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Madison, Wisconsin

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Dear Joe:

Welcome back. Of course, I wonder if you were able to find your way back since I understand that Syracuse is buried under 40 or 50 feet of snow. But assuming that this gets to you, having penetrated not only through the snow but through the pile on your desk, I just thought that you would be interested in an account of a new anticancer drug or group of drugs that Charlie Heidelberger gave at a seminar last night. He has apparently been collaborating with Hoffman LaRoche on the synthesis of these materials and they are now in the course of clinical trial.

The key compound in his study has been 5-fluorouracil, analogous to the bromo-uracil which various people have found to be an analogue of thymine in the synthesis of DNA. I think that Charlie went after fluorouracil on the supposition that this would be an even better analogue of thymine, but it turns out that he got interesting results for entirely wrong reasons. For example, I found myself that fluorouracil inhibited E. coli and that this inhibition was reversed not at all by thymine or thymidine but very efficiently by uracil. It thus appears that fluorouracil if anything corresponds to the uracil in RNA rather than the thymine of DNA. Charlie and his collaborators have done a number of studies with ascites tumors and other tissues and one of my own students did some work with E. coli which lead to the conclusion that a small amount of fluorouracil is actually incorporated into RNA and none at all into DNA. Fluorouracil has in fact been found to have rather definite anticancer activity, Charlie having even succeeded in curing a substantial fraction of mice receiving certain transplantable tumors. The response is quite different from one tumor to another as one might expect, but the results were good enough to warrant clinical investigation and I gather that fluorouracil may end up to be an agent of approximately the same limited but definite sort of usefulness as mercaptopurine. Like the other anticancer drugs, fluorouracil is extremely toxic, and as one of clinicians put it last night, its application may be more a matter of prolonging the "terminal state" than life itself. Of course, as in any clinical investigation, the trials with this new material have had to be confined to the most difficult risks and the not entirely satisfactory results perhaps should not be taken entirely at face value.

It looks as if fluorouracil has two points of action, or perhaps even three: (1) A small amount is incorporated into RNA. Whether or not this is deleterious to the function of the RNA is not known. (2) In some fashion, it inhibits the incorporation of uracil into RNA so that one would assume that inhibitory levels are in a synthesis at large will be blocked. This I suspect is the main carcinostatic reaction. (3) A small amount of administered fluorouracil does turn up as the fluoro analogue of dioxyuradylic acid. This fluoro dioxyuradylic acid is apparently analogous to the natural nonfluoro compound and seems to function in preventing the methylation of the uracil residue

in the formation of thymidylic acid. There is, therefore, with fluorouracil and especially with the dioxynucleotide itself, very substantial inhibition of DNA synthesis. This, to my mind, may turn out to be the most interesting part of the story. The report on the nucleotide was part fragmentary as they have had to prepare it entirely by isolation and they have not had large enough amounts to do very much with it. However, it is active in the inhibition of tumors and of the biochemical mechanisms I have just mentioned at extremely low dilutions of the order of 10^{-9} molar for the inhibition of incorporation of formate carbon into thymine, for example. So there may be indeed a very promising approach on this last point. Whether it will result in a selective toxin is of course another question that can be decided only by experiment.

These observations lead me back again to my hobbyhorse of substitutional chemotherapy. I hope I have made it clear that I thought that the cancer problem was the most intriguing point of application of these notions. For obvious reasons, one would like to try it out on a simpler system, for example, bacterial antibiosis. However, you would want to make the executive decision yourself whether to proceed step-wise or to jump right in into the cancer field. My explicit proposal at the present time is to use cancer cells themselves, in fact, the same tumor that you are using for test purposes later on as the source of your natural metabolites, and to subject these crude materials to the range of substitutional and modificational reactions that we have already discussed. If you had included a reaction that would result in the fluoridation of uracil residues, you yourself would have discovered the effects of fluorouracil that we have just described. Actually, there is at least some possibility that considerably greater selectivity might be discovered if you had substitutions of small di- or tri-nucleotides. We don't know very much about the penetration of such materials into cells but simply because there are a great many different ones there is the opportunity of greater differential activity with such materials. This is, of course, a half-way step to try to feed an entirely fraudulent gene to the target cell.

I am still not sure but that the bacterial system may not still be the more appropriate for pilot studies. There is every reason to pursue the analogy between bacterial and cancer growth in the first stages of which one cannot hope to find a great deal of selectivity. You can, however, hope to find a class of compounds and ways of producing them which can then be applied in a larger scope to your experiments with tumor cells.

I am, of course, anxious to hear how far the seeds that I am trying to drop are germinating.

To avert to a slightly different theme, I would like to know whether you do have any concrete plans with regard to the production of analogues of neuraminic acid. I have in mind particularly the nitrophenol derivative which I had proposed to you earlier as a likely good homogenic substrate for the neuraminidase activity of influenza and other viruses. The production of such a material would be a tremendously valuable tool in fundamental virus studies and in addition might have a rather wide application for diagnostic purposes. I think I need not enlarge on this much further than pointing out how desirable it would be to have a good sensitive chemical or enzymatic test for virus activity. Such a test could be made specific by virtue of the specific inhibition of the virus by antibody. Whether the diagnostic applications of

such a material would have a sufficient market to justify commercial interest, I do not know, but I have no doubt about the extent of scientific interest. If, therefore, you do not have tangible plans for the exploration, I hope you will let me know about it so that I can try to encourage other people to get into it. There are some possibilities, some of them being, in fact, commercial ones. Since this particular area is, as I have indicated, of marginal commercial interest, it is a little hard to predict what your reaction to that would be.

One of my colleagues here, Steve Kubie, has told me that he made a reasonably good preparation of nitrophenolgalactacide by the straight fusion reaction, simply fusing the two reactants without very much more fuss or bother. I suspect that the unreacted nitrophenol could then be extracted by ether or other measures that your chemical friends will know better about. The same procedure might just happen to work reasonably well with colominic acid as a source of the neuraminic acid residue.

Another trick I'd like to try out of my own curiosity sometime, would be to see if one can make nitrophenol conjugates of cell walls or even intact cells by similar methods with a view again to using such preparations as specific substrates for wall splitting enzymes. The only reason I bring this up is that you may sometime happen to have some nitrophenolated cells as a by-product of some other reactions along this line, and if any such come up I'd be delighted to see whether bacteria phage had any specific effect on such a substrate.

Joe, I wonder if it happens that you are a member of the New York Academy of Sciences? If not, I would strongly recommend it to you as the Academy puts on quite a number of symposia each year which deal with topics within your professional interest. I will be very happy to sponsor your membership, if you would be interested. The dues are $15 a year and you would certainly get a remarkable series of publications for it.

For example, I just had a notice that there is going to be a conference on "Screening Procedures for Experimental Cancer Chemotherapy" next March 13-15 in New York. Looking at the program, I would judge that this is one of the sort of meetings that you would be quite interested to attend yourself. I believe that in general these meetings are not open to the public except by invitation. As a sort of postscript, Joe, I wonder if you can add $30 to my next check on account of expenses for servicing the dictating machine, supplies, and a case.

With all the best.

Yours,

Joshua
s/ Joshua Lederberg

JL:ac

P.S. I hope you have been following the publications from Art Kornberg at Washington University, St. Louis, and his group very closely. The last word is that under certain conditions, some of his enzyme preparations are able to

incorporate unnatural nucleotides into DNA. As I think most people will agree this may ultimately prove to be one of the most promising approaches towards antiviral and anticancer chemotherapy. I would doubt that any part of it is quite ready for detailed commercial exploitation, unless you were in a position to do some very elaborate enzyme work indeed. But I strongly advise you to keep your eyes open.

That's it again.

Josh.