

A COMPARATIVE INVESTIGATION OF BULIR'S FERMENTATION TEST AT 43° C. AND THE STANDARD AMERICAN TEST AT 37° C.

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IN our previous paper in this *Journal* it was shown that the temperature of 46° C.¹ recommended by Eijkman and Bulir in their fermentation test is excessively high, as in water cultures it has a destructive effect on *Bact. coli*. This was in accordance with the previous work of Brown and Skinner (1930) and Skinner and Brown (1934). At the same time we were able to show that in cultures in Bulir's medium at 43° not only is the destructive effect of the excessively high temperature on *Bact. coli* removed but the reproduction and fermentation activity of this organism is distinctly stimulated. At 43° Eijkman's and Bulir's tests effectively distinguish between true *Bact. coli communis* and other strains.

The American standard fermentation test on bouillon containing 0.5 per cent. lactose is considered to be a model specimen of a sensitive fermentation test, being sensitive even in detriment to its own specificity (Leiter, 1929; Horwood and Heifertz, 1934; Winslow, 1934; and others).

For the purpose of a comparative valuation of Bulir's method with our temperature modification we compared it with the American standard method. We made 100 parallel investigations of water from the polluted Fontanka River in Leningrad, applying both methods.

Quantities of 1 c.c. of the undiluted water and dilutions of 1/10 to 1/1,000,000 in sterile water were introduced into test-tubes containing 5 c.c. of Bulir's medium (1 per cent. mannite) or 5 c.c. of American bouillon (0.5 per cent. lactose), with Durham's tubes to indicate the presence of gas. Each water sample was introduced into three rows of test-tubes: into one row containing Bulir's medium, and into two rows containing lactose bouillon. Each dilution was introduced with a separate pipette, but with the same pipette into corresponding test-tubes of all three rows. Cultures in Bulir's medium were grown at 43° (according to a verified thermometer); one set of cultures in lactose bouillon were grown at 43°, the other at 37°. The results were recorded after 24 and after 48 hours of growth. Control inoculations were made in 24 hours

¹ Temperatures in ° C.

on Endo's medium from the two maximum dilutions in each row which had produced gas. When gas production began after more than 24 hours, additional control inoculations were made after 48 hours. Colonies of the *Bact. coli* type which developed on Endo's medium were identified by their inability to grow in Simmons' medium and their capacity to produce gas in Bulir's medium at a temperature of 46°. Bacteria of the *Aerobacter* and *Citrobacter* types were therefore excluded.

Table I clearly shows that Bulir's fermentation test at 43° is more specific than the American standard lactose bouillon test at 37°, as the latter does not exclude *Aerobacter* and *Citrobacter* strains.

A summary of the results of our experiment is given in Table I.

Table I. 100 samples of water from the Fontanka River taken in January and February 1935

Stages of investigation	Fermentation tests	Number of positive tests which produced titre in c.c.						Average titre in c.c.	
		1	1/10	1/100	1/1000	1/10,000	1/100,000		1/1,000,000
. Fermentation titre (presumptive test):									
	Lactose bouillon at 37°	—	—	3	32	43	19	3	0-00066
	Lactose bouillon at 43°	—	—	3	29	51	17	0	0-00060
	Bulir at 43°	—	—	6	27	48	18	1	0-00100
. Confirmed <i>coli-aerogenes</i> titre without differentiation of <i>Bact. coli</i> and <i>Bact. aerogenes</i> :									
	Lactose bouillon at 37°	—	—	3	33	44	18	2	0-00067
	Lactose bouillon at 43°	—	2	6	26	52	14	0	0-00291
	Bulir at 43°	—	1	7	26	47	18	1	0-00200
. Confirmed <i>coli</i> titre with the exclusion of <i>Bact. aerogenes</i> from the record:									
	Lactose bouillon at 37°	1	1	5	32	46	14	1	0-01190
	Lactose bouillon at 43°	—	2	6	31	47	14	0	0-00295
	Bulir at 43°	—	1	10	24	46	18	1	0-00240

Remark. The increase of *coli* titre (gas production) by one succeeding decimal dilution after 24 hours of growth was recorded in 5 cases (5 per cent.) on Bulir's medium, and 3 times (3 per cent.) on lactose bouillon both at 37° and 43°.

When fermentation tests were made on lactose bouillon at 43° their specificity noticeably increased, bringing them nearer to Bulir's medium. The above experiment confirms once more the correctness of our opinion (Minkevich *et al.* 1928), that when Bulir's fermentation test is used in water examinations, the necessity of additional tests for *Bact. coli* is practically removed.

In the following experiments we established that in lactose bouillon, as well as in Bulir's medium, incubation at 43° stimulates gas production by *Bact. coli*.

Minimum quantities of *Bact. coli* cultures were introduced into test-tubes containing an equal volume of lactose bouillon (10 c.c.), and were grown in the closed fermentation apparatus described in our preceding paper (pp. 55-57) at 37° and 43°. Each parallel row consisted of 4-5 sets of apparatus.

Table II records the results of these experiments, the numerators showing the quantity of gas produced after the lapse of 24 hours, the denominators in 48 hours.

Table II. *Bact. coli strain, No. 12*

Exp. No.	Temp. ° C.	Apparatus No.				
		1	2	3	4	5
1	37	—	$\frac{5.6}{7.4}$	$\frac{5.7}{8.0}$	$\frac{5.2}{7.4}$	$\frac{5.5}{7.4}$
	43	—	$\frac{6.8}{7.1}$	$\frac{7.0}{7.0}$	$\frac{5.7}{5.7}$	$\frac{7.0}{7.0}$
2	37	$\frac{6.2}{7.1}$	$\frac{6.2}{7.2}$	$\frac{5.9}{7.0}$	$\frac{6.1}{7.0}$	$\frac{6.1}{7.0}$
	43	$\frac{7.7}{7.0}$	$\frac{7.0}{7.0}$	$\frac{7.7}{7.7}$	$\frac{8.2}{8.2}$	$\frac{7.6}{7.6}$

The figures in Table II indicate that at 43° *Bact. coli* produces a maximum quantity of gas within the first 24 hours, whereas at 37° gas production still continues throughout the following 24 hours and equals or may exceed that of tests at 43° only at the end of 48 hours of growth.

CONCLUSIONS

1. Bulir's fermentation test at 43° is specifically more sensitive than the American standard test at 37° for discovering *Bact. coli communis* in water.
2. Carrying out the American standard test at 43° increases its sensitiveness.

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