shaped cells are now clearly distinguishable as fibroblasts, with oval nuclei and several nucleoli. There is a tendency at this time for the fibroblasts to circumscribe clusters of fat cells. At 72 hours (Fig. 3), reaggregation of fat cells into islands surrounded by fibroblasts is well established. The lobulation is similar to that of the mature parent tissue. Numerous sudanophilic droplets interspersed with clear vacuolated areas are visible in the cytoplasm of the epithelioid cells (Fig. 4).

Addition of insulin to the culture medium (20 μg/ml at 48 hours) produced a marked change in organization of the adipose cell islands. The fat cells enlarged, almost obliterating the fibroblastic septa, (Fig. 5). The cytoplasm became more basophilic and the fat droplets increased in size and number (Fig. 6). Aside from mechanical compression, no obvious cytoplasmic changes were seen in the fibroblasts.

Discussion. These preliminary observations show that the technique of monolayer cell culture lends itself to the study of adipose cells and their response to insulin. Cells of the brown fat body show the same ability to reaggregate after dissociation as Moscona(7) and Steinberg(8) described in other tissues. No conversion of multilocular to unilocular fat cells was observed, although this occurs normally in the interscapular fat pad *in vivo*. Transformation of fibroblasts to fat cells *in vivo* has been a controversial topic. In this study, fibroblasts were not converted to fat cells regardless of insulin level. Mitotic figures were found in early cultures, but could not be assigned with certainty to either cell category. The increase in total cell numbers, however, suggests that fat cells do divide, at least before they have acquired a full complement of fat droplets.

The addition of insulin to cultures markedly enhanced lipogenesis in these cells. The low level of fat formation in control cultures could have been due either to the presence of some insulin adsorbed on the parent cells or introduced in the serum, or may represent a low intrinsic activity.

Summary. Dissociated interscapular fat pads of 1-3-day-old rats were studied in monolayer cultures. The fat cells were observed to form islands of epithelioid cells which were clearly distinguishable from fibroblasts. Addition of insulin to cultures induced hypertrophy of adipose cells and increased the number and size of their fatty droplets. The fibroblasts were unaffected.

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Prevention of Tuberculosis and Reversal of Tuberculin Reaction in Guinea Pigs.* (30095)

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A significant number of infected guinea pigs showed a complete reversal of tuberculin reaction following treatment with isoniazid (INH). In addition, the minimal infective dose of *Mycobacterium tuberculosis* employed in this study failed to produce disease in animals treated before inoculation. The design of this experiment differs from other studies in several respects(1-3). Each animal was followed individually with periodic tuber-

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culin tests; an identical minimal inoculum was given subcutaneously; INH was administered orally; and the experiment extended over a 2-year period.

The clinical implications of changes in tuberculin reactivity are extremely important (1,2). The primary purpose of this study was to evaluate further the problem of reversal or instability of the tuberculin reaction.

Materials and methods. Tuberculin-negative adult male albino guinea pigs, weighing 300 to 600 g, were evenly distributed according to weight into 3 major groups, 27 each, and a 4th drug-control group, 8 in number. The animals were followed individually and housed in cages, singly or 2 per cage. A commercial guinea pig diet,† supplemented with ascorbic acid (0.25 mg per ml of water), was fed to all animals. Greens were supplied only once weekly to insure adequate drug intake since INH was also administered in the drinking water.

M. tuberculosis strain H37Rv was grown on Lowenstein-Jensen medium for 3 weeks. The method for preparing the mycobacterial suspension has been described(4). One ml of a 2×10^7 dilution of the bacillary suspension was inoculated subcutaneously into the right groin of each animal in the 3 major groups. Colony counts showed that each guinea pig received approximately 6 viable units.

Each animal in the 2 experimental groups and in the drug-control group was given a daily oral dose of 25 mg of INH‡ per kg of body weight. The daily dose of drug was dispensed in drinking bottles with a maximum volume of 75 ml per animal. Results of a preliminary experiment indicated that an adult guinea pig drank approximately 75 ml of water within a 24-hour period.

The primary prophylactic and the drug-control groups were started on INH 48 hours before inoculation; the duration of treatment was 4 months for the primary group and 14½ months for the controls. Animals of the secondary prophylactic group were started on therapy individually after developing a positive tuberculin reaction of 10 mm induration or greater. INH was discontinued when the animal exhibited complete reversion of tuberculin hypersensitivity on 2 consecutive monthly tests. The minimum duration of treatment was 4 months; the maximum duration was one year.

Beginning 2 weeks following inoculation, animals were tuberculin tested at weekly intervals for 6 weeks, at the 10th week, and then at monthly intervals until termination of the experiment. All guinea pigs were tested repeatedly with the exception of the untreated infected control animals. Each guinea pig was injected intradermally with 5 mg of Old Tuberculin§ (O.T.) in a 0.1 ml volume. Different sites on the lateral regions of the back were chosen for successive skin tests. All tests were read after 48 hours by one person who was unaware of the animal's identity. Reactions were graded as recommended in Diagnostic Standards(5) and in addition, degree of induration was measured according to the method of Goddard *et al*(6).

Animals which died or were sacrificed were autopsied by one person. The extent of macroscopic diseases was graded according to the method proposed by Steenken and Wolinsky(7). Representative tissues from all animals were placed in 10% formalin for histopathologic study. One-half of each spleen showing 2+ or less involvement was homogenized in a Tenbroeck grinder for smear, culture, and guinea pig inoculation.

Results. None of the animals of the primary prophylactic and drug-control groups developed a positive cutaneous reaction to 5 mg of O.T. A few reactions, however, graded as less than 1 plus, were observed at irregular intervals in 12 of 27 guinea pigs in the primary group.

Seventeen of 27 guinea pigs in the secondary prophylactic group and 16 of 27 infected controls showed a positive tuberculin skin test by the 5th week after inoculation. All animals in both groups developed a 2 plus (10 mm) or greater reaction by the 10th week with the exception of one animal which

† Dave Clarke Feeds, Paramount, Calif.

‡ Generously supplied by Panray/Parlam Corp., Englewood, N. J., Squibb Institute for Medical Research, New Brunswick, N. J., and Hoffmann-LaRoche, Inc., Nutley, N. J.

§ Wyeth Laboratories, Marietta, Pa.

TABLE I. Complete Reversal of Tuberculin Reaction.

Duration of isoniazid administration (wk)	Cumulative total No. of animals	Cumulative %
17	1	4
18	2	7
21	3	11
22	4	15
27	7	26
32	8	30
36	10	37
39	12	44
40	14	52
41	15	56
42	16	59
48	17	63
50	18	67
52	19	70

did not exhibit a 2 plus reaction until the 14th week. Although INH administration was initiated when a 2 plus reaction was found, 11 of the 27 animals in the secondary prophylactic group showed a 4 plus (necrosis) skin test (2 at the 6th week, 3 at the 7th, and 6 at the 8th week).

Twenty-four of 27 animals in the control group developed a 4 plus reaction by the 10th week after inoculation. Necrosis was not observed in one guinea pig until the 54th week; 2 animals failed to develop a 4 plus reaction during 17 months of observation.

As shown in Table I, 19 of 27 animals (70%) in the secondary prophylactic group showed complete reversal of tuberculin reactivity for at least 2 consecutive months while on INH. The duration of treatment which induced complete reversal ranged from 17 to 52 weeks with a median of 36 weeks. Eight guinea pigs showed a decrease in reaction but did not revert completely during the maximum treatment period (one year); 4 of these animals died before 2 consecutive negative skin tests were observed.

After INH was discontinued, 23 of the 27 animals in the secondary prophylactic group were followed with skin tests for a period of 4 to 85 weeks. With the exception of 2 guinea pigs, reactions were read as less than 1 plus or negative at all test periods. One of the 19 animals which reverted completely and 1 of the 4 incomplete reverters showed a single positive reaction at the 13th and 26th post-treatment week, respectively.

Mortality and post-mortem findings of the experimental and control groups are summarized in Table II. Twenty-three of 27 guinea pigs in the control group died with progressive tuberculosis; death occurred from the 16th to the 70th week with a median of 37 weeks. There were no tuberculosis deaths in the primary and secondary groups. However, in the secondary prophylactic group, 4 survivors, killed 24 months after inoculation, showed evidence of visceral tuberculosis. Two positive cultures were susceptible to isoniazid.

Discussion. The effectiveness of INH prophylaxis in experimental and clinical tuberculosis is well-documented(2,3,8-10). One of the major differences in this study from that of several other investigations was the small infective dose. Results indicate the significance of size of inoculum. Primary INH prophylaxis completely protected guinea pigs inoculated with 6 viable units of *M. tuberculosis*. This favorable result may be due in part to the large dose of INH employed. When INH was initiated after development of a positive tuberculin reaction, sterilization

TABLE II. Postmortem Findings.

Group	Observation period (months post-inoculation)	No. of animals	No. of deaths	Gross visceral tuberculosis No. of animals Positive	Negative
Primary prophylactic	17	27	10	0	27†‡
Secondary prophylactic	24	27	15	2*	25*‡§
Infected non-treated control	17	27	23	27†	0
Drug control	17	8	2	0	8†‡

* Includes survivors killed 24 months postinoculation.
† " " " 17 " "
‡ Spleen homogenates were negative for *M. tuberculosis* by culture and guinea pig inoculation.
§ Histopathology of splenic sections of 2 guinea pigs were compatible with tuberculosis.

of tissues was not achieved in all animals.

Reversal of positive tuberculin reactions in animals subjected to single or multiple drug therapy postinoculation has been reported by several investigators(1,11-13). Observation of reverters following cessation of treatment indicated that a positive reaction may again occur(12,14). In the present study, a positive reaction (following a previous reversal) occurred in only 2 guinea pigs. The possibility that some of the animals may have reverted as a result of repeated tuberculin tests cannot be definitely answered. The data in this study, however, would not support this concept.

Culturable organisms were not demonstrated in tissues of animals with negative gross pathology although histologic evidence of tuberculosis was found in 2 guinea pigs. Viable tubercle bacilli have been recovered from grossly negative tissues(12). The observation that drug-treated animals with negative or intermittent weak reactions may still exhibit macroscopic disease(13) is in agreement with the findings reported here.

Summary. Reversal of the tuberculin reaction occurred in 70% of animals treated with INH following development of a positive test. One year of INH treatment failed to sterilize the tissues in 15% of the animals. Evidence is presented that INH completely protected guinea pigs from tuberculosis when treatment was initiated prior to inoculation of a minimal dose. All non-treated controls developed visceral tuberculosis.

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Antagonism of the *D*-Alanine Reversal of *D*-Cycloserine Action by *L*-Alanine in *Mycobacterium acapulcensis*. (30096)

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During a study of the effect of *D*-cycloserine on growth of *Mycobacterium acapulcensis* it was found that *D*-alanine reversed the growth inhibition produced by this antibiotic, but surprisingly enough *DL*-alanine was not so effective. This paper reports on the characteristic of the antagonism of *L*-alanine over the *D*-alanine reversal of *D*-cycloserine action. In addition, a mutant resistant to *D*-cycloserine was isolated and studied.

Methods. *D*-cycloserine was kindly supplied by Hoffmann-LaRoche (Basel, Switzerland). *D*-alanine and *DL*-alanine were purchased from California Corp. for Biochemical