August 4, 1945

Dear John;

Enclosed you will find some comments on the data you were kind enough to send on to me. By now you have probably seen and answered the points and questions I raised.

Things are moving rather slowly with me, except for the laboratory. As I may have already told you, I have definitely decided to get back into a physiology department or one where I could feel freer to develop along general cellular physiology. I had a rather nasty experience at Wisconsin mainly because it was so unexpected. The head of the department and the next ranking member granted all my demands as to salary, rank and working conditions but the dean of the Med School there turned it down with these reported words; 'I have one Jew already on my faculty'. Apparently the department head was entirely unaware of the existing prejudice and got quite excited about it and went to the president, Fred. Under the circumstances I wouldn't accept any appointment in the Med. Sch. there,except the deanship. They are however trying to get me an appointment in the graduate faculty in some other school. There is one setup under Huskens new head of the Botany department I would consider but I am rather pessimistic and besides the whole business has left a pretty bad taste in my mouth.

Fortunately for my morale when I got back from Madison, I met Harrison who is dean of biological sciences at Chicago who told me that they were definitely interested in getting me up there and that I could expect a bid when things settle down there. By the way, did you know that they have recently Urey, Fermi and Mayer are away from Columbia and they are now going after von Neumann at Princeton ? As you know, we have acquired a new chancellor (none other than Compton) who is expected to make things hum around here when and if he gets here. They are talking of creating a biophysical division which has the support of Steinbach, Hamburger and the head of the physics department, Hughes. The latter is by the way the power on the campus now because of his friendship with Compton who trusts his judgment implicitly. But all this will take time, probably a year.

In any case I am sick and tired of scientific politics and sure would like to get to a place where I could shut the doors and windows of my laboratory and forget all about it. I am in an unfortunate position in that I cannot accept a minor position and everybody knows it. The next position I accept must not only be permanent but must also give me the opportunity to build a laboratory and personnel to push the problems I have been outlining in the last few years. Funny part about it is I can get all the money I want and in fact have had to turn down some in the past few months. Well, so much for that. I shall try my darndest to get us together in the same lab as soon as I can. Too much damn time is being wasted.

How are things with you ? What is happening to Visscher's department ? I noticed that he just lost two of his younger workers ?

Give my fondest to Buena and Isobel.

Yours,

Comments, answers, questions.

Many of your questions dealt with the peculiarities observed in following the R.Q. during adaptation. The fall obtained in the early period in undissimilated cells is probably due to a very small amount of a fermentable contaminant. That this would not be observed in dissimilated cells is to be expected. Such cells store over 80 % of any carbohydrate presented to them so that very little of this substance whatever it is makes its appearance as CO_2 via the fermentative system.

The use of the R.Q. alone in following the course of the adaptation is fraught with many difficulties. Crowding effects, the accumulation of incompletely oxidized metabolites, and other factors poorly defined influence the R.Q. even in cells when the complete fermentative system is present. Thus, suspension which contain less than 2 mg. dry weight per cc. show little aerobic fermentation (as far as the R.Q. is concerned) even with glucose, maintaining R.Q.'s of unity for long periods of time, which after about 4 hours begins to exceed one. A very informative and simple experiment to perform in this connection is to take the same culture and make up several suspensions of different densities (ranging from about 1 mg. to 10 mgs. dry weight per cc. and measure the R.Q. while metabolizing 2 % glucose. These experiments emphasize the necessity of always using the same densities when depending on R.Q. determinations and indeed if feasable more than one density should be run in an experiment. I have use the R.Q. extensively in helping me to determine when adaptation has occurred but these were always checked by a measurement. And in determining rates of adaptation the only safe thing to do is to take samples at intervals and run anaerobic CO_2 curves. It is more laborious but you are far more certain of what you are measuring.

The inverse Pasteur phenomenon you have observed is apparently not a general concomitant of the synthesis of additional fermentative enzymes. I have not seen it occur in any other strain but the one which you are using, which by the way is not a cerevisiae but is Saccharomyces carlsbergensis. I sent you that particular one because it can adapt in nitrogen and seemed more suitable for your heat measurement experiments. What the explanation is I for the life of me cannot figure out. The first thing one would think of is a competition for some common substrate at the point of divergence of the two enzyme systems and since the fermentative systems is more effective in binding substrate it becomes successful. But as you can see there are several objections to such an explanation. One would, in the first place, expect to find the phenomenon much more widespread than it is. In the second place, fermentation per se gives rise to substrates which are perfectly utilizable by the oxidative systems and for which the fermentative enzymes would not compete. It may be that in this particular strain the oxidative mechanism is particularly sensitive th inactivation by the products of fermentation. Why not try a little alcohol for its effect on oxygen consmption in this strain?

Your experiments with inhibitors and substrates are interesting and look promising. Again, however I would suggest that their effects on adaptation could be more surely tested if the is taken as the measure. I would include hydrolyzed ATP, i.e. adenylic acid in all experiments in which the effect of the former is being tested. In connection with azide, your lower concentrations were not employed in our experiments. In the literature 0.001 M is usually the lowest tried the more usual one being 0.005 M. In connection with these experiments there is one peculiarity which might be worth following. According to your fig. 14 the concentrations of azide could raise the R.Q. Of what ? Certainly not the galactose metabolizing system. The fermentative system for this sugar does not as yet exist in these cells and so a 'pasteur phenomenon' cannot be invoked. If the cells you used for these experiments are undissimilated then I believe I have the explanation. Some time ago I tested the effect of cyanide, azide, and dinitrophenol on the nature of the dissimilation of the endogenous polysaccharide reserves. I did this because I knew that the latter two were able to prevent the deposition or synthesis of such reserves from

exogenous carbohydrate. I found that dinitrophenol and to some extent azide could somehow shunt the endogenous reserves into the fermentative pathway. Although I have no convincing proof as yet I believe that this is similar in nature to the onset of the fermentation of the endogenous carbohydrate in injured cells. Both the azide and the cyanide some way interfered with the maintenance of the glycogen as such, it breaks down and the fermentable hexoses can diffuse over to the fermentative enzymes. I think it would be worthwhile to repeat these experiments with both dissimilated and undissimilated cells with and without added substrate. The question could be easily answered.

The preliminary experiments on the products of galactose metabolism which you report are extremely important for the problem and I would suggest that you prosecute them with all possible vigor especially since you are in a laboratory where that kind of analysis is already fairly well established.

In connection with some of your speculations; it has always seemed to me very unlikely, and even more so now, that the adaptation involves any thing at the triose level e.g. pyruvate. After all, since the asymmetry of galactose is in the H ; OH position on the 4th carbon, the trioses you would get by splitting galactose would not differ from those which you would get by splitting glucose. It seems to me that the problem the cell has to solve in metabolizing galactose resides in the first two or at most three steps, i.e. until glucose-P is formed. Beyond that everything is the same. In the last few months I have been able to prove that an enzyme similar to hexokinase is formed during the adaptation. I have called it 'galactokinase' and it phosphorylates galactose only, no other hexose. I am trying to pin down the carbon position of the P group and it looks now like Galactose-1-P. These experiments will once and for all lay low the 'biochemical boys' who wistfully and vaguely looking for the accumulation of 'necessary intermediates' as the explanation of the adaptive period. I never could, and still cannot, understand the rational behind such reasoning. Don't they have any faith in their own concept of enzyme specificity ? Do they really believe that the same enzymes can handle galactose which ordinarily handle glucose without modification ? It is clear that in addition to the galactokinase at least one other enzyme must be formed which is probably an isomerase of some kind. The next step is going to be a great deal more difficult. Primarily I am not interested in the details of the biochemistry of galactose metabolism I agree with you however in that we must know them but I sure wish some of the biochemists would do that job for us. I believe that we have more important things to worry about.

One of the things that has me fascinated, due mainly to the devil in me, is the following; the galactokinase only appears in cells after adaptation has occured and does not occur in the unadaptable S. Ludwigii no matter how long it is grown on galactose as the sole source of carbon. How in the name of Meyerhof, Cori and all the other P-greates does the unadapted and unadaptable cell oxidize the galactose, which it does very nicely ? I gave a lecture up in Chicago to Evans' group and drove them frantic with this pretty problem.