Langdon and Bloch have postulated that $\Delta^7$-cholestenol may be an intermediate in the biosynthesis of cholesterol(13). If this should be true, the large amounts of $\Delta^7$-cholestenol in rodent skin would imply a less effective conversion to cholesterol in this tissue than in the rest of the body or in the skins of other species.

*Summary.* 1. The concentration of $\Delta^7$-cholestenol in rat skin sterols increased rapidly from birth to 5-6 weeks of age but remained fairly constant thereafter. Skin sterols from adult male rats contained from 35-40% of $\Delta^7$-cholestenol while skin from females contained 25-30%. These proportions did not change in severe pantothenic acid deficiency nor in hypothyroidism. Hyperthyroidism, however, appeared to increase the concentration of $\Delta^7$-cholestenol in sterols from female rats. 2. The total fast-acting sterol in the skins of the wild rat and cotton rat were similar to those of the laboratory rat, while sterols from the skins of the mouse and guinea pig contained about 15% of a fast-acting component. The amounts of $\Delta^7$-cholestenol were low in the skins of the pig, rabbit, dog, calf, chicken, and cat. A sample of human skin and a human atheromatous aorta contained negligible amounts of the new sterol.

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## Effect of 5-Bromouracil on Utilization of Thymidine by *Streptococcus faecalis.** (20952)

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The introduction of a halogen in substitution for a hydrogen or an alkyl group in a metabolite not infrequently results in the formation of a compound with antimetabolite activity. 5-Bromouracil has been reported by Hitchings *et al.*(1) and by Weygand *et al.*(2) to inhibit the growth of *L. casei* and *S. faecalis*, respectively, in basal media supplemented with thymine. In addition, Hitchings *et al.* (1) have observed that 5-bromouracil, in the absence of thymine or folic acid, supported limited growth of *L. casei*. In view of evidence that thymine and its desoxyriboside are incorporated by different mechanisms in these microorganisms(3), studies have been made of the effect of 5-bromouracil on the growth of *S. faecalis* in a medium supplemented with either thymidine or thymine.

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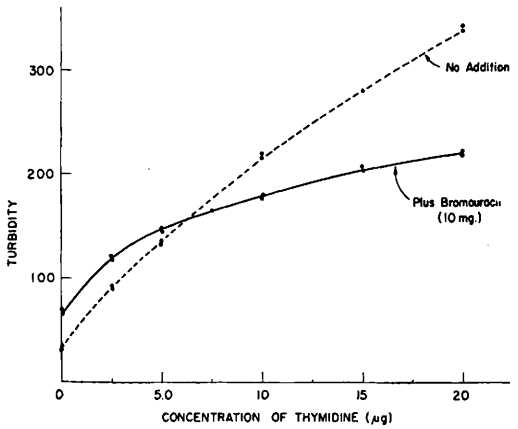


FIG. 1. The effect of 5-bromouracil on the growth of *S. faecalis* in a medium supplemented with thymidine.

*Experimental. Studies with growing culture.* The organism used in these studies was *Streptococcus faecalis* (ATCC 8043) which was grown in a standard basal medium(4). 5-Bromouracil‡ (10 mg) was placed in one of 2 series of assay tubes, to which increasing amounts of thymidine were added (final volume: 10 ml). The tubes were inoculated and then incubated for 22 hours at 37°C. Growth was measured as turbidity in a Klett-Summerson photoelectric colorimeter (Filter No. 66). The results are shown in Fig. 1. At this level, bromouracil significantly stimulated the growth of *S. faecalis* when thymidine was present in amounts of 5 μg or less per 10 ml of medium, but as the concentration of thymidine was increased, the inhibitory effect of bromouracil became evident; this was not abolished even by 300 μg of thymidine.

In a second type of experiment increasing quantities of 5-bromouracil were added to 2 series of tubes, one of which contained 10 μg of thymine and the other 20 μg of thymidine. They were inoculated and incubated at 37°C for 20 hours. The results are shown in Table I. In agreement with the investigations of Weygand *et al.*(2), 5-bromouracil was able completely to inhibit the growth of *S. faecalis* in the medium supplemented with thymine; however, when thymidine replaced thymine the pattern was changed remarkably, although definitely inhibitory, bromouracil depressed growth to only 50% of maximum, even at a concentration of 1%. There was no relationship between the method of addition of the halogenated pyrimidine to the medium and its effect on the growth of this organism. Bromouracil, when sterile-filtered and added aseptically to previously autoclaved media, or when autoclaved in the media, inhibited the growth of *S. faecalis* to the same extent.

*Studies with resting cells.* Medium (500 ml) supplemented with thymine (2.5 mg) was sterilized in one-liter Erlenmeyer flasks for 20 minutes at 15-lb pressure. After inoculation with *S. faecalis,* the flasks were incubated at 37°C for 20 hours. The cells were harvested by centrifugation and twice resuspended in saline and recentrifuged. Thymidine was incubated with the washed resting cell suspensions in the presence and absence of 5-bromouracil. The composition of the incubation mixtures (total volume, 1.0 ml) was as follows: thymidine, 1 μM; phosphate buffer, λ 0.5 M, 0.1 ml, pH 8.1; and wet bacterial cells, 50 mg, either in the presence or absence of 5-bromouracil, 25 μM. After an incubation period of 3 hours at 37°C the reaction was stopped by the addition of 0.1 ml 25% $HClO_4$(5). After cooling in an ice bath for 10 minutes the acidified suspensions were centrifuged in the cold and analyses for total desoxyribose by the Stumpf reaction(6), free pentose by the Nelson reaction as modified

TABLE I. Effect of 5-Bromouracil on Growth of *S. faecalis* in Media Supplemented with Thymine or Thymidine.*

| Conc. of 5-bromouracil (mg) | Growth | |
|---|---|---|
| | Thymine (10 μg) | Thymidine (20 μg) |
| 0 | 340 | 338 |
| 5 | 122 | 226 |
| 10 | 95 | 226 |
| 20 | 72 | 225 |
| 30 | — | 220 |
| 40 | — | 212 |
| 50 | 54 | 204 |
| 60 | — | 192 |
| 70 | — | 180 |
| 80 | — | 166 |
| 90 | — | 158 |
| 100 | — | 150 |

* Growth measured as turbidity in a Klett-Summerson colorimeter (filter #66).

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by Manson and Lampen(7), and unreacted thymidine by microbiological assay with *Leuconostoc citrovorum* (ATCC 8081) were performed.

Either in the presence or absence of bromouracil, thymidine as such completely disappeared. Although all desoxyribose was lost in the absence of the halogenated pyrimidine, in its presence the desoxypentose was retained completely and in a bound form. These results strongly suggest that the desoxyriboside of 5 -bromouracil had been formed in a manner similar to the conversion of 6-azathymine to the corresponding desoxyriboside(8).

*Discussion.* 5-Bromouracil in an otherwise unsupplemented medium not only supports the growth of *S. faecalis* to a limited extent, but in addition is incorporated into the nucleic acid of this organism(11). Therefore, an increase in the amount of growth when bromouracil was added to media which contained suboptimum concentrations of thymidine, was expected. However, it was not anticipated that a non-competitive inhibition of the growth of *S. faecalis* would be caused by the analog even in the presence of very high concentrations of the normal metabolite, thymidine.

It would appear, therefore, that there are at least 2 pathways for thymidine utilization, and that the major one is not inhibited by bromouracil. Under conditions where the supply of thymidine is minimal, the nucleoside may be shunted to this major pathway exclusively, and hence bromouracil not only does not exert any inhibition, but is also utilized to some extent for growth. The overall effect is one of an increase in growth beyond that observed with thymidine alone. When the thymidine concentration is increased beyond minimal levels, a second or minor pathway may be utilized. If the minor pathway involves a cleavage of thymidine to free thymine, then the inhibition produced by bromouracil would be understandable. Although *S. faecalis* possesses a nucleosidase which could catalyze this reaction(8), evidence has been presented previously which indicates that the cleavage of thymidine to free thymine, probably is not the major pathway for thymidine utilization in this microorganism(3).

Evidence suggesting the formation of the desoxyriboside of 5-bromouracil with resting cell suspensions of *S. faecalis* has been presented. It is conceivable that this compound also may be formed during conditions of ordinary growth. Normally, the higher the ratio of bromouracil to thymidine, the larger would be the amount of the unnatural desoxyriboside formed. However, in the growth experiments, when the ratio of thymidine to bromouracil was increased, not only was there no reversal of inhibition, but there appeared to be an increase in the degree of antagonism. This would appear to eliminate 5-bromouracil-desoxyriboside as the causative agent in the observed inhibition. The corresponding riboside of bromouracil, 5-bromouridine, has been reported reversibly to inhibit the growth of Neurospora (No. 1298) in a medium supplemented with uridine or cytidine(9). However, 5-chlorouridine(9) and 5-iodouridine (10) have no effect on the growth of *S. faecalis* in media supplemented with folic acid or thymine and, in addition, the iodinated derivative exerted no inhibition of growth in the presence of thymidine. Investigations of the effect of halogenated derivatives of the *desoxyribosides* on the growth of organisms which have a thymidine requirement are indicated.

*Summary.* 1. Whereas 5-bromouracil competitively inhibited the growth of *S. faecalis* in a medium supplemented with thymine, an incomplete, and non-competitive inhibition was observed in the presence of thymidine. 2. 5-Bromouracil prevented the disappearance of desoxyribose when resting *S. faecalis* cells are incubated with thymidine, and this is interpreted as signifying the formation of the desoxyriboside of 5-bromouracil. 3. Possible pathways of thymidine metabolism in *S. faecalis* are discussed.

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## Microbiological Activities of Iodoorotic Acid and Iodouridine.* (20953)

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In a previous paper it was shown that neither 5-iodoorotic acid-$I^{131}$ nor 5-iodouridine-$I^{131}$ is concentrated by the regenerating liver of the rat or by normal or certain neoplastic tissues of rats or mice(1). For several species of microorganisms, however, certain halogenated pyrimidines have been described as antimetabolites(2-4) or have exerted limited activity as growth factors(2). In fact, 5-bromouracil appears to be incorporated into the nucleic acids of *Streptococcus faecalis*(5), and to interfere markedly with the utilization not only of exogenous thymine, but also of a related compound formed endogenously in the presence of derivatives of folic acid(6). These results with bacteria suggested a reinvestigation of the effect of iodoorotic acid and iodouridine on the growth of certain microbial species.

*Experimental. 5-Iodoorotic acid-$I^{131}$ and 5-iodouridine-$I^{131}$.* These compounds were synthesized by previously published methods (1). *Determination of radioactivity.* The concentration of $I^{131}$ in the cells was determined with the aid of a well-type scintillation counter constructed by MacIntyre(7). Since $I^{131}$ emits highly penetrating gamma radiations, the cellular concentrations of $I^{131}$ could be determined directly without fractionation or isolation. *Apparent metabolic activity of iodoorotic acid.* Increasing amount of iodoorotic acid were added to 2 series of assay tubes (final volume: 10 ml); of these, each tube of one series was supplemented with 50 μg of orotic acid. The tubes were autoclaved at 120°C for 15 minutes, inoculated with *L. bulgaricus* 09, and incubated for 72 hours at 37°C. Growth was measured turbidimetrically in a Klett-Summerson photoelectric colorimeter using filter No. 66, or by titration with 0.1 N NaOH.

TABLE I. Effect of Iodoorotic Acid on Growth of *L. bulgaricus* 09.

| Iodoorotic acid, mg | 0.1 N acid/10 ml media* | |
|---|---|---|
| | No addition | Plus orotic acid (50 μg) |
| | ml | ml |
| 1.0 | 16.1 | 15.6 |
| .10 | 12.9 | 15.5 |
| .01 | 5.9 | 8.6 |
| 0 | 3.5 | 7.8 |

* Acid-production after 72 hr growth.

The *results,* presented in Table I, indicate that iodoorotic acid not only did not antagonize the growth of *L. bulgaricus* 09 in the presence of orotic acid, but, in the absence of the latter, enabled maximal growth to be attained.

*Activity of autoclaved versus sterile-filtered solutions of iodoorotic acid.* The growth of

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