NOTES

INFLUENCE OF SODIUM THIOSULFATE ON THE SURVIVAL OF COLIFORM ORGANISMS IN STORED SAMPLES OF UNTREATED LAKE WATER[1]

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Sodium thiosulfate ($Na_2S_2O_3$) favors the survival of coliform organisms in untreated lake water samples. Seasonal variation in the temperature of source water at the time of sampling also influences their survival. These two factors may be important in relating the losses observed in the coliform content of stored water samples to the initial level as determined by Standard Methods procedure.

Two master samples of Lake Michigan untreated water from the Chicago 68th street crib were collected on each of 215 days from July 1, 1951, to June 30, 1952. One master sample was promptly distributed into two subsamples, A1 and A2, and the other master sample promptly distributed into subsamples B1, B2, C1, and C2.

Sodium thiosulfate was added to each of two subsamples, B1 and B2, to give a concentration of 0.029 g per 100 ml. These and two subsamples without thiosulfate, C1 and C2, were immediately placed in storage at 5–6 C for 18 hours. Each of the remaining two subsamples, A1 and A2, was tested for the most probable number (MPN) of coliform organisms in 100 ml, by the Standard Methods procedure. After storage, subsamples B1, B2, C1, and C2 were similarly tested.

The source temperature at the time of sampling varied from an average of 0.1 C in February to 20.1 C in August. The average temperature of the source water and number of master samples collected are shown for each month in table 1. Each value under A, B, and C represents the average of a series of duplicate MPN's for each month. The difference between A and C shows a consistent loss of coliform bacteria when these samples were stored at 5–6 C for 18 hours. The

TABLE 1

Effect of $Na_2S_2O_3$ on survival of coliform bacteria (average MPN) in samples of Lake Michigan untreated water stored for 18 hours at 5 C

Month	Avg. Source Temp C	No. of Master Samples‡	Unstored (Initial)	Stored 18 hr 5–6 C		Loss	Loss
				$Na_2S_2O_3$ present	$Na_2S_2O_3$ absent	$Na_2S_2O_3$ present	$Na_2S_2O_3$ absent
			Series A	B	C	A–B	A–C
1951							
July	18.1	20	40.6	35.9	3.6	4.7	37.0†
Aug.	20.1	23	173.3	76.5	5.4	96.8†	167.9†
Sept.	18.9	19	9.9	7.4	1.7	2.5*	8.2†
Oct.	14.1	20	62.2	53.3	16.0	8.9	46.2†
Nov.	4.5	18	34.2	28.1	8.1	6.1	28.1†
Dec.	2.9	15	49.8	37.1	14.9	12.7†	34.9†
1952							
Jan.	1.0	18	13.2	10.5	6.4	2.7	6.8†
Feb.	0.1	19	8.1	6.1	2.5	2.0	5.6†
Mar.	1.1	15	29.0	18.3	8.4	10.7	20.6†
Apr.	5.1	17	24.2	8.0	0.7	16.2	23.5†
May	10.4	14	11.0	1.0	0.7	10.0*	10.3†
June	14.6	17	39.7	8.8	4.2	30.9	35.5†

* Significant at the five per cent level.

† Significant at the one per cent level.

‡ Six subsamples were distributed from each master sample. One MPN was determined from each subsample.

MPN's were transformed to logarithms for analysis of variance as earlier work had shown that the log MPN's from such samples were approximately normally distributed. The F-test was used to measure the significance of the difference between the A and C series for each month. This difference was highly significant in

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every case, indicating a serious risk of underestimating the level of the coliform population in water examined if samples are stored under refrigeration for 18 hours before planting.

The loss in the B series of subsamples, containing $Na_2S_2O_3$, was usually much less severe than in the C series where no $Na_2S_2O_3$ was added. Only in September, January, February, and May were the losses or differences between A and B significant.

Sodium thiosulfate appears to favor the survival of coliform bacteria in stored samples of Lake Michigan water. Information regarding the mechanisms of this survival should be obtained from a study of the physiological effects of $Na_2S_2O_3$ on coliform bacteria. Until such information can be utilized to decrease coliform losses on storage, the practice of storing water samples for several hours before inoculating presumptive media should be discontinued.

THE STIMULATED GROWTH OF VIBRIO FETUS BY THE USE OF HORMONES

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Vibrio fetus is one of the causes of early abortions and lowered fertility in the bovine and ovine species. Since its discovery in the United States in 1918 by Smith (J. Exptl. Med., **28**, 701, 1918) research workers have found it to have exacting requirements for propagation in the laboratory. Several media have been developed that give growth in 24–72 hours. However, such growth is slow and limited, and many workers feel that the requirements of this organism have not been fulfilled. The senior author theorized that, in the *in vivo* environment provided by the gravid uterus, hormone stimulation possibly plays an important role in the multiplication of *V. fetus*. To test this theory a series of experiments was devised, using a bovine strain of *V. fetus* isolated in this laboratory. Semisolid thiol and fluid thioglycolate media commonly employed in the propagation of the microorganism were used.

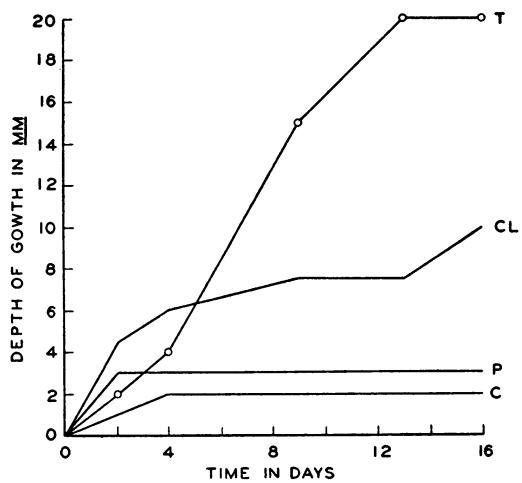

Figure 1. Growth of *Vibrio fetus* in semisolid thiol medium containing progesterone (P), corpus luteum (CL), and testosterone (T). The growth in semisolid thiol medium without hormones is shown by (C).

The following hormones when added to these basic media were found to enhance markedly the growth of *V. fetus*: corpus luteum extract, progesterone, and testosterone (figure 1).

Previous work has established the pleomorphic character of *V. fetus* cultures maintained on laboratory media, wherein long spiral forms and coccoid forms predominate. Initial isolation of *V. fetus* from aborted fetuses, however, shows predominately a highly motile, short, comma-shaped, microorganism. There seems to be, from present studies, a definite correlation of morphological form to the hormone used.

Further studies are underway designed to evaluate the possible influence of the hormones associated with reproduction on the morphology, pathogenicity, and other characteristics of *Vibrio fetus*.