TABLE I. Effect of Dog Blood Collected Daily Following Irradiation on Platelet-Poor Hemophilic Plasma and Whole Hemophilic Blood.

Days after irradiation	Test series 1: .5 ml irradiated dog whole blood* .7 ml platelet-poor hemophilic plasma†		Test series 2: .5 ml irradiated dog whole blood* 1.2 ml hemophilic whole blood*‡	
	% prothrombin utilized in 1 hr	No. platelets per mm³ mixture	% prothrombin utilized in 1 hr	No. platelets per mm³ mixture
2	90	243000	90	432000
3	90	240000	82	404000
4	85	161000	90	356000
5	81	138000	90	308000
6	79	61300	90	302000
7	17	12750	90	204000
8	28	2300	90	198000
9	18	1275	90	218000

* Whole blood freshly drawn, no anticoagulant, immediate mixing. Irradiated dog same as in Fig. 1.

† Prepared by centrifugation at 5°C at about 14000 g for 2 hr, silicone technic, no anticoagulant.

‡ Equivalent to .7 ml plasma.

tivity of dogs subjected to total body irradiation remained at normal levels. 2. Whole hemophilic blood with a full complement of platelets accelerated the clotting of irradiated dog's blood, while platelet-poor hemophilic plasma did not.

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Studies on Purine and Pyrimidine Requirements of a Strain of *Streptococcus faecalis*. (19199)

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In an earlier paper(1) it was reported that a strain of *Streptococcus faecalis*, subsequently designated *S. faecalis* AB, required uridine or other pyrimidine nucleosides and nucleotides for optimum early growth. In that study, the authors used a basal medium containing free purine and pyrimidine bases to obtain maximum growth response to uridine. Snell *et al.*(2-4) reported that purine and pyrimidine bases had no stimulatory effect on the growth of *S. faecalis*, while Barton-Wright (5) and Luckey *et al.*(6), without discussion

of specific requirements, included these compounds in a basal medium for microbiological assays with this organism.

In order to determine whether the inclusion of uridine in the medium modified the utilization of other pyrimidines or purines by *S. faecalis* AB, a more detailed study of purine and pyrimidine requirements was undertaken.

Experimental. The materials and methods used are similar to those described previously (1) except that all purines and pyrimidines were omitted from the basal medium. Individual nucleic acid derivatives* were added to the culture tubes separately and with uridine in order to compare growth responses in each case. Supplements were assayed at five levels ranging between 0.7 x 10^{-5} M and 7.4 x 10^{-5} M. Since no inhibition of growth was noted with more than the optimum quantity of supplement in the basal medium, the concentration selected for the presentation of comparative data is that (3.7 x 10^{-5} M) which produced the maximum response with the least active compound. In a preliminary analysis and purification by methods of Cohn(7) and Carter(8), it was found that only guanine and guanylic acid showed a difference in microbiological response between the commercial and purified preparations. Therefore, with the exception of guanine and guanylic acid, commercial products were used in subsequent experiments without further purification. Growth in triplicate tubes was determined turbidimetrically and the average reading recorded at incubation times which showed maximum differences in growth rates, usually 16 and 24 hours at 30°C.

Results. The data obtained from the addition of individual purines and pyrimidines to the synthetic basal medium are shown in Table I. Of the compounds tested, none of the purine bases or their ribosides and ribotides gave a significant increase in growth as compared to the basal medium. Of the pyrimidine bases tested, only orotic acid stimulated growth while all of the pyrimidine ribosides and ribotides stimulated growth above that obtained with the basal medium.

TABLE I. Comparative Growth Responses of *S. faecalis* AB to Purines, Pyrimidines and Related Compounds. 23 hr incubation at 30°C.

Supplement (3.7×10^{-5} molar)	Growth: optical density × 100
None	11.5
Adenine	11.4
Guanine	12.1
Adenine + guanine	13.1
Hypoxanthine	10.3
Thymine	14
Cytosine	12.6
Orotic acid	21
Uracil	13.2
Uracil + adenine + guanine	12.9
Uracil + xanthine + cytosine	12
Xanthine	12.1
Adenosine	11.1
Guanosine	13.8
Uridine	21.4
Cytidine	19.8
Thymidine	13.5
Adenylic acid	11.2
Guanylic acid	12.2
Uridylic acid	22
Cytidylic acid	21.2

TABLE II. Growth Response of *S. faecalis* AB to Combinations of Purine and Pyrimidine in Presence of Uridine. Incubation at 30°C.

Supplement* (3.7×10^{-5} molar)	Growth: optical density × 100 16 hr	24 hr
None	1.2	12.2
Uridine	5.2	37.4
" + cytosine	4.9	36.2
" + thymine	4	35.7
" + uracil	5.3	36
" + " + cytosine + xanthine	12.5	36.1
Uridine + uracil + cytosine + xanthine + adenine + guanine	16.2	36.2
Uridine + adenine	16.2	39.2
" + guanine	16.1	38
" + hypoxanthine	18.2	39.5
" + xanthine	14.3	39.4
" + adenosine	16	37.4
" + guanosine	15.8	38.2
" + adenylic acid	12.2	39.2
" + guanylic acid	13.9	39.6

* Uridine added at a level of 2 μg per tube (approximately 8×10^{-7} molar).

The growth responses obtained with uridine in combination with purines and pyrimidine bases are listed in Table II. It is apparent during early growth (16 hours at 30°C) that all the purine compounds tested show a synergistic action in the presence of uridine and increase growth significantly above that ob-

* Cytosine supplied by Dougherty Chemicals, orotic acid supplied by Sharp & Dohme; all others supplied by Schwarz Laboratories.

TABLE III. Comparative Growth Responses of *S. faecalis* AB to Varying Concentrations of Uridine and Orotic Acid. 16 hr incubation at 30°C.

Supplement	μg/tube	Growth: optical density × 100
Uridine	2	16.2
"	1	12.7
"	.2	4.4
Orotic acid	10	16.4
" "	5	11.8
" "	1	4.5

tained with uridine alone. No synergism was observed with the pyrimidine bases tested.

Orotic acid can replace uridine although it is less active than uridine as shown in Table III. The amount of orotic acid required for equivalent growth is approximately 8-fold greater than uridine on a molal basis.

Discussion. Since *S. faecalis* AB maintains approximately 30% maximum growth at 24-hour incubation when repeatedly subcultured in a synthetic medium containing no purines or pyrimidines, it appears that the organism can synthesize these compounds at limiting rates.

It has been demonstrated that pyrimidine ribosides and ribotides increase the rate of growth of the test organism, while the free bases with the exception of orotic acid, are not utilized for growth. It has also been shown that purines further increase the rate of growth in the presence of uridine. This synergism is contrary to the findings of Loring and Pierce(9) who observed that the growth of a mutant strain of *Neurospora* requiring uridine or cytidine was inhibited by a molal equivalent quantity of adenosine.

Accepting the general view that purines and pyrimidines are incorporated into nucleic acid by separate metabolic pathways(10-12), the synergism between purines and pyrimidine ribosides or ribotides suggests that growth is initially limited by the slower rate of uridine synthesis, and that the increased growth rate in the presence of adequate uridine again becomes limited by inadequate synthesis of purines.

Hammarsten and coworkers(13,14) and Mitchell and Houlahan(15,16) have postulated alternative pathways of pyrimidine synthesis. Using relative activity or utilization of a metabolite as a basis for postulating the sequence of intermediates in biological synthesis, the data presented here indicate that orotic acid is a precursor of uridine. Similarly, the observation that uridine is most readily utilized(1) suggests that it is the immediate precursor for nucleic acid pyrimidines.

Paege and Schlenk(17) have discussed possible reaction schemes involving orotic acid in pyrimidine metabolism. They were unable to demonstrate either direct decarboxylation of orotic acid to uracil or the formation of orotic acid riboside by enzymes of *Escherichia coli*. In our work with *S. faecalis* AB, it appears that orotic acid (or its riboside) is the probable intermediate decarboxylated since uracil cannot replace either orotic acid or uridine. A recent publication by Michelson *et al.*(18) reporting the isolation of orotic acid riboside from *Neurospora* gives further support to the theory that the biosynthesis of uridine, in some species at least, does not involve a direct coupling of ribose to uracil.

Summary. (1) A study was made of the purine and pyrimidine requirements for maximum rate of growth of a strain of *Streptococcus faecalis*. (2) Orotic acid, though less active, will replace uridine in promoting optimum early growth. (3) All of the purine compounds tested showed no growth stimulation *per se*, but provided a synergistic increase in growth rate in the presence of uridine. (4) The implications of these results for pyrimidine metabolism are discussed.

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Lack of Effect of Cortisone on Inhibitory Action of Antigonadotropic Sera. (19200)

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Numerous clinical and laboratory reports have indicated that cortisone and pituitary adrenocorticotropin (ACTH) may affect normal immune responses by altering the normal interaction between antigen and antibody(1-4).

The formation of an antigonadotropic substance in the serum of patients and laboratory animals after prolonged injection of pituitary gonadotropic extracts has been extensively studied(5). The exact relationship between this antigonadotropic substance and other types of antibodies is as yet obscure(6). In view of the recent observations suggesting an alteration in antigen-antibody reaction by cortisone, we have tested the effect of this compound upon the inhibitory properties of potent antigonadotropic sera.

Several batches of a concentrate of hog pituitary gonadotropin, prepared according to the method of Meyer and McShan(7), were employed as antigen. Young female sheep were injected subcutaneously daily with 200 mg of this powdered antigen in saline suspension for 700 days. Serum was prepared in the usual manner and stored in the frozen state until used.†

The same pituitary preparations used as antigen were also employed in testing the inhibitory potency of the sheep serum. For this purpose, weanling female rats of the Holtzman strain were given subcutaneously 3.3 or 5 mg of the antigen in 0.5 cc water daily for 3 days. A saline suspension of cortisone acetate (0.25 cc) was administered subcutaneously daily for 5 to 11 days beginning 2 to 8 days before the administration of the antigen or antiserum. The antisera were administered subcutaneously daily at a dosage of 0.5 cc or 1 cc as indicated in Table I. All animals were autopsied 24 hours after the last injection.

The data in Table I show that both sheep sera employed were highly effective in suppressing the gonadotropic response in the test rats. The addition of cortisone, even when administered for as much as 8 days prior to the antisera did not reduce the inhibitory effect of the antigonadotropic sera. Thus, there appeared no interference with expected interaction between antigen and antiserum as suggested by other studies.

Summary. Cortisone administration did not interfere with the antigonadotropic effectiveness in the rat of inhibitory sheep sera

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