112 Ransom Rd.,
Falmouth, MA 02540.
(508)548-8903 (until Sept. 6).

Aug. 26, 1989.

Dr. Thomas D. Brock,
1550 Linden Dr.,
Madison, WI 53706.

Dear Tom,

Enclosed are the pages with corrections from the rest of your ms. In general, it seems to me that you are not as close to the more molecular, later material as to the earlier developments, and so you will find more suggestions than in the first batch. If I may take the liberty of suggesting something of mine to read (in addition to the reprints that I sent), the micro. text of which I am a coauthor has a good bit of history, and you might find the chapters on protein synthesis and on regulation, in e/3, interesting; in a few places my interpretation differs from yours.

My largest general criticism is that Avery is undervalued. Your postponing of transformation until after mating and transduction results in diminishing the importance of his discovery; and when you propose that transformation really contributed little to the advance of bact. genetics you fail to distinguish the importance of discovering the role of DNA from the limitations of the technique for further exploration. The fact that Avery had a strong aversion to speculating doesn't mean that he was really floundering or had no idea of the possible implications for genetics. The main reason his work didn't have the impact of Watson and Crick is that it didn't suggest a mechanism for gene function, nor did it automatically lead to a broad range of expts., as their discovery did. Perhaps the surprising thing is that his discovery didn't stimulate more people to get into studying the biochemistry of DNA -- the gap between the biochemistry and the genetics must have seemed insuperable.

Nevertheless, the smart people, some of whom you quote, rapidly recognized the significance of identifying the material of heredity. It was clearly what led Josh to quit med school and seek the same in a more amenable organism. You give Hershey and Chase a great deal of credit for making the role of DNA credible, but even before that the work of Taylor and of Hotchkiss -- which must be considered an extension of Avery's work, in the same lab -- had made it clear that transformation was not a peculiarity of type specificity but could be carried out with any identifiable gene. The Hershey-Chase expt. was definitely confirmatory, and quantitatively far cruder; it seemed so important only because of the reluctance of the phage people to concede that their logical approach had been upstaged by accident by a group of medical bacteriologists. If the H-C expt. had not been done it would have had little impact on the acceptance of the importance

of DNA, for within a year the Watson and Crick discovery would have clinched it for even the most extreme skeptics. I find it a bit odd for you to ask what would have happened without transformation, rather than asking what would have happened w/o Hershey and Chase.

Incidentally, since you enjoy digging out inadequately recognized priority in other cases, I would think it worthwhile to discuss why Avery's discovery didn't lead to a Nobel Prize, though he lived for 11 more years. McCarty's book discusses Mirsky's unfortunate role dispassionately, and I discuss additional factors in the BioEssays reprint that I sent you. I might add that in my opinion Avery should be credited with initiating bacterial genetics as well as molecular genetics, since he provided the essential breakthrough of gene transfer. I think you focus too much on the fact that the 1944 paper didn't bring this out clearly. Before then, what was reported about transformation had no hint of being related to genetics; the 1944 paper now made that connection reasonable, even though it was suggested only tentatively; and the fact that the strengthening of the evidence for gene transfer came gradually rather than dramatically does not diminish its importance (again, consider the springboard for Josh's work). On the whole, I think you give Griffith's confused interpretation more detailed attention than it deserves, relative to Avery.

I would like to see a little discussion of the strong parallel between transformation and generalized transduction (both requiring double <u>exchange</u> with part of the introduced fragment), compared with the several techniques for <u>adding</u> genes in a replicon. Transformation led to the discovery that plasmids and naked phage DNA also could be introduced, inefficiently, by a similar process, and so it was revived as an important technique by the recomb. DNA revolution. Terminology here is not uniform; in our text we decided to use transfection not only for phage DNA but for an intact plasmid, and transformation for introducing a fragment of naked DNA.

I was surprised not to see more on plasmids; their importance and their nature were well recognized before the time when you draw the book to a close. At least a section on the resistance plasmids would seem in order.

Two additional points about transformation. It might be appropriate to note that in Haemophilus the uptake of DNA is much more specific than in E. coli, and the two organisms also differ in how they treat the strands. This topic could be linked to a discussion of how transformation appeared at first to be an artefact (i.e. DNA creeping into a rare, damaged cell) but it turned out to be an evolved mechanism, since it has been shown to involve specific enzymes.

In the discussion of lysogeny there could be more emphasis on the importance of recognizing that immunity is not an additional consequence of lysogenization, as it first appeared to be;

instead, the same mechanism keeps both the prophage and any entering phage from initiating vegetative multiplication.

The discovery of transduction involved a bit of serendipity that is not widely recognized and might be worth noting. Moving from E. coli to Salmonella, Lederberg and Zinder continued to use double auxotrophs to eliminate the background of reversion to prototrophy, but they soon found that the process in this organism was usually limited to transferring one gene. If you look back at their paper you will find that they started with a double auxotroph for Phe and Tyr, assuming that these were independent mutations. But this was almost certainly a single, leaky mutation in the common aromatic pathway, for I had found that as mutants in this pathway turned up with increasing leakiness they lost successively the requirement for p-hydroxybenzoate (discovered later), PAB, and Trp; the leakiest were doubles for Phe + Tyr. The irony is that if L & Z had started with an honest double mutation they might never have discovered transduction!

Incidentally, I'm not sure that the picture of how transduction works, with an agent that is filtrable but could not be detected in the filtrate of either partner, comes across. The recipient carries the prophage and rarely releases infectious particles; when these infect the non-immune donor they give rise to a burst of phage; and these particles, returning to the recipient, occasionally transduce it (because immunity to reinfection does not prevent mechanical introduction of the DNA). Also, in discussing lysogenization I'm not sure you make it clear that a phage infecting an indicator strain initially causes vegetative multiplication in most cells, as in zygotic induction, while the rare survivors have become lysogenized and immune.

Finally, I'm attaching a possible footnote, on a piece of history that may be too obscure for you to feel appropriate to include; but on rereading my paper in the Henry Ford Symp. I think I had the problem pretty well figured out; Monod was then still a bit ambivalent, as you could find in the Disc. on p. 47. Monod's running away with all the credit is very similar to the overshadowed Vogel discovery of repression, which has intrigued you. Incidentally, Vogel worked in my lab, where I had begun to get interested in regulation, and we first reported on repression together, in an abstract. I foolishly did not pursue it further because biosynthetic pathways were still so profitable.

I hope you find my comments helpful. Incidentally, because of one late chapter the next ed. of the Davis et al. text will unfortunately be too late for classes starting this fall. The publication date is now set at Oct. 15.

Sincerely,

Bernard D. Davis

Possible footnote on permease:

Bernard Davis has provided the following:

Before Cohen and Monod began to work on B-galactoside uptake I had observed that Aerobacter auxotrophs for glutamate, which were blocked in citrate synthetase, could use citrate instead of glutamate in the absence of glucose but not under the usual conditions of testing, i.e. aerobic growth in the presence of glucose. Since the enzymes for using citrate were constitutive it seemed evident that the variable response of the auxotroph revealed induction of the formation of a transport system, and its repression, like that of B-galactosidase, by glucose.

Howard Green, a post-doctoral fellow whom I encouraged to pursue this lead, then showed that wild-type Aerobacter could metabolize radioactively labeled citrate, after a lag, in the absence of glucose but not in its presence: a typical diauxic response. The conclusion seemed inescapable that the organism formed an inducible and repressible transport system for citrate.

I reported these findings shortly thereafter at the Henry Ford Hospital Symposium (ref.) It may be difficult today to imagine how hard it then seemed to accept the idea of a variety of specific transport systems in the tiny bacterial cell. At that time Krampitz was having difficulty convincing microbial biochemists, and even Krebs, of the existence of the Krebs tricarboxylic acid cycle in E. coli, because the ability of extracts to carry out all its reactions left unexplained the inability of intact cells to utilize citrate. My proposal of inductive changes in a morphological entity such as a membrane would seem even more radical, and Dr. Green refused to risk his

reputation, as a beginning scientist, by attaching his name to this conclusion; I could therefore only acknowledge his contribution in a footnote to the data.

Monod (with whom I had worked for a few months) came through New York on the way to the same symposium and he told me of his discovery, with Georges Cohen, that E. coli could concentrate a non-metabolizable B-galactoside. In their initial, brief publication in the C. R. Soc. Biol. they did not choose between active concentration, which seemed too much to expect of a tiny bacterium, and stoichiometric attachment to induced "hooks" in the cell. Monod favored the latter, and I tried hard, without success, to convince him that the answer had to be a transport system. (Pappenheimer, in whose apartment we met, eventually pushed me out the door so they could resume work on a joint manuscript.)

At the symposium in Detroit I presented my strong conclusion, while Monod presented an ambivalent interpretation of his data. However, in the later, published version he had become more receptive to the idea of active transport. Subsequently, as this book describes, he and Georges went on to an elegant analysis of their system. Though I continued to work on citrate for a while this work was not fruitful, for it lacked a non-metabolizable analog that could be actively transported, and there was no background of genetic studies in Aerobaacter. Nevertheless, when Monod gave the Dunham Lectures three years later at Harvard, as a guest of the department to which I had meanwhile moved, I found it painful that he devoted a lecture to

"permeases" without mentioning my work on citrate. As in other episodes described in this book, he did not share important discoveries willingly.

Reference:

Davis, Bernard D. Relations between enzymes and permeability (membrane transport) in bacteria. pp. 509-522 in Gaebler, O. H. (editor), Enzymes as Units of Biological Structure and Function (Henry Ford Hospital Symposium). Academic Press, New York.

Tom: if you find this interesting but too detailed please feel free to use the material as you wish. If you use it as a direct quote I suppose I ought to see any altered version.