May 3, 1953

Dear Luca:

I have been able to make a hasty review of your draft. As you say, the writing is very rough, but I have no qualms about your ability to write excellent English prose (viz. your WHO paper). While I would welcome an opportunity to go over the final paper, do not hesitate to submit it at your own judgment if necessary. I have a little difficulty in viewing the paper as a whole, and my comments now are on particulars.

p. 1: the term genetic adaptation is satisfactory and simple; I would think "pre-adaptation" was more expressive if coupled with your explanations. In fact, one may contrast genetic pre-adaptation with genetic and physialogical post-adaptations respectively.

p. 2: I think too much weight is given (as an immediate response, no doubt) to Yudkin's position. I think it can be expressed as an attempt to obliterate the distinctions by postulating so unstable a genotype that ~~genetic~~ local changes could not be perceived. I would give it the status of an ad hoc compromise, rather than equal weight with I and III. Your comment on II and III as oversimplifications would be a good introduction to the necessity for detailed analysis: "III can also be phrased in terms which would include the pre-adaptation theory. If the population is studied as a whole, and no attempt made to resolve its elements, the enzyme balance of the mass will doubtless be found to show adaptive adjustments of the kind subsumed under this hypothesis. For an understanding of the genetic basis of such changes, howver, we must ~~xxxxxxx~~x must examine the adaptive responses of individual cells, and we shall find that the apparently directed, postadaptations of whole populations are best interpreted in terms of pre-adaptive changes of small numbers of its constituent cells.

p.2 bottom or intra- vs. inter-clonal variation

p.3 The suggested figure is an excellent idea. However, I would distinguish 1A and 1B vs. 2A and 2B (instead of 1,2,3, 4) to make later reference easier. It should be ~~pin~~- pointed out that, with reference to survival in presence of drug, 1A and B are not meaningfully different, but that other criteria may ultimately be found by which they can be distingusihed (elg. responses to much higher levels of drug). Eagle's work would be put on p.3, if anywhere, as a possible case approaching B. I would ignore Sevag, as gibberish. Instead of "distinction between models 3 and 4 may seem possible", elementary.

p.3 Clonal appearance of mutants: pedigree— along lines of Zelle's study of smooth-rough variation in Salmonella typhimurium (J. Inf. Dis. 71:131-152 1942)

p.4 Why not "variance analysis", and a "crude variance analysis" in place of "fluctuation test"
I would not say "fully vitiated by"--*subject to* is a sufficient criticism.

Is not the denial of a Poisson distribution seimply a formal satement of Hinshelwood's objection? In constructing the Poisson series, one does not have to postulate an equal a priori probablity, but simply a low probability and one that is independent of neighbours (environmental effects) and sibs (heritable). I.E., when I sample the populations, I have no concern for the a priori probabilities, but need only consider the result. With large humbers of cells, and a low expectation per trial, can one not ignore the a priori probabilities and simply consider the total successes/ total trials? I am not expressing this very well, and may perhaps be wrong in my thinking (of which I must do some more on this question). There has been no discussion of this in the presehb context [perhaps becuase of the considerations given], but

there is something very much like it in the discussion of mechanisms of exponential disinfection: See Irwin, 1942 J HYg. 42:328; Chick 1930 in Brit. M.R.C.: System of Bacteriology, and discussion by Topley&Wilson, Princ. Bacteriology & Immunity,1946ed. p.140. I do not believe there has been a serious attempt to combine the stochastic and distributional approaches, as a realistic theory perhaps should try.

AM May 4 After a night's relaxation, I am fairly sure that my objection is correct. The "fluctuation test" is a comparison of intra- and inter-culture variances. In the absence of some correlating factor (heredity or environment) these variances should be the same. The problem would then reduce to the question of a non-Poissonian distribution of samples from the same culture. It is beyond the immediate limits of my ingenuity to devise a distribution of cells from which properly drawn samples will show other than sampling error. If a non-Poisson distribution were found, we would immediately suspect either the sampling process, or the assay method as the source of the heterogeneity. One would, I presume, get a negative binomial distribution if the ~~samples were~~ sample sizes were not uniform, but distributed in a logarithmic series. However, one can get a negative binomial out of almost any data, since one has an extra parameter. I do not propose that this analysis is incorrect, but only that it is a restatement of the others.

I am a weak biometrician, and perhaps for this reason I am always suspicious of the generality of any biometric test. (This applies to kinetics as well). One can often disqualify the null hypothesis, but it is often difficult to predict whether a major or a trivial modification of the null hypothesis will be needed. The present discussion (i.e. of Luria&Delbruck)is an excellent example.

To return to p.4: I do not think Newcombe's method should be discussed at all as a fluctuation test, since it is not the variance that is important, but the more direct demonstration of statistical clones. Of course the spread plates will show a high variance, and the unspread a low one, but ~~is~~ it is not the difference in variance, but the difference in mean, which is emphasized.(This is unimportant). I do not see that the objection can be taken very seriously (effect of spreading): the inoculum is so large that there is a confluent growth whether or not the plate is respread, although I suppose there is still some local inhomogeneity. Would it helped the argument to have added the phage first, and respread after, perhaps, 20 minutes when the ~~seen~~ sensitive cells can be shown to be already doomed? My only reservation to this proof is that it still relies on a statistical demonstration of the clones, and does not isolate them as such.

p5:Unless we are referring to different work, Law(Nature 4/12/52),as I recall, studied resistance in <u>vivo</u>, and showed that a cluster of resistant cells occurred in one line of an unselected tumor. My memory may not be right.

p6. I would prefer not to describe the other methods summarized here as "indirect selection", although this would be perfectly legitimate. Sib selection would have been perhaps a better name for the replica-plating result. For the present, could you write "Drug-resistant variants have also been secured by selection for correlated characters .... in the absense of the drug"

Should one add here experiments which might be done based on the linear accumulation of mutants in the chemostat (Novick and Szilard?). Unfortunately, there is very little with drug resistance along this line, Bryson and Szybalski having disregarded this opportunity to use their turbidostat.

p.7 I am greatly disappointed that Roger Stanier should have quoted this work of Doudoroff's in the present context. If one considers that the toxic stimulus is not salt concentration, but <u>rapid change</u> in salt concentration, there is no meaningful physiological adaptation at all. This has been borne out more recently by Anderson's experiments on osmotic shock with phage (Ann Inst Pasteur Jan. 53). It is doubtful whether a free phage particle could be thought of as using an adaptive

mechanism. I would either leave out this reference, or explain the detailed interpretation.

To 7, after Dauermodifikationen, I would add: Some caution must be exercised in postulating similar mechanisms for bacteria, etc.* It is important to keep in mind that the distindtive feature of Dauermod., the gradual loss of resistance on later cultivation .... is here a property of individual lines of descent, and that experiments of a similar sort but based on unresolved populations may reflect a gradual change of the population, but a sudden, discrete mutational change in a few individuals subsequently selected* But Perhaps you make this point sufficiently later on.

p7 bottom: I would prefer you not quote my very casual experiment with chloraminobenzoate (Strandskov) since Demerec' published statament is available. If you like, you can quote my review as dealing with the general question.

p8 I would put Eagle in after Baskett on p.7 with the proviso that no test was made of the heritability of the adaptation. In fact, Doudoroff's case can be used as a good example where two mechanisms are operating (though the physiological can be viewed as either simple or spurious), as may well be the case also with Eagle, and a good deal of Hinshelwood's material.

p8: direct genetic effects.... I am rather fond of this section, and pleased you are able to work it in. Would it be better to put Dauermodifikationen after this, instead of as above?

p9:line 32 *of mating between compatible cells* instead of fusion.... I would omit sentence on inheritance of F+, but if you like the JGM paper can be inserted as an addnl. reference for the whole story. Lwoff will not like expression "lyogenic phage"-- I don't care. temperate may do as well. In fact, "lytic" phage will also transduce, if the recipient is protected by previous infection with the temperate.

p 10. middle on S^r...: Excellent!

11: azide: but $Az^r \times S^r$ has been used.

13: I had forgotten about colicin as an antibiotic. I think it would be worth while to say two or three lines, especially about the qualitative specificyt of the different resistance types in recombination tests. Almost anything is a modified phage these days; I shouldn't be surprised if streptomycin is claimed to be such sometime!

13: Tatum long ago worked out the inhibition of strain K-12 by Valine, and its antagonism by isoleucine (a better statement than complete medium), had isolated valine-resistant mutants and done just a few crossing experiments with them. Unfortunately, I do not have the details, and doubt if they have been published. One locus was found, I think, to the right of TL but I haven't heard any more about it in 5 years. Probably, it would be better not to quote any of this without a reference, but for my own curiosity I will question Tatum about it.

13: In Salmonella S^r transduction ca 100-1000X control.

I am very much disturbed by the repeated citation of the pneumococcus transformation as an example of directed mutation, by way of a justification of drug-induced adaptations (cf. the SGM symposium; Hobby in Bact. Revs. March 1953; originally perhaps largely due to Dobzhansky: see Amer. Natur. 87:123, v. recently, as well as his book). For this reason, as much emphasis as possible should be given to the pn.tr. as a rare species of genetic transfer. I think this is already implicit in your discussion; I do not know whether more should beput in in the beginning.

15: -Hughes- OK. ~~Ciri~~ critique of technique should perhaps be toned down, but not excised. Could some of his variations have occurred in subsequent clones to the initial isolation?

16: I am not sure if I understand the last 6 lines. Have you noticed S^r reversion to S^s in W-1177? Was it not before there were only 3% S^r that you noticed a discrepancy in

counts with and without sm? [Don't put this in paper, but replica-plating is of course ideally suited to finding this kind of mutant.] Have you studied such reversions genetically? One could add that S^r grades continuously into S^d; it would be worth seeing whether a small amount of streptomycin would accelerate the growth rate. If so, there would be no special point to studying reversion to S^s in the difficult case, when more typical S^d would give a more direct answer. ~~Since~~ $S^d \rightarrow S^s$ has been measured in several cases (Demerec; Bertani; Catlin) and, I think, Newcombe).

17 P2: This is a very important paragraph. I fully agree with your conclusions. The emphasis might be that we should by no means ignore the more difficult cases, but that it has not been shown that any of these are in fundamental contradiction with the genetic theory. I think it is questionable whether extra-nuclear effects play an appreciable rule in adaptations of the protozoa. One tends to emphasize the unique.

18 blenorrhage = gonorrhea?. I do not think we have to quote specific cases, but this depends on whether drug-resistance will be considered at the sympoisum from any other viewpoints.

18-19 In the April 1953 Jour. Bact. Morton and Klein have an extensive discussion of synergism (which they claim is <u>always</u> genetic [and I think doubtful]) and ~~anat~~ antagonism (always, of course, physiological). Unless you know of an earlier paper of theirs to quote on the same theme, I think it would be best to regard this as coming too late.

This has all the makings of an interesting work, Luca, and I am only sorry that so many circumstances impose this rush. Perhaps we should, after completing this, undertake to do a more thorough job together. But let us postpone discussion of it until we can do so in person.

Back to p. 5: in Dittrich's expt: membrane filters/ cellophane sheets.
In second paragraph, the wording is especially difficult. I suggest:
"Another approach, based on clonal ~~appearance~~ occurrence of resistant mutants, depends on secondary differences sometimes observed among ~~different~~ independent mutations for resistance to the same drug./ If resistants appear in clones, such differences...."

You will have noticed a minor error at the heading of one of our tables (− should be + or ±, I forget which. It can be corrected easily in reprints; perhaps you should notify editors to include it in Corrigenda. In view of the symmetry of the table, it should confuse no one who goes as far as to study it.

Again, alas, hastily,

Joshua Lederberg