virus to produce CPE with inclusions in mouse embryonic fibroblasts were not significantly altered. Pathogenicity for mice, which was of a high order after the first 3 or 4 passages, was completely lost after about the 36th passage.

Attempts to produce plaques on other cell types. Using the procedure described above for obtaining virus directly from the salivary glands of infected mice, attempts were made to produce plaques on chick embryo cells, HeLa cells, and L cells. These attempts were all unsuccessful; neither plaques nor inclusions were observed after 6 days' incubation in any of these cell types.

Summary. Using a double agar overlay, MSGV produced discrete spherical plaques in mouse embryonic fibroblasts, but not in chick embryo, HeLa or L cells. Plaques appeared about 36 hours after seeding as single large rounded inclusion-laden cells, surrounded by wide clear zones. Centrifugal expansion by progressive involvement of adjacent cells resulted in plaque diameters of 0.2 to 0.8 mm by the 6th day. Fluid medium from infected bottle cultures commonly contained 1×10^7 PFU/ml. A linear relationship was found between plaque counts and virus dilution. The virus was markedly stable when stored for 5 hours in PBS with 2% calf serum, but rapid loss of titer occurred if serum was omitted. Virus titer and CPE production *in vitro* were not significantly altered during 120 passages, but pathogenicity for mice was lost after about 36 passages.

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Effect of B-3-Thienylalanine on Antibody Synthesis. II. Relation of Time of Drug Feeding to Inhibition of Synthesis.* (28606)

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It has previously been reported that B-3-DL-thienylalanine, (B-3-TA), when fed to rats by stomach tube, would inhibit antibody formation if feeding was started 2 days before antigen injection and continued until sacrifice of the animals(1). No inhibition was seen if feeding began on the third day after immunization, even though similar amounts of drug were fed. Addition of the drug during synthesis of antibody *in vitro* showed no depressing effect on the synthetic process as measured by incorporation of radioactive glycine into antibody.

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Wissler *et al.*(2) showed that there is a reduction in the splenic cellular proliferation which accompanies antibody formation following B-3-TA feeding. The present work was undertaken in an effort to: a) establish the optimal time of B-3-TA feeding in relation to antigen injection necessary to achieve maximum inhibition of antibody synthesis and b) to investigate further the effect of B-3-TA on the cellular changes which occur in the spleen of the immunized rat(3).

Materials and methods. Sprague-Dawley, young adult, male, albino rats were used in these experiments. Diets were prepared as described by Hruban *et al.*(4). The rats received their total ration in 3 separate feed-

ings approximately 6 hours apart. For 3 days before the beginning of each experiment all animals received a liquid diet with the amino acid mixture replaced by casein hydrolysate to accustom them to the tube feeding process. They were then switched to the experimental diet which contained B-3-TA in varying concentrations so that each animal received the same total dose irrespective of length of drug administration. In diet prepared for rats which were fed for the entire duration of the experiment ("maximally inhibited," fed from day −2 to day +4), phenylalanine was replaced by an amount of B-3-TA equivalent to 2.5 times the phenylalanine on a molar basis. Phenylalanine was omitted from diets containing the analogue. Immunization was carried out by one intravenous injection of 1.0 ml of sheep erythrocytes (2% in normal saline). All animals were sacrificed by exsanguination under light ether anesthesia; blood was collected, serum was obtained by centrifugation a few hours following sacrifice and stored at −20°C until titrated. The techniques employed for antibody titration have been described(5). Spleens were taken at sacrifice and fixed in Carnoy's fluid. They were embedded, sectioned and stained with methyl-green pyronin according to the techniques employed previously(6).

Results. Experiments were carried out to test the possibility already considered(1), that the most effective time for the inhibitory action of B-3-TA was the early period following antigen injection: the induction phase(7). This period has been termed by several authors as the "induction period" or "inductive phase" of antibody synthesis(8,9). It is known that no antibody synthesis is detectable during this phase(8). It is apparent, however, that something takes place during this short time which is very important to set the stage for the synthesis of antibody protein(10,11). In the first 2 experiments, groups of 5 rats each were fed with analogue for the following periods: throughout the experiment, from the beginning to the first or the second day after immunization, and from day 1 or 3 after antigen to the day of termination of experiment. The results (Fig. 1) show that feeding with analogue during a period which includes the induction period of antibody synthesis will considerably reduce the amount of antibody present in the serum. This phenomenon was tested again in 2 further experiments in which the different groups of rats were fed for the entire length of the experiment, until day 2 after antigen administration and on days 3 and 4 after immunization. While the former two groups showed moderate to high inhibition of synthesis, Group 3 showed a titer comparable to that of control animals (Fig. 2). In all experiments up to this point feeding of the analogue was always begun 2 days before or on the same day after antigen injection. It seemed interesting to examine the effect of feeding B-3-TA according to the following schedule: from the day on which antigen was administered to end of experiment; during the 2 days following antigen injection only; during the 2 days preceding antigen injection. This was compared to the group known to have maximum inhibition (−2 to +4) and minimum inhibition (+3 to +4). One could thus also determine whether feeding of analogue during the pre-immunization period (namely, from day −2 to day 0) was necessary to obtain maximum inhibitory effect. The data in Fig. 3 show clearly that while drug feeding before immunization does not interfere with antibody formation, feeding for only 2 days after antigen injection leads to marked lowering of titer. A second control group was introduced in these last experiments, consisting of rats receiving regular laboratory chow instead of the usual control liquid diet. It is not clear whether animals fed control liquid diet respond to the antigenic stimulus as well as animals on regular laboratory chow. Study of histologic sections of spleen in all groups of animals revealed some differences in the appearance of the red pulp at time of sacrifice, 5 days after antigen injection. Fig. 4 illustrates the aspect of the red pulp of the spleens from an animal in a "maximally inhibited" group and an animal in a "minimally inhibited" group. It is apparent that the number of pyronin positive cells observed during antibody synthesis and believed to be antibody forming cells is only moderately de-

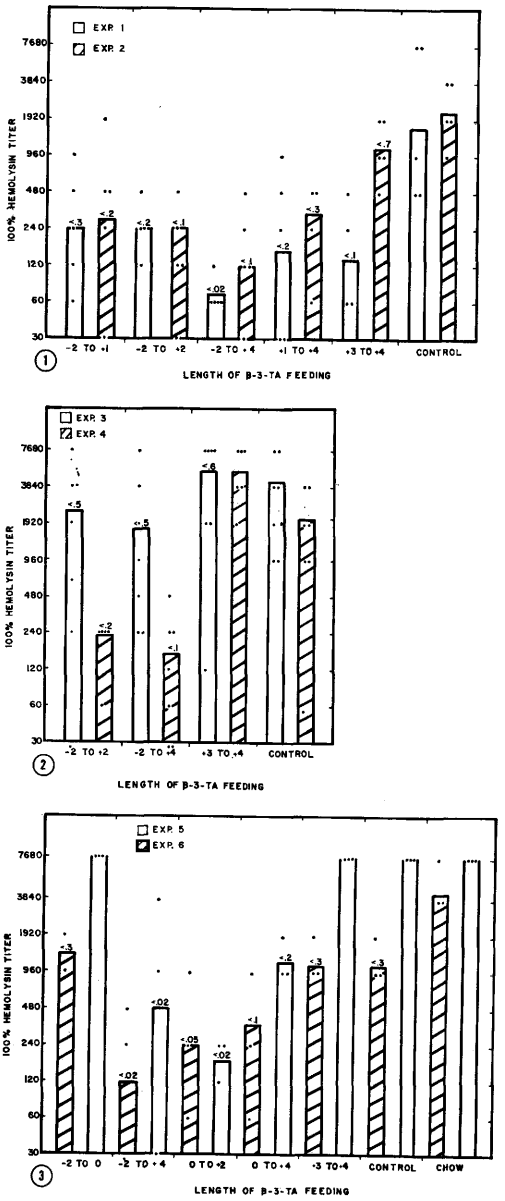

FIG. 1. Hemolysin serum titer of rats killed on day 5 after antigen injection in 2 experiments in which B-3-TA was fed for periods shown on abscissa. Ordinate represents maximum dilution giving 4+ hemolysis as read visually. P values are indicated at top of each bar. Individual bars represent arithmetic mean of individual titers indicated by the single dots.

FIG. 2. Hemolysin serum titer of rats killed on day 5 after immunization and fed B-3-TA for varying periods. Ordinate represents maximum dilution giving 4+ hemolysis as read visually. P values indicated at top of each bar. Individual bars represent arithmetic mean of individual titers indicated by the single dots.

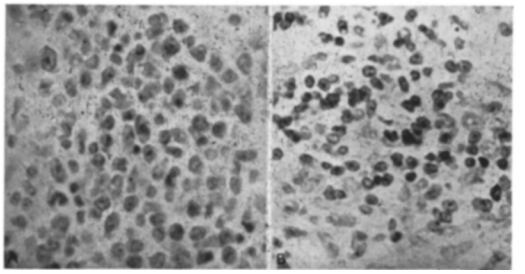

FIG. 4. Splenic red pulp of 2 rats from representative minimally inhibited (A) and maximally inhibited (B) groups. Cell types represented are similar, although pyroninophylic cells in B are less numerous and there appear to be more mature forms as compared to A.

creased in the treated animal as compared to the other animal.

Discussion. There seems to be a definite relationship between time of analogue feeding, as related to time of antigen injection, and amount of antibody present in the serum. In fact, antibody production is maximally inhibited by feeding B-3-TA only if the time of feeding includes the induction period of antibody synthesis (day one and two after antigen injection in the system employed here). This early phase of antibody synthesis is still one of the very obscure problems in immunogenesis(8,9,10,11). Antibody formation appears to be blocked by the action of this analogue on some processes which take place during the early post-immunization period, since B-3-TA does not exercise any inhibitory action if given only during the pre-immunization period. In view of the model recently proposed by Nossal(12) which considers cell division before antigenic stimulation as a necessary event in synthesis of an antibody, one could conclude that the action of B-3-TA on cell division is of minor importance. This seems in agreement with recent findings of Hruban(13) showing that cell division seems to be only relatively affected by this analogue. Alternate possible explana-

FIG. 3. Hemolysin serum titer of rats killed on day 5 after immunization and given B-3-TA for different periods. It is apparent that feeding before immunization (column 1) does not appreciably affect antibody titer. Ordinate represents maximum dilution giving 4+ hemolysis as read visually. P values are indicated at top of each bar. Individual bars represent arithmetic mean of individual titers indicated by the single dots.

tions for the observations reported here are: It is possible that B-3-TA does not inhibit antibody synthesis but merely inactivates the antibody in such manner as to make it non-reactive. This seems very unlikely since one would expect the antibody to be inactivated regardless of time of administration of analogue. Furthermore, the study of analogues has revealed a definite inhibitory action on the synthesis of certain proteins, which in many cases has a selectivity for one stage only of the synthetic mechanism, much as the one seen here. Novick(14) has observed that B-3-TA will inhibit the synthesis of permease and block concentration of inducer in *E. coli*, but not the synthesis of B-galactosidase once the inducer has been concentrated inside the organisms. On the other hand the inhibitory effect might be exercised at the level of antibody synthesis regardless of time of feeding of the analogue. The observation that antibody synthesis is not affected even by addition to an *in vitro* antibody forming system of 7 times the amount of drug normally used definitely contradicts this possibility(1). This lack of inhibition has recently been confirmed by Hruban(13).

Summary. The time of action of B-3-TA on antibody synthesis has been further narrowed to the first 2 days following antigen injection. A study of the histological reaction observed in the red pulp of the spleen following immunization has revealed only slight decrease in number of pyronin positive cells at 5 days after administration of antigen.

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Comparative Actions of 1,10-Phenanthroline and Quinidine on Normal and Fibrillating Isolated Atria.* (28607)

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The chelating agent, 1,10-Phenanthroline (1,10-P), has been used successfully in experimentally induced atrial and ventricular fibrillation in rabbits(1). To study further the action of 1,10-P, the effects of 1,10-P and quinidine on fibrillating and normal rabbit atria were compared. The effects of equal concentrations of 1,10-P and quinidine on the action potential of rat atria were also compared.

Methods. The atrial preparation. Male albino rabbits weighing 2-3 kg were killed by a blow on the neck and bled, the heart quickly removed, and the atria dissected free from ventricles and surrounding tissues. The right atrial appendage was impaled on a platinum electrode and the left atrial appendage

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