thyroxine compounds are given orally, is shown in Fig. 1. It can be seen that to inhibit the effect of PTU, the strength of the dose necessary is 2.5-3.5 μg/100 g of body weight. Fig. 2 is the dose-weight ratio when the thyroxines are given subcutaneously. The amount of the hormone required to maintain the weight of the glands within a normal range is 2.0-2.5 μg/100 g of body weight which is somewhat lower than when the compounds are given orally.

Discussion. The data for the most part are self-explanatory, but two points need further consideration. The first is that oral administration requires a larger dose of thyroxine to bring the propylthiouracil treated thyroid weights into line with normal weights than do subcutaneous injections of the same compounds. Adsorption of the thyroxine in the intestine is a factor in dose requirement (Fig. 1 and 2). The thyroid weight in most of the groups shows a large standard error. Despite the large standard error values, further statistical analysis shows that these values are not too significant because of the great differences between the mean thyroid weight of the control as compared to the mean thyroid weight of the experimental groups. This fact is further emphasized in that the P values for all the groups are less than 1×10^{-4}. A P value of 5×10^{-2} is statistically significant in most experiments.

Summary and conclusions. Experiments, using 110-120 g Sprague-Dawley male rats, were conducted to compare the activity of L-Thyroxine, sodium-L-Thyroxine and disodium-L-Thyroxine administered by subcutaneous and oral routes. There are no significant differences in the antipropylthiouracil effect of the three synthetic thyroxine compounds when administered by the same route. Considering that the gavage method permits some technical error, the amount of thyroxine necessary to inhibit the effect of propylthiouracil is approximately 50% more by the oral method than that necessary by subcutaneous injection.

Addendum. Since this paper went to press, a report of clinical work with sodium L-thyroxine has been published (Salter, William T., and Rosenblum, Ira, *Am. J. Med. Sc.*, 1952, v224, 628). An assimilation by the patients of 67% of the orally given sodium L-thyroxine was reported.

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Effect of Arginine on Growth and Formation of Arginine Dihydrolase in *Streptococcus faecalis.**† (19982)

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Gale(1) showed in strain ST of *Streptococcus faecalis* that cells formed during the first few hours of incubation on an arginine "rich" medium contained a more active arginine dihydrolase enzyme system than cells formed at any later stage of the growth cycle. However, Slade and Slamp(2) found in strain D10 of *S. faecalis* that the first formed cells possessed very little dihydrolase activity. Enzyme formation in this strain commenced after

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TABLE I. Growth and Dihydrolase Formation by ST on 'Low' Arginine Media.

Time, hr	Qco_2*	% arginine removed	% glucose removed	Cells, dry wt/100 ml	E units†	pH
4	210	69	10	3	630	7.5
5	138	94	19	17	2340	7.4
6	131	99	33	31	4060	7.2
8	70		62	61	4270	6.5
12	50		95	82	4100	6.6‡
25	45		96	80	3600	6.6

* Determinations made with l-arginine as substrate.
† E units = Qco_2 × dry wt cells/100 ml medium.
‡ pH adjusted from 5.8 to 6.6 with N NaOH at 10.5 hr.

a short lag period and increased progressively during the growth of the culture. The formation of arginine dihydrolase by this organism was typical of an adaptive process. It is the purpose of this investigation to examine the process of formation of the dihydrolase enzyme system in these 2 strains.

The complete dihydrolase reaction in cell-free extracts of *S. faecalis* occurs in at least 2 steps with citrulline as an intermediate (Slade)(3). 1) arginine + $H_2O \rightarrow$ citrulline + NH_3; 2) citrulline + $H_2O \rightarrow$ ornithine + CO_2 + NH_3. The dihydrolase enzymes will be referred to as (a) arginine desimidase and (b) citrulline ureidase(3).

Materials and methods. Procedures for the culture of the organisms, preparation of cell-free extracts. chemical determinations, and estimation of enzyme activity have been given (2,3). Strain ST was obtained from E. F. Gale of Cambridge University. The chemically defined medium of Slade *et al.*(4) was employed where indicated; however, the concentration of salts A was increased 3 times. The "low" arginine medium consisted of 0.4% casein digest (Difco Casitone), 1% yeast extract (Difco), 0.6% glucose, and 1% Na_2HPO_4. The "high" arginine medium was the same as the "low" arginine medium except that it contained an additional 0.7% arginine. l-Arginine • HCl, dl-ornithine • HCl, and dl-citrulline were used in all cases. They were obtained from commercial sources. Citrulline was also synthesized from dl-ornithine by the method of Kurtz(5). The rate of arginine desimidase has been shown (3) to be approximately the same as the rate of citrulline ureidase in strains D10 and ST of *S. faecalis* when grown on arginine. Therefore in these experiments, manometric measurements of the rate of CO_2 evolution from arginine are a measure of the complete arginine dihydrolase enzyme system. Rates of ureidase activity in cells grown on citrulline were made with citrulline as substrate.

Results. Growth requirement for arginine. Strains ST and D10 showed an absolute growth requirement for arginine. No significant growth was obtained in the absence of arginine on extended incubation and repeated transfer in the defined medium. However, citrulline, the intermediate compound in the breakdown of arginine, was able to replace arginine in both strains. Total growth in the presence of citrulline was approximately one-fourth that obtained with arginine.

Enzyme formation on "high" and "low" arginine media. Slade and Slamp(2) have shown with D10 that it was necessary to add arginine to a casein digest medium in order that significant dihydrolase formation occur. In the absence of added arginine no significant quantities of enzyme were formed at any stage in the growth cycle. Table I shows that the ST cells formed during the first 4 hours of growth on "low" arginine media possessed high dihydrolase activity (Qco_2 210). A similar result was obtained on "high" arginine medium. Results on the latter medium confirm the observations of Gale(1). A pronounced decrease in enzyme activity followed the disappearance of arginine in both cases. Both media had been largely depleted of arginine in an incubation period of 5-7 hours. Each strain used arginine at approximately the same rate on high arginine medium. A lag period of about 8 hours occurred in strain D10 before utilization of arginine reached the

TABLE II. Effect of Arginine on Dihydrolase Formation by ST in a Defined Culture Medium.

Arginine added, mg/100 ml medium	Cells, mg dry wt/100 ml medium	Q_{CO_2}
1	<2	120
5	<2	150
10	3	160
50	7	150
100	7	203
500	8	204

Cultures incubated 7 hr. Q_{CO_2} determinations made with l-arginine as substrate.

TABLE III. Comparative Activity of Arginine- and Citrulline-Grown Cells.

Activity (Q_{CO_2})			
Whole cells		Cell-free extract	
Arginine	Citrulline	Arginine	Citrulline
Arginine-grown cells			
120	<1	64	52
Citrulline-grown cells			
<1†	<1†	5*	33*

* Culture medium included .1% yeast extract (Difco).

† Activity determined by assay for the presence of citrulline.

Strain ST cells grown on chemically defined medium 16-18 hr. l-arginine was replaced by twice its wt of dl-citrulline.

Q_{CO_2} values of cell-free extracts are per mg dry wt of the extracts.

maximum rate. In ST, however, a lag period of only 4 hours occurred. The Q_{CO_2} of the ST culture decreased between the 6th and 8th hours of incubation to about 60% of its maximum activity. Total growth doubled during the same period. In contrast, enzyme activity in D10 was initially low and increased as growth progressed. Growth was largely completed when approximately 75% of the arginine had been removed. No decrease in enzyme activity occurred during the D10 culture cycle(2).

Quantities of arginine required for enzyme formation. Table II shows that quantities as small as 10 μg arginine/ml resulted in a high level of enzyme formation in young cells of ST on a defined medium. In D10, about 1200 μg arginine/ml medium did not result in significant enzyme formation(2). Consequently, more than 100 times as much arginine was required for the synthesis of a comparable quantity of enzyme in D10 as was required in ST.

Enzyme formation on citrulline. Analysis of cells grown on citrulline offered an opportunity to determine whether arginine desimidase was formed in response to the presence of citrulline as well as arginine. Table III shows that whole cells harvested from a synthetic medium containing citrulline in place of arginine did not show significant arginine desimidase or citrulline ureidase activity. The inability of these cells to metabolize citrulline at an appreciable rate is due principally to lack of permeability of the cell wall to citrulline. This is clearly illustrated (Table III) when whole cells and cell-free extracts from arginine-grown cells are compared. Due to the weak growth response obtained with citrulline it was necessary to add yeast extract to the synthetic medium in order to obtain sufficient cells to prepare a cell-free extract. These extracts did not possess significant arginine desimidase activity (Q_{CO_2} 5). The small value obtained was probably due to the arginine present in the yeast extract. In contrast, the activity on citrulline was 6.5 times that obtained with arginine. Consequently, it was not possible to determine whether cells grown on citrulline possessed arginine desimidase. Also attempts to replace arginine with ornithine plus CO_2(1) in the synthetic medium were not successful.

Discussion. It is possible that the widely differing arginine dihydrolase activity of young cells of strains ST and D10 may be due to a difference in inherited ability to utilize arginine. The lag period required before ST was able to utilize arginine at maximum rate was one-half that required by D10. Also, the weight of cells formed by ST after 6 hours was twice that formed by D10 in the same period. In addition, the total dihydrolase in the former culture was 4 times that present in the latter case.

The relationship of growth to the removal of arginine also differed in the 2 streptococcal strains. In ST the 2 processes occurred concurrently and at a similar rate during the entire culture cycle. The growth of strain D10 preceded the removal of a significant quantity of the arginine present. Ninety per cent of the cells had been formed while only 2.5% arginine was removed. It seems likely in

strain ST that the synthesis of arginine dihydrolase, and consequently the utilization of arginine, is directly connected with growth. In spite of the fact that both cultures require arginine for growth, a highly active arginine dihydrolase enzyme system is not required by D10 for *maximum cell formation* to occur.

A possible explanation of the sharp drop in dihydrolase activity with ST is also evident from these data. The decrease did not begin until practically all the arginine had been removed. Tests for the presence of citrulline during the period of decreasing enzyme activity showed that sufficient citrulline (or a citrulline-like compound) was present to supply the requirement. The cells formed during this period (in the absence of arginine) did not contain arginine desimidase. Consequently, desimidase-active cells formed during the period of arginine fermentation were diluted with desimidase-inactive cells. The total desimidase content (E units) of the ST culture at the start of the phase of decline (6 hr) was the same as the quantity present at the end of the phase (12 hr). The decline in enzyme activity on high arginine media was also due to dilution of the active cells.

The opposite situation existed in strain D10. All cells produced during the culture cycle were formed in the presence of excess arginine. Accordingly, there was no opportunity for ureidase-positive, desimidase-negative cells to be formed.

The formation of arginine desimidase and citrulline ureidase in the presence of either arginine or citrulline as a substrate is in agreement with the concept of "simultaneous adaption" (6).

Summary. 1. Strains D10 and ST of *S. faecalis* were shown on a synthetic culture medium to require arginine for growth. Citrulline, the intermediate compound in the hydrolysis of arginine by these organisms, was found to replace arginine. Citrulline, however, produced only one-fourth as much growth. 2. Strain ST, in contrast to D10, possessed the ability to produce in very young cells a strong arginine dihydrolase enzyme system in the presence of small (10 μg/ml) quantities of arginine. Approximately 100 times as much arginine was required by D10 to produce a comparable enzyme activity. 3. Serial fermentation analyses of cultures of the 2 strains are presented in relation to the rate of synthesis of the arginine dihydrolase enzyme system by the cells. 4. No significant quantities of arginine desimidase were formed when citrulline was used as a substrate for growth. 5. It is concluded that the synthesis of arginine dihydrolase in strain ST is directly connected with growth. Although both ST and D10 require arginine for growth, a strong dihydrolase enzyme system is not required by D10 for maximum cell formation to occur.

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An Activator of Plasminogen in Normal Urine.* (19983)

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The presence of a potent fibrinolytic agent, similar to plasmin, in normal urine (1) has recently been confirmed (2). Further investigations have shown, however, that this agent is an activator of plasminogen and is not a fibrinolytic enzyme.

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