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The Normal Mouse in Experimental Chemotherapy

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Immunologically intact mice are suitable for drug screening, the purpose of which is to determine the ability of a certain concentration of a drug to inhibit multiplication of *Mycobacterium leprae* or to kill them. However, the population of *M. leprae* in the intact mouse, even after maximal multiplication, is very much smaller than that of the untreated lepromatous patient (approximately 10^6, compared to 10^{10} or more). Therefore, one could not use the *M. leprae*-infected, immune-competent mouse as a model of the lepromatous patient undergoing chemotherapy. So small a population of *M. leprae* is unlikely to include drug-resistant organisms (by analogy with *M. tuberculosis,* the maximal frequency with which drug-resistant mutations occur is probably not greater than $1:10^6$). Moreover, the immunologically intact mouse does not tolerate even this small population (10^6) of *M. leprae,* but rapidly kills the organisms. In intact mice, one could not allow the organisms to multiply, then administer treatment for some duration and, finally, withdraw treatment and study the sub-population of persisting *M. leprae.* Nor could one study in intact mice the development of drug resistance, which occurs only in large populations ($\geq 10^6$) of *M. leprae,* although one could demonstrate the presence of drug-resistant organisms (which requires only $10^{3.7}$–10^4 *M. leprae,* with the potential to multiply only to 10^6) in intact mice.

Thus, because of the small bacterial population, one could not study in immune-competent mice either of the two phenomena—drug resistance and microbial persistence, both phenomena characteristic of much larger populations of *M. leprae*—that appear to determine the outcome of chemotherapy of a patient with lepromatous leprosy. In short, one could not study the intact mouse as if it were the patient. Such studies may be carried out only in *M. leprae*-infected, immune-deficient rodents, which tolerate much larger bacterial populations, and which may therefore serve as models of the lepromatous patient for studies of chemotherapy.

On the other hand, one may frame certain chemotherapeutic questions in such a fashion that their answers may be approached experimentally by means of studies in *M. leprae*-infected, immunologically normal mice. In fact, several sorts of studies have been successfully carried out in normal mice: a) studies of the minimal inhibitory concentration (MIC) and the minimal effective dosage (MED); b) studies of the intermittent administration of drugs; and c) studies of action mechanisms of a variety of drugs.

MIC and MED. The MIC and MED of dapsone (DDS) were measured simply by administering DDS, incorporated into the diet in several concentrations, to *M. leprae*-infected, immunologically normal mice and rats, examining the effect of each diet on the multiplication of the organism, and, at the same time, measuring concentrations of the drug in the plasma. As the result of these studies, the MIC of DDS for *M. leprae* was determined to lie in the range 1–10 ng per ml, and the dietary concentration of DDS producing these concentrations in mice lay in the range 0.00003–0.0001 g per 100 g ([14, 18, 19]).

Measurement of the MIC of clofazimine (CLO) for *M. leprae* was not so simple a task because of storage of the drug in the tissues; the antimicrobial effect of this drug does not appear to be directly related to the concentration of the drug in the plasma. On the other hand, the MED of CLO against *M. leprae* in the mouse, measured as was that for DDS, appears to lie in the range 0.0001–0.0003 g per 100 g ([4, 8]).

Intermittency. Drugs effective when administered intermittently lend themselves to supervised administration, an important consideration in the design of drug regimens to be employed in the control of leprosy. It is simply not practical, even in developed countries, to supervise the administration

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TABLE 1. *Measurement of the bactericidal effects of intermittently administered ethionamide by means of the "proportional bactericide" technique.**

Drug	No. AFB inoculated per mouse				Proportion of viable *M. leprae*
	10^4	10^3	10^2	10	
	No. mice showing multiplication/no. inoculated				
None	5/5	5/5	5/5	0/5	0.024
Ethionamide 0.1% continuously	5/5	0/5	0/5	0/5	0.0002
Ethionamide 500 mg/kg three times weekly	5/5	4/5	1/5	0/5	0.0017
Ethionamide 500 mg/kg once weekly	5/5	5/5	3/4	0/5	0.013
Dapsone 0.01% continuously	4/4	3/5	1/5	0/5	0.0011

* Adapted from reference no. 1.

to outpatients of drugs that must be administered daily. In the same vein, the longer the permissible interval between successive doses of an intermittently administered drug, the better the drug lends itself to supervised administration. Clinical studies had already demonstrated that both rifampin (RMP), CLO, and acedapsone could be administered at intervals of one month or longer with little or no loss of efficacy, compared to that of the drugs administered daily. The remaining bactericidal agents currently available, ethionamide (ETH) and prothionamide (PTH), are toxic; thus, if these drugs could be administered intermittently, one might be able both to minimize toxicity as well as to ensure supervisability of their administration.

In an attempt to answer the question of whether a thioamide could be administered intermittently, Colston and his co-workers administered ETH to *M. leprae*-infected normal mice with different degrees of intermittency, and, by means of the proportional bacteriocide technique, determined bactericidal activity. As shown by their results ([1]), some of which are presented in Table 1, three-times-weekly administration of ETH was about as effective as continuous administration, whereas administration of the drug once weekly was almost without effect. Although this is by no means the final answer, these first results suggest that ETH (and also PTH, by extension) does not lend itself to intermittent administration.

Mechanism of action. Several kinds of studies of action mechanism have been carried out in immunologically normal mice. One kind of study involves testing the efficacy of analogs of active drugs, in attempts to recognize the functional groups and structural features essential to drug activity, and to identify active metabolites, if such there be. Examples of this experimental approach are comparative studies of the activity of structural analogs of DDS ([6]) and CLO ([7]) on *M. leprae*. In the case of the DDS analogs, none of those tested was active, although the analogs were administered in a dosage equivalent to 100 times the MED of DDS. In the case of the CLO analogs, all four of the analogs tested were active against *M. leprae*; however, none of the analogs was as active as clofazimine, despite the fact that two of them were as long lived as CLO. On the other hand, the two most active analogs, like CLO, contained chloro-substituents in the *para*-positions of the two benzene rings.

Another application of the *M. leprae*-infected normal mouse to study of the action mechanisms of drugs is represented by published work on DDS and work in progress on RMP. DDS appears to exert a bactericidal effect when it is administered in full dosage to previously untreated patients with lepromatous leprosy, who do not display primary resistance to the drug, whereas this drug and its close relatives, the sulfonamides, appear to be only bacteriostatic when tested on susceptible organisms *in vitro*. In normal mice infected with *M. leprae*, DDS behaves only as a bacteriostatic drug, or as a weakly bactericidal drug, depending primarily on the concentration in which it is administered ([2, 3, 5, 15]). In addition, when the drug is administered in the MED, the onset of its action is delayed, suggesting that *M. leprae* ordinarily possess a pool of some precursor of tetrahydrofolic acid.

RMP has appeared to be far more active against *M. leprae* in the lepromatous patient, as measured by inoculation of mice, than when the drug is administered to *M. leprae*-infected mice, although the pharmacokinetics of the drug are more favorable in mouse than in man. This apparent discrepancy may have implications of enormous importance for leprosy control; the regimens recommended by the WHO Study Group ([20]) were based on the demonstrations of intense bactericidal activity of the drug in the course of clinical trials. The possibilities that the effect measured in man has resulted from one or another artefact, or reflects a (metabolic?) difference between *M. leprae* in the patient and those during the plateau phase in the normal mouse must be considered. Does inoculation of mice with organisms recovered from skin-biopsy specimens obtained from multibacillary patients underestimate the proportion of viables, because of a host-versus-graft reaction induced by the human antigens included in the suspensions of *M. leprae*? Or is this possibly the case for organisms that have been damaged by RMP? Is the proportion of viable organisms in a biopsy specimen decreased during shipment of the specimen to a distant laboratory? Or are *M. leprae* that have been damaged by RMP less resistant to the effects of chilling and storage for several days (during shipment) than are organisms not so damaged? These questions and others are currently being examined in a series of experiments in immunologically normal mice.

A final application of the *M. leprae*-infected, immunologically normal mouse to studies of action mechanism is represented by studies of the activity of antithyroid drugs and interferon inducers. Methimazole had been reported therapeutically active in leprosy patients in a paper ([17]) in which the author found evidence that the hypothyroid state was beneficial to such patients. In a series of studies in mice, however, it was shown ([11, 13, 16]) that methimazole and propylthiouracil were active in dosages too small to produce hypothyroidism, or when co-administered with thyroid substance, whereas ablation of thyroid activity by means of ^{131}I-iodide had no effect on multiplication of the organisms. Moreover, methimazole displayed antimicrobial activity against a number of cultivable mycobacteria *in vitro*.

Polyinosinic-polycytidylic acid (poly IC), a synthetic interferon inducer, had been shown to protect experimental animals against infection by a variety of bacterial pathogens, an activity thought not to depend upon induction of interferon. In a series of experiments ([12]), poly IC, injected locally in a dosage that produced interferon *in situ*, prevented multiplication of *M. leprae* in normal mice. So, however, did polyinosinic acid, which does not induce interferon. And, on the other hand, local injection of mouse interferon was no more effective than was local injection of saline.

In an initial study ([9]), tilorone, another synthetic inducer of interferon, was found to inhibit multiplication of *M. leprae* in mice, at the same time that it enhanced the diseases in mice of the same strain produced by *M. lepraemurium* and *M. marinum*. Further studies ([10]) showed that, when tilorone was administered in a reduced dosage, or beginning late during logarithmic multiplication, the drug only enhanced multiplication of *M. leprae*. Confirming a dual action of tilorone are unpublished studies showing inhibition of several strains of cultivable mycobacteria *in vitro*.

Thus, although the *M. leprae*-infected, immunologically normal mouse is unsuitable for studies in experimental chemotherapy of leprosy requiring large populations of the organisms, many questions remain that may be answered by work in this experimental system.

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