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Dear Jacque,

This is in reply to your very kind letter of March 14 in which you extended an invitation to participate in the coming International Congress on Biochemistry which is to be held in Paris in 1952. Let me say that I should very much like to participate in this Congress and will make every effort to do so. As you can well imagine, I cannot at the present be completely certain that this will be possible. In this connection, let me raise a pertinent issue. In view of the fact that the Congress will not be able to pay travel expenses, it will be necessary for me to obtain some assistance in that direction from other sources. It is very likely that I will be able to do so. It would be of great help to me, however, if I could receive from you or from Professor Wurmser an official letter inviting me to present a paper at this Congress. Such a letter could be very useful and would probably result in my obtaining the necessary funds from our University here.

Needless to say, I noted with great interest your remark that you were in a position to demonstrate rigorously that adaptation does not involve combination of enzyme and substrate. We have evidence on the same question which leads to the same conclusion. I am sure, however, that your experiments are probably more conclusive than ours. Let me briefly describe what we have done along these lines. We used the system which I described previously which demonstrated that a non-utilizable analogue (-methyl glucoside) can be used to induce the maltose system. This made it easier to examine substrates concentration effects since the adapting substrate does not disappear under the conditions of our test since it is not utilized. Using this system we determined the dissociation constant of the adapting substrate with the enzyme-forming system by measuring rates of adaptation at different adaptive substrate levels. These experiments indicated that the combining constant, K_s, was equal to 0.002. We then determined the combining constant of -methyl glucoside with the adaptive enzyme system by using it as an inhibitor of maltose utilization. The combining constant so measured was at least a thousand times greater if not more. Apparently this substance has a very low affinity for the enzyme induced. Unless there is some complication

which we are not aware of, it would appear from these experiments that the inducing substrate combines with something else other than the enzyme. It could, of course, be argued that enzyme-substrate combination still occurs but that when the enzyme is combined with the enzyme-forming system the dissociation constant with substrate is markedly modified. However, it seems to me that this would introduce an unnecessary complication. The peculiar feature is that the enzyme-forming system should have so much greater an affinity for substrate than the enzyme itself, but nevertheless this is the conclusion we are forced to draw. I shall be interested to hear about your experiments.

Other features of our work have progressed very satisfactorily in certain directions. We have continued to analyze the long-term adaptation to galactose and have performed the type of bud analysis which Ephrussi has used so effectively in his "petite" mutation. Our results which we are now preparing for publication completely confirm in quantitative detail the conclusions we drew from our analysis of the mass reversion phenomenon which we described in Proceedings paper. There is, however, one additional piece of information which was not contained in the paper which you have read. We had supposed that the distribution factor between parent cell and bud would be of the order of 1/10. Our detailed analysis of the kinetics of reversion led us to the rather surprising conclusion that this distribution factor would have to be $\frac{1}{3}$. This conclusion was confirmed quite directly by the bud analysis experiment. There are some obvious explanations for this which I won't detail in the present letter.

This long-term adaptation phenomenon has also been extremely useful in studying the "adaptin" problem since it turns out that the adaptin fraction can convert slow adapting cells to "fast." These positives, however, are not permanent and revert in the absence of substrate in the same way that normal positives do. The great advantage, however, is that the phenomenon of slow adaptation provides us with an excellent assay of the active material, much more accurate than anything that was available to us previously. It has now become possible to perform meaningful experiments which relate the active principle to its chemistry. Thus far, we have not found anything inconsistent with our earlier, though crude, conclusions about the nature of the active substance.

One other aspect of our work I believe you will find of some interest. We have found, surprisingly enough, that when a cell adapts to maltose it makes not only an enzyme specific for maltose but apparently a series of enzymes which are specific for synthetic -glucosides. These enzymes are indeed different and can be isolated and separated. What they are doing in the cell, I don't know. There are obvious possible interpretations involving the concept that the -glucosidase enzyme-forming system in attempting to make maltase also through "errors" makes a whole series of specific -glucosidases. Thus far, we have been able to isolate two such highly specific enzymes and we are well convinced on other grounds that there may be indeed many more. It may well be that these series of observations are going to very profoundly alter our views about enzymatic adaptation in general

I have just received Pollock's paper today and have not had the opportunity to go through it. I shall write to you subsequently about it.

I do wish it were possible for us to get together since I think that we are both, as has happened in the past, arriving at the same, more or less, general conclusions. I should deeply appreciate it if you would, when you find the time, send me your comments on our long-term adaptation paper. I know they will prove very helpful.

Sincerely yours,

S. Spiegelman

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