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Growth of *E. coli* B in High PO_4 , YE, Dextrose Medium.

Composition of Medium

Antifoam A ~ 2 mg.
 K_2HPO_4 - 21.2 gm.
 KH_2PO_4 - 17.0 gm.
 Difco YE - 10.0 gm.
 Dist. H_2O to 900 ml - Autoclave

10% Dextrose - 100 ml - Autoclave
 Add dextrose to phosphate - YE solution aseptically. Inoculate with 2.0 ml of 24 hr broth culture of *E. coli* B.

2 x 15 liter cultures in 5 gal Pyrex bottles grown in fermenter at ~ 34° for 16-18 hrs. Harvested separately in Sharples. First labelled "A", 2nd "B"
 Yield "A" - 103 gm; "B" - 115 gm.

Sonoration:

Suspend cells from "A" in total volume of 412 ml in GG 1/20, 7.0, sonorate (70 ml each time) 10' in Raytheon, centrifuge 20' at 90 in Serval, collect supernatant. This is 10' sonorate. Residue has oil droplets in it & a good deal of black material so it was decided to keep this extract separate from "B" until first step in purification has been carried out & assay done.

Suspend cells from "B" in total volume of 460 ml in GG 1/20, 7.0, sonorate (70 ml each time, ~40 ml lost) 10' in Raytheon, centrifuge as above, collect supernatant. This is 10' sonorate "B".

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Protein Determination:		1660	mg/ml
"A"	1:20 - Urea .05 ml	.293	28.2
"B"	1:20 .05 ml	.303	30.0
St. -	1.0 mg/ml of Bovine Ser. Alb. .05 ml	.204	(1.0)

1000 ml / -
+*

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Growth of P^{32} labelled coli B in Glycerol-Lactate Medium.

Preparation of Medium

In 6 l. flask 920 ml of distilled water, then 0.3 ml of 1% K_2PO_4 , pH 7.0.

Add 1.5 ml of Oak Ridge P^{32} (~ 7.0 mCi/ml on 9/3/56).

Dilute 0.1 ml \rightarrow 100.0 ml -

On the 30
SF - 1150 cpm

$$\text{cpm/0.1 ml} = 1130$$

$$\text{cpm/ml undiluted} = 1130 \times 10 \times 1000 = 1.13 \times 10^7$$

$$\text{When diluted to 1000 ml cpm/ml} = 1.13 \times 10^7 \times \frac{920}{1000} = 1.04 \times 10^7$$

When medium is complete P content is $\sim .62 \mu\text{M/ml}$

$$\text{So sp. activity will be } \frac{1.04 \times 10^7}{.62} = 1.68 \times 10^7 / \mu\text{M}$$

Autoclave 15' at 16#, cool, add aseptically 40 ml of sol A + 40 ml of sol B. mix, inoculate with 1.0 ml of 8 hr. broth culture of *E. coli* B, incubate for 18 hr. at 37°. Inoculate at 10 PM 9/9/56

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10³⁰ AM - Determination of phosphate uptake by cells.

Dilute culture 1:500, centrifuge, dilute supernatant 1:500, count 0.1 ml of each.

SF - 1130
8Cr - 7, 8

cpm/0.1 ml of 1:500

Total - 2278, 2263

Supern. 990, 1014

Reincubate.

3⁵⁰ PM

Make same dilutions as above of culture + supernatant

SF - 1100
8Cr - 8

Total.

cpm/0.1 ml of 1:500

2335, 2268

Corr.

2230

Corr.

2222

Supern.

677, 663

670

662

Supernatant Uptake of P^{32} =

$$\frac{662 \times 100}{2262} = 29\%$$

$$\text{Uptake} = 100 - 29 = 71\%$$