May 15, 1948.

Dr. Norman Horowitz,
Kerkhoff Laboratories,
Caltech,
Pasadena, California.

My dear Horowitz,

Thanks very much for your sample of canavanine, which arrived safely today. Some rather encouraging results have been obtained with hydroxyaspartic (anti-aspartic) and valine (anti-isoleucine), but I'll have to collect some more equipment before going much further. This promiseseto eventuate soon.

I'm sure you don't want a complete account by letter of my recent work; it would take quite a few pages to present the data in detail. Briefly, I have focussed my interest on a single enzyme (system) in E. coli, namely lactase, looked for independent occurrences of lactase- mutations (by plating on EMB-lactose medium as described in my paper in Genetics) and, having collected a couple of hundred of them, am comparing them genetically and phenotypically. Correlatively, and in correspondence with Monod, lactase has been extracted from the wild type by grinding or autolysis, and appears to be a simple hydrolytic enzyme. Nearly a dozen loci seem to be represented among the mutants, two or three of them being the most numerous; the others occur very occasionally. About half the mutants are essentially specific for lactose (some being methyl-b-galactoside -; others +); the others seem to be pleiotropic, in which components of the maltase, glycolytic , or gluconic-fermenting systems are affected also. Some of these have been tested rather rigorously for monogenicity; the same patterns have recurred on several occasions, they behave as a unit in crossing-over and in reverse mutation, so that as far as can be tested, a unit change is responsible for thencompound effects. However, suppressor mutations at

distinct loci have been picked up which may restore individual components of the pattern. For example a number of mutants have been identified with a locus to be called Lac_3-. These are all glucose, lactose, and maltose-negative. In addition reverse mutations to the wild type Lac_3+, selection from large populations in glucose or lactose medium results in G+M-L- or G-M-L+ phenotypes. When crossed with wild type these yield the Lac_3- phenotype as well as the parentals. Of particular interest is what appears to be a temperature sensitive allel of Lac_3 which is wild type at 30°, Lac_3- at 40°, but has distinct critical temperatures for sorbitol; glucose, mannitol, mannose, fructose or maltose; and lactose within that interval.

There does not seem to be any simple picture that willcover these data. Of course one can say that in some unknown way the effects are all indirect; but what procedures are available by means of which one could decide, for example, from which if any of these loci the enzyme receives its specificity, and which indirectly affect enzyme production. Please do not consider these remarks an "attack" on the one-to-one theory, especially the very credible and sober version which you quoted to me. I do wonder about the kind of evidence on which it is based. You suggest for example that 75% of the gene mutations encountered in Neurospora probably bear a unique relationship to the enzyme they affect. Aside from the rather moot question of selection for non-pleiotropic mutants, It occurs to me that some of the discussion of this question has been circular. That is, monogenic control is used as an argument for a primary effect (viz. the treatment of the pyrimidine-thiazololess mutant). Of course, monogenic control should be an incentive to look very hard for a single primary effect, since this possibility always exists; to my mind this is the greatest value of the 1:1 theory, but in terms of your letter, a given mutant might be one of the other 25%.

I have a more concrete inquiry concerning the Neurospora work. Have you accumulated in any system a number of recurrences phenotypically affecting a chemical

step your faith in the simplicity of which is, a priori, sufficiently strong that it would not be immediately abandoned if bigenic control were discovered? And have these recurrences been studied genetically and shown to be allelic (within reasonable doubt) ? It must be obvious that this is almost the only kind of genetic evidence that would bear on a 1:1 relationship for any particular enzyme. It was this question that motivated the work I am now doing, and I must admit that I was very much surprised to find what I did. Of course, one can argue that the gene-enzyme relationship is coli is not the same as in other organisms, that lactase is an exceptional case, or that the terminology of gene, allelism, crossing-over and so forth has a different material basis in coli (although no such contradiction has yet appeared). It may therefore be important to duplicate this kind of study with other enzymes, and with other organisms.

Aside from direct evidence, the 1:1 theory has the appeal that no alternative can feasibly be set up now. Enzyme specificity must be transmitted from one generation to the next somehow! But there may perhaps be some use in looking at the analogous problem of antibody specificity. Noone (I think) would argue that this is conferred by the gene, but there is considerable evidence that the competence of an animal (or its gamma-globulin or whatnot) to react to a given antigen is genetically controlled (e.g. rabbit antibody to human M-substance). If serological response could be analogized with enzymatic adaptation (as has been done before) one might draw the rather vague picture that genes might control in some indirect and possibly interdependent way the production of enzyme precursors which are competent to react with substrate to produce the enzyme. I am not sure that this says anything.

This discussion has gotten out of hand, and I had perhaps better stop at this point. Your remarks, or those of any of your group, would be appreciated.

As to reprints, I have rather lazily been sending GWB a dozen or so replicates of each for local distribution, with most of them earmarked. Is this inefficient or inconvenient? If so, let me have a list of the names of the men who would be interested to be circularized.

Best regards from Esther, and to your colleagues,

Sincerely yours,

Joshua Lederberg