# Effect of Rifampin on the Immune Response in Mice

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In an investigation of the effect of rifampin on the immune response in mice, the cellular immunity was evaluated with the split-heart allograft technique. The survival time of the heart in animals treated with rifampin at a dose of 20 mg/kg per day from the day of the transplantation until the graft was rejected was longer (33.7 days, $P < 0.001$) than that of animals not treated with antibiotics (14.5 days). When rifampin was given at a dose of 5 mg/kg per day for the same period, the mean survival time of allografts was 19.5 days. The number of demonstrable plaques of hemolysis and the humoral antibodies to sheep erythrocytes were also reduced by a human therapeutic dose (20 mg/kg per day). However, the suppression of the humoral immune response was probably of more limited biological significance, suggesting a differential sensitivity to rifampin. In contrast to rifampin, benzylpenicillin had no noteworthy inhibiting effect on the cellular or humoral immune response.

Rifampin, an antibiotic that inhibits bacterial deoxyribonucleic acid dependent ribonucleic acid polymerase and is readily soluble in lipids (10, 16), has previously been controversially described as an immunosuppressive agent (1, 3, 9, 21). Recent in vitro studies in our laboratory have demonstrated that this antibiotic markedly inhibits the chemotaxis of human polymorphonuclear leucocytes as well as the lymphoproliferative response of the human lymphocytes stimulated with mitogens (7, 8).

The purpose of the present study was to elucidate the effect of rifampin on both the humoral and the cellular immune response in mice. Rejection of tissues or organs transplanted in allogenic recipients has been generally regarded as an example of cellular immunity, implying that it is probably lymphocytes arising in the thymus that are mainly responsible for initiating such rejection. In the investigation of cellular immunity, the split-heart allograft technique has been used (23). In the study of the humoral immune response, immunization was performed with sheep erythrocytes (SRBC), and the number of antibody-producing cells was determined as well as levels of agglutinating and hemolytic antibodies.

## MATERIALS AND METHODS

**Animals.** Mice of the CBA $H$-$2^k$ (Anticimex AB, Evelund, Sollentuna, Sweden) strain were used. They were 8 weeks old when first treated. Donor hearts were obtained from 12- to 36-h-old DBA/2J (G1; Blomholtgård Ltd., Denmark).

**Antibiotics.** Rifampin (kindly supplied by A. B. Ferrosan, Malmö, Sweden) was dissolved in 10% dimethylformamide and then diluted in sterile water. The antibiotic was given intraperitoñeally (i.p.) in the

morning at a daily dose of 20 mg/kg (human therapeutic dose, HTD) or 5 × HTD (100 mg/kg) or 1/4 HTD (5 mg/kg). Penicillin (benzylpenicillin, Kabi) was injected i.p. in a single dose of 200 mg/kg per day.

**Determination of rifampin serum level.** The serum concentration of rifampin was determined microbiologically by gel microdiffusion by using a strain of *Streptococcus faecalis* (12).

**Antigen and immunization.** SRBC were collected and stored in Alsever solution at 4°C for 1 week before use. The cells were washed four times in isotonic balanced salt solution (BSS) (pH 7.4) and diluted 1:4, and 0.2 ml of the suspension was injected i.p. (14).

**Agglutinating antibodies.** Antisera were serially diluted in BSS, and each dilution was mixed with an equal volume (25 µl) of 2% washed SRBC. The tests were performed in microtiter plates (Sterilin, Teddington, Middlesex, England). Agglutination was read after 45 min at 37°C. Sera from treated but unimmunized mice were used as controls (14).

**Hemolytic antibodies.** Serial dilutions of antisera in BSS were incubated with equal volumes (25 µl) of 1.5% SRBC and guinea pig complement. Hemolysis was determined after 45 min at 37°C. Sera from treated but unimmunized mice were used as controls (14).

**Antibody-producing cells.** The splenic production of antibodies to SRBC was measured by a direct plaque-forming cell assay by using a modification of the Jerne-Nordin method (4, 11). Lymphocytes were prepared from spleens removed 4 days after immunization with SRBC by passage through a metallic membrane filter (Millipore Corp., Bedford, Mass.) and isolated by density gradient centrifugation on Lymphoprep (Lymphoprep, Nyegaard and Co. A/S, Oslo, Norway). A solution of 0.5% agar (Difco Laboratories, Detroit, Mich.) and 0.05% diethylaminoethyl dextran (Pharmacia Fine Chemicals, Inc., Uppsala, Sweden) in 250 µl of BSS at 46°C was mixed with 25 µl of SRBC diluted 1:5 in BSS, 25 µl of guinea pig serum (previously absorbed with SRBC for 20 min at 4°C) diluted

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1:4 in phosphate-buffered saline, and 100 μl of the spleen lymphocytes in a concentration of $10^6$ lymphocytes per ml. Three separate 100-μl drops of the mixture were placed on a petri dish, and a glass cover slip (24 by 32 mm) was immediately placed on each drop. Plates were incubated for 3 h at 37°C, and plaques were counted under a stereomicroscope (Wild M5, Wild Heerburg, Inc., Heerburg, Switzerland). Representative plaques were checked for a central lymphocyte.

**Transplantation.** Heart grafts were transplanted with a modification of the Judd and Trentin method (13, 23). Hearts from newborn DBA mice were sectioned along the ventricular septum and each half was inserted into a subcutaneous pouch in the dorsal part of the ear of the recipient CBA mouse. The survival of the transplant was monitored by measuring the electric activity with a Mingograf 61 with an integrated amplifier. The activity of the host hearts was also recorded. This activity differed from that of the transplanted hearts and did not confuse measurements.

**Statistics.** Statistically evaluated tests were performed to determine whether the results obtained might be significant. Student's $t$ test was used.

## RESULTS

**Toxicity of rifampin.** Rifampin was apparently well tolerated by the mice when given in an HTD or 1/4 HTD. However, when rifampin was given in a larger dose (5 HTD), signs of toxicity appeared, such as loss of body weight.

**Determination of rifampin serum level.** The serum concentration of rifampin was determined microbiologically 2 h after injection. In mice given 5 mg of rifampin per kg per day (1/4 HTD), the serum concentration of the antibiotic ranged from 6 to 13 μg/ml, and in mice given 20 mg/kg per day (HTD), it ranged from 17 to 28 μg/ml.

**Survival time of allografts.** As reference, untreated transplanted mice were used. Figure 1 illustrates the effect of rifampin given i.p. on the survival time of split-heart allografts from DBA mice in CBA mice. Daily antibiotic treatment was given from the day of transplantation until rejection of the grafts was detected with an electrocardiograph. A remarkable and significant ($P < 0.001$) delay occurred in the rejection of the grafts in the mice given rifampin in an HTD (20 mg/kg per day) (Fig. 1). The mean survival time ± standard deviation (MST ± SD) was 33.7 ± 9.6 days, compared with 14.5 ± 3 days in the reference group. When rifampin was given in a dose of 5 mg/kg per day (1/4 HTD) for the same period, an MST ± SD of 19.5 ± 4.1 days was detected, which also differed significantly from the reference group ($P < 0.01$). Figure 1 also illustrates that penicillin given i.p. for the same period did not prolong the survival of the grafts (15.9 ± 3.9 days).

A marked prolongation ($P < 0.001$) of survival of allografts was also noted in mice given rifampin at an HTD from 20 days and 10 weeks before transplantation and every day until rejection (Fig. 2). The MST was 25.2 ± 6.2 days and 24.2 ± 5.7 days, respectively. Figure 2 also shows that in mice treated only from the day of transplantation, the rejection was later (33.7 days) than in the pretreated groups, but the difference was not significant ($P > 0.4$).

Figure 3 illustrates the effect of an HTD of rifampin on the survival time of allografts when given daily from the day of transplantation until day 7 or from day 9 after transplantation until rejection. In both groups survival was significantly prolonged ($P < 0.01$). However, the effect
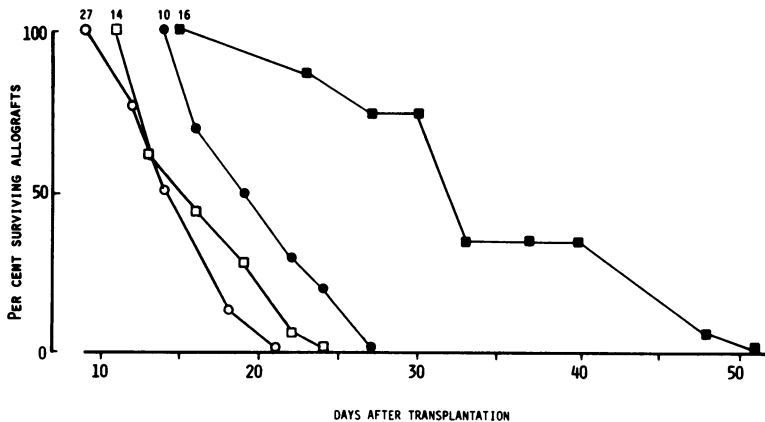


DAYS AFTER TRANSPLANTATION

FIG. 1. *Percentage of surviving split heart DBA allografts in CBA mice treated daily with an HTD of rifampin (■), a 1/4 HTD of rifampin (●), or an HTD of benzylpenicillin (□) from the day of tranplantation until the rejection of the grafts. Untreated transplanted mice (○) served as a reference. Numbers at the top of each curve denote the number of transplantation experiments in each group.*
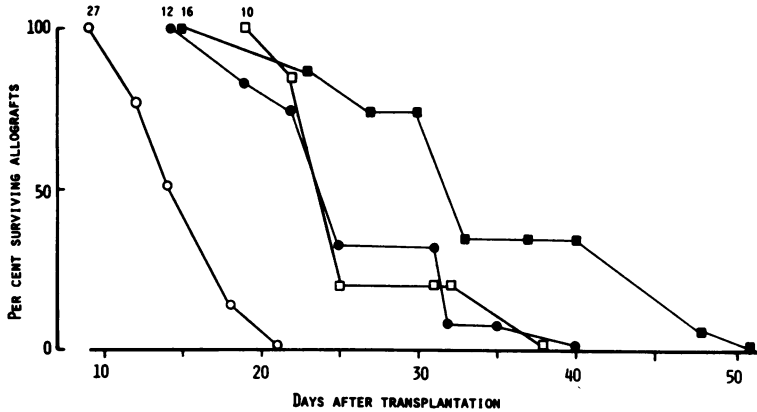
FIG. 2. *Percentage of surviving split-heart DBA allografts in CBA mice treated daily with an HTD of rifampin from the day of the transplantation until the rejection of the grafts (■) and in addition for 20 days (●) or 10 weeks (□) before the transplantation. Untreated transplanted mice (○) served as a reference. Numbers at top of each curve denote the number of transplantation experiments in each group.*



FIG. 3. *Percentage of surviving split-heart allografts in CBA mice treated daily with an HTD of rifampin from the day of the transplantation until the rejection of the grafts (■), from the day of transplantation until day 7 after transplantation (□), or from day 9 after transplantation until rejection of the grafts (●). Untreated transplanted mice (○) served as a reference. Numbers at top of each curve denote the number of transplantation experiments in each group.*
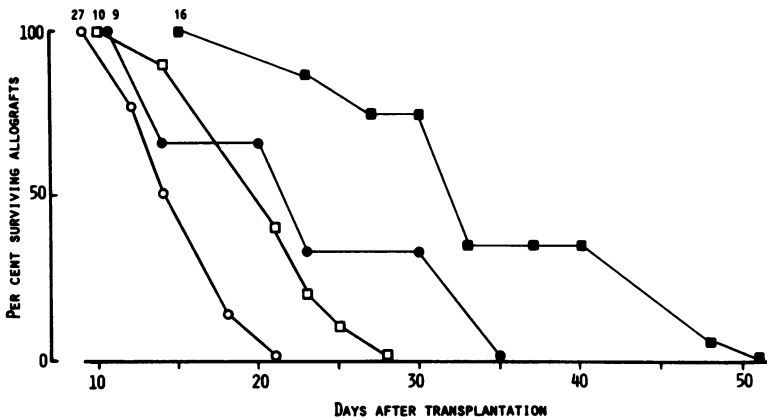
of the drug was less pronounced ($P < 0.02$) in these groups of mice than in the group given rifampin from the day of the transplantation until the rejection of the allografts (Fig. 3).

**Antibody-producing cells.** The direct plaque-forming cells from spleens of mice immunized with SRBC and treated with rifampin or penicillin from the day of immunization with or without pretreatment, were analyzed. When the mice were treated with rifampin in a dose of 20 mg/kg per day from the day of immunization until day 3, only 52% ± 5% of the number of plaques of hemolysis obtained with the spleens of untreated immunized mice was detected ($P < 0.001$) (Fig. 4). The primary antibody response to SRBC was also curtailed when treatment

with the HTD was given from day 30 until day 3 (39% ± 8% of the control values). Animals pretreated for 7 days and then daily until day 3 after immunization (62% ± 10% of the control values) and animals treated from 1 day after immunization until day 3 (77% ± 10% of the control values) also had a reduced number of plaque-forming cells. Only a slight decrease (21%) in the number of plaques was observed in mice given rifampin in a dose of 5 mg/kg per day from the day of immunization until day 3. However, penicillin did not interfere with the assay.

**Agglutinating and hemolytic antibodies.** The titers of the agglutinating and hemolytic antibodies of mice immunized with SRBC and treated with rifampin or penicillin were meas-

ured. The antibody titers of normal mice immunized with SRBC were used as a reference. Table 1 shows that mice treated with rifampin at an HTD from the day of immunization until day 3, 4 days after immunization had an agglutinating antibody level ($P < 0.01$) as well as a hemolytic antibody level ($P < 0.02$) significantly lower than the reference mice. Also in sera from mice treated for the same period and, in addition, for 7 days or 30 days before the transplantation, the titers tended to be lower. A difference, although of lower statistical significance, was also found in animals treated with a 1/4 HTD of rifampin. Penicillin given to immunized mice did not interfere with the primary antibody response to SRBC.

## DISCUSSION

Rifampin, a widely used antibiotic, inhibits the synthesis of deoxyribonucleic acid-dependent ribonucleic acid polymerase in bacteria (10) and readily diffuses into human cells (16). However, in 1970 Paunescu (19) reported an immunosuppressive effect of rifampin on delayed hy-
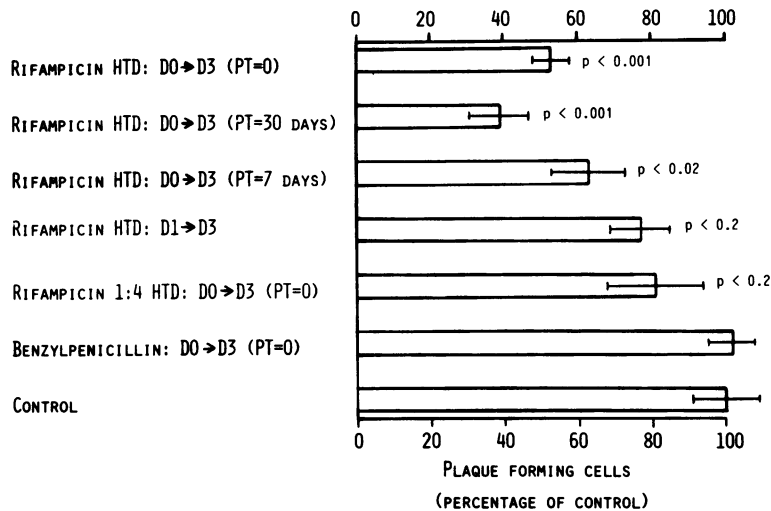


FIG. 4. *Direct plaque-forming cells from spleens of mice immunized with SRBC and treated daily with rifampin (HTD, 1/4 HTD) or benzylpenicillin (HTD) from the day of immunization (D0) or one day after immunization (D1) until day 3 (D3) with or without pretreatment (PT). Each group consisted of 7 to 10 mice. The results denote the mean ± SE expressed as a percentage of the control. The number of plaque-forming cells in the control group was 73 ± 6/10^5 lymphocytes.*

TABLE 1. *Agglutinating and hemolytic antibodies from sera of mice immunized with SRBC[a]*

| Status | Agglutinating titers | $t$ | $P$ | Hemolytic titers | $t$ | $P$ |
|---|---|---|---|---|---|---|
| Normal | 46 ± 7 | | | 768 ± 161 | | |
| Rifampin HTD: day 0 to day 3 (PT = 0) | 23 ± 5 | 2.673 | 0.02 | 256 ± 90 | 2.774 | 0.01 |
| Rifampin HTD: day 0 to day 3 (PT = 30 days) | 33 ± 9 | 1.140 | 0.3 | 473 ± 160 | 1.299 | 0.2 |
| Rifampin HTD: day 0 to day 3 (PT = 7 days) | 22 ± 11 | 1.840 | 0.1 | 282 ± 126 | 2.377 | 0.02 |
| Rifampin HTD: day 1 to day 3 (PT = 0) | 35 ± 13 | 0.745 | 0.4 | 334 ± 195 | 1.716 | 0.1 |
| Rifampin ¼ HTD: day 0 to day 3 (PT = 0) | 26 ± 5 | 2.324 | 0.05 | 405 ± 75 | 2.043 | 0.1 |
| Benzylpenicillin HTD: day 0 to day 3 (PT = 0) | 41 ± 10 | 0.409 | 0.4 | 581 ± 91 | 1.011 | 0.3 |

[a] Mice were treated daily with rifampin or penicillin (HTD, ¼ HTD) from the day of immunization or 1 day after immunization until day 3 with or without pretreatment. Agglutinating and hemolytic antibody titers from untreated but immunized mice served as references. The results are expressed as the mean ± SD of the titers. Each group consisted of 10 mice. Student's $t$ test was used in the statistical analysis. PT, pretreatment.

persensitivity in rabbits and guinea pigs and the production of antibodies after injection of serum albumin. Forsgren et al. (6, 7) recently found that rifampin inhibits the chemotaxis of human polymorphonuclear leukocytes in vitro and ascribed the effect to inhibition of the protein synthesis necessary for chemotaxis of the leukocytes. Forsgren et al. (5) also observed an inhibition of the lymphoproliferative response of human peripheral lymphocytes incubated with 25 $\mu$g of rifampin per ml and stimulated with mitogen, an observation in agreement with data given by Paunescu (19, 20) and Nilsson (17).

The present study shows that rifampin significantly delays rejection when given daily in an HTD (20 mg/kg per day) from the day of the transplantation. The immunosuppressive effect appeared weaker when treatment with rifampin was started before the transplantation, presumably because the mice became accustomed to the antibiotic, probably owing to the induction of deacetylating liver enzymes (18). Treatment performed only before the transplantation or discontinued on day 7 after transplantation inhibited the immune response less, which contrasts with the usual lympholytic immunosuppressants, such as corticosteroids (2). Treatment started day 9 after transplantation also notably delayed the rejection of the allografts, an effect which might prove clinically useful in the prevention of graft rejection. Serrou et al. (21) have reported a significantly longer survival time of skin allografts in rabbits treated with rifampin. Dajani et al. (3) observed that the cutaneous reaction to *Mycobacterium bovis* BCG and DNCB in guinea pigs was markedly reduced during treatment with rifampin in an HTD. The reactions were restored when the antibiotic was withdrawn. Dajani et al. (3) also observed a progressive reduction in the skin test reaction to stabilized purified protein derivatives in 14 patients with active pulmonary tuberculosis undergoing treatment with rifampin. Also on these patients the immunological reactions were restored after withdrawal of the antibiotic. But according to Smith et al. (22), certain rifamycin derivatives have a stronger toxic effect on fresh human leukemic blood cells than on normal blood cells. However, Smith et al. (22) claimed that rifampin does not affect normal lymphoproliferation.

Bassi et al. (1) noticed a depression of the primary antibody response in mice only when large doses were given i.p. In our investigation, however, the primary antibody response to SRBC, as judged from number of demonstrable plaque-forming cells, was significantly ($P <$ 0.001) inhibited by daily treatment of the animals with an HTD, during the period of sensitization (day of immunization to day 3) with or without pretreatment. Mice given a 1/4 HTD of rifampin exhibited a reduced immune response, but the reduction was not statistically significant. The level of agglutinating, as well as hemolytic, antibodies was also influenced by rifampin.

Our finding on the prolongation of heart grafts during rifampin treatment seems biologically highly significant. However, the reduction of antibody-forming potential, while statistically significant, is probably of a more limited biological significance. A 50% reduction of plaque-forming cells represents a reduction of a single round of multiplication of B-cells. The serology experiments showing a reduction with only one to two twofold-dilution steps can be interpreted similarly. The experiments thus seem to show a differential sensitivity of the cell-mediated response versus the antibody response to rifampin. Further experiments are required to elucidate this possibility.

The mechanism of interaction of rifampin with the responding cells is obscure. There is general consensus that macrophages are present in an allograft bed and are necessary for the primary antibody response to T-cell-dependent antigen (15). One might therefore imagine that the immunosuppressive effect demonstrated is related to an inhibition of the phagocytic activity (1). However, the immunosuppressive properties of rifampin can be ascribed to its ability to diffuse into human cells, owing to its ready liposolubility and to its inhibition of protein synthesis in lymphocytes. The availability of an antibiotic with immunosuppressive additive effect when used in combination with immunosuppressants can be useful in clinical situations for treatment of patients with inflammatory diseases and for transplanted patients. However, the immunosuppressive properties of rifampin may also be an important adverse effect of the drug.

## LITERATURE CITED

1. **Bassi, L., L. Di Bernardino, V. Arioli, L. G. Silvestri, and E. L. C. Ligniere.** 1973. Conditions for immunosuppression by rifampicin. J. Infect. Dis. **128**:736–744.
2. **Blomgren, H., and B. Andersson.** 1971. Characteristics of the immunocompetent cells in the mouse thymus: cell population changes during cortisone-induced atrophy and subsequent regeneration. Cell. Immunol. **1**: 545–560.
3. **Dajani, B. M., J. E. Kasik, and J. S. Thomson.** 1973. Effect of rifampin on the immune response in guinea pigs. Antimicrob. Agents Chemother. **3**:451–455.
4. **Fauci, A. S., and K. R. Pratt.** 1976. Polyclonal activation of bone-marrow derived lymphocytes from human peripheral blood measured by a direct plaque-forming cell assay. Proc. Natl. Acad. Sci. Immunol. **73**:3676–3679.
5. **Forsgren, A., and G. Banck.** 1978. Influence of antibiotics on lymphocyte function in vitro. Infection **6**(Suppl. 1):91–97.

6. **Forsgren, A., and D. Schmeling.** 1977. Effect of antibiotics on chemotaxis of human leukocytes. Antimicrob. Agents Chemother. **11:**580–584.

7. **Forsgren, A., D. Schmeling, and G. Banck.** 1978. Effect of antibiotics on chemotaxis of human polymorphonuclear leucocytes in vitro. Infection **6**(Suppl. 1):102–106.

8. **Forsgren, A., A. Svedjelund, and H. Wigzell.** 1976. Lymphocyte stimulation by protein A of *Staphylococcus aureus*. Eur. J. Immunol. **6:**207–213.

9. **Grassi, G., and E. Pozzi.** 1972. Effect of rifampin on delayed hypersensitivity reactions. J. Infect. Dis. **126:**542–544.

10. **Hartman, G., K. O. Honikel, and J. Knüsel.** 1967. The specific inhibition of the DNA-directed-RNA-synthesis by rifampicin. Biochim. Biophys. Acta **145:**843–844.

11. **Jerne, N. K., and A. A. Nordin.** 1963. Plaque formation in agar by single antibody producing cells. Science **140:**405.

12. **Juhlin, I.** 1973. The determination of gentamycin concentration. Acta Pathol. Microbiol. Scand. Sect. B **81**(Suppl. 241):48–53.

13. **Judd, K. P., and J. J. Trentin.** 1971. Cardiac transplantation in mice. I. Factors influencing the take and survival of heteropic grafts. Transplantation **11:**298–302.

14. **Ljungström, I., and G. Huldt.** 1977. Effect of experimental trichinosis on unrelated humoral and cell mediated immunity. Acta Pathol. Microbiol. Scand. Sect.

C **85:**131–141.

15. **Lohmann-Matthes, M. L.** 1976. Induction of macrophage-mediated cytotoxicity, p. 463–486. *In* David S. Nelson (ed.), Immunobiology of the macrophage. Academic Press Inc., New York.

16. **Mandell, G. L.** 1973. Interaction of intraleukocytic bacteria and antibiotics. J. Clin. Invest. **52:**1673–1679.

17. **Nilsson, B. S.** 1971. Rifampicin: an immunosuppressant? Lancet **ii:**374.

18. **Otten, H., M. Plempel, and W. Siegenthaler.** 1975. Rifampicin, p. 551–573. *In* A. M. Walter and L. Heilmeyer (ed.), Antibiotika Fibel. 4. Auflage. Georg Thieme Verlag, Stuttgart.

19. **Paunescu, E.** 1970. In vivo and in vitro, suppression of humoral and cellular immunologic response by rifampicin. Nature (London) **228:**1188–1190.

20. **Paunescu, E.** 1971. Action de la rifampicin sur la mitose de la cellule lymphoide stimulée. Ftiziologia **20:**49–53.

21. **Serrou, B. C., L. Solassol, H. Karcenty, X. Joyeux, and H. Pujol.** 1972. Action immunodepressive de la rifampicin. Nouv. Presse Med. **1:**1295.

22. **Smith, R. G., J. Whang-Peng, R. C. Gallo, P. Levine, and R. C. Ting.** 1972. Selective toxicity of rifamycin derivatives for leukaemic human leucocytes. Nature (London) New Biol. **236:**166–171.

23. **Svehag, S. E., and W. Schilling.** 1973. Synergism between alloantibodies or Fab₂ and prednisone in the survival of murine split heart allografts. Transplantation **15:**345–347.