obtained from tissue under bullae in lungs of 4 older men are also included. Statistical analysis of this graph shows a highly significant increase in relative amount of elastin with age ($t = 3.87$, $p > 0.001$). The regression slope shows an increase in elastin content of the lung of 0.816% per decade of life. On superficial examination of the graph, women over 50 appeared to have more pulmonary elastin than men in this age group. However, when subjected to statistical analysis, the difference in this small series was found to be insignificant ($t = 0.75$, $p = 0.4$). Comparison of the elastin content of sub-bullous tissue with that of normal lung parenchyma of older men revealed a highly significant decrease in the former ($t = 5.09$, $p > 0.001$).

Histological studies of the elastin isolated by our method showed that the end-product was composed only of elastic tissue. However, one preparation contained traces of collagen deep within the walls of a pulmonary arteriole.

Discussion. It has been generally believed that the elastic tissue content of the aging lung is reduced. Our measurements must be viewed critically and are simply a prelude to further investigation. Although the method was well standardized and corrected for errors due to foreign material in the lung, results obtained from lungs of different ages may not be entirely comparable. Pulmonary elastin may change physically and chemically with age as has been demonstrated in elastin from the aorta(4), and these changes may affect the efficiency of isolation and separation. Confirmatory studies of elastin content by other methods are needed. Simultaneous measurement of pulmonary collagen is contemplated in view of the close relationship between collagen and elastin. It is of considerable interest that concentration of elastin in the lung is relatively high. Of the tissues thus far analyzed, only the aorta and the ligamentum nuchae contain more elastin than the lung(1,2).

Summary. 1. An alkaline digestion method for quantitative determination of elastin has been adapted for study of lung parenchyma. 2. Concentration of elastin in the adult human lung has been determined over a wide age span, and has been found to increase with age. 3. Concentration of elastin in lung parenchyma subjacent to areas of bulla formation is significantly reduced.

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Effect of 6-Mercaptopurine on Antibody Production.* (24281)

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Since immunologic mechanisms participate in the rejection of homografts by experimental animals(1), some modification of the immune response is necessary in order to achieve a "take" of the grafted tissue. Alteration of antibody production may occur naturally, as in agammaglobulinemia(2), or may be induced by means of X-radiation(3) or cortisone(4). Protein(5) or vitamin(6) deficiencies obtained by means of special diets (7) or by the action of amino acid analogues (8) may lead to suppression of antibody formation and to prolonged survival of skin homografts(9). In the special case of tumor

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homografts, treatment of the recipient with cortisone may result in a "take"(10). Since bone marrow transplantation has been suggested as a possible treatment for radiation injury, aplastic anemia and some neoplastic diseases(11), our laboratory has become interested in altering antibody formation by various means. The purpose of this paper is to present data which indicate that an antagonist of nucleic acid metabolism, 6-mercaptopurine (6-MP), can abolish the antibody response to a purified antigen.

Method. White New Zealand strain rabbits weighing about 3 kg and maintained on a stock *ad libitum* diet were used. All animals were injected intramuscularly with 50 mg of crystalline bovine albumin (Armour) 3 times weekly for 2 weeks. The first day when antigen was injected was counted as day 0; time prior to this was designated by a (−) sign and time subsequent to day 0 was designated by a (+) sign. The experimental animals were given 6-MP, 3 mg/kg/day according to the following schedule; Group 2M (10 animals): from days 0 to +11; Group 3M (5 animals): from days 0 to +21; Group 4M (5 animals): from days −7 to +4; Group 5M (5 animals): from days +7 to +18; Group 6M (5 animals): from days +18 to +29. There were 10 animals in the control group. Bleedings were made from a marginal ear vein at the times indicated by Fig. 1. Antibody was measured by the tannic acid hemagglutination technic of Stavitsky(12). Results are expressed as the $\log_2$ of the highest serum dilution which gave a +1 pattern.

Results. The course of the experiment is depicted graphically in Fig. 1. Low titers of antibody could be detected in the control group by day +4, with the peak titer at about day +16; thereafter a plateau persisted through the last bleeding at day +38. No antibody could be detected in Group 2M, given 6-MP from days 0 to +11 until 7 days after the antimetabolite had been discontinued; a peak titer of antibody was reached 14 days after this time.

Group 3M, given 6-MP from days 0 to +21, made no detectable antibody during the 38 days of the study. Clinical evidence of toxicity was apparent by day +14, and was manifested by weight loss, poor appearance of fur and diarrhea. One animal died during the study on day 20. Two animals suffered spontaneous abortion. All animals of this group were neutropenic by day 14.

Group 4M, given 6-MP from days −7 to +4 had no detectable antibody on day +4, but by day +14 antibody production was nearly equal to the control values. Group 5M, which received 6-MP from days +7 to +18 showed an apparent cessation of antibody production between days +4 and +14. Four days after the last injection of 6-MP, however, antibody could again be detected and thereafter the titers rapidly approached the control levels. Administration of 6-MP during the height of antibody response, as in Group 6M, had no effect on antibody production.

Discussion. The data reported herein demonstrate that 6-MP, a powerful nucleic acid antimetabolite, abolishes the rabbit's immune response to bovine serum albumin when given simultaneously with the antigen. Although the site of action of 6-MP with respect to antibody formation is unknown, it seems unlikely that suppression of protein formation is an important factor, since administration of the drug during the height of the immune response had no effect on the titer of antibody in the serum. It may be inferred from the data that 6-MP had no effect on the anamnestic response, but did have a pronounced effect on the primary response. It is probable that 6-MP interferes with the utilization of purines for nucleic acid synthesis(13) and, if antibody producing tissue can be assumed to be in a hypermetabolic state, then such interference could have a profound and relatively selective action on the immune response. The presumed organization of "templates" for antibody formation is intimately concerned with the nucleic acid metabolism of the cell (14), and it is possible that 6-MP disrupts either the "information center" (DNA) or the actual template (RNA-protein) of antibody forming cells, thus leading to depression of the primary response. Studies to determine the mechanism and site of action of 6-MP in

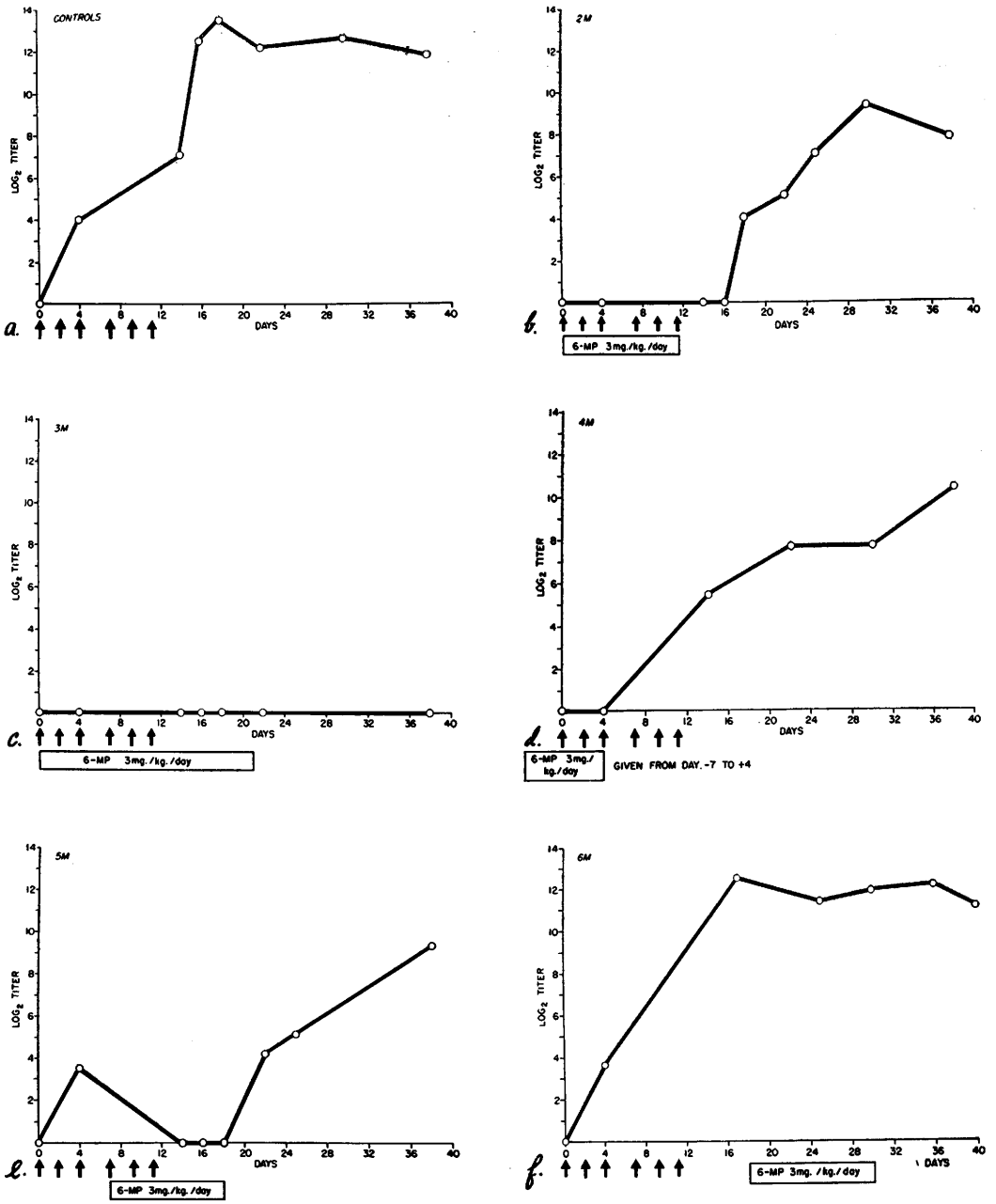

FIG. 1. Course of immune response in control and 6-MP treated rabbits. Each vertical arrow represents antigen inj. Time of administration of 6-MP is represented by the clear block. Each value depicted in the graph represents avg titer for the group.

regard to antibody formation, as well as its effects on the immune response to cellular antigens, are in progress.

Summary. 1. The effect of 6-MP on antibody response of hyperimmunized rabbits has been studied. 2. When given simultaneously with the antigen, 6-MP profoundly suppresses the formation of humoral antibody. When given prior to antigenic stimulation, the effect of 6-MP on antibody production is slight. There is no effect on amount of antibody in the serum when 6-MP is given during the

height of antibody production. 3. It has been tentatively concluded that the action 6-MP is on the primary antibody response, rather than on the anamnestic response.

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Sensitivity of Mice to Endotoxin after Vaccination with BCG (*Bacillus Calmette-Guérin*)* (24282)

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The establishment of tolerance to endotoxins derived from gram negative bacteria upon repeated injection is attributed to increased activity of the reticulo-endothelial system (RES). Tolerance is effective against many manifestations of endotoxin activity, such as fever, leukopenia, shock, and Shwartzman reactions(1) and may last in mice for 4 months(2). Single or repeated injections of endotoxin result in increased clearance of particles or large molecules from the blood stream by the RES(3). Similarly, infection with virulent tubercle bacilli or vaccination with BCG were found to result in increased clearance by the RES(4). In view of these findings it appeared possible that BCG vaccination might induce tolerance to endotoxin. This view is supported by the fact that a single injection of endotoxin increases resistance to tuberculous infection(5) indicating some relationship of the host's reaction to these 2 biologically active agents. Experiments were therefore undertaken to study the reaction of mice infected with tubercle bacilli to endotoxin derived from gram negative bacteria.

Methods and materials. Swiss albino mice of the Webster strain were used. Lipopolysaccharide was prepared according to the technic described by Westphal and Lüderitz from a strain of *E. coli B*(6). Preparations were also obtained from the Difco Laboratories, Detroit, Mich. (Lipopolysaccharide *E. coli* 026:B6) and from Wander S. A., Berne, Switzerland (Pyrexal Wander).‡ The lipopolysaccharide was suspended in physiological solution of sodium chloride and was injected intraperitoneally. For vaccination 0.2 ml of a BCG§ culture grown for 7 to 10 days in the liquid tween-albumin medium was injected intravenously. In some experiments cord factor derived from virulent tubercle bacilli‖ was

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