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 ($4.0 \times 10^8$ 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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**References and Notes**

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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6. Supported by grants from the U.S. Public Health Service.

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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-

ceptus was a teratoma. (ii) Female PR-2054 partially aborted a fetus at 5 months. An enlarged head caused a fetal dystocia which was manually relieved. The head was nearly twice normal size. There was hypogenesis of both ear auricles (anotia). The enlarged head was most likely due to internal hydrocephaly associated with a defective foramen magnum similar to the Arnold-Chiari malformation in man. The interparietal portions of the occipital bone were present, but the portion of the occipital bone forming the posterior half of the foramen magnum was absent. The neural arches of the first and second cervical vertebrae were also missing. The left and right anterior limbs were absent (amelia). The posterior extremities were macerated by the chewing of the mother and therefore could not be assessed. (iii) Female 374 had a grossly normal fetus that was taken by Caesarean section. This monkey was mated during each of two menstrual cycles. She was treated with thalidomide after the second mating but was not treated after the first mating. On the basis of weight, size, and ossification of the skeleton, the fetus was judged to be 4 months old. Therefore, conception must have occurred at the time of the first mating, when the female was not treated. (iv) Female 815 had a fetus taken by Caesarean section. It was grossly malformed and had a facial capillary hemangioma over the bridge of the nose and on the cheeks. The right anterior extremity consisted of a stub of soft tissue connected to a single digit. The finger contained a distal and intermediate bony phalanx. The left anterior limb was absent (amelia). Only a tiny nub of soft tissue was present to represent an extremity. The left posterior extremity had a stub of soft tissue intervening between the trunk and a foot. Small plaques of bone, thought to be a femur, tibia, and fibula, were in the stub of tissue representing the thigh and leg. A tarsal region was apparent but it was not possible to ascertain whether the cartilage primordia of the tarsal (ankle) bones were present. Five metatarsal bones were observed. The second and third were half the length of the others. This resulted in a retraction of their associated (second and third) digits, the toes appeared to be shortened in relation to the others. Each of the five digits had three bony phalanges. The first digit was not the hallux (big toe) because it was not in a position of apposition to the other digits and contained three bony phalanges instead of the usual two. The right posterior extremity was similar to the left except that only the left second metatarsal bone was shorter than the others. This resulted in a retraction of the associated digit causing the second toe to appear shortened in relation to the others. The only grossly abnormal finding of the viscera was dilated ureters.

The malformations described in these two monkeys were anatomically identical to the deformities reported in children (*2*) whose mothers had taken thalidomide during pregnancy. The anomalies in the monkeys reported here are of such distinctive character that it would be highly unlikely that they could have occurred, even in these limited cases, merely by chance. The birthrate of anomalous offspring among monkeys (*Macaca rhesus*), according to Lapin and Yakovleva (*9*), constitutes 0.48 percent. They reported that defective cardiac development is the most common congenital malformation in the monkey. Their findings would further strengthen our position that thalidomide produced the skeletal defects in our monkeys. The skeletal system in both man and the monkey was the primary target for malformations induced by thalidomide.

*Note added in proof*: Two additional, typically malformed fetuses were recovered from the thalidomide-treated monkeys (2062 and 2063). After 3 months of pregnancy, female 1162 aborted a teratoma. The drug treatment period for monkeys 2063 and 1162 was from day 34 to 40.

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## Transplantation Tolerance Induced in Adult Mice by Protein Overloading of Donors

Abstract. *Treatment of donor animals with unrelated antigens can regularly inhibit reactivity of spleen cells against the host, with subsequent induction of specific immune tolerance in the recipient animals if the barrier to histocompatibility is weak. Mice made tolerant in this way accept skin grafts from donor strain animals. In the case of strong differences (in $H_2$ locus) or heterologous (rat-mouse) combinations, the inhibition of the reactivity of the transferred lymphoid cells against the host is partial, and the skin-graft survival time is significantly prolonged.*

As a result of the treatment with protein antigen, adult animals become immunologically unresponsive to the antigen. Moreover, the treated animals neither produce antibodies nor develop delayed type hypersensitivity to a different antigen if the latter is given a few days after the beginning of the treatment with the first antigen (*1*). In such treated animals, rejection of the homografts is significantly delayed (*2*), and when spleen cell donors are similarly treated, the rate of mortality due to the reaction of these cells against the recipients is reduced by 50 percent (*3*). These observations were recently confirmed by Miller, Martinez, and Good (*4*) who showed that administration of five strong bacterial antigens to the donors of spleen cells reduces the reactivity of these cells against the host.

The finding that the severity of the reaction of the grafted cells against the host (graft-host reaction) could be reduced is particularly meaningful because of the possibility of inducing permanent tolerance in adult animals to foreign tissues. If an irradiated animal transfused with foreign lymphoid cells does not succumb to the graft-host reaction, it may then become tolerant to the tissues of the donor animals, if an appropriate amount of cells has been injected (*5*).

To test this possibility, a three-step procedure was followed: (i) treatment of the donors with large doses of an antigen; (ii) transfer of the spleen or bone marrow cells of the treated animals into irradiated recipients; (iii) graft of the recipients with skin of the donor strains. Three combinations were used: Balb/c spleen cells injected into DBA/2 mice (weak histocompatibility barrier), C57Bl spleen cells injected into C3H