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Influence of Certain Purines, Pyrimidines, and Pterins on Synthesis of "Folic Acid" by *Aerobacter aerogenes*.

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Snell and Peterson[1,2] reported that *L. casei* requires for growth a hitherto unrecognized nutrilite which they termed the *Lactobacillus casei* eluate factor. They noted that the material possessed many of the properties expected of a naturally occurring purine. Subsequent papers from other laboratories have corroborated the purine-like nature of the material. Thus Stokstad[3] found that a combination of guanine and thymine possessed some growth-promoting property in lieu of concentrates of the eluate factor. Mitchell, Snell, and Williams[4] found that *Strep. lactis R* requires an essential growth factor, concentrates of which would likewise promote growth of *L. casei*. The factor was concentrated to a state approaching purity and was named "folic acid." Thymine and uric acid each had some slight "folic acid" activity.[5] Recent evidence indicates that there are several naturally occurring materials which vary in their effectiveness as growth factors for *L. casei* and *Strep. lactis*.[6,7]

In experiments with surviving rat liver it has been shown that following the incubation of rat liver with xanthopterin more "folic acid" is found on microbiological assay of the digestion mixture than is present in a similar amount of rat liver alone.[8,9] This finding appears to indicate that under these conditions xanthopterin in some manner influences the synthesis, destruction, or other reactions of "folic acid." Conceivably xanthopterin or some closely related derivative either constitutes one moiety of the "folic acid" molecule, or, by virtue of structural similarity, interferes with "folic acid" metabolism. Totter and Day[10,11] have also provided evidence for a relationship between "folic acid" and xanthopterin.

In an attempt to obtain further information on the structure of "folic acid," the influence of various purines, pyrimidines, and pterins on the synthesis of "folic acid" by *Aerobacter aerogenes* has been investigated. This organism was found[12] to produce large amounts of "folic acid," when grown aerobically in a medium of glucose, hydrolyzed vitamin-free casein, and inorganic salts.

The media used were prepared as follows:

	A	B	C
Glucose	4 g	4 g	4 g
Salts A [13]	1 ml	1 ml	1 ml
Salts B [13]	1 "	1 "	1 "
Salts 1 [14]	0.2 "	0.2 "	0.2 "
Salts 2 [14]	0.2 "	0.2 "	0.2 "
Hydrolyzed casein (SMA Corp.)	0.2 "	0.8 "	2.0 "
Water to	200 "	200 "	200 "

1 Snell, E. E. and Peterson, W. H., *J. Biol. Chem.*, 1939, **128**, xciv.

2 Snell, E. E., and Peterson, W. H., *J. Bact.*, 1940, **39**, 273.

3 Stokstad, E. L. R., *J. Biol. Chem.*, 1941, **139**, 475.

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5 Mitchell, H. K., and Snell, E. E., *The University of Texas Publication*, 1941, **4137**, 36.

6 Keresztesy, J. C., Rickes, E. L., and Stokes, J. L., *Science*, 1943, **97**, 465.

7 Stokstad, E. L. R., *J. Biol. Chem.*, 1943, **149**, 573.

8 Wright, L. D., and Welch, A. D., *Am. J. Med. Sci.*, 1943, **206**, 128.

9 Wright, L. D., and Welch, A. D., *Science*, 1943, **98**, 179.

10 Totter, J. R., Shukers, C. F., Kolson, J., Mims, V., and Day, P. L., *Fed. Pro.*, 1943, **2**, 72.

11 Totter, J. R., and Day, P. L., *J. Biol. Chem.*, 1943, **147**, 257.

12 Thompson, R. C., *The University of Texas Publication*, 1942, **4237**, 87.

13 Snell, E. E., and Wright, L. D., *J. Biol. Chem.*, 1941, **139**, 675.

14 Williams, R. J., and Saunders, D. H., *Biochem. J.*, 1934, **28**, 1887.

TABLE I.

Influence of Certain Purines, Pyrimidines, and Pterins on Synthesis of "Folic Acid" by *Aerobacter aerogenes*. *Strept. lactis R* was used as the assay organism.

Exp. No.	Medium	Test material	Turbidity	"Folic acid," mγ/ml	Biotin, μγ/ml
1	A	None	112	3.5	—
		5γ xanthopterin	113	2.5	—
		10γ "	119	2.2	—
		20γ "	112	1.5	—
		40γ "	117	1.5	—
2	A	None	128	6.3	—
		37.5γ xanthopterin	129	3.7	—
		75.0γ "	129	3.7	—
		187.5γ "	129	3.7	—
3	A	None	116	4.0	54
		37.5γ xanthopterin	119	2.0	48
		75.0γ "	117	1.8	53
		187.5γ "	113	2.0	45
		375.0γ "	119	2.2	58
4	A	None	121	2.5	19
		100γ xanthopterin	121	1.0	19
		100γ adenine, cytosine, guanine, uracil, or xanthine	120-125	2.5	15-18
5	A	None	132	4.0	—
		100γ xanthopterin	135	1.8	—
		100γ leucopterin, iso-xanthopterin, or thymine	131-134	4.2	—
6	A	None	118	5.3	—
		100γ xanthopterin	120	2.0	—
		100γ thymine, uric acid, hypoxanthine, or allantoin	116-124	5.0-6.0	—
7	A	None	150	4.0	—
		100γ xanthopterin	151	2.5	—
	B	None	214	8.8	—
		100γ "	212	2.7	—
	C	None	248	12.7	—
		100γ "	246	4.7	—
8	C	None	212	8.8	22
		100γ xanthopterin	240	3.0	20
		100γ leucopterin, iso-xanthopterin, thymine, adenine, cytosine, guanine, uracil, xanthine, uric acid, hypoxanthine, or allantoin	216-248	9.2-11.7	18-22

The pH was adjusted to neutrality before use. Medium C is that used by Thompson.[12] Medium C permits luxuriant growth of *A. aerogenes*, medium B permits good growth, while only moderate growth results with medium A because of the small amount of nitrogen it contains. The cultures for microbiological assay were grown in 125 ml Erlenmeyer flasks. The materials to be tested were added in 10 ml of water. Of the medium (double strength), 10 ml were used and the flasks sterilized by autoclaving for 15 minutes at 15 lb pressure. One ml of a 24-hour culture of *A. aerogenes* in medium A was used for inoculation of the flasks. The cultures were incubated at 37°C for 8-24 hours, depending on the rate of growth of the organisms. After incubation the cultures were autoclaved, the bacterial density determined turbidimetrically, and the "folic acid," and occasionally the biotin, content of the combined cells and medium determined microbiologically. Enzymatic digestion of the cultures to liberate any bound "folic acid" or biotin was not employed since

Thompson has shown[12] that when *A. aerogenes* is grown aerobically most of the "folic acid" and biotin produced appears in an uncombined form in the medium and is not retained within the cells.

Bacterial density was determined in the Klett-Summerson photoelectric colorimeter. A 540 μ filter was used. The instrument was adjusted to read 0 against distilled water. Uninoculated medium read about 40. "Folic acid" was determined either with *Streptococcus lactis R* as the test organism according to the method described by Mitchell and Snell[5] or with *Lactobacillus casei* ϵ as the test organism, using the method of Landy and Dicken.[15] A concentrate of "folic acid" (about 200 Snell-Peterson units of "folic acid" per milligram) kindly furnished by Dr. E. L. R. Stokstad served as the "folic acid" standard. The standard has now been assayed, with the two organisms, against a sample of a crystalline folic acid from liver, kindly furnished by Dr. E. L. R. Stokstad, and the results have been expressed in terms of millimicrograms (10^{-9} g) of liver "folic acid" per ml of combined cells and medium.

Biotin was determined by the method of Landy and Dicken[15] using *L. casei*, or by the method of Snell and Wright[13] using *L. arabinosus*.

The xanthopterin, leucopterin, and isoxanthopterin used were kindly prepared by Drs. A. H. Land and J. M. Sprague of these laboratories, according to published methods.[16,17,18] Dr. F. S. Daft of the National Institute of Health kindly supplied the sample of thymine used. The other materials tested were commercial products.

Table I presents the results obtained.

In its effect xanthopterin was unique among the compounds tested. Although the addition of xanthopterin to cultures of *aerogenes* was without effect on the growth of the organism, or on the amount of biotin produced, the amount of "folic acid" found was invariably decreased significantly. Although the results were qualitatively similar whether performed with *S. lactis* or *L. casei*, higher values for "folic acid" content were obtained with the latter test organism. This probably indicates that the "folic acid" produced by *A. aerogenes* is more active for *L. casei* than for *S. lactis*. (Stokstad[7] found that a folic acid isolated from yeast was about twice as active for *L. casei* as for *S. lactis*.) Such an effect with xanthopterin was also observed both in the presence of rapid luxuriant growth (medium C) and in the presence of slow moderate growth (medium A). Other experiments, not reported in detail here, have shown that the xanthopterin influenced the amount of "folic acid" formed by *A. aerogenes* and that it did not inhibit the growth or acid production of the assay organisms. Derivatives as closely related to xanthopterin as isoxanthopterin (2-amino-6,9-dioxypteridin) or leucopterin (2-amino-6,8,9-trioxypteridin) were without effect on the "folic acid" production of *A. aerogenes*. None of the other purines or pyrimidines tested (Table I) had any effect on the growth of *A. aerogenes* or on its "folic acid" or biotin production.

By independent methods it has been concluded that xanthopterin, or a material closely related to it is similar in structure to one portion of the "folic acid" molecule. An explanation for the phenomenon observed might be found in the following alternatives: (1) under some conditions xanthopterin is able partially to replace "folic acid" in metabolism, (2) xanthopterin is similar enough in structure to an intermediate in the microbiological synthesis of "folic acid" that it effectively inhibits the utilization of the intermediate yet in itself is incapable of ready conversion to "folic acid." The first hypothesis is supported by the fact that xanthopterin shows no toxicity for *A. aerogenes*, as evidenced by the growth of the organism or by the biotin which it produces. The second possibility is in general agreement with much of the available information concerning vitamin antagonism where a derivative closely related to an active metabolite may prevent the normal action of the metabolite yet in itself be incapable of replacing the vitamin. Under such conditions, however, and contrary to the findings pre-

15 Landy, M., and Dicken, D. M., *J. Lab. and Clin. Med.*, 1942, **27**, 1086.

16 Purrmann, R., *Ann.*, 1940, **546**, 98.

17 Purrmann, R., *Ann.*, 1940, **544**, 182.

18 Purrmann, R., *Ann.*, 1941, **548**, 284.

TABLE II.
Comparison of "Folic Acid" Production of *Aerobacter aerogenes* as Influenced by the Presence of Various Pterins when Assays Were Performed with *Strept. lactis R* and *L. casei* ε

Exp.	Medium	Test material	Turbidity	"Folic acid" produced: *Strept. lactis* assay mγ/ml	"Folic acid" produced: *L. casei* assay mγ/ml	Biotin produced μγ/ml
1	C	None	321	9.2	16.8	15
		100γ xanthopterin	325	2.5	5.4	15
		100γ leucopterin	320	9.0	17.0	17
		100γ isoxanthopterin	321	9.2	17.6	15

sented, large amounts of the antimetabolite might be expected deleteriously to influence the growth of the organism or nearly completely to inhibit the formation of the metabolite. Obviously, an adequate explanation for the depressant effect of xanthopterin on the production of "folic acid" by *A. aerogenes* cannot be offered at this time. It is suggested, however, that these experiments supply additional evidence for relating the structure of xanthopterin to that of "folic acid." Available data on the empirical analysis of crystalline folic acids[7,19] appear to substantiate this suggestion.

Summary. When xanthopterin was added to cultures of *A. aerogenes* only small amounts of "folic acid" were synthesized by the organism. Xanthopterin was without effect on the resulting turbidity of the cultures or on the amount of biotin produced. Several other purines, pyrimidines, and pterins were without effect on the growth of the organism or on the production of "folic acid" or of biotin. It is suggested that xanthopterin either may partially replace "folic acid" in metabolism or its structural similarity to an hypothetical intermediate in the microbiological synthesis of "folic acid" enables it to inhibit the synthesis or utilization of the intermediate.

[19] Pfiffner, J. J., Binkley, S. B., Bloom, E. S., Brown, R. A., Bird, O. D., Emmett, A. D., Hogan, A. G., and O'Dell, B. L., *Science*, 1943, **97**, 404.

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Muscle Glycogen as Influenced by Castration, Adrenalectomy, and Treatment with Testosterone and Desoxycorticosterone Acetate.

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Schumann[1] has reported that castration of male rats and rabbits leads to a depletion of muscle glycogen. Testosterone propionate or desoxycorticosterone acetate were said to be effective in restoring the muscle glycogen in either castrated or adrenalectomized animals to a level even above that found in unoperated controls. Furthermore, injections of testosterone into normal rats led to muscle glycogen values higher than those in the controls. His results, if they should be confirmed, would constitute the first clear evidence for the participation of the testicular hormone in carbohydrate metabolism in skeletal muscle. Previous attempts to link the secretion of the testis with general metabolic processes have been, as Koch[2] has pointed out, inconclusive.

[1] Schumann, H., *Z. f. d. ges. exp. Med.*, 1938, **103**, 117; *Ibid.*, 1939, **105**, 577; *Pflüger's Arch. f. d. ges. Physiol.*, 1940, **243**, 686; *Ibid.*, 1940, **243**, 695; *Klin. Wchnschr.*, 1940, **1**, 364.

[2] Koch, F. C., *Physiol. Rev.*, 1937, **17**, 153.