that is, at a low pH, calcium would be essentially ionized in the presence of strong chelates and perhaps could then move across the intestinal barrier in that form and no differences would be seen. At a higher pH, calcium would begin to form strong associations with anions, such as citrate and phosphate; the behavior of the absorbed $Ca^{++}$ would then be dependent upon the modifying effect of the complexing agent. However, considerably more data are required to establish a relationship between the above observations and a hypothetical complexing agent. Also, the role of plasma citrate, known to be elevated by vit. D(9), must be evaluated in respect to the present findings.

*Summary.* The effect of pH of the dosing solution on the relative tibia deposition of $Ca^{47}$ absorbed from the duodenum of rachitic and vit. D-treated chicks was examined. Vit. D had its usual enhancing effect on $Ca^{47}$ absorption; however, it was observed that the percent absorbed $Ca^{47}$ deposited in tibia varied with intraduodenal pH and vit. D-status of the chick. At low pH values (1.9, 2.0), there were no differences in the percent of duodenally absorbed $Ca^{47}$ accumulated by tibia in rachitic or vit. D-treated chicks whereas, at high pH values, proportionally less of the absorbed $Ca^{47}$ was deposited in rachitic tibia; pH was without effect on uptake of $Ca^{47}$ by tibia in the vit. D-treated birds.

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## Effect of Several Anti-Leprosy Drugs on Multiplication of Human Leprosy Bacilli in Foot-Pads of Mice. (27293)

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When inoculated into the foot-pads of mice leprosy bacilli multiply slowly (generation time 20 to 30 days) to a level of $10^5$ to $10^7$ bacilli per foot-pad(1,2). The bacilli are then detectable in histologic sections. The incubation period (time from inoculation until development of significant lesion in the sections) varies with the number of bacilli inoculated; with an inoculum of $5 \times 10^3$ bacilli, the incubation period is 5 to 10 months.

This system, though slow, enables one to work experimentally with the multiplication of leprosy bacilli outside of the human patient. We wish here to report on its suitability to drug testing. The experimental design was one thought likely to produce clear-cut results; the drugs were fed at a high (but sub-toxic) level, and the bacilli were inoculated at the lowest dose that seemed likely to give consistently positive results in the controls.

*Materials and methods.* The inoculum was from the second passage of a strain (B2409) that was derived from a skin specimen removed at biopsy of a patient with typical lepromatous leprosy. The number of bacilli in-

* Leonard Wood Memorial (American Leprosy Foundation).

TABLE I. Effect of Drugs on Multiplication of *M. leprae* in Foot-Pads of Mice. Mice were inoculated with $5.0 \times 10^3$ leprosy bacilli, and fed the diets shown (DDS = 4,4'-diaminodiphenyl sulfone; INH = isoniazid; PAS = para-aminosalicylic acid; CS = cycloserine). Each month a mouse was killed and foot sections examined (0 = normal; 3+ = mycobacterial lesion occupying 12 × 45 × to 12 × 20× field; 4+ = mycobacterial lesion occupying 12 × 20 × field or greater). For the harvests 2 mice were killed, their foot-pads removed, minced, vibrated with glass beads, and No. of acid-fast bacteria (AFB) counted.

| Diet | Sections (mo) | | | | | | | | Harvest (AFB/mouse) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 8 mo | 11 mo | 13 mo |
| Control | 0 | 0 | 0 | 0 | 3+ | 3+ | 3+ | 4+ | $4.2 \times 10^5$ | $3.8 \times 10^6$ | $1.4 \times 10^6$ |
| DDS, .1% | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | $<3 \times 10^4$ | $<3 \times 10^4$ | $<3 \times 10^4$ |
| INH, .1% | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | $<3 \times 10^4$ | $<3 \times 10^4$ | $<3 \times 10^4$ |
| PAS, .6% | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | $<3 \times 10^4$ | $<3 \times 10^4$ | $<3 \times 10^4$ |
| CS, .5% | 0 | 0 | 0 | 0 | 0 | 0 | 3+ | 0 | $<3 \times 10^4$ | $2.4 \times 10^4$ | $4.1 \times 10^5$ |

oculated per mouse was $5.0 \times 10^3$.

The methods are described elsewhere in greater detail(1,2). In brief, leprosy bacilli were inoculated into the right hind foot-pad. The mice were then placed in cages containing jars of the diets described below. At monthly intervals, a mouse from each group was sacrificed, and sections cut of the decalcified foot.

After the incubation period there was observed in the untreated controls an area containing acid-fast bacteria, with or without granuloma formation. At this time more mice were sacrificed, the foot-pads washed, cut off, thoroughly minced, and the tissue vibrated in the Mickle apparatus with 3 mm amplitude with about 20 3 mm glass beads and 2 ml Hanks' balanced salt solution. The number of acid-fast bacteria was counted by the "rapid" modification of a microscopic procedure described elsewhere(1,3). The lower limit of detectability, corresponding to one organism seen during the microscopic search, is about $3 \times 10^4$ acid-fast bacteria per foot-pad. Counts on the same suspension agree within about 20% if there are enough organisms present to minimize sampling variation. Portions of the suspensions were cultured at 33° for 4 months on Loewenstein-Jensen, 25% blood agar, and 7H9 media. Although occasional nonacid-fast bacteria were grown, acid-fast bacteria were not observed.

Four drugs were tried in this first experiment. They were mixed with ground commercial chow in the concentration shown. The controls were given the ground diet only. There were 20 mice in each group.

*Results.* The results of examinations of the tissue sections and harvests of acid-fast bacteria are given in Table I. DDS, INH, and PAS gave complete suppression. Cycloserine appeared to slow the growth of leprosy bacilli for a few months, but the bacilli were later harvested in somewhat reduced numbers. The controls behaved in the normal pattern in all respects, incubation period, histology of tissue response, number of acid-fast bacilli harvested, and negativity of cultures for mycobacteria.

*Discussion.* The work described here was an attempt to learn whether the experimental design was adequate to reveal chemotherapeutic activity of drugs, at least some of which are known to have activity against *M. leprae* in the natural human disease. The differences between treated and control groups were distinct, and further major adjustments of the experimental conditions do not appear necessary. In work in progress more antimycobacterial drugs are under study, and modifications are being tried to see whether the answer can be obtained more rapidly, or with fewer mice.

It would be desirable to compare the activity of drugs in this experiment to their efficacy in treatment of the natural disease. However, drug trials in human leprosy are difficult to carry out with any high degree of precision, because the disease is so chronic, and the organism is so durable in the human tissues. DDS and related sulfones are the most commonly used drugs in treatment of leprosy, and the clinical impression that sulfones are effective is widespread. In the clinical evaluation studies of the Leonard Wood Memorial, in which patients are assigned to

treatment on a random basis, DDS was found to have therapeutic value in lepromatous leprosy as compared to untreated controls(4). Cycloserine was later found about as effective as DDS(5). Results with PAS(4) were ambiguous (PAS was found to be as effective as diasone and less effective than DDS in the one group of patients in which PAS was tried; in other groups diasone and DDS had the same activity). Other published clinical opinions of PAS are not unanimous and appear to be based on experience with small numbers of patients without concurrent controls.

Davidson(6) reported that lepromatous patients responded favorably to INH for 6 to 9 months and then either failed to improve or deteriorated. This course has been interpreted as the result of selection of resistant forms of *M. leprae*. The very great numbers of bacilli present in lepromatous patients and the long period of observation in clinical trials would be more favorable to the observation of resistant forms than are the small number and shorter time in the experiment described here.

The critical question as to whether the acid-fast bacilli in this experimental system are indeed leprosy bacilli has been discussed elsewhere(1,2). The evidence is most simply explained by the hypothesis that they are leprosy bacilli (*i.e.*, that they are the acid-fast bacteria of the patient's tissues). Other hypotheses would need to be unattractively complex. For example, a hypothesized contaminant from the patients would need to be endowed with the following properties: non-cultivable, present in all patients' tissues and present in numbers proportional to the number of leprosy bacilli, capable of slow growth in mouse foot-pads to produce a large round cell response without necrosis and with occasional invasion of nerves.

*Summary.* 1. A comparison of the activity of 4 drugs was carried out in mice inoculated into the foot-pad with human leprosy bacilli. The drug was administered in the diet, starting on the day of inoculation. 2. Multiplication of the bacilli was completely suppressed by DDS, INH, and PAS, and it was delayed and partially suppressed by cycloserine.

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## Impairment of Net Synthesis of Glutathione in Mouse Liver After Tourniquet Trauma.* (27294)

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Numerous metabolic imbalances occur as a part of the syndrome induced by tourniquet trauma. One of the imbalances studied in this laboratory(1,2,3,4) has been the depletion of the non-protein sulfhydryl (NPSH) fraction of various tissues: liver, kidney and muscle. A lowered incorporation of $S^{35}$-L-cysteine into the major non-protein sulfhydryl component of liver, *i.e.*, glutathione (GSH), has been observed(3,4). This led directly to the present work. Decreased incorporation of L-cysteine into GSH might be due to decreased GSH synthesis, to increased GSH degradation, or to both. Synthesis, relatively

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