coliform organisms were present in the environment was excluded by the observation that generous contamination of food and water with suspensions of mouse feces containing very large numbers of coliforms did not significantly increase the mortality following penicillin.

Following a one-month period during which no penicillin was used in the animal room, the mortality was again observed to be in the range of 50–80 per cent.

Discussion

The fatal effect of small doses of penicillin in guinea pigs has been studied by many investigators, but a clear elucidation of the pathogenesis of the disease has not been presented. Our experiments have indicated

that this syndrome was initiated by changes produced by penicillin in the composition of the intestinal flora. Within 12 hours after the parenteral injection of penicillin a 100-fold decrease in the total number of cultivable organisms in the cecum (predominantly anaerobic gram-positive cocci) occurred. Between 24 and 48 hours after penicillin an explosive proliferation of gram-negative coliform organisms occurred in the cecum, and the number of these organisms rapidly rose from insignificant levels ($<10^2$) to 10^8 to 10^9 per gm. In some animals even larger numbers of gram-negative anaerobic bacteria (Bacteroides) were found. Associated with overgrowth of gram-negative organisms was severe inflammation of the cecum and a somewhat less severe ileitis. Acute inflammation and bacteria were present in the regional lymph nodes. Bacteremia occurred in a high percentage of animals that became clinically ill. Thus the shift in intestinal flora was followed by an infection of the intestinal wall, which ultimately extended beyond this organ and caused the death of the animal.

Although some investigators have suggested that this disease is the result of a direct toxic effect of penicillin on the animal,[5] several lines of evidence contradict this hypothesis: (1) penicillin in doses lethal for conventional guinea pigs is not toxic for germ-free animals,[8,9] (2) a similar syndrome can be produced by several other chemically unrelated antibiotic agents, including bacitracin, which is not absorbed from the gastrointestinal tract,[4,10,11] and (3) the syndrome can be prevented by the administration of antibiotic substances effective against gram-negative bacteria.

De Somer, Van de Voorde, Eyssen and Van Dijck [4] first clearly postulated that the lethality of penicillin for the guinea pig was due to suppression of the normal gram-positive intestinal flora, overgrowth of coliform bacteria, and overwhelming toxemia caused by absorption of bacterial toxins from the intestine. They were able to reproduce the clinical syndrome by parenteral injection of *E. coli* culture filtrates. Because they were consistently unable to demonstrate bacteremia, they later modified this conclusion [12] and stated the belief that death was due to fluid and electrolyte losses into the bowel lumen and metabolic changes associated with the sudden shift in the intestinal flora. We have found severe inflammation of the ileum and cecum, infection of the regional lymph nodes and bacteremia in a large proportion of animals, and believe that uncontrolled infection is an adequate explanation of the illness observed in these animals. The bacteremia appears to be a significant event because it occurs at the time when the animals become obviously ill.

The morphologic alterations in guinea pigs following penicillin administration have not been adequately documented previously. Others have alluded to the presence of enteritis but this has not been clearly

described or illustrated. Our studies of serially sacrificed guinea pigs following penicillin administration showed that the development of enteritis and regional lymphadenitis correlated well with the clinical and microbiologic findings. We have not, however, been able to explain the mild acute ileitis that occurred in the first 24 hours after penicillin administration, before the appearance of coliform bacteria in the cecal content.

Experiences with other antibiotic agents substantiate the view that the lethal effect of small doses of penicillin in the guinea pig is due to suppression of gram-positive organisms. Methicillin,[13] erythromycin,[14] spiramycin[15] and bacitracin[4,10,11] are all effective chiefly against gram-positive bacteria, and all kill guinea pigs in relatively small doses. Tetracyclines, which are more effective against gram-positive than against gram-negative bacteria, also produce a similar syndrome.[16,17] Streptomycin, a potent agent against both gram-positive and gram-negative organisms, has produced varying results. Eyssen, de Somer and Van Dijck[10] found it to be toxic for the guinea pig, while Fischer[18] found that it protected against the lethal effect of penicillin. Chloramphenicol,[10] neomycin and polymyxin B, which are highly active against gram-negative bacteria, do not appear to be lethal for the guinea pig, and in our experiments neomycin and polymyxin B provided protection against the effect of penicillin.

At least two important questions concerning the pathogenesis of the enteritis remain unanswered: (1) what is the role of the normal intestinal flora in keeping the normal guinea pig intestine free, or nearly free, of coliform bacteria, and (2) what factors allow these bacteria, which are normally present in large numbers in most animal species, to invade the intestinal wall of the guinea pig and produce a fatal infection? Recent studies by two groups of investigators[19,20] have indicated that the presence of acetic and butyric acids, produced by normal intestinal bacteria, may be the most important deterrent to the multiplication of salmonellas in the cecum of the mouse. Of particular pertinence is the additional suggestion by Meynell[19] that there are inhibitory substances in the normal mouse gut which keep the numbers of coliform bacteria within certain limits. Studies are currently in progress to determine if such substances can be demonstrated in the intestine of the guinea pig. Formal and colleagues[21] and Sprinz and associates[22] found that *E. coli* was much less pathogenic than *Shigella flexneri* for the germ-free guinea pig. A small proportion of their animals died, however, following monocontamination with *E. coli;* morphologic study of the intestine was not carried out earlier than one week after contamination.

The pseudomembranous enterocolitis which occurs in human patients receiving broad spectrum antibiotic therapy appears to be somewhat

analogous to this experimental disease. Most cases have been associated with proliferation of *Staphylococcus aureus* in the intestine,[23] but typical cases have occurred in which this organism could not be found. *Proteus vulgaris, Pseudomonas aeruginosa* and *Candida albicans* have also been associated with cases of persistent diarrhea following antibiotic therapy.[23] These complications of antibiotic administration occur unpredictably, and it is difficult or impossible to gain information about their exact pathogenesis because they are recognized only after the important changes in the intestinal bacterial ecology have taken place. Further study of the experimental model described here may provide a better understanding of the determinants of the human disease, and perhaps contribute to the development of rational preventive measures.

Summary

We have studied the pathogenesis of the fatal illness produced in guinea pigs by a single intramuscular dose of 50,000 units of penicillin G. In the first 12 hours after penicillin administration the total number of culturable organisms (predominantly gram-positive bacteria) in the cecum fell to less than 1 per cent of the pretreatment level. Between 24 and 48 hours a 10-million fold increase in the number of coliform bacteria in the cecum occurred. An even greater increase in the number of anaerobic gram-negative rods was also seen in some animals. These changes in the intestinal flora were accompanied by a severe cecitis, moderate ileitis and acute regional lymphadenitis. A high incidence of bacteremia due to coliform organisms was found in animals that appeared ill. This syndrome could be prevented by the administration of nonabsorbable antibiotic agents effective against coliform bacteria.

We believe that this disease is an example of bacterial superinfection induced by alterations in the microbial flora of the intestine, and may prove to be a useful experimental model for the further study of this phenomenon.

References

1. Hamre, D. M.; Rake, G.; McKee, C. M., and MacPhillamy, H. B. The toxicity of penicillin as prepared for clinical use. *Amer. J. Med. Sci.*, 1943, 206, 642–652.
2. Boyd, E. M., and Fulford, R. A. The acute oral toxicity of benzylpenicillin potassium in guinea pigs. *Antibiot. Chemother. (Wash.)*, 1961, 11, 276–283.
3. Schneierson, S. S., and Perlman, E. Toxicity of penicillin for the Syrian hamster. *Proc. Soc. Exp. Biol. Med.*, 1956, 91, 229–230.
4. DeSomer, P.; Van de Voorde, H.; Eyssen, H., and Van Dijck, P. A study on penicillin toxicity in guinea pigs. *Antibiot. Chemother. (Wash.)*, 1955, 5, 463–469.
5. Koch, E.; Bohn, H.; Heiss, F., and Schneider, R. Über Penicillinintoxikation. Ihre cerebral ausgelösten Klinischen Standardsyndrome. Penicillinaseaktivitat des Gehirns und weitere Schutzmechanismen. *Naunyn Schmiedeberg Arch. Exp. Path.*, 1953, 220, 157–183.

6. CRECELIUS, H. G., and RETTGER, L. F. The intestinal flora of the guinea pig. *J. Bact.*, 1943, **46**, 1–13.
7. SMITH, H. W., and CRABB, W. E. The faecal bacterial flora of animals and man: its development in the young. *J. Path. Bact.*, 1961, **82**, 53–66.
8. FORMAL, S. B.; ABRAMS, G. D.; SCHNEIDER, H., and LAUNDY, R. Penicillin in germ-free guinea pigs. *Nature (London)*, 1963, **198**, 712.
9. NEWTON, W. L.; STEINMAN, H. G., and BRANDRISS, M. W. Absence of lethal effect of penicillin in germ-free guinea pigs. *J. Bact.*, 1964, **88**, 537–538.
10. EYSSEN, H.; DESOMER, P., and VAN DIJCK, P. Further studies on antibiotic toxicity in guinea pigs. *Antibiot. Chemother. (Wash.)*, 1957, **7**, 55–64.
11. EFFENBERGER, E., and SEELIGER, H.P.R. Die toxische Wirkung des Penicillins und anderer antibiotischer Mittel auf Meerschweinchen. *Arzneimittelforschung*, 1961, **11–1**, 38–41.
12. DESOMER, P. Die Bedeutung der Darmflora für die Toxizität der Antibiotika beim Meerschweinchen. *Zbl. Bakt.*, 1957–58, **170**, 338–340.
13. SEELIGER, H.P.R., and WERNER, H. Die toxische Wirkung des Methicillins (2, 6-Dimethoxy-benzamidopenicillin-Natrium) auf Meerschweinchen im Vergleich zu seiner atoxischen Wirkung bei Ratten. *Path. Microbiol. (Basel)*, 1962, **25**, 153–169.
14. KAIPAINEN, W. S., and FAINE, S. Toxicity of erythromycin. *Nature (London)*, 1954, **174**, 969–970.
15. BOYD, E. M., and PRICE-JONES, M. A. The comparative acute oral toxicity of spiramycin adipate in mice, rats, guinea pigs, and rabbits. *Antibiot. Chemother. (Wash.)*, 1960, **10**, 273–284.
16. ROINE, P., and ETTALA, T. Toxicity of aureomycin to guinea pigs. *Nature (London)*, 1952, **169**, 1014.
17. AMBRUS, C. M.; SIDERI, C. N.; JOHNSON, G. C., and HARRISSON, J.W.E. Toxicity of penicillin and aureomycin in guinea pigs. *Antibiot. Chemother. (Wash.)*, 1952, **2**, 521–527.
18. FISCHER, G. W. Beitrag zur Ursache des Penicillin-Todes bei Meerschweinchen. *Zbl. Bakt.*, 1955, **164**, 230–233.
19. MEYNELL, G. G. Antibacterial mechanisms of the mouse gut. II. The role of Eh and volatile fatty acids in the normal gut. *Brit. J. Exp. Path.*, 1963, **44**, 209–219.
20. BOHNHOFF, M.; MILLER, C. P., and MARTIN, W. R. Resistance of the mouse's intestinal tract to experimental salmonella infection. I. Factors which interfere with the initiation of infection by oral inoculation. *J. Exp. Med.*, 1964, **120**, 805–816.
21. FORMAL, S. B.; DAMMIN, G.; SPRINZ, H.; KUNDEL, D.; SCHNEIDER, H.; HOROWITZ, R. E., and FORBES, M. Experimental Shigella infections. V. Studies in germ-free guinea pigs. *J. Bact.*, 1961, **82**, 284–287.
22. SPRINZ, H.; KUNDEL, D. W.; DAMMIN, G. J.; HOROWITZ, R. E.; SCHNEIDER, H., and FORMAL, S. B. The response of the germ-free guinea pig to oral bacterial challenge with *Escherichia coli* and *Shigella flexneri*. *Amer. J. Path.*, 1961, **39**, 681–695.
23. PROHASKA, J. V.; MOCK, F.; BAKER, W., and COLLINS, R. Pseudomembranous (staphylococcal) enterocolitis. Int. Abstr. Surg., *Surg. Gynec. Obstet.*, 1961, **112**, 103–115.

We are greatly indebted to Colonel Helmuth Sprinz and Dr. Samuel B. Formal for their guidance and criticism.

LEGENDS FOR FIGURES

Photomicrographs were prepared from sections stained with hematoxylin and eosin.

FIG. 1. Ileum of normal guinea pig. × 185.

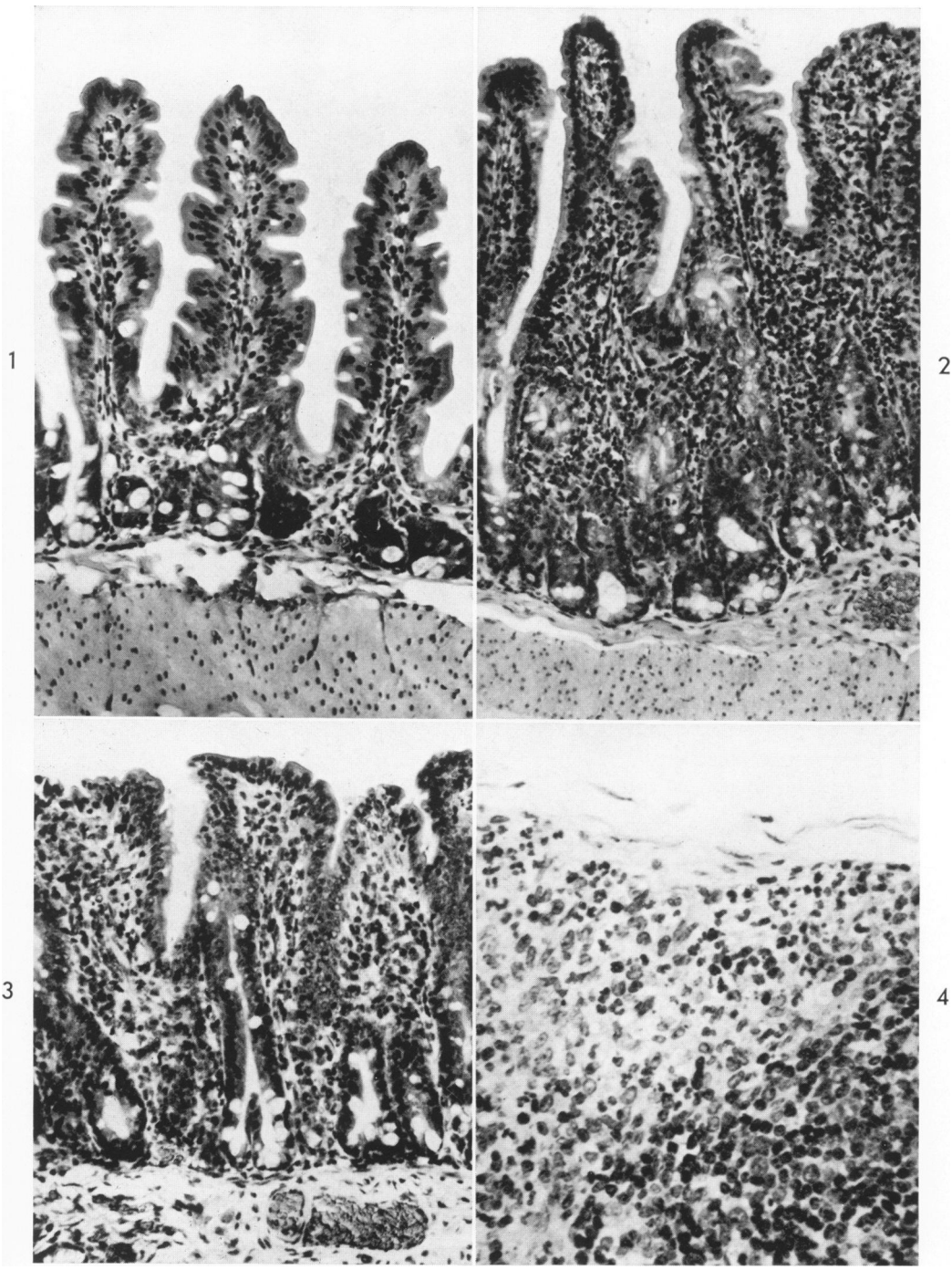
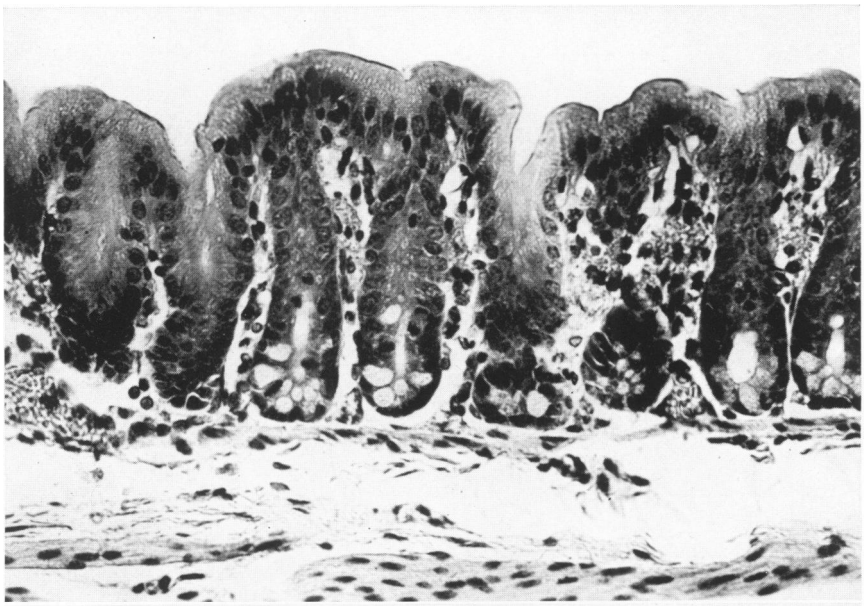


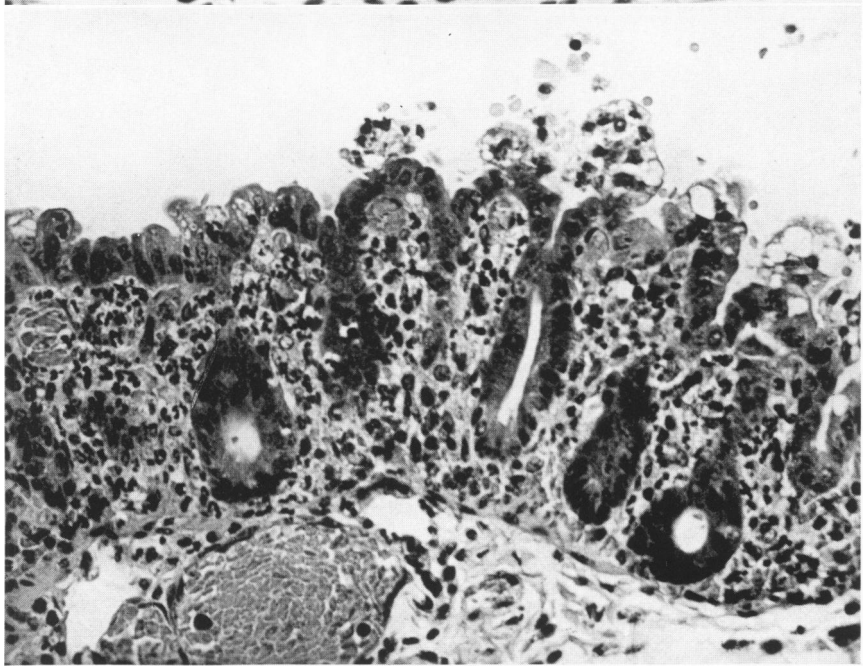
FIG. 2. Ileum, 24 hours after penicillin administration. Villi are expanded by a neutrophil infiltration in the lamina propria. The crypts are slightly lengthened. × 150.

FIG. 3. Ileum, 72 hours after penicillin administration. The epithelium is hyper-plastic and there are greatly elongated crypts and very short villi. The lamina propria contains a moderate number of chronic inflammatory cells. This change involved the distal ileum and was less severe in some animals. × 170.

FIG. 4. Mesenteric lymph node, 72 hours after penicillin administration. Neutrophils are evident in the peripheral sinus. × 330.



5



6

FIG. 5. Cecum of normal guinea pig. × 260.

FIG. 6. Cecum, 72 hours after penicillin administration. The epithelial cells are greatly reduced in number and are cuboidal. Sloughing degenerate cells appear on the surface. The lamina propria contains a mixed acute and chronic inflammatory cell infiltrate. Congested vessels and scattered inflammatory cells are evident in the submucosa. This type of change involved the cecum diffusely and was present but less severe in more distal parts of the colon. × 260.