UTILIZATION OF PYRIMIDINES AND PYRIMIDINE DEOXYNUCLEOSIDES BY *THERMOBACTERIUM ACIDOPHILUM* (*LACTOBACILLUS ACIDOPHILUS*)

SØREN LØVTRUP AND DAVID SHUGAR

Department of Histology, University of Göteborg, Göteborg, Sweden, and Institute of Biochemistry and Biophysics, Academy of Sciences, Warsaw, Poland

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ABSTRACT

LØVTRUP, SØREN (University of Göteborg, Sweden) AND DAVID SHUGAR. Utilization of pyrimidines and pyrimidine deoxynucleosides by *Thermobacterium acidophilum* (*Lactobacillus acidophilus*). J. Bacteriol. **82**:623–631. 1961.—The utilization of pyrimidine deoxynucleosides was investigated by means of deoxyribosides of unnatural pyrimidines, especially by halogen-substituted uracil derivatives. All investigated deoxyribosides could be used, except that of *N*-methylthymidine. It was concluded that this substance cannot be a substrate for the enzyme *trans*-*N*-deoxyribosylase, which has been shown to be active in the utilization of deoxyribosides in this microorganism. With uracil as the only pyrimidine source, the halogen-substituted deoxyuridines had a certain inhibitory effect on growth.

Contrary to previous findings, it was observed that normal growth occurs in the presence of thymine as the only pyrimidine source. The utilization of this substance is less efficient than that of uracil; a 1:10 dilution leads to a decrease in the extent of growth with the former, but not with the latter. From these results, complemented with experiments in which halogen-substituted uracil derivatives and the corresponding ribosides or deoxyribosides were used as inhibitors, it has been possible to account for most of the metabolic interconversions of pyrimidines in the investigated microorganism.

Thermobacterium acidophilum (*Lactobacillus acidophilus*) Orla Jensen strain R26 will grow only in the presence of deoxyribosides. For this reason the organism has been used in a microbiological assay for deoxyribonucleic acid (DNA) and its hydrolysis products (Hoff-Jørgensen, 1952, 1954). During work on the development of this method, Hoff-Jørgensen also had to establish the general nutritional requirements of the bacterium. Some other reports have been published concerning the pyrimidine requirements of this strain (Jeener and Jeener, 1952; Siedler, Nayder, and Schweigert, 1957; Løvtrup and Roos, 1957*a*).

Two aspects of pyrimidine metabolism have been investigated in the present work: the utilization of (i) deoxyribosides and (ii) free pyrimidines. Both problems have been attacked mainly by the supplementary use of a number of unnatural pyrimidines and pyrimidine deoxyribosides.

MATERIALS AND METHODS

The microbiological technique used in this study has been described previously (Løvtrup and Roos, 1957*a*), and additional modifications have also been published (Løvtrup and Roos, 1959). A further modification introduced here is the addition of guanine as the only purine source in the double strength basal medium. Unless otherwise stated, deoxyguanosine was used as the deoxyribose bond source. The criterion for utilization of added substances was growth, as measured turbidimetrically. All experiments reported here, in which an effect, enhancing or inhibiting, was observed, have been repeated several times, to establish the significance of the results.

Most of the compounds were obtained from commercial sources and, where considered necessary, checked by chromatography or absorption spectroscopy. *N*-Methylthymidine was prepared by methylation of thymidine with diazomethane, according to Miles (1957).

All the deoxyribosides and other inhibitors tested were added in a concentration corresponding to the highest deoxyriboside concentration in our standard curves, i.e., 4 mμM. In a few instances, the effect of a higher concentration has been investigated. In these cases the concentration 40 μg per ml double strength basal medium, the concentration of pyrimidines in the basal medium, was used for convenience.

RESULTS

Utilization of deoxyribosides. A number of deoxyribosides were tested with respect to their ability to support growth of our bacteria. Solutions of the substances listed in Table 1, and of the same concentration as those of deoxyribosides at the highest point on our standard curves (4 mμM), were prepared. The pyrimidine content was varied as indicated, and the concentration of the individual pyrimidines was 40 μg per ml of double strength basal medium.

It will be seen that the bacteria are quite unable to utilize the deoxyriboside bond in *N*-methylthymidine. The remaining compounds may all support growth, although there is a significant difference in efficiency between thymidine, which gives the highest, and 5-bromodeoxyuridine, which gives the lowest, growth. The results with the unnatural deoxyuridines make it possible to distinguish between two types of inhibition. When the results with deoxyuridine and its iodo derivative are compared, it is seen that growth proceeds to the same extent in the presence of either thymine plus uracil or thymine alone. The deoxyriboside bond of 5-iododeoxyuridine may thus be freely utilized. This does not hold for the fluoro and bromo derivatives, which under the same circumstances permit somewhat lower growth.

Another kind of inhibition is observed when the results with uracil as the only pyrimidine source are considered; in this case inhibition occurs, which is specific for uracil. This inhibition is seen to decrease as the atomic weight of the halogen substituent increases.

It has been observed that 5-bromouracil, a thymine analogue, may be incorporated into the DNA of bacteria in place of thymine. Once the unnatural DNA has been formed, further multiplication of the bacteria may be inhibited. We have investigated this question by repeated subcultivation of our bacteria on 5-bromodeoxyuridine. The first three subcultures gave growth responses corresponding to 233, 223, and 245, respectively, indicating no increased inhibition of the bacteria.

Utilization of pyrimidines. In a medium supplied with deoxyguanosine, various natural and unnatural pyrimidines were added, in two different concentrations. It appears (Table 2) that only three of the seven tested substances

TABLE 1. *Utilization of natural and unnatural deoxyribosides by Thermobacterium acidophilum (μg/ml of medium)*

	Thymine + uracil	Thymine	Uracil	No addition
N-Methylthymidine	32	40	37	25
Thymidine	398	379	384	27
5-Fluorodeoxyuridine	316	304	48	38
5-Bromodeoxyuridine	292	276	82	31
5-Iododeoxyuridine	361	334	122	30
Deoxyuridine	358	349	357	56

TABLE 2. *Utilization of pyrimidines by Thermobacterium acidophilum*

	Pyrimidine concn per ml of medium	
	40 μg	4 μg
Orotic acid	323	285
Thymine	316	95
Uracil	325	308
5-Bromouracil	53	37
Cytosine	44	39
5-Methylcytosine	33	36
5-Hydroxymethylcytosine	43	39

TABLE 3. *Effect of various combinations of pyrimidines on the growth of Thermobacterium acidophilum. Thymine in low concentration (4 μg/ml) in all cases*

5-Bromouracil	Cytosine	5-Methylcytosine	5-Hydroxymethylcytosine	Growth response
				μg/ml
+	−	−	−	64
−	+	−	−	128
−	−	+	−	83
−	−	−	+	93
+	+	−	−	69
+	−	+	−	65
+	−	−	+	57
−	+	+	−	141
−	+	−	+	123
−	−	+	+	110
+	+	+	−	87
+	+	−	+	76
+	−	+	+	76
−	+	+	+	155
+	+	+	+	79

may sustain normal growth, namely, orotic acid, thymine, and uracil. Of these, thymine is least efficient; growth is substantially decreased when the thymine concentration is decreased from 40 to 4 μg.

To test the possible effect of combining two or more of the inefficient pyrimidines, these were added (Table 3) to a medium containing thymine in the lower concentration (Table 2), which gave a low but significant growth response. The concentration of the other pyrimidines was 40 μg per ml of basal medium. Growth was inhibited in all cases where 5-bromouracil was added. Apart from this, it appears that none of the combinations will sustain full growth. Cytosine gives a significant stimulation, and a slight effect is apparently also obtained with the combination of the two other cytosine derivatives.

Unless a synthetic pathway exists, entirely different from what is at present known for other organisms, it must be concluded from the results in Table 2 that cytosine may be synthesized from both thymine and uracil. To estimate whether the synthesis of cytosine may limit growth, an experiment was performed in which cytosine was added to tubes containing one or two of the other pyrimidines. Thymidine, deoxyuridine, deoxycytidine, and deoxyguanosine were added in the respective experiments and two different concentrations of pyrimidines were used (Table 4).

These results will be dealt with in the Discussion; it should only be mentioned here that deoxycytidine is seen in some instances (thymine + uracil and uracil in low concentration, uracil in high concentration + cytosine) to give maximal growth. This does not agree with previous observations concerning the efficiency of different deoxyribosides (Løvtrup and Roos, 1959). It should be noted, however, that in the previous experiments the medium contained guanine, adenine, uracil, and cytidylic acid, but no thymine, all at the high concentration. It is likely that this difference suffices to explain the observed discrepancy.

TABLE 4. *Growth response of Thermobacterium acidophilum to various combinations of pyrimidines and deoxyribosides (μg/ml of medium)*

Concn per ml medium	Pyrimidine	Thymine + uracil + cytosine	Thymine + uracil	Thymine + cytosine	Thymine	Uracil + cytosine	Uracil	Cytosine	No addition
μg									
40	Thymidine	328	279	305	269	317	307	42	39
	Deoxyuridine	320	280	288	280	301	290	83	66
	Deoxycytidine	332	315	306	310	360	300	44	36
	Deoxyguanosine	314	283	278	278	295	296	35	27
4	Thymidine	332	315	114	99	333	291	42	37
	Deoxyuridine	340	353	145	135	331	283	73	69
	Deoxycytidine	395	399	136	125	373	333	39	40
	Deoxyguanosine	312	323	86	86	288	248	30	27

TABLE 5. *Comparison of the inhibitory effect of 5-fluoro- and 5-bromodeoxyuridine on the growth of Thermobacterium acidophilum (μg/ml)*

Concn per ml of medium	Pyrimidine	Thymine + uracil + cytosine	Thymine + uracil	Thymine + cytosine	Thymine	Uracil + cytosine	Uracil	Cytosine	No addition
μg									
40	5-Fluorodeoxyuridine	359	316	320	304	54	48	34	48
	5-Bromodeoxyuridine	319	306	300	276	149	82	42	31
4	5-Fluorodeoxyuridine	65	81	48	47	49	39	37	34
	5-Bromodeoxyuridine	167	168	73	61	94	80	38	30

Similar results were observed when 5-fluoro- and 5-bromodeoxyuridine were used (Table 5).

One difference to be noted was the inferior utilization of the deoxyriboside bond in the bromo derivative. In addition, specific uracil inhibition was observed with both substances. In this respect 5-fluorodeoxyuridine is seen to be the most potent inhibitor.

When an unnatural deoxyriboside is added, the deoxyribose moiety will be transferred to other bases, liberating the unnatural pyrimidine during the course of this reaction. The observed inhibitions might therefore equally well be due to the free base. Some experiments were designed to test this question. With either deoxyguanosine or mixtures of this with pyrimidine deoxynucleosides as the deoxyribose source, 5-bromouracil was added in two different concentrations, namely, that of the pyrimidine additions (40 μg per ml of medium), and that of the deoxyribose addition (4 mμM per ml or about 0.8 μg per ml of medium). The results are shown in Table 6.

A comparison of the effect of the inhibitor in low concentration with those of 5-bromodeoxyuridine (Table 5) shows directly that, as far as the specific uracil inhibition is concerned, the free base is much less potent than the deoxynucleoside. When the inhibitor concentration was raised, however, considerable inhibition was found, and this occurred even in the presence of thymine. A rather large effect of cytosine addition was observed, even in the presence of thymine, except when deoxycytidine was present. Such a distinct stimulation was not observed in the presence of thymine in any of the results reported above, and may thus be taken to indicate that 5-bromouracil may interfere with the synthesis of cytosine from thymine. With dexoyguanosine, and uracil in high concentrations and inhibitor in low concentration, growth was considerably stimulated by cytosine addition. A similar, but slighter effect was observed when thymidine was also present, but not when deoxycytidine was added.

With fluoro-substituted derivatives we have been able to compare not only base and deoxyriboside, but the riboside as well, with regard to their inhibitory ability. The results of these experiments, in which the inhibitors were added

TABLE 6. *Effect of 5-bromouracil on the growth of Thermobacterium acidophilum in the presence of various combinations of pyrimidines and deoxyribosides (μg/ml of medium)*

Pyrimidine concn per ml of medium	Substrate	5-Bromouracil concn per ml of medium	Thymine + uracil + cytosine	Thymine + uracil	Thymine + cytosine	Thymine	Uracil + cytosine	Uracil	Cytosine	No addition
μg										
40	Deoxyguanosine	40 μg	157	102	169	110	53	44	36	31
		4 mμM	329	280	335	286	283	142	36	24
	Deoxyguanosine (3/4) + thymidine (1/4)	40 μg	170	146	167	122	73	63	48	48
		4 mμM	288	259	308	265	266	212	36	33
	Deoxyguanosine (3/4) + deoxycytidine (1/4)	40 μg	116	121	110	117	35	57	39	43
		4 mμM	300	290	311	297	226	201	27	35
	Deoxyguanosine (1/2) + thymidine (1/4) + deoxycytidine (1/4)	40 μg	148	159	137	151	55	68	45	50
		4 mμM	308	315	313	340	270	278	29	32
4	Deoxyguanosine	40 μg	49	40	58	53	58	49	44	37
		4 mμM	252	180	73	61	73	61	31	26
	Deoxyguanosine (3/4) + thymidine (1/4)	40 μg	63	68	57	64	60	49	53	46
		4 mμM	260	219	75	72	209	191	27	33
	Deoxyguanosine (3/4) + deoxycytidine (1/4)	40 μg	44	50	40	52	43	45	43	47
		4 mμM	216	211	123	97	146	149	25	30
	Deoxyguanosine (1/2) + thymidine (1/4) + deoxycytidine (1/4)	40 μg	51	78	55	68	54	65	41	46
		4 mμM	260	272	79	82	185	217	27	29

TABLE 7. *Comparison of the effects of a free pyrimidine base and the corresponding riboside on the growth of Thermobacterium acidophilum (μg/ml of medium)*

Pyrimidine concn per ml of medium	Substrate	Thymine + uracil + cytosine	Thymine + uracil	Thymine + cytosine	Thymine	Uracil + cytosine	Uracil	Cytosine	No addition
μg									
40	5-Fluorouracil	348	260	298	291	56	45	47	47
	5-Fluorouridine	321	286	327	302	105	50	48	34
4	5-Fluorouracil	101	103	58	51	56	48	48	41
	5-Fluorouridine	265	212	89	87	67	70	42	39

TABLE 8. *Effect of 6-methyluracil on the growth of Thermobacterium acidophilum (μg/ml of medium)*

Pyrimidine concn per ml of medium	6-Methyluracil concn per ml of medium	Thymine + uracil + cytosine	Thymine + uracil	Thymine + cytosine	Thymine	Uracil + cytosine	Uracil	Cytosine	No addition
μg									
40	40 μg	322	227	330	228	313	279	31	28
	4 mμM	343	297	351	283	328	262	37	24
4	40 μg	282	265	82	73	246	195	27	24
	4 mμM	330	254	84	81	257	170	29	23

only in the low concentration, are shown in Table 7.

A comparison with Table 6 shows that 5-fluorouracil is a more potent inhibitor than the corresponding bromo derivative, which agrees with the findings for the deoxyribosides reported in Table 5. Both the free base and the nucleoside give nearly complete inhibition with uracil, hence it is rather difficult to decide which is the more potent inhibitor. Only with thymine + uracil in low concentration was a difference observed, showing that the deoxyriboside has the strongest effect. This result agrees with that found for the corresponding bromo compounds. In contrast to this, 5-fluorouridine was seen to exert a much slighter inhibiting effect than the other two substances.

The influence of the thymine isomer, 6-methyluracil, was also investigated (Table 8). This compound is seen to have little influence on the growth of the bacteria, and the slight inhibition observed affects the utilization of both thymine and uracil for synthesis of cytosine. The former effect is seen most clearly in the experiments with high concentration of both inhibitor and pyrimidines (thymine + uracil and thymine).

TABLE 9. *Influence of aminopterin on the growth of Thermobacterium acidophilum*

	Aminopterin (1.6 μg per ml of medium)			
	+ Cytosine		− Cytosine	
	+	−	+	−
Thymine	129	150	353	337
Uracil	45	50	356	312
5-Methylcytosine	33	25	33	23
Thymine + uracil	125	127	343	340
Thymine + 5-methylcytosine	142	93	301	210
Uracil + 5-methylcytosine	79	59	296	232

The synthesis of thymine from uracil is presumed to involve a methylation in which tetrahydrofolic acid takes part. To test whether this reaction occurs also in our microorganism, some experiments were performed (Table 9) with aminopterin, the folic acid inhibitor. The pyrimidine concentration was 40 μg per ml of basal medium.

Growth on uracil was suppressed by aminopterin, but this inhibition was partly

relieved by the addition of thymine. Even the addition of 5-methylcytosine had a slightly stimulating effect. Aminopterin was also seen to exert an inhibitory influence on the conversion of both thymine and uracil to cytosine. The influence on growth of 5,6-dimethyluracil was also investigated. No effect whatsoever was observed.

DISCUSSION

Utilization of deoxyribosides. Recent work has demonstrated that in mammalian cells deoxyribose compounds are formed from the corresponding ribose compounds, on the nucleotide level (review by Reichard, 1960). Such a metabolic link between ribonucleic acid (RNA) and DNA is evidently absent in this microorganism. As shown by Hoff-Jørgensen (1952), only deoxynucleotides or deoxynucleosides will support the growth of *T. acidophilum*. Neither deoxyribose nor deoxyribose 1-phosphate could replace these substances.

The added bound deoxyribose must therefore be distributed between the various bases in DNA in the correct pattern. The mechanism of this distribution in the present microorganism and other lactic acid bacteria was investigated by MacNutt (1952). He demonstrated an enzyme, deoxyribose *trans-N*-glycosylase, or *trans-N*-deoxyribosylase (Roush and Betz, 1958), which catalyzes the transfer of the deoxyribosyl group from one purine or pyrimidine base to another. The specificity of this enzyme was found to be rather low, since its substrates include various bases which normally do not occur in deoxyribosides. The activity of the enzyme may account for the observed utilization of unnatural deoxyribosides, although there may be a somewhat less efficient utilization of the deoxyriboside linkage. Since *N*-methylthymidine cannot support growth, it appears that this substance cannot be a substrate for the enzyme.

The utilization of uracil, as opposed to that of thymine, was more strongly inhibited by the halogen-substituted deoxyribosides than by the corresponding free bases. The effect is thus presumably exerted at the deoxynucleoside level, i.e., the exchange between uracil and the halogen derivative is inhibited to a much greater degree than the corresponding exchange with thymine. This finding seems to show that the added pyrimidine source is not transformed to the remaining pyrimidines at the ribonucleoside or nucleotide level, the bases thus formed being liberated and incorporated into deoxynucleosides by the enzymatic reaction suggested above. The most likely mechanism for utilization of the desoxyriboside is therefore that a *trans-N*-glycosylation occurs with the pyrimidines added, and that transformation to other pyrimidine deoxynucleosides occurs on the deoxyriboside level.

The slight inhibition of growth by 5-fluoro- and 5-bromodeoxyuridine in the presence of thymine without cytosine addition is of the same order as that observed with the free bases, and may therefore be an effect of the free halogen pyrimidines liberated by the *trans-N*-glycosylation.

It has been reported by Dunn and Smith (1957) and by Zamenhof, Rich, and DeGiovanni (1958) that 5-bromouracil may be incorporated into bacterial DNA. By this incorporation the bacteria may lose their ability to multiply. The subcultivation experiments which we have carried out show that no such loss occurs.

Metabolism of pyrimidines. The original medium employed by Hoff-Jørgensen (1952, 1954) included adenine, guanine, thymine, and cytidylic acid. In later work it was found by Jeener and Jeener (1952) that even uracil had to be supplied to achieve bacterial growth. The same result was reported by Siedler et al. (1957) and by Løvtrup and Roos (1957*a*). It was found in the latter work that omission of guanine and uracil (in the absence of thymine) led to inhibition of growth, whereas the absence of adenine, cytidylic acid (cytosine), and thymine (in the presence of uracil) had no serious effect. A systematic study of the pyrimidine requirements was not carried out at that time. The present results show, however, that the bacteria may grow in the presence of thymine as the only pyrimidine source. The reason for the repeated observation of the necessity of uracil is obscure. It remains undisputed, though, that in a medium supplied with uracil and cytosine, addition of thymine generally has a very slightly stimulating effect; in many cases it may even depress growth to some extent.

It is thus obvious from our results that thymine and uracil are interconvertible, and that they both may give rise to cytosine. (It is assumed in

the present discussion that no pyrimidines other than these three are present in the bacteria.)

Before discussing the metabolism of pyrimidines, the consequences of the fact that these substances are present in both DNA and RNA may be considered. As mentioned previously, there is reason to believe that the synthetic pathways of the two nucleic acids are completely separated. The transformations of DNA pyrimidines which must occur probably take place at the nucleoside level (Reichard, 1960). Thus, if uracil is added, the deoxyuridine formed will give rise to thymidine and deoxycytidine; if thymine is added, only the formation of deoxycytidine is needed. With RNA, the added pyrimidine(s) is presumed to be transferred to ribosides or ribotides, at which level similar transformations may occur (uracil → cytosine or thymine → uracil + cytosine).

This circumstance obviously obscures the interpretation of the inhibition experiments carried out by us, because a limited supply of RNA pyrimidines may inhibit growth just as well as the supply of deoxyriboside (Jeener and Jeener, 1952; Siedler et al., 1957). This fact must be taken into account in the interpretation of our results.

The separation of DNA and RNA metabolism is indicated by the experiments with uracil (Table 4). From the high concentration experiments (without cytosine), it was seen that growth is independent of the added deoxyriboside. Addition of cytosine had no significant effect on growth, except with deoxycytidine. It thus appears that in the presence of cytosine, the synthesis of deoxycytidine limits growth; and in the presence of deoxycytidine, the supply of cytosine is the limiting factor. This shows that added cytosine is not easily transformed to deoxycytidine, i.e., the metabolism of cytosine in RNA and DNA occurs along separate pathways.

Another fact which emerged from the results shown in Table 4 was the much more efficient utilization of uracil as compared to thymine. When the lower concentration of pyrimidine was used, growth was significantly reduced in the presence of thymine, but not when uracil was added.

Certain suggestions as to the possible pathways of pyrimidine conversion may be obtained from these experiments, particularly from those with low pyrimidine concentration (Table 4). First of all, irrespective of the source of deoxyriboside, thymine + cytosine always gave much lower growth than uracil + cytosine. This must mean that thymine → uracil occurs, but limits the growth. It is not possible to demonstrate that the conversion uracil → thymine is limiting. The results with thymidine and thymine show that thymine → cytosine may be limiting, and all the experiments with uracil show that uracil → cytosine limits growth. Particularly, the results with addition of deoxycytosine show that it is at the RNA level that this limitation occurs.

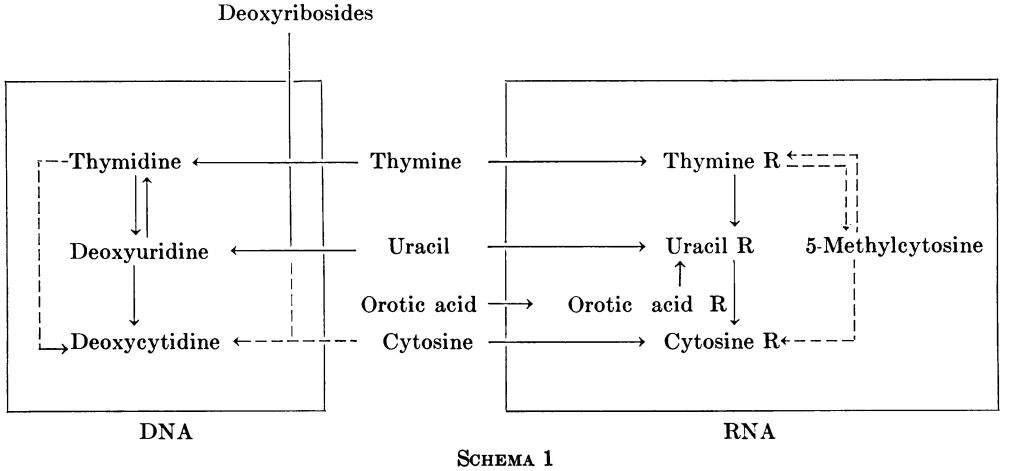
It is not possible to decide with certainty whether the transformation thymine → cytosine occurs via uracil, but the results do suggest an intermediate position of uracil. When the thymine requirements are fulfilled (thymine + uracil), deoxyuridine is seen to be a much more efficient source of deoxycytidine than is thymidine. In the absence of thymine (with or without cytosine), they are equally efficient, but here deoxyuridine must supply two other deoxyribosides, whereas thymidine has to take part only in the production of deoxycytidine. Even at the DNA level there is much to be said in favor of the uracil derivative taking an intermediate position.

The conversion cytosine → uracil is not possible, because if it occurred to only a very slight extent growth would occur in the presence of cytosine and a deoxyriboside. Such growth occurs only with deoxyuridine, demonstrating the extremely efficient utilization of this pyrimidine.

There remain many unanswered questions about the pyrimidine metabolism of *T. acidophilum*, but the main outline of the possible conversions is probably as represented in Schema 1. (The symbol R indicates that the pyrimidines react either as ribosides or ribotides. The broken lines represent uncertain reactions.)

With halogenated deoxyuridines, a specific inhibition of uracil utilization was observed. The combined results of Tables 1 and 5 showed that this inhibition increased with decreasing molecular weight. A similar regularity was not observed when the chloro, bromo, and iodo derivatives act as thymine inhibitors in thymineless strains of *Escherichia coli* (Zamenhof et al., 1958; Dunn and Smith, 1957).

As the halogenated bases are liberated by



SCHEMA 1

the action of *trans-N*-deoxyribosylase, some experiments were performed to test the action of 5-bromouracil. It was found that the free pyrimidine was less active than 5-bromodeoxyuridine. The inhibitory effect was antagonistic mainly to uracil, but with high inhibitor concentration even thymine utilization was affected. In the experiments we added not only deoxyguanosine, but also three mixtures of deoxyribosides. The results of these experiments give some information about the reactions which were inhibited. In the experiments with low uracil and low 5-bromouracil concentrations, it was seen that the synthesis of thymidine, particularly, was affected. When this substance was added, a substantial increase in growth was achieved. Addition of cytosine had no effect, and even the addition of deoxycytidine gave only very slight stimulation.

With the fluoro derivatives a comparison was made of the activity of both the free pyrimidine and the riboside with that of the deoxyriboside. As with the bromo derivatives, the deoxynucleoside was a stronger inhibitor than the base, whereas the riboside was less efficient. This latter observation indicates that the free base is only partially liberated from the riboside, or not at all. The inhibition by the deoxyribosides, over and above that of the free pyrimidines, must presumably hit the DNA synthesis proper. This effect cannot, however, be a mere inhibition of the utilization of the deoxyribose linkage in the *trans-N*-glycosylation reaction, since 5-fluorodeoxyuridine, which was the strongest specific inhibitor, was better utilized in the enzymatic deoxyribose transfer.

In our experiments with 6-methyluracil, we found this substance to possess very little inhibitory action. The effect seemed to be concerned particularly with synthesis of cytosine, from both thymine and uracil.

Friedkin and Kornberg (1957) have shown that the transformation uracil → thymine in *E. coli* occurs at the deoxyriboside level, in a tetrahydrofolic acid-dependent reaction. Folic acid is a necessary component of the medium for *T. acidophilum* (Hoff-Jørgensen, 1952; Løvtrup and Roos, 1957*b*). In the presence of uracil alone, growth was completely blocked by the folic acid antimetabolite, aminopterin. This inhibition was partly relieved by thymine, showing that among the reactions influenced by aminopterin must be the formation of thymine. Also, the addition of 5-methylcytosine counteracted, to a very slight extent, the inhibition by aminopterin. This suggests the possibility that 5-methylcytosine is an intermediate between thymine and cytosine, if, as is not quite certain from our results, there is a pathway between these two substances not involving uracil.

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