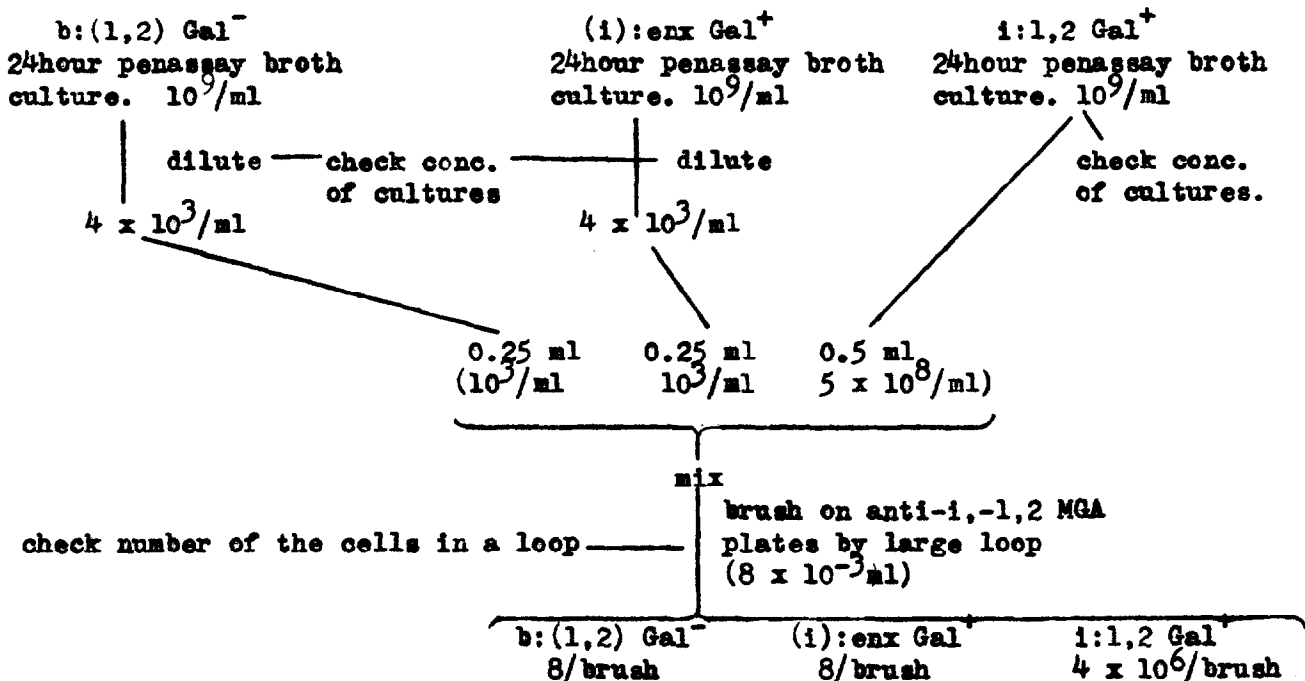# List of the Projects of Experiments.

(Oct. 4, 1955)

Tetsuo Iino

I. Experiments on the effect of the difference of phase on the H-antigen transduction.

1. Reconstruction experiment.

b:(1,2) Gal$^-$
24hour penassay broth
culture. 10^9/ml

(i):enx Gal$^+$
24hour penassay broth
culture. 10^9/ml

i:1,2 Gal$^+$
24hour penassay broth
culture. 10^9/ml

dilute —— check conc.
of cultures ———— dilute

check conc.
of cultures.

4×10^3/ml

4×10^3/ml

0.25 ml
$(10^3$/ml

0.25 ml
10^3/ml

0.5 ml
5×10^8/ml)

mix

check number of the cells in a loop ————

brush on anti-i,-1,2 MGA
plates by large loop
$(8 \times 10^{-3}$ml)

b:(1,2) Gal$^-$
8/brush

(i):enx Gal$^+$
8/brush

i:1,2 Gal$^+$
4×10^6/brush

2. Transduction from <u>Sal. abony</u>(Fla$^+$, b;enx) to TM-2 Fla$^-$-5 (i:12).

 a). Obtain Gal$^-$ mutant from TM-2 Fla^a-5.

 b). Obtain single phase culture of TM-2 Fla$^-$-5.

 c). Transduction experiment:

  <u>Sal. abony</u> Fla$^+$, b:(enx) ————x 1:1 mixture of $\begin{cases} \text{TM-2 Fla}^-\text{-5,i:(1,} \\ \text{Gal}^-. \\ \text{TM-2 Fla}^a\text{-5,(i):1} \\ \text{Gal}^+ \end{cases}$

  <u>Sal. abony</u> Fla$^+$, (b):enx ————x   "   "

Screen Fla$^+$ swarms on MGA-plates, and test antigen.

3. Transduction between single phase cultures (antiserum selection).

a). $\left\{\begin{array}{l} \text{TM-2 i} \longrightarrow x \\ \text{TM-2 1,2} \longrightarrow x \end{array}\right.$ 1:1 mix of Sal. abony b, Gal$^-$ and enx, Gal$^+$.

                                    "   "   " .

b). $\left\{\begin{array}{l} \text{TM-2 i} \longrightarrow x \\ \text{TM-2 1,2} \longrightarrow x \end{array}\right.$ "   b, Gal$^+$ and enx, Gal$^-$.

                                 "   "   " .

c). $\left\{\begin{array}{l} \underline{\text{Sal. abony}}\ b \longrightarrow x \\ \text{"} \qquad \text{enx} \longrightarrow x \end{array}\right.$ 1:1 mix of TM-2 i, Gal$^-$ and 1,2, Gal$^+$.

                                 "   "   " .

d). $\left\{\begin{array}{l} \underline{\text{Sal. abony}}\ b \longrightarrow x \\ \text{"} \qquad \text{enx} \longrightarrow x \end{array}\right.$ "   i, Gal$^+$ and 1,2, Gal$^-$.

                                 "   "   " .

II. Experiments on the i:b:enx-strains

4. Recover unstable i^+b^+/i^+b^- from original culture of i:b;enx-strain.

5. Compare changed reactivity of b and i in parent cultures as function of medium.

$\left.\begin{array}{ll} \text{TM-2} & \text{i} \\ \underline{\text{Sal. abony}} & \text{b} \\ \text{"} \qquad \text{"} & \text{b,i} \\ \text{"} \qquad \text{"} & \text{(b),i} \\ \text{"} \qquad \text{"} & \text{i} \end{array}\right\}$ transfer to $\left\{\begin{array}{l} \text{Penassay broth} \\ \text{EMB-agar} \\ \text{Minimal agar} \end{array}\right.$

Compare the intensities of agglutination for anti-b and anti-i.

6. Alternative phase of i^+b^-.

a).Brush the culture of i^+b^- on MGA containing anti-i or anti-i + anti-enx,

b).Test the antigen types of the swarms developed.

8. Transduction of H_1 from i:b:enx.

a). Prepare lysate of i:b:enx. If U.V. is not efficient to induce phage, use penicillin.

b). Transduction: i:r:enx $\longrightarrow x$ Sal. abony b:enx (select by anti-b and -enx)

c). Transduction: Fla+i:r:enx $\longrightarrow x$ Sal. paratyphi SW666 Fla$^-$ b

8. Test of the locus specificity in H_1-transduction to i:r:enx.

a). <u>Sal</u>. <u>sendai</u> a:1,5 ———x i:r:enx

b). <u>Sal</u>. <u>altendorf</u> c:1,7 ——x   "

c). <u>Sal</u>. <u>zega</u> d:z^6 ———x   "

In each case, brush on MGA containing anti-r and anti-enx, and test the types

of antigen of swarms developed.