that these chromosomes might be useful as a diagnostic character in distinguishing different species of mosquito.

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PURINES AS GROWTH REQUIREMENTS OF SPIRILLUM SERPENS

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The recognition of the purine and pyrimidine bases as factors affecting the growth of microörganisms has been reported with increasing frequency during the past few years. Richardson[1] in 1936 found that under certain conditions uracil was essential for the growth of *Staphylococcus aureus*. Möller[2] showed that adenine was required for the growth of *Streptobacterium plantarum*, while Pappenheimer and Hottle[3] found adenine to be necessary for the growth of a strain of group A hemolytic streptococci. In the latter case adenine could be replaced by hypoxanthine, guanine, xanthine, guanylic acid or adenylic acid. Furthermore, it was observed that purines were not required by this organism if the carbon dioxide tension above the medium was maintained sufficiently high. Snell and Mitchell[4] have reported the requirements of several lactic acid bacteria for purine and pyrimidine bases. *Streptococcus lactis* was found to require adenine and thymine for growth. Guanine was found to be essential for the growth of *Leuconostoc mesenteroides*, while uracil stimulated the growth of the latter organism and of *Lactobacillus arabinosus*. Robbins and Kavanagh[5,6] have recently described the effect of guanine and hypoxanthine on the growth of *Phycomyces*. In the present communication we wish to report the essential nature of several purine bases for the growth of a strain of *Spirillum serpens*.

Investigation of the cultural requirements of this organism (carried by American Type Culture Collection as No. 8084) showed that although it could not be grown in simple synthetic media the addition to such media of small amounts of natural extracts caused growth to occur. The active principle of such extracts was isolated and was found to consist of purine bases. Hypoxanthine, adenine and guanine were found to affect the growth of the organism.

Experimental.—The basal medium used had the following composition:

	AMOUNT PER LITER OF MEDIUM
Asparagine	5.0 g.
Inorganic salts:	
KH_2PO_4	500 mg.
K_2HPO_4	500 mg.
$MgSO_4 \cdot 7H_2O$	200 mg.
NaCl	10 mg.
$FeSO_4 \cdot 7H_2O$	10 mg.
$MnSO_4 \cdot 4H_2O$	10 mg.

The pH of the medium was adjusted to 7.2. The addition of the proper purine bases to this medium completely met all requirements of the organism. None of the "B-vitamins" was required.

Cultures were grown in 50 ml. Erlenmeyer flasks. Solutions of materials to be tested were pipetted into the flasks and the volume in each was adjusted to 5.0 ml. with distilled water. To each flask was then added 22.0 ml. of the basal medium described above (27 ml. cultures were required to fill the absorption cell used in determining extent of growth). The flasks were plugged with cotton, sterilized by steaming for 15 minutes, cooled and inoculated. Inoculum was prepared from 24 hour cultures of the organism in beef extract-peptone broth. The cells of such a culture were centrifuged out aseptically, resuspended in sterile water, and one drop of the resulting suspension was used to inoculate each flask.

The growth of the organism was measured by quantitatively comparing turbidities in a thermoelectric turbidimeter.[7] Growth was expressed directly as galvanometer readings.

TABLE 1

HYPOXANTHINE AND GUANINE

(Growth Expressed as Turbidimeter Galvanometer Readings)

μG. HYPOXANTHINE PER 27 ML. CULTURE	μG. GUANINE ADDED PER 27 ML. CULTURE 0	10	200
0	29.7*	....	..
3	38.2	30.5	28.2
6	48.5	37.8	29.0
12	59.9	60.0	30.0
30	64.0	64.0	33.0
100	65.8	..	..
250	65.8	..	65.0

* Absorption cell filled with distilled water gave a reading of approximately 25.

The effect of the addition of hypoxanthine to the basal medium is shown in table 1. This compound alone is able to replace all of the growth-promoting activity of natural extracts. In the third and fourth columns of table 1 is shown the effect of the addition of guanine to cultures containing hypoxanthine. Guanine inhibits the growth stimulated by hypoxanthine, provided the concentration of guanine is greater than that of hypoxanthine. If the amount of hypoxanthine present is in excess of the amount of guanine, the latter factor has no effect on growth.

TABLE 2

HYPOXANTHINE AND ADENINE

(Growth Expressed as Turbidimeter Galvanometer Readings)

μG. HYPOXANTHINE PER 27 ML. CULTURE	μG. ADENINE ADDED PER 27 ML. CULTURE 0	2	5	10	50	100	200
0	28.0	..	..	..	..	..	..
2	33.5	38.3	35.1	29.8	..	..	..
5	41.0	45.8	47.8	39.1	28.0	28.0	..
10	50.5	52.2	54.8	52.5	29.5	28.0	..
15	55.8	..	..	..	..	..	..
100	67.0	..	..	67.0	66.0	51.0	33.8

The effect of the addition of adenine to cultures containing hypoxanthine is shown in table 2. It can be seen that if the concentration of adenine is equal to or less than the concentration of hypoxanthine, growth greater than that produced by the hypoxanthine alone occurs. If the adenine concentration is greater than that of hypoxanthine, toxic effects are observed, and if the ratio of adenine to hypoxanthine becomes large, the physiological activity of the latter compound is entirely masked. It should be noted that when the concentrations of both factors are large, the toxic effect of adenine is apparent at lower adenine-hypoxanthine ratios than at lower concentrations.

Uric acid, xanthine and uracil have no effect on the physiological activity of hypoxanthine.

TABLE 3

PHYSIOLOGICAL ACTIVITY OF ADENINE AND GUANINE

(Growth Expressed in Turbidimeter Galvanometer Readings)

μG. GUANINE PER 27 ML. CULTURE	μG. ADENINE PER 27 ML. CULTURE 0	5	10	20	50	100	250
0	27.0	32.5	31.1	30.0	29.5	29.0	28.7
5	25.9	55.0	56.1	55.0	34.8	30.8	29.4
10	25.8	46.5	58.0	60.5	55.0	34.8	30.0
20	26.0	32.9	56.0	63.0	60.0	53.0	31.0
50	25.8	30.0	36.8	56.7	63.5	60.0	44.1
100	26.0	30.1	31.8	45.0	57.5	54.2	44.3
250	26.0	28.9	30.1	37.9	55.0	53.5	51.0

Table 3 shows the effect of the addition of various combinations of adenine and guanine to the basal medium. Neither adenine nor guanine alone is active, but a mixture of approximately equal parts of the two is able to supply the requirements of the organism. If either component of the mixture is appreciably in excess of the other, toxic effects are observed, and if the imbalance is extreme, the physiological activity is completely masked. Neither the purine, xanthine nor the pyrimidines, uracil, cytosine or thymine, have any effect on the action of adenine or guanine. Adenosine and yeast adenylic acid cannot take the place of adenine.

Discussion.—The growth requirements of *Spirillum serpens* are completely met in a medium composed of asparagine and inorganic salts supplemented with hypoxanthine or an equimolecular mixture of adenine and guanine. The organism thus differs from other organisms which have been reported to require purines in that all of the latter organisms have rather complex requirements in addition to the purines.

The reversible inhibitory action of certain purines on the physiological activity of others has been described. A similar relationship has not hitherto been reported in the case of the purine requirements of microörganisms, but several examples of a similar phenomenon with other nutrilites have been reported. McIlwain[8] showed that pyridine-3-sulfonic acid or its amide interfered with the growth of organisms requiring nicotinic acid. *p*-Aminobenzoic acid in small amounts has been reported to overcome the bactericidal effects of sulfanilamide and its derivatives.[9] Snell[10] has reported that the sulfonic acid analogue of pantothenic acid inhibited the growth of organisms which require this vitamin. The addition of excess pantothenic acid reversed the inhibition.

The toxic effects of the pyridine-3-sulfonic acid, the sulfanilamide compounds and the pantoyl taurine are believed due to the fact that they are structurally very similar to the corresponding naturally occurring nutrilites. The inhibitory substances thus appear to be able to fit into the biochemical patterns normally occupied by the essential nutrilites. They are not, however, able to carry out whatever vital functions are performed by these compounds and the metabolic processes of the organism are blocked at this point.

This picture can be applied in the present instance, but is complicated by the fact that adenine and guanine, which are inhibitory under certain conditions, contribute to the growth of the organism under other conditions. In seeking to rationalize these results the following speculation is suggested.

Assume that the purines function in a physiologically essential complex that contains two purine bases per molecule. (Several important coenzymes are known to have a dinucleotide structure.) Then, according to the results obtained, this complex can function only if (1) both purines are hypoxanthine, (2) one purine is hypoxanthine and the other adenine or (3) one purine is adenine and the other guanine. The complex cannot function physiologically if (1) both purines are adenine, (2) both purines are guanine or (3) one purine is hypoxanthine and the other guanine. Xanthine and uric acid do not compete for places in the complex. Such an hypothesis is in agreement with all of the observations, and is useful in indicating possible answers to a number of questions. For example, on this assumption it can be seen how it is possible for an excess of adenine or guanine to be toxic while an equimolecular mixture of the two promotes growth, and how it is possible for an excess of adenine to inhibit the action of hypoxanthine while a lower concentration adds to growth.

In line with the above hypothesis it is interesting to recall that Stockstad[11] found *Lactobacillus casei* to require what appeared to be a dinucleotide isolated from liver. This dinucleotide could be partially replaced by a mixture of purine and pyrimidine bases, but only by amounts much greater than required of the dinucleotide.

Summary.—Hypoxanthine or an equimolecular mixture of adenine and guanine is essential for the growth of *Spirillum serpens*. Adenine or guanine alone does not support growth, and under certain conditions these compounds are toxic to the organism. No other vitamin-like compounds affect the growth of the organism.

An hypothesis is suggested concerning the toxic effects of adenine and guanine.

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* Standard Brands Fellow for 1941–1942.

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