completely disappeared and no lines can be attributed to it). The patterns suggest the possibility of stacking fault or "double *c*-axis" structures similar to those existing in the rare earths or, alternatively, suggest a mixture of phases resulting from a "disproportionation" of the Cs III. Such a mixture of phases could conceivably have a lower free energy than a single phase. Rate considerations might also favor the formation of a mixture of solid phases rather than a single phase on passing from the Cs III to "Cs IV" region. Assuming "Cs IV" to be approximately close-packed, and considering only one diffraction line that could be attributed to a face-centered-cubic structure, we have calculated relative compressions for "Cs IV" at pressures between 43 and 55 kb at 27°C.

These compressions are included in Fig. 2 which gives the compressibility of Cs as we have measured it by x-ray diffraction techniques. For comparison, the results Bridgman obtained by volume displacement methods are also shown. With the above assumption, v/v_0 for "Cs IV" at the Cs-III-"IV" transition is calculated to be 0.404. This corresponds to a volume decrease of about 2.4 percent. This, when added to the 9.0 percent decrease occurring at the CsII-III transition, gives an overall change of about 11.4 percent which agrees well with the value determined by Bridgman.

In our most successful procedure for preparing these specimens, so difficult to handle, for simultaneous diffraction-resistance measurements, use was made of a two-piece polyethylene cell. The two mating parts were designed to form a rectangular cavity of dimensions approximately 0.16 × 0.24 × 0.015 cm. At each end of the 0.24-cm wide portion of the cell were tiny reservoirs for Cs. A fine, pointed, copper wire was pushed through the polyethylene in each reservoir area to make contact with the Cs for the resistance measurements. Liquid Cs was injected into the cell with a small hypodermic needle. The outside dimensions of the polyethylene cell were 0.48 × 0.24 cm. This cell was centered in a 2.54 cm (on edge) tetrahedron composed of boron-filled, phenol-formaldehyde resin. The corresponding triangular anvil faces were 1.90 cm on a side. The press was used with the x-ray tube in position "B." The primary beam of x-rays was directed perpendicularly to the 0.015-cm thick Cs specimen.

We experienced difficulty because of the presence of relatively large crystals in all the Cs phases. This is undesirable in powder diffraction work. Various schemes, such as cycling through the various phase changes, were sometimes successful in breaking up the large crystals. In the main, however, samples not containing some large crystals were only obtained by chance. This made it necessary to prepare and run many samples in order to obtain usable patterns.

The Cs (*15*) comes in contact with polyethylene and with copper during the experiments. There seems to be no evidence of contamination that would affect the results. Diffraction patterns taken before and after an experiment, both taken at low pressure, give the same result.

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Action of Erythromycin on "Protoplasts" in vivo

Abstract. *Bacteria may persist in "protoplast" form in kidneys after treatment of experimental enterococcal pyelonephritis with penicillin. Erythromycin, while ineffective against the bacterial form of infection, was able to kill "protoplasts" in vivo. This finding is consistent with the hypothesis that intact cell wall interferes with the ingress of erythromycin into the cellular area in which it acts.*

We reported recently that bacteria may persist in "protoplast" form (*1*) in kidneys after treatment of experimental enterococcal pyelonephritis with penicillin (*2*). Our observation has prompted speculation concerning the possible role of these forms in the pathogenesis of chronic pyelonephritis. It is possible that protoplasts per se account for the chronicity and frequent recurrence of renal infection in man. This could explain some of those instances of progressive disease characterized by the apparent absence of infection as indicated by the failure of bacteria to grow when urine or tissue biopsy were cultured on standard media. Alternatively, chronicity of pyelonephritis might be related to reversion of protoplasts to bacterial forms, secondary to changes in host-parasite relationship. The animal model described in this report provides a means of studying host-parasite-drug relationships in protoplast infection.

Penicillin has been shown to induce protoplast formation in vitro and accounted for the production of these forms in vivo after the treatment of enterococcal pyelonephritis in rats. Since penicillin functions by inhibiting cell wall synthesis, it would not be expected to kill protoplasts. Therefore, an attempt was made to treat this type of renal infection with an antibiotic which has a different mode of action.

Erythromycin, a member of the macrolide group of antibiotics, was found by Brock and Brock to inhibit protein synthesis (*3*). Wolfe and Hahn confirmed this observation and inferred that the mechanism involved may be an interaction between erythromycin and one or several categories of RNA that mediate protein synthesis (*4*). Taubeneck had reported earlier that stable L-forms of *Proteus mirabilis* were more susceptible to erythromycin and

Table 1. Average of the logarithm of the number of bacterial and protoplast forms, per gram of kidney, isolated from treated and untreated pyelonephritic rats. The numbers in parentheses indicate the proportion of animals with one or both kidneys infected.

Treatment	Culture medium	
	Standard	0.3*M* sucrose
Saline	5.22 (29/29)	5.66 (29/29)
Erythromycin	4.94 (27/27)	5.22 (27/27)
Penicillin	0.26 (4/28)	2.32 (19/28)
Penicillin + erythromycin	0.26 (3/28)	0.53 (9/28)

other macrolides than the parent strains were (*5*). This selective susceptibility was attributed to the absence of cell walls in the L-forms and would account for the easier entrance of the antibiotic into the cell. Thus, in intact animals, it might be expected that erythromycin would not affect bacteria but would kill protoplast forms.

To test this hypothesis, groups of male Wistar rats, weighing 100 to 115 g, were injected intravenously with 1.0 ml (4.0×10^8 bacteria) of an 18-hour broth culture of *Streptococcus faecalis*. One day later some of these animals were treated with 100,000 units of procaine penicillin injected intramuscularly twice daily for 3 weeks. One week later, when protoplasts were expected in kidneys of animals treated with penicillin, groups of rats were given erythromycin (10 mg intramuscularly twice daily for 2 weeks). Controls consisted of infected animals which were treated with (i) erythromycin alone, (ii) penicillin alone, or (iii) saline. The methods used have been described (*2*) and may be briefly summarized. When the animals were killed, the kidneys were removed aseptically, cut into two pieces in a transverse plane, and weighed. One piece from each kidney was homogenized and serially diluted in distilled water. Measured portions of appropriate dilutions were incorporated into blood agar base (BBL) pour plates. The remaining part of each kidney was cultured in a medium designed to support protoplasts. Since this form of an organism requires a hyperosmotic environment for survival and reversion to the bacterial form, the kidney tissue was homogenized and serially diluted in heart infusion broth (Difco) to which 0.3*M* sucrose was added (final osmolarity, 750 milliosmols/liter). Blood agar base pour plates made from portions of these dilutions also contained 0.3*M* sucrose. Colony counts were made after the plates had been incubated at 37°C for 48 hours. The colony count on the blood agar base medium without sucrose represented bacterial forms only, the protoplasts having been destroyed in the distilled water. Alternatively, the colony count on the blood agar base medium with sucrose represented both bacterial and protoplast forms, the latter having been protected from osmotic lysis during homogenization and dilution by addition of 0.3*M* sucrose. The difference in colony counts obtained by these two methods was taken as the number of protoplasts present.

The results are shown in Table 1. Comparison of results obtained in groups treated with penicillin alone and penicillin plus erythromycin indicated that when 0.3*M* sucrose medium was used significant differences were noted in numbers of animals infected (19 versus 9, $X^2 = 7.14$; $p < .001$) and in quantity of infection (2.32 versus 0.53, $t = 4.48$; $p < .001$). However, when standard medium was used there were no significant differences between numbers of infected animals (4 versus 3, $X^2 = .16$; $p =$ N.S.) or quantity of infection (0.26 versus 0.26, $t = .04$; $p =$ N.S.). Thus, while erythromycin had little effect on the bacterial form of the infection, it was successful in killing protoplasts in vivo. This finding is consistent with the hypothesis that intact cell wall interferes with the ingress of erythromycin into the cellular area in which it acts.

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1. As used in this paper, "protoplast" refers to an osmotically fragile bacterial cell in which the amount of cell wall present has not been determined.
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Thalidomide Syndrome in Monkeys

Abstract. *Pregnant monkeys were treated with thalidomide after implantation but before formation of the fetal limbs. Two fetuses that were recovered from the treated females had congenital abnormalities. The thalidomide syndrome in the monkeys was manifested by amelia, phocomelia, internal hydrocephaly, facial capillary hemangioma, hypogenesis of the metatarsal bones, and anotia.*

Since the reports of McBride (*1*) and Lenz (*2*) of congenital malformations from thalidomide, there have been numerous attempts to reproduce the human malformations in experimental animals. Somers (*3*), using the rabbit, was the first to produce fetal abnormalities with thalidomide in an experimental animal. Other workers (*4–6*) have used a variety of other animals. The rabbit (*3, 4*) and the mouse (*6*) have been the only animals in which gross malformations of the fetus were observed. Lucey and Behrmann (*7*) showed that treating monkeys before implantation of the zygote resulted in no live births. He advanced the hypothesis that the drug killed the embryo prior to its implantation. Our study reveals that the typical thalidomide syndrome, as observed in man, can be induced in the monkey.

Fourteen female Cynamologus monkeys (*Macaca irus philippinensis*) were used in our study. Day 1 was recorded as the first day of menses. The females were placed with males on days 10 through 17. Daily vaginal washings to detect spermatozoa were performed after the female was exposed to a male to indicate when mating took place. Thalidomide (10 mg/kg) was given by an oral tube from day 32 to 42. These days were chosen for treatment because implantation does not occur until 9 to 11 days after mating (*8*). Treatment before this time will prevent nidation (*7*). The dosage schedule took into account that limb development occurs 26 to 26.5 days after conception (*8*). After two monkeys (PR-2052 and PR-2054) aborted, we performed Caesarean sections on pregnant females 374 and 815 in order to avoid losing any fetuses by unobserved abortions. All fetuses were examined grossly, and the internal viscera were examined for malformations. The eviscerated fetuses were skinned, fixed in 80 percent ethanol, and cleared with 1 percent potassium hydroxide. The skeleton was stained with 0.5 percent alizarin red.

Four pregnancies have occurred. (i) Female PR-2052 aborted spontaneously during the 3rd month of pregnancy. Gross dissection revealed that the con-