This finding is important with reference to biological assay of INH in patients' plasma. For this assay the plasma usually is kept frozen or refrigerated for variable periods preceding testing. Adding the error caused by bacteriological technic of dilution it appears questionable whether the bioassay carries any value. Whether the factor contained in plasma is still active at the dilution resulting from distribution in the culture medium, cannot be answered because the chemical method is not sensitive enough for dilutions in that range. Should it be the case, INH would decrease in concentration during process of incubation, which usually lasts about 3 weeks. The presence of the postulated factor in plasma in larger or smaller quantities may explain why some patients show quicker inactivation of INH than others.

TABLE IV. 10 and 20 mg/cc INH Respectively Added to Pooled Rabbit Plasma.

	Found μg/cc after					
Temp.	4 hr	1 day	3 days	7 days	9 days	14 days
25°	7.2		4.4		2.2	2.0
37°	7.1		4.8		.3	.0
7°	7.9		5.9		3.2	3.0
	18.4	17.3	13.6	7.5		6.7
frozen	5.2		4.7		3.5	3.4

TABLE V. 10 mg/cc INH Added to Human Plasma.

		Found μg/cc after time				
	Temp.	0	1 day	3 days	7 days	21 days
R	25°	9.3	6.8	4.4	4.2	.9
R	37°	9.3	6.0	4.8	3.0	.0
J	7°	9.3	7.2	4.9	4.4	4.0
R	7°	8.8	7.2	6.1	5.8	5.1
R	frozen	9.3	8.0	6.9	6.0	5.7

Summary. INH is stable for several weeks in aqueous and buffered solution at pH values below 8 and at low temperature. It is unstable in human and rabbit plasma whether added to pure plasma or present after oral or parenteral administration. Instability is enhanced by increased temperature, but is quite marked at refrigerator temperature. The presence of a factor in blood causes changes in chemical state of INH so that it no longer reacts with reagent used in the assay method.

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Effect of β-3-Thienylalanine on Antibody Synthesis. I. Preliminary *in vitro* and *in vivo* Studies.* (25908)

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Experiments of Wissler *et al.* have shown that feeding β-3-thienylalanine (β-3-TA) in absence of dietary phenylalanine results in almost complete inhibition of antibody response to a single intravenous dose of particulate antigens in rats(1). In these experiments the amino acid analogue was incorporated in a liquid diet and fed by stomach tube for a period of 6 days beginning 2 days before antigen injection and continuing up to day before sacrifice. Antibody titer at this time was always considerably lower than that of control animals. The histologic transformations in rat spleen which accompany synthesis of antibody(2) were consistently absent in animals fed β-3-TA. Our experiments originated during study of antibody production in tissue culture(3). The purpose was to study the effect of β-3-TA on antibody synthesis *in vitro*

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TABLE I. Incorporation of Glycine 1-C^{14} into Antigen Bound Material. Ratio of radioactivity incorporation of experimental over control animals.

No. of spleens cultured	Days of β-3-TA feeding after antigen	β-3-TA added to culture	Exp. / Control
6	0,1,2	—	1.4
6	1,2	—	2.8
6	2	—	5.4
18		+	5.3
24		—	6.6

All rats inj. 3 days before sacrifice. All spleens cultured 3 days. β-3-TA added to tissue culture 12 hr after beginning of incubation. Amount of β-3-TA added was 50 γ/ml of medium (15 γ/mg of tissue present in culture).

and to compare this to inhibition of antibody synthesis observed *in vivo* by Wissler and coworkers(1).

Materials and methods. Young adult male albino rats of the Holtzman strain were used throughout. Tissue culture methods, manner of antibody titrations, of antibody determination in tissue culture fluids, and histological technics employed have been described(3-5). The antigen used in *in vivo* experiments was a 1% suspension of washed sheep erythrocytes; 1 ml of this suspension was administered intravenously. Tube-feeding of β-3-TA, diet preparation, etc. were done according to the methods described by Wissler *et al.*(1). Rats to be prepared for tissue culture were injected intravenously 72 hours before beginning of culture with 1.0 ml of *S. typhi* vaccine. Tissues to be cultured were always explanted 72 hours after antigen injection. β-3-TA added to the medium 12 hours after beginning of culture. The medium to which β-3-TA was to be added was prepared without phenylalanine.

Results. The initial experiments tested the effect of β-3-TA added directly to the tissue culture medium on synthesis of antibody as measured by incorporation of radioactive glycine into antigen-binding material. As shown in the 2 bottom lines of Table I, β-3-TA added to a tissue culture system in which spleen fragments were synthesizing antibody did not alter appreciably final antibody output.

The fact that spleens for tissue culture were explanted 3 days after injection of antigen suggested the possibility that β-3-TA did exercise its depressing effect during earlier stages of antibody synthesis. Thus, β-3-TA would not depress the response to antigens once this response had been established. Experiments were therefore set up in which this hypothesis could be tested both *in vitro* and *in vivo*.

Table I summarizes results obtained in several tissue culture experiments, conducted as follows: β-3-TA was fed to rats for varying periods of time after antigen injection. Spleens were explanted 72 hours after antigen was administered and culture was allowed to proceed for 48-72 hours in normal medium. There is a marked depression of antibody synthesis if β-3-TA feeding is started on day of antigen injection or one day after (Table I). If feeding is begun on second day after antigen injection (1 day before starting tissue culture) or added to culture medium in which spleens from animals not receiving β-3-TA are explanted, there is only a very slight depression of antibody synthesis. In one experiment up to 7 times the amount of β-3-TA normally used was added to the culture medium and no more effect was noted.

To test further the possibility that β-3-TA was active only during early stages of antibody synthesis *in vivo* experiments were set up in which groups of rats were fed β-3-TA starting at different times in relation to antigen injection and continuing to day of sacrifice. Thus, it was hoped to be able to determine the period during which β-3-TA was most active in its depressing effect. Fig. 1 summarizes results obtained by this experimental approach.

If β-3-TA feeding is started after the end of induction period, there is no significant depressing effect on hemolysin synthesis. On the other hand, a considerable depression of the synthetic process is evident if β-3-TA is administered during the days immediately following antigen injection and an even greater depression exists with earlier feeding of the analogue.

To minimize the possibility of error arising from toxic phenomena due to β-3-TA all rats should receive the same amount of analogue. Thus rats in groups fed only for shorter times

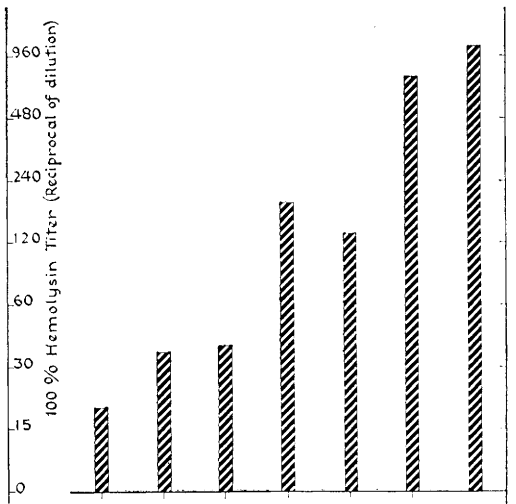

FIG. 1. Effect of β-3-TA feeding begun at different times before and after sheep erythrocyte inj. on 100% hemolysin titer in rats.

received a large dose of analogue over a shorter period of time. One may wonder whether under these conditions a stimulatory effect could be elicited on the synthesis of antibody. This seems very unlikely since no such effect was noted in tissue culture experiments in which β-3-TA was added to the *in vitro* system.

Discussion. Formation of antibody can be depressed or abolished by several physical(6) as well as chemical(7,8) agents. More recently reports have appeared which stress the fact that considerable inhibition or complete suppression of antibody synthesis can be obtained by use of substances which are analogues of normal metabolites(1,9-15). Only one of these papers(14) deals with the time at which the analogue seems more effectively to antagonize the synthetic process and bears out the same relationship between administration of analogue and inhibition of antibody production observed in the work reported here.

The so-called induction period of antibody formation is still a relatively poorly understood phenomenon. It is known that after a single antigenic stimulation, formation of antibody does not begin until approximately 48-72 hours after the stimulus has been introduced(16). During this time no appreciable antibody synthesis takes place as shown by studies of incorporation of labeled amino acids into antibody protein(17). The fact that β-3-TA seems to depress synthesis of antibody only when administered during induction period suggests that this substance may interfere with preparation of the so-called "antibody forming mechanism" or of a part of it. Sterzl's study in which 6-mercaptopurine(14), an analogue of natural purines, was used indicates that there may be two areas in preparation of this mechanism. In fact 6-mercaptopurine is known to interfere with synthesis of nucleic acids(18); β-3-TA seems to exercise its inhibitory function only at the level of protein synthesis(19). Further experiments are needed, and are being carried out at present, to clarify the level in the antibody synthesizing process at which β-3-TA acts. These studies may contribute greatly to a better understanding of the early stages of antibody synthesis.

Use of β-3-TA in experiments of this nature may be made somewhat cumbersome by the need to administer the analogue by stomach tube. This disadvantage may be compensated by the fact that β-3-TA affords a tool which has no equal because of its lack of toxicity for normal cells at the level used in these experiments(1). The possibility of β-3-TA administration by a route other than oral in the hope of further simplifying its use is being investigated.

Summary. When β-3-thienylalanine, an analogue of phenylalanine, is fed to rats for appropriate time before and after antigen injection it will markedly inhibit antibody response. This analogue added to an antibody synthesizing tissue culture system gave no inhibition of antibody synthesis under these conditions. It appears that unless β-3-TA is fed during induction period, its inhibitory effect will not be manifested. It is possible then that β-3-TA is active only in inhibiting the "setting up" of the antibody forming mechanism.

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Immunization Studies with Living Vaccine of *Salmonella typhimurium*. (25909)

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Production of artificial immunity to Salmonella infections in both man and animals is a problem which for the most part remains unresolved. Only recently has a properly controlled field study shown that typhoid vaccination confers some level of protection against infection in man(1). In laboratory animals, some information indicates that recovery from the disease yields best immunity to reinfection. In the past, such results were explained on basis of selection of innately resistant animals initially present(2). However, Hobson(3) recently showed that mice infected with relatively avirulent strain of *Salmonella typhimurium* developed a resistance to reinfection with virulent strains without appreciable deaths occurring from initial infection. Studies using killed *S. typhimurium* vaccines to immunize against mouse typhoid demonstrated that these vaccines offer only low level of protection against infection under natural conditions(4) and are only somewhat more satisfactory against challenge by intraperitoneal route(5). In fowl typhoid, where recent studies have indicated that naturally recovered chickens are immune to reinfection with *Salmonella gallinarum*(6), Smith(7) showed that dead vaccines had little or no protective effect while good immunity was produced by live vaccines. In view of these findings, and since our previous attempts to protect against mouse typhoid with killed vaccines have been unsuccessful, a living avirulent mutant of *S. typhimurium* was sought for further investigation. Isolation of streptomycin-dependent bacterial strains by Miller and Bohnhoff(8) suggested a technic which could be employed to obtain an avirulent mutant of *S. typhimurium*. Herzberg and Elberg(9,10) reported isolation and characteristics of such mutant of *Brucella melitensis* and on use of this variant in immunization studies. This communication gives results in which living vaccines were employed to protect mice against intraperitoneal challenge with a virulent culture of *S. typhimurium*.

Materials and methods. Cultures. An antigenically complete culture of *S. typhimu-*