November 23, 1950.

Dr. Margaret Lieb,
Rerckhoff Laboratories,
California Institute of Technology,
Pasadena 4, California.

Dear Peg:

Thank you for sending your thesis, which I return herewith. Have you sent it to Genetics?

Your letter invited comments, so here goes: (from particulars to generalities)

Unclear points: p. 13- which work of R & S (unpubl.)
Summary: "maximum of 200x" 200x what?
Literature cited: Lederberg 1949 b (heterozygotes) missing (p.13)
Ryan 1950 is an improper reference, as MGB is not a publication. (Ask Nelson about just this point). The method has been published before.
What is your policy concerning initials or names in your citations? You should be consistent. A generally good policy is to use initials only for men, and the full first name for women, except where there are many authors. Zentr. Bakt. does not have be be spelled out at such length., but the Abt. (A or B) and Originale or Referate should be indicated (abbr.)

Arguable points: Newcombe (1948).... is rather inane, since the "formula" does not detect ~~anything~~ anything. You might write: the recovery of mutants may be expressed as the ratio $\frac{r_2/n_2}{r_1/n_1}$ (or $\frac{r_2 n_1}{r_1 n_2}$)

At best, the quoted arrangement is inverted for the use you want to make of it in table 3.

"the entrance of histidine into the gene itself"... Tsk! (Shades of "pantothenate impinging on the gene"!) Of course you might well and properly mean that protein building blocks, including histidine, are necessary fpr the building up of h/ function. But this sudden lamarckian note (i.e. direct relationships between gene constitution and biological effect) is a little surprising.

General discussion: 1) Phenotypic lag in spontaneous mutation. Newcombe's argument is on the whole very weak (although his conclusions are acceptable) because he presupposes a specific model of the mutation process. The discrepancies between Methods I as against II and III hinge on the average clone size, i.e., ratio of mutant cells to mutant clones with one or more effective mutants. This ratio ("d" in the attached) can, of course beinfluenced by phenotypic lag, but

it also depends on the model you use (as you have pointed out for the possibility of nuclear segregation). The Luria-Delbruck model supposes that each cell has a smooth probability of mutation between each fission, at a rate proportional to the fission rate. The individual cell is assumed to increase smoothly, rather than discretely, from one to two, at an exponential rate. A mutation occurring early in an interfission interval thus has a yield of 2 mutants at the end of the interval, while one which occurs late has a yield of 1, the average yield being $\sqrt{2} = 1.4$. Another (and perhaps simpler)model would be that mutation occurred only at fission, with a yield of 1.0 (i.e., one nonmutant; one mutant progeny - theory of mutation as copying error) or of 2.0. These differences will be reflected in a comparable change in the theoretical clone size. (I forgot to mention above that you ought to clarify, omit, or otherwise modify p.8 line 6-8). Another, less critical consideration, is that it is invalid to strike an arithmetic mean of method II - but apparently you did not do this. The discrepancies of Newcombe's data are exaggerated almost 2-fold by this error (which arises from the assumptions of the "likely average" approach of L&D - one has to pool all the data somehow, and use an overall "G" for the series). I think you would have done better to use Lea and Coulson's maximum likelihood method in view of the critical use you wish to make of your data. While you are justified in concluding that no discrepancy (which might be based on phenotypic lag) could be detected, I don't think that you can say with even mild assurance that there is no phenotypic lag for the spontaneous mutations, which your summary at least might misimply, (as well as the statement " only induced h/ mutations
p33 exhibit phenotypic lag "). You probably do not intend to convey this impression, but I suggest that you look out for it very carefully.

Enclosed is a mimeographed summary, modified from L&D which I've used in classes. It shows the discrepant result of the model of mutations at fission only. The discrepancies between I and III can be most objectively phrased in d.

Another passing point: how were the data of your tables weighted ? (By number of observations, or by $1/SD^2$ or $1/sd^4$).

A point of considerable importance concerns the dominance of h/. I don't think you are entitled to make a very strict inference from the behavior of K-12 heterozygotes. You don't really know whether the mutation from h/ to h- is a "loss" or a "gain". But in any event, if you define h/ as dominant, it is inconsistent then to refer to phenotypic development being obscured by the time required for segregation. The h- phenotype could only begin to develop after segregation was completed, if h/ is dominant in your sense.

Along the same lines, I am not clear how far you are trying to generalize your results on the relative rates of mutation from and to h/. Your discussion is cagey, but your introduction leads one to look for a general comparison. It might be worthwhile to emphasize that (p.29) the spontaneous mutation rates of different stocks from h- to h/ straddle the rather consistent rates from h/ to h-, p28.

Your final conclusion (that I can discuss), that induced h- have no lag is somewhat disturbing. Your suggestion p.31 that the h/ may be effectively h- to begin with is probably correct, since the cells were prepared so as to be in lag phase. One must also consider that both UV and penicillin may actually accelerate the loss of h/ phenotype. Would it be possible to determine whether uv treated h/ are more resistant to penicillin initially than untreated, or does the problem of liquid sensitives rule out a determination?

On the whole, the problem and treatment are very interesting, but it is unfortunate that the complexity of the material makes a conclusive determination almost beyond reach, for most of the really interesting questions. That makes critique easy, but proofs difficult.