~~Test H213 for partial segregation; hemizygosity of lac.~~

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Na^+ reaches equilibrium rapidly between water space of cells and environment. K^+ concentrated: 2-15 mg/ml K bound inelegant; also diffusible K in equilibrium. "After initial equilibration there is a further slow uptake of K even in resting cells suspended in a medium with no energy source. This appears to be due to the residual metabolism of the cells."

When glucose is added, K is taken up at a minimum rate of 1 mg K/min/ml cells. Bound K (low K medium for growth) is not readily lost. Free K is lost upon washing. By metabolism, cells exchange K rapidly (5%/min.) Cell membrane must be highly permeable.

2.3 ± 0.3 atoms K taken up per mole glucose.

Iodoacetate inhibited K-exchange but not P-loss. DNP prevented K turnover. Azide inhibited P uptake. Excess PO_4 partially

Attempts to isolate K compounds failed. K was released by suspending cells a) in NaCl pH 9 2) Et_2O; water 3) freezing + thawing, 4) extn. 50% EtOH. Implied that K-compounds are extremely unstable + destroyed when extracted. Uptake with D-1-P accelerated.

See Lebowitz & Kepermitz.

Potassium metabolism in Escherichia coli II Metabolism in the presence of carbohydrates & their metabolic derivatives JCP 34: 259 - 291.
Roberts, Cobb 1.2, + ...

Rb behaved like K and could be used as a tracer.
K-uptake inaffected by UV or by penicillin.

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+ : aerobacter, coli, para B, typhi, enteritidis

− : typhi, paratyphi, cholera-suis, dysenteriae.

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Hershey + Bronfenbrenner.

① China-Blue – Rosolic Acid Indicator medium.

Toluene supposedly inhibits oxidation but not hydrolysis. after Kacstrom.
No activity in autolysates.

Deere et. al 1936. – lactose is not removed from broth by lac–.
Measured lactase by increase in total reducing power caused by toluene or thymol-treated cells. Thymol sterilizes in 1 hour.

Substrate: .5% lactose in 1% acacia + .1M Phosphate at 7.0-7.2.
Samples dried by vacuum distillation. Dried cells (20-30 mg.), suspended in 25 cc 2% acacia, 10-20 mg thymol & incubated. After 1 hr, 25 cc 1% lac added. Dil. c̄ .01% $CuSO_4$ to stop enzyme action.
Activities: small activity noted in <u>unadapted</u> cells! .1-.2% hydr/mg cells.
This increased to about 4.5%

No specific statements on <u>no-cell</u> controls in lactose-acacia system.
Acacia might be hydrolyzed! 12 hour incubation period. No statement on contamination! [10 mg thymol / 50 cc.] Dried + Non-dried cells had similar Activity.

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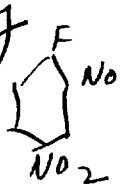
Only 19 of the 32 ϵNH_2 (lysine) of β-lactoglobulin react with  (FDNB) unless denatured. All can be acetylated.

W 327. ~~Mal S_M+ T-L-B_1-~~ ×

$M_1 + M_3 - S_M +$ T-L-B_1- × S_M- M_1- B-M- H.

$S_M - M_1 - M_3 +$ B M T L B_1 ....

S_M+ S_M+ M_3- ——— —

S_M	M_1	M_3	Glu	Mal
−	−	+	+	−
−	−	−	−	−
+	−	+	+	?
+	−	−	−	?
+	+	−	−	+
+	+	+	+	+
−	+	−	−	−
−	+	+	+	+

If suppressor affects M_1 −

$S_M + M_1 - M_3 +$ and $S_M + M_1 - M_3 -$ have to be identified from ++ and − + (wild types). Need progeny tests of Mal+ Mal+

(1) Measure "K_m" of adaptation and compare ~~c~~ K_m for the enzyme.

(2) Determine u.v. absorption spectrum of [illegible] + [illegible] (unadapted) for spectrophotometric evidence of complex formation. Do. enzyme + OMPG in presence of inhibitor - $Mg \cdot F \cdot PO_4$ (?)

If $S_M \rightarrow Mal_1-$ in $S_M + M_1 - M_3 +$.

$S_M \pm M_1$

Wild types vs. $S_M + M_1 - M_3 +$. Cross segregants $\overline{\text{Glu+ Mal+}}$ to wild type and look for Mal- recombinants.

If $S_M \rightarrow Mal_1-$ in $S_M + M_1 - M_3 - [\text{Glu} - \text{Mal}+]$, must be distinguished from $S_M \pm M_1 + M_3 -$. Take M_3+ papillae and cross $\bar{c}$ wild type ....

Glu-Mal+ is index of $S_M + M_3 -$.

Cross W108 – Mal+ – Glu+ : $S_M + Mal_3 + Mal_1 +$ x $S_M - Mal_3 + Mal_1 -$

and look for Mal segregation. Diploid, Mal+ Type.

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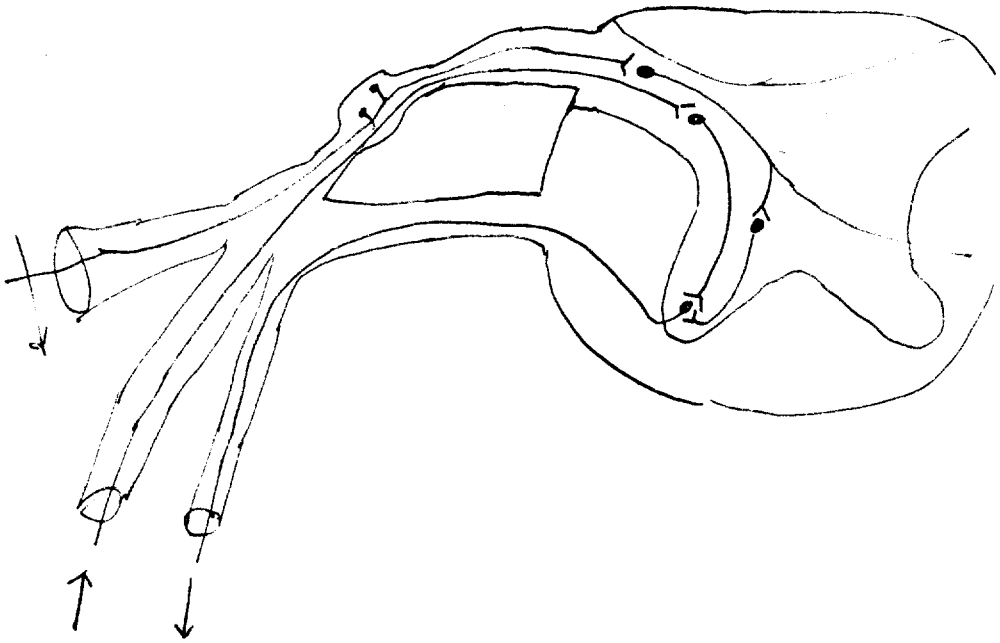
J. Neurophys. 8 (1945)

Intermuscular disturbances + pain.

post tibial nerve stim, measure tib. ant. response Spinal cat. Stim n. at each respiration. (artificial). Pain produced by acid in stim muscle fields. Response increased. No response to conc. reflex stimuli.



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$\phi N(CH_3)_2$ (DMA) 47 ml + CS_2 50 ml are mixed in a 500 ml suction flask and in ice bath in hood. Add 9.1 ml. $ClSO_3H$ dropwise. Add 13.9 g p-nitrophenol rapidly. Stir one hour & let stand overnight.

Add 100 ml .4 M. KOH → yellow crystals. Stir thoroly. Evaporate CS_2 at 80° in vacuo. Recrystallize crude product 3-4 x in 80% EtOH. [Method from J. Ch. S. 1:684 (1926)].

Formed activity measurable in 10 hours. opt. at pH 6.12 in acetate N/2. $K_m = 7 \times 10^{-5}$ M. from talse diastase.

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