July 22, 1953

Experiments on the Production of the "Compound" Using Double-Auxotrophs

These experiments were carried out with a mutant of B/lt which requires histadine and another mutant of B/lt which requires proline.

The histadine-requiring mutant of our tryptophane-less strain is grown in the chemostat with tryptophane as a controlling growth factor at a generation time of two in = hours. In the stationary state, due to the presence of the compound, the supernatant from the growth tube has an optical density of 0.630 at (280) and an absorption of 0.347 at (250). Tryptophane is then added at time zero both to the tank and the growth tube to bring up the tryptophane concentration in the tank to 2.5 milligrams per liter and in the growth tube to 2 milligrams per liter.

The bacterial density increases after this from 0.1 to 3 (Beckman at 350) to 0.185. After hours the bacterial density is practically stationary at this higher level. The chemostat now rises with histadine as the controlling growth factor and the tryptophane concentration in the growth tube may be appreciable but is certainly below 2 milligrams per liter. The optical density of the supernatant at 280 and 250 is determined for a number of points in time after the tryptophane has been added. This optical density has to be corrected for the absorption of the tryptophane which we added (see note), and in the following we shall refer always to optical densities which have been so corrected. After the adding of the tryptophane, the optical density at 280 and 250 falls in the chemostat and after 47 hours reaches a value of 0.260 at (280) and 0.300 at (250). The corresponding corrected values are 0.208 at (280) and 0.274 at (250). If we assume that this absorption is due to some substance which the bacteria produce when they grow histadine limited, we then can determine for each

point in time the amount of compound present from the observed absorption at 280 and 250, after first correcting these values for the absorption of tryptophane present in the growth tube, and then apply to these corrected values the formula

The absorption of the compound present at 280, which is given by this formula, is shown in the semi-logarithmic plot in Figure 1. It is a straight line falling with a slope of $\frac{1}{7 \text{ hrs.}}$. This is in fairly good agreement with the generation time at which the chemostat was running of 6.4 hours. Our experiment is, therefore, consistent with the assumption that, when the tryptophane concentration is raised in the growth tube to a value of less than 2 milligrams per liter, the bacteria cease producing the compound, which is then washed out from the growth time with a generation time of 6.4 hours.

NOTE: In correcting for the absorption of the tryptophane added, we assume that all the tryptophane added is present except the amount of the added tryptophane which goes into the increase of the bacterial density that occurs after the addition of the tryptophane. This does not mean that the tryptophane is present in an unchanged form, but means only that if the tryptophane is changed, it is changed into a compound which has the same optical density at 280 and 250. In order to verify this assumption, a special experiment was made in which we added to a chemostat in which the histadine strain was growing, with tryptophane as a controlling growth factor, ten milligrams per

liter of tryptophane. The bacterial density rose from 0.133 (Beckman at 350) to 0.157. The generation time was 5.4 hours. The absorption of 10 milligrams per liter of tryptophane is .300 at (280), or corrected for gunk

Extracted with ether, an absorption of 0.271 at (280) remains in the water. This shows that only a small amount of tryptophane was converted into indol. The bioassay of the supernatant for tryptophane showed no growth, but the control of looking for growth after adding tryptophane was not made so that we do not know whether the bulk of the absorption is due to tryptophane or to some compound having the same absorption as tryptophane other than indol.

The histadine concentration in the above experiments was milligrams per liter. The experiments with the proline-less strain were quite similar. The curves obtained are shown in Figure 2. The correction formula used was as follows:

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July 22, 1953

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These experiments were carried out with a mutant of B/lt which requires histadine and another mutant of B/lt which requires proline.

The histadine-requiring mutant of our tryptophane-less strain is grown in the chemostat with tryptophane as a controlling growth factor (sorbic) at a generation time of ~~two~~ $\tau = 6.4$ hours. In the stationary state, due to the presence of the compound, the supernatant from the growth tube has an optical density of 0.630 at (280) and ~~an absorption~~ of 0.347 at (250). Tryptophane is then added at time zero both to the tank and the growth tube to bring up the tryptophane concentration in the tank to 2.5 milligrams per liter and in the growth tube to 2 milligrams per liter.

The bacterial density increases after this from ~~0.1 to 3~~ 0.123 (Beckman at 350) to 0.185. After 4 hours the bacterial density is practically stationary at this higher level. The chemostat (then runs) ~~now rises~~ with histadine as the controlling growth factor and the tryptophane concentration in the growth tube may be appreciable but is ~~certainly~~ below 2 milligrams per liter. The optical density of the supernatant at (280) and (250) is determined for a number of points in time after the tryptophane has been added. This optical density has to be corrected for the absorption of the tryptophane which we added (see note), and in the following we shall refer always to optical densities which have been so corrected. After the adding of the tryptophane, the optical density at 280 and 250 falls in the chemostat and after 47 hours reaches a value of 0.260 at (280) and 0.300 at (250). The corresponding corrected values are 0.208 at (280) and 0.274 at (250). If we assume that this absorption is due to some substance which the bacteria produce when they grow histadine limited, we then can determine for each

point in time the amount of compound present from the observed absorption at 280 and 250, after first correcting these values for the absorption of tryptophane present in the growth tube, and then apply to these corrected values the formula

$$\sigma_{280\,(comp)} = \frac{\frac{274}{208}\sigma_{280\,corr} - \sigma_{250\,corr}}{\frac{274}{208} - \frac{\sigma_{250(comp)}}{\sigma_{280(comp)}}}$$

where $\frac{\sigma_{250(comp)}}{\sigma_{280(comp)}} = 0.5$

The absorption of the compound present at 280, which is given by this formula, is shown in the semi-logarithmic plot in Figure 1. It is a straight line falling with a slope of $\frac{1}{7\ hrs.}$. This is in fairly good agreement with the generation time at which the chemostat was running of 6.4 hours. Our experiment is, therefore, consistent with the assumption that, when the tryptophane concentration is raised in the growth tube to a value of less than 2 milligrams per liter, the bacteria cease producing the compound, which is then washed out from the growth time with a generation time of 6.4 hours.

NOTE: In correcting for the absorption of the tryptophane added, we assume that all the tryptophane added is present except the amount of the added tryptophane which goes into the increase of the bacterial density that occurs after the addition of the tryptophane. This does not mean that the tryptophane is present in an unchanged form, but means only that if the tryptophane is changed, it is changed into a compound which has the same optical density at 280 and 250. In order to verify this assumption, a special experiment was made in which we added to a chemostat in which the histadine strain was growing, with tryptophane as a controlling growth factor, ten milligrams per

liter of tryptophane. The bacterial density rose from 0.133 (Beckman at 350) to 0.157. The generation time was 5.4 hours. ~~The absorption of 10 milligrams per liter of tryptophane is .300 at (280), or corrected for gunk~~ After 21 hours the optical density at (280) is 460 *

Extracted with ether, an absorption of 0.271 at (280) remains in the water. This shows that ~~only a small amount~~ about 3 mgm/l may have been of tryptophane ~~was converted~~ into indol. The bioassay of the supernatant for tryptophane showed no growth, but the control of looking for growth after adding tryptophane was not made so that we do not know whether the bulk of the absorption is due to tryptophane or to some compound having the same absorption as tryptophane other than indol.

The histadine concentration in the above experiments was 1.5* milligrams per liter. The experiments with the proline-less strain were quite similar. Proline conc. 1.5 mgm/l The curves obtained are shown in Figure 2. The correction formula used was as follows:

Bact. density rose from 0.130 to 0.180 in 4 hours (Beckman at 350)

460 − 260 (from experiment here described with 2 mgm/l tryptophane gives 0.200 which is o. density of 6 about 7 mg/l tryptophane (instead the expected 8 mgm/l)

Histidine

	τ_{280}	τ_{250}	$\tau_{280\,corr}$	$\tau_{250\,corr} - .052$
Ohm	.630	.347	591	.591
4¾ h	469	340	340	.288
10¼ h	334	274	203	.151
23¼	255	286	061	009
28¼	257	309	037	—
47¼	260	300	052	—

Assume $\frac{1}{8}$ of tryptophane left = .052 at 280
.026 at 250

Hence final gunk $\left\{ \begin{array}{l} \tau_{280} = .208 \\ \tau_{250} = .274 \end{array} \right.$

Correction formula $\tau_{280\,(corr)} = \dfrac{\frac{274}{208}\tau_{280} - \tau_{250}}{\frac{274}{208} - 0.5}$

$= \dfrac{1.317\tau_{280} - \tau_{250}}{0.817}$

Prolene 5/29/53 $t = 6$ hr

	σ_{280}	σ_{250}	σ_{280}(corr)	σ_{280}(corr) − .052
Ohr	685	339	699	647
4 3/4	478	340	340	~~288~~
10 1/4	317	254	184	132
23 1/4	217	214	069	17
28 1/4	210	217	053	—

Final Bunk $280 = 210 - .052 = .158$
$250 = 217 - .026 = .191$

Correction formula

$$\sigma_{280\,corr} = \frac{\frac{191}{158}\sigma_{280} - \sigma_{250}}{\frac{191}{158} - 0.5}$$

$$= \frac{1.20\,\sigma_{280} - \sigma_{250}}{0.70}$$

9.10
4.73
435