ACADEMIC MEDICINE

PRESENT AND FUTURE

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CYCLES AND FASHIONS IN BIOMEDICAL RESEARCH

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My task was to collect some of the threads comprising the fabric of fundamental biology and to comment on the health and medical applications thereof. As shorthand for that conception, the elaboration of biology and pathology from first principles of chemical and physical structure, I will caption it a reductionist or reductive model.

The starting point of my own thought was very well stated by Drs. Kennedy and Lehninger, who talked about the promissory notes that reductive biology had been tendering for a number of years. Dr. Kennedy quoted Dr. Charles Huggins: "Whose lives have been saved by a Warburg apparatus?" I suspect that is not such a difficult question to answer. My variant is, "How many lives have been saved in the last twenty years by the 'double helix'?"—an expression that stands as proxy for all of modern reductive biology.

In 1944 Avery, MacLeod, and McCarty reintroduced DNA to the

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biologists' consciousness. This development stood against the presumption of the prior two decades that proteins were everything: they were enzymes, and they were sources of such exquisite specificity in every other realm, why not in the genetic material as well? But as is well known, the experiments of these investigators gave the first and eventually irrefutable, direct evidence that genetic specificity resided in the chemical structure of DNA. In the brief interval from 1944 to the beautiful elaboration of the structure of the DNA molecule, the double helix, by Watson and Crick in 1953, thinking and experiments in biology were unassailably revolutionized. Little biological research today is not deeply informed by these conceptions.

Nevertheless, until just now, one might have sought in vain for important public health or specific therapeutic applications of that knowledge. As a geneticist, I would be the first to recall many important applications of chromosome and cell biology, e.g., the delineation of genetic syndromes and the further illumination of pathogenetic processes. Starting with Garrod's insights, the development of medical genetics followed soon upon the rediscovery of Mendelism in 1900. It is all the more paradoxical that hardly anybody's health for twenty-five years after 1953 depended on knowing that the DNA structure was a double helix. How can such a revolutionary and fundamental insight of reductive science have had such a delayed impact on our major health problems?

Today we are just beginning to see a flood of practical applications in the pipeline, and one or two have materialized. The molecular genetic prenatal diagnosis of sickle cell disease is one of the first medical applications that explicitly depends on the knowledge of DNA structure: Y. W. Kan's work is an epitome of the DNA revolution.

The biotechnology industries that are founded on recombinant DNA likewise depend on that reductive base. Even with appropriate skepticism about the pace of development of these industries in the next year or two, no one doubts the large number of forthcoming therapeutic innovations. Human proteins such as pituitary hormones, interferon, insulin—and many others today unknown—are accessible in no other fashion.

So the texture of my question has changed in the last few years—an authentic turning point in our perspective of history of this phase of medical science. Let me state it a bit differently: the phase of application having arrived, why did it take so long? or need it have taken so long? Some people think such a question is both impatient and petulant, but I think it ought to be addressed.

Over the last thirty or forty years of medical history, one can, of course, trace a host of important innovations. The whole style of medical practice has sharpened, and it is far more attuned to critical scientific inquiry. Physiological and metabolic inquiry, to understand disease process and

management of the care of the patient, from the informed perspectives from immunology and endocrinology as well, is a new common standard. Looking for more specific indicators, I have had some trouble trying to authenticate the most important specific changes in medical practice during that period of time. Once one gets past the antibiotics, which may be regarded as the culmination of the last prereductive era of medical science, it is hard to find a predominant single item in the modernization of medical care.

The use of steroids ranks high, despite caveats about iatrogenic complications. As is typical of many innovations, these complications now loom far larger than first expected. In any event, the initial discovery of the use of steroids in medicine, as with other advances, was closer to serendipity than reductionist planning.

My own conjecture is that one of the most important changes in medical practice is the management of the body fluids. I have had some difficulty, however, in getting quantitative data on the history of medical practice with respect to routine fluid infusion therapy. Few will question that this therapy has been a life-saving addition to the armamentarium, if only for the infantile diarrheas. Drinking saline water may in fact become an equally efficacious medical technology!

Water does not sound like a very sophisticated medical entity. There are a few things one puts into the water, but they are not particularly complex from a chemical standpoint, and I doubt that one would invoke reductive biology as the route of discovery in this field. But it is all the more reason to seek the different threads that have informed medical practice. We do lack the kind of critical history that would enable us to judge what has happened there, as well as in many other important changes in practice. Paul Beeson's comparison of textbooks of medicine is an indispensable way of looking at medical history; but it is almost too comprehensive, and few people will take on the assessment of the most important improvements. In my own view, we are seeing, in this decade, the completion of two cycles of medical science and practice. With the DNA revolution we are well into the third.

The first cycle rested on the scientific foundations of medical microbiology laid just a century ago. This was based on the specific recognition of germs as living organisms and as agents of disease: the methods that we owe to Pasteur and Robert Koch, the taxonomy of microorganisms, obtaining them in pure culture and identifying them as etiological agents, the development of vaccine prophylaxis, and antimicrobial therapy. This cycle represented a revolutionary scientific as well as medical finding. It took from 1880 till the 1940s and 1950s to approach an asymptote (Table 1).

We well know how mortality from infectious disease has changed since the turn of the century. While, indeed, much of that change can be

TABLE I

Three Major Cycles of Biomedical Progress

Cycle	Dates	Description	Developments
Infectious disease	1880–1940 . . .	Reductive—germ theory	Vaccines Antibiotics
Human physiology	1922–1980 . . . . . convergence 1980s	Reductive in aim; semi-empirical in practice	Insulin Cortisone Diuretics Psychotropics
Molecular biology	1944–1980	Reductive!	Enzyme inhibitors DNA diagnosis — Atherosclerosis Cancer Transplant rejection

attributed to larger cultural and social developments, it is not a question of "either/or"; and one can hardly dispute the importance of scientific knowledge about what is contaminating our water supplies, or about which vaccines would be effective. Our standards and expectations are much higher today. Even if, after earlier successes, the opportunities for rapid public health improvement are less today than sixty or eighty years ago, we do not want to stop now. Just think how deprived we would be if we had to rely on these very general measures of sanitation and vaccination, and were barred from the much-derided high technology of medical care.

The second cycle I would date to about 1922. It evokes the names of D. D. Van Slyke and J. L. Gamble, i.e., systematic application of human physiology and chemistry in medicine. Many of the specific interventions that are part of medical and surgical practice stem from physiology: the understanding of what the various organs of the body do and how they communicate with one another. Physiology, like much of biology, is informed by medical observations and vice versa. It deserves more honor than it now gets, judging from the departmental arrangements at many of our medical schools. Perhaps just because so much physiology has been incorporated in internal medicine, there is a structural problem fitting physiology as basic science into the organization of many medical schools.

If one then looks back at the variety of important introductions into management over this period of time and asks what the role of reductive science was, one can see that critical exploitation rather than initial discovery has been more significant. An outstanding example is seen in contemporary psychiatric medicine. One cannot describe the development of the now indispensable agents used in the treatment of schizophrenia and depressive illness as having stemmed in any way from a reductive model. Quite the contrary! The empirically demonstrated efficacy of agents like chlorpromazine and lithium then demanded the attention of investigators into the biochemical foundations of the mode of action of the drugs. Their discovery was empirical and preceded the neurochemical theory that is just now emerging.

It is a consequence of our successes against infections that now our priority health problems are heart disease, cancer, and psychiatric illness. The inherent intricacy of these problems, which are rooted deeply in the molecular and cellular structure of the human organism, outreaches the existing base of applicable scientific knowledge. This ignorance has frustrated the building of a theoretical program for the control of these killers comparable with the advances in the golden age of bacteriology.

This frustration is partly obscured by a number of valuable piecemeal advances in all of these fields, by the proliferation of high-technology diagnostic machines, and by the development of scientifically trained, sophisticated specialties to make these accessible to patients. This technological revolution has also carried a heavy price tag, and there now exists some political pressure for cost-reduction that would better be directed to benefit-improvement. The training of these specialists has been the main contribution of academic basic science institutions to today's "half-way technology" in medical care. As I have indicated, most of the important new drugs of the past thirty years have been discovered through empirical, not rational, procedures and in industrial, not academic, laboratories. Empirical as they were, these discoveries also depended on an infrastructure of scientific knowledge to calibrate how aspirin, chlorpromazine, or thiazides could best be employed. Equally important, a host of spurious remedies would be firmly planted in our medicine cabinets without the critical authentication of efficacy and safety that must be informed by the most rigorous scientific judgment.

In the exploitation of such new drugs, we have had to call upon every resource that science could offer, from the analytical methodologies to the critical frame of mind. We have had to organize new kinds of experiments, to define the proper scope of these interventions, to search for their side effects, and so forth. This ramification is, in a way, as indispensable as the initial discovery. It is reaching down toward the development of a reductive infrastructure for medicine, rather than having built on a deductive foundation for the initial discovery of these useful

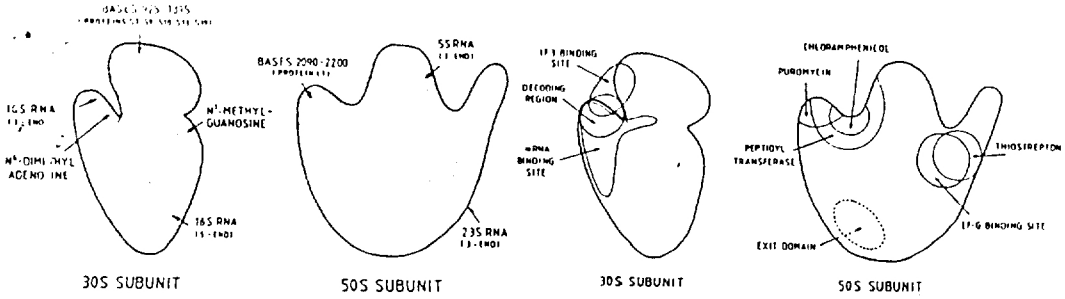
agents. With the exception of prenatal diagnosis, which did start from first principles of genetics and the cytogenetics, very few medical advances have been conceived from prior knowledge of the biology of the organism.

My question about the double helix relates to the third cycle, just at its zenith of scientific accomplishment and burgeoning potential for application. In times past, I might have leaned on the problematical structural relationships of basic sciences to clinical medicine, to account for the imputed delay. The question, it has become apparent, understated the complexity of the task.

To illustrate an essential cellular organelle, the ribosome of *Escherichia coli* (none of this my own work) is sketched in Fig. 1. These cartoons show the structure of the ribosome from four quarters. The important point is that the ribosome is composed of no less than 55 different protein subunits. Ribosomes tend to fall apart into a 30 S and 50 S major component. The *S*'s and *L*'s are on the two respective columns. At this point, every one of those has now been isolated. The amino acid sequence of the majority has been worked out, at least in some degree. Especially revealing is the self-assembly of this organelle: if you mix the different protein constituents with three molecules of specific ribosomal RNA, the ribosomes will self-assemble from these parts. Whatever magic is in the structural organization of the cell derives from the chemistry of its parts. But what complex chemistry!

The extraordinary effort that has been required in order to get to this stage of knowledge has involved: the mechanical labor of developing methods for the purification of these particles; the separation of their protein constituents in ways that do not chemically alter them; and the analysis of these particles, one by one, in order to determine their chemical composition—always in such a way that their biological integrity would not be degraded. Rather than being impatient about it taking from 1953 until now, one marvels that it has been possible to go that far in the molecular dissection of this very important particle.

So it was not enough to proclaim that the structure of DNA was a double helix and to learn the code by which protein structure was determined. That was the revolutionary opening of the door to a vast array of further investigations of the amazing variety of structures in the cell. From these, one can then expect to see a variety of applications in human pathology. We already know of genetic diseases of bacteria that result from mutations in different ribosome constituents. Environmental factors also influence ribosomal structure and function. Analogous human diseases are bound to become evident, following the same principles. Unfortunately, there remains a host of technical problems in trying to do the same thing with the ribosomes of eukaryotes. A few of the units have been found. The general structure of the ribosomes is not fundamentally different, but in this case, we must fish these things out of cells that have



Ribosomal Proteins

Proteins of 30S Ribosomal Subunits			Proteins of 50S Ribosomal Subunits		
Designation	Mol. Wt.	Binding	Designation	Mol. Wt.	Binding
S1	65,000		L1	22,000	
S2	27,000		L2	28,000	+
S3	28,000		L3	23,000	
S4	25,000	+	L4	28,500	
S5	21,000		L5	17,500	
S6	17,000		L6	21,000	+
S7	26,000	+	L7	15,500	
S8	16,000	+	L8	19,000	
S9	17,500		L9		
S10	17,000		L10	21,000	
S11			L11	19,000	
S12	17,000		L12	15,500	
S13	14,000		L13	20,000	
S14	15,000		L14	18,500	
S15	13,000	+	L15	17,000	
S16	13,000		L16	22,000	+
S17	10,000		L17	15,000	+
S18	12,000		L18	17,000	+
S19	14,000		L19	17,500	+
S20	13,000	+	L20	16,000	+
S21	13,000		L21	14,000	
			L22	17,000	
Sum	405,000		L23	12,500	+
			L24	14,500	+
			L25	12,500	+
			L26[c]	12,500	
			L27	12,000	
			L28	15,000	
			L29	12,000	
			L30	10,000	
			L31		
			L32		
			L33	9,000	
			L34		
			Sum	549,000	

FIG. 1. Ribosomal subunits of *Escherichia coli*, reproduced with permission of authors and publishers. The four illustrations are from H. G. Wittman. "Architecture of Prokaryotic Ribosomes," *Annual Review of Biochemistry* 52(1983):in press. The tabular material is from D. E. Metzler. *Biochemistry. The Chemical Reactions of Living Cells.* New York: Academic Press, Inc.,1977, p. 929. (A plus sign in table indicates direct binding to ribosomal RNA.)

a lot of aggressive enzymes, which tear things apart as soon as they are taken out of their normal niche.

I conclude that it is asking too much to expect reductive advances in medical practice until we can fill in the infrastructure between information that is in the DNA, and the way the cell is finally designed and built.

Without correctly assembled ribosomes, proper protein synthesis in the cells cannot continue. Ribosome assembly also presents an exciting challenge from the standpoint of its regulatory mechanisms. Here there are fifty-five different proteins, whose synthesis is precisely coordinated. One finds hardly any unassembled leftover constituents within the *E. coli* cell under a very wide range of conditions. Some of the protein constituents are able to turn off the synthesis of others at various levels, some at transcription and others at translation, and in that way the system is kept in elegant balance. The details of these interactions again involve intricate geometrical and physical patterning of the reacting macromolecules.

Our knowledge of this organelle is matched in some measure by what we know of how cell membranes and several other organelles are put together. However, the cell membrane is not a homogeneous, chemically consistent structure, and thus it presents still further challenges to elucidating its adaptations to the various roles it must play for different kinds of cells in their own circumstances.

Further glimpses into "complexity" come from work on a single bacterium, *E. coli*. Again, a very important part of the message is that in a comprehensive presentation, the details are unreadable. Figure 2 shows the *E. coli* genomic map as of two years ago. About 1,000 genetic factors have been identified in *E. coli*, each known well enough to admit the name of a protein or some enzymic or regulatory function. Most of the morphogenetic variants in the human species would not qualify so well, because of ignorance of the protein or regulatory process involved.

This map is organized into 100 intervals called "minutes," in the *E. coli* jargon. The reason for such a unit is that the process of fertilization, i.e., the transfer of genetic information from a male cell to a female cell, is rather prolonged in *E. coli*; it takes about 100 minutes for entry, from the beginning of the chromosome to the end. Jacob and Monod showed us how to use the time of entry of a gene for mapping. Finer methods which, in increasing measure, comprise the direct examination of DNA sequences are available today.

These hundred minutes of *E. coli* correspond to about 4 million base pairs: it would take about 1,000 pages of this book to inscribe them one by one. So far, we know sentences, here and there, adding up to about a dozen pages. We can infer from the local density of the map that the *E. coli* genome has sufficient information to encode about 5,000 different

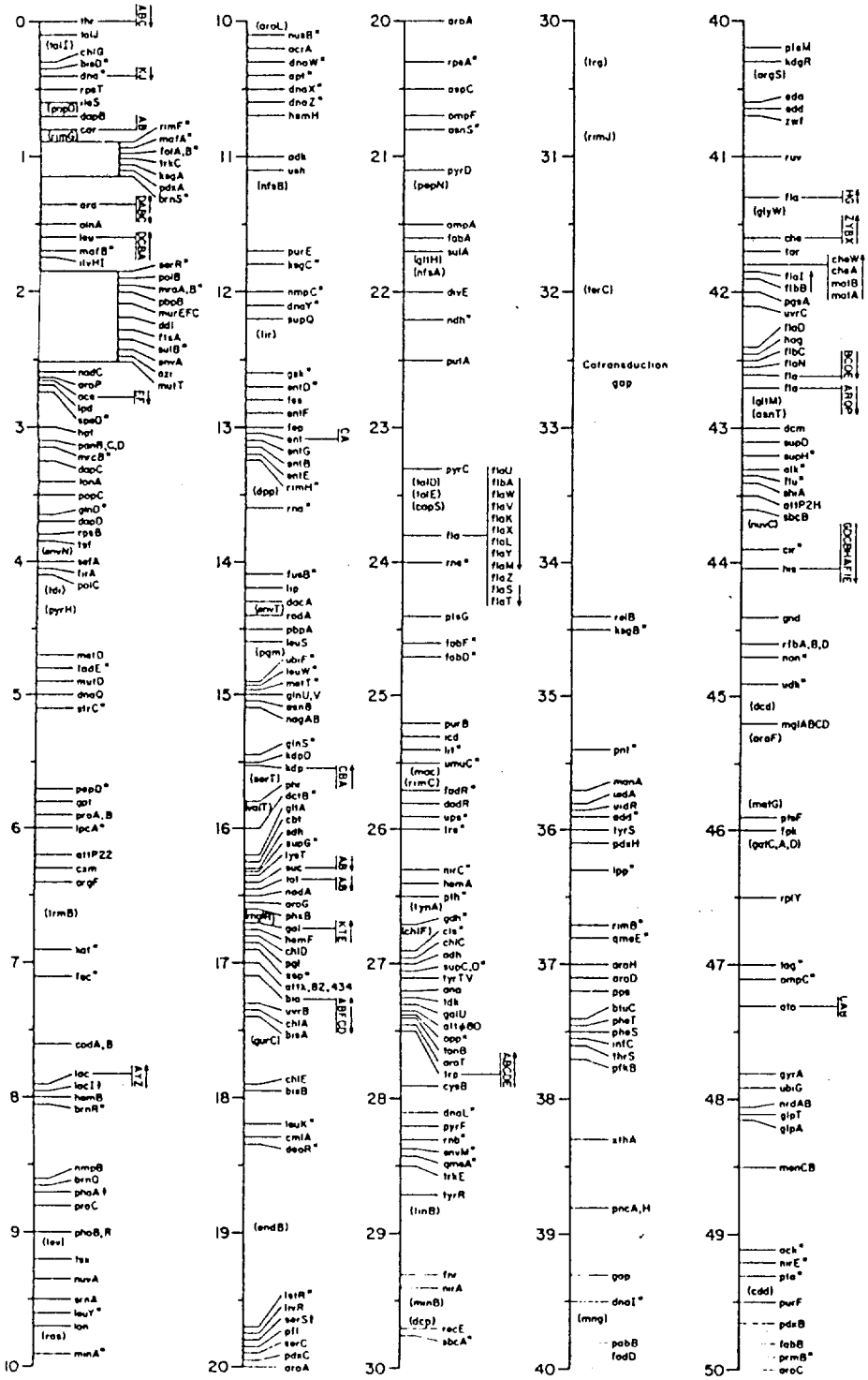


FIG. 2. Linkage map of *Escherichia coli* K-12. Reproduced with permission from Bachmann and Low, *Microbiological Reviews* 44(1980):1–56.

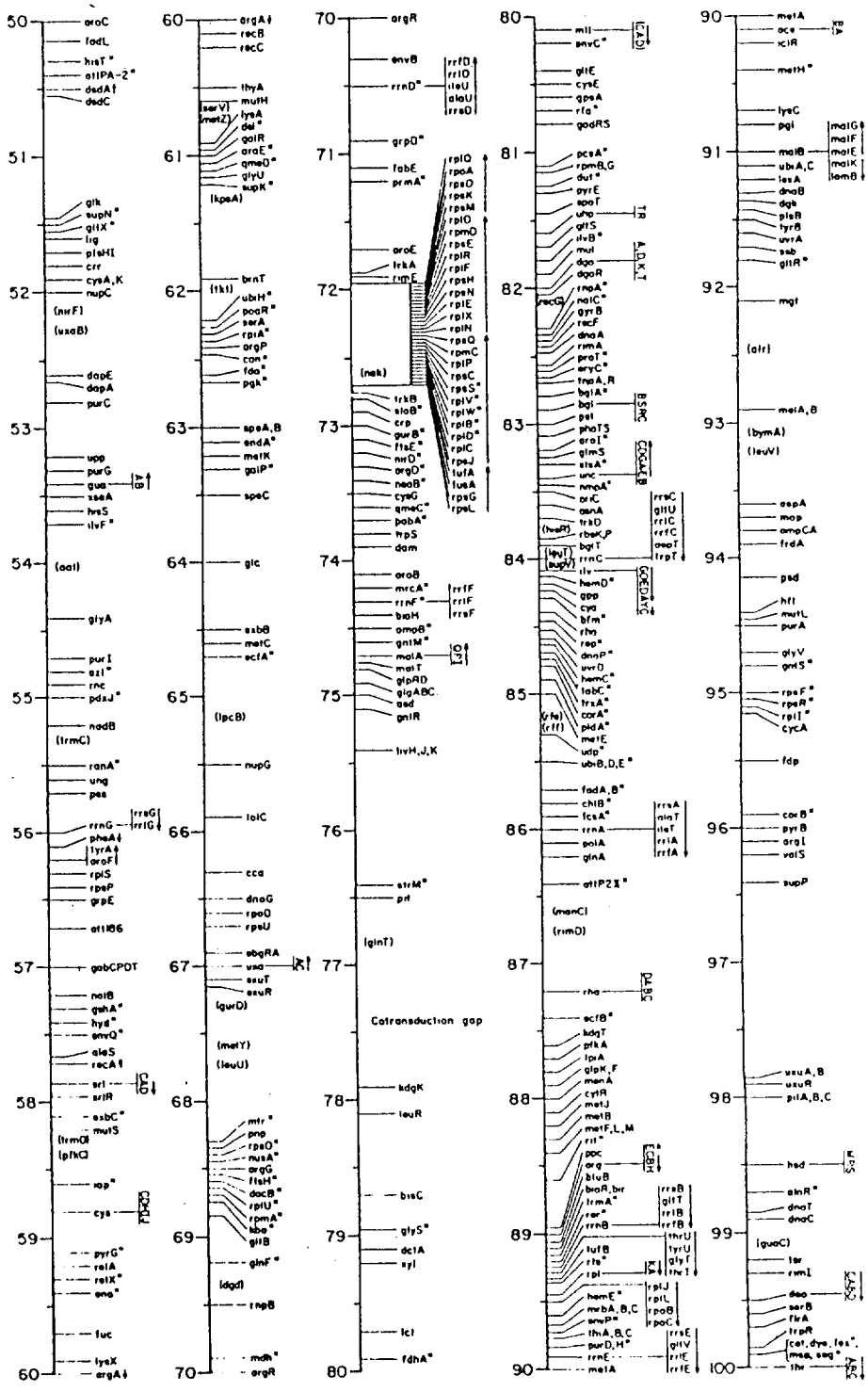


FIG. 2 (*concluded*)

protein chains. As I have indicated, about a fifth of those have now been mapped. The map also embraces about 100 known regulatory sites (there are doubtless many more). These are responsible for the rate at which specific genes are expressed. We know the sequences of some, and the picture is beginning to hang together. About 200 or so of these chains (generally 1,000 nucleotides or less) have now been sequenced, i.e., their DNA is fully known. These chains represent somewhat less than 1 percent of the map.

We might consider some other interesting objects whose complexity has been examined. Figure 3*a* shows the title head of a fascinating paper that appeared in *Nature* just about a year ago. There are almost as many authors as elements in the article—a reflection of the complexity of the enterprise they had undertaken. The paper itself is almost unreadable, but that is a compliment! Its main content is restated in Fig. 3*b*.

Obviously, print is an unsatisfactory medium for transmitting this sort of information. The figure is printed from a computer data base of DNA sequence data, courtesy of the SUMEX computer facility at Stanford University. As shown on the title page of the paper, the mitochondrial human genome comprises 16,569 base pairs. The polymorphism within the human species is already giving rise to some very interesting discussion about our ancestral lineages.

Study of the mitochondrial genome shows that there are 5 protein chains that have been previously recognized, and we know where these are. Eight other sequences also produce messenger RNA and putatively code for structural proteins, but we do not know what those are. There are 22 transfer RNAs, and there are two ribosomal RNA components as well. Thus, the structure of the mitochondrion is about half worked out in terms of the allocation of particular proteins, thoroughly worked out in terms of its DNA sequences.

Recall that the mitochondrion is about 30 seconds of *E. coli*, about a half percent of the size of the bacterial genome. Of course that means it is 1,000-fold less by comparison with the human genome! It is still not the most complex entity so far studied: phage T7 has almost 40,000 nucleotides, recently fully sequenced by J. J. Dunn and his colleagues at Brookhaven.

Here now the reductive program can be laid out. The human genome has about 3 billion nucleotide units in it. The DNA of each cell, when unpacked, is about two meters long, about the height of the person. If all that information were structural, it would encode for 10 million genes: the information content of the *Encyclopaedia Britannica*. These are large but quite finite numbers. Modern biology has given us an opportunity for the first time to measure the complexity of our challenge and examine the implications of the reductive strategy that has been so successful in

unlocking the fundamentals of living processes.

Most people now believe that about 1 percent of the genome is actively coding DNA. Hence, to get a reductive understanding of the human body, we must investigate about 100,000 different protein entities. So far, there are about 1,000 to which we could attach names. Of the ones that we can name, about 100 have been isolated from human sources. Talking about the amino acid sequence of a protein is proxy for a depth of understanding of the relationship of structure to function like the heading of a chapter, one for hemoglobin, another for collagen, and so forth.

To elucidate 100,000 proteins is then a $100 billion enterprise, with present day technology. That figure will be mitigated with further technological advances, but mere purification is already tedious and costly. Some proteins will be elusive, perhaps vanishingly scarce, although still very important in the economy in certain kinds of cells. We are now skimming the cream in terms of what is most accessible, abundant, stable, and so forth. We may wonder whether we will ever be able to afford to go through this entire reductive base. Regardless, does anyone advocate delaying further attention to specific medical problems until the reductive base is complete?

This measure of the size of the enterprise demands a sense of priorities as to which part of the landscape has the most important treasures. (We are not always going to guess right, because of the unpredictability of the insights that most rapidly lead to important applications.)

In this setting I am preaching to the choir about the need to promote better mutual understanding of the problems and methods of clinical observation and fundamental laboratory investigation. Part of the answer is the scientific training of clinically oriented people. The converse, I believe, is equally important but has been neglected even more: that is, the exposure of biological scientists to health problems. This should not be thought of solely as a way to accelerate practical results, although I believe it is an indispensable part of that mission. The history of science is replete with examples of the testing of reductive theories by confrontation with facts and observations from nature, sometimes with revolutionary impacts on the narrowly structured models that science must use. Today's natural history is clinical observation: recall that Avery's work on DNA was impelled by his effort to systematize pathogenic strains of pneumonia, each of which demanded a unique vaccine.

Robust biological theory is an urgent requirement for our understanding of environmental hazards and for the establishment of economically viable policies of regulation. We face the perplexing challenge of predicting hazards to human health before they materialize; and this goal can only be realized with much more solid predictive methods with which to interpret laboratory experiments and translate these into quantitative

standards of exposure for regulatory purposes. To do this will require a vast extension of comparative toxicology as a biological discipline. In the long run, this application of reductive biology to preventive health may be even more productive than anything likely to emerge in therapeutic medicine.

Perhaps the greatest difficulty with the long-standing promissory notes is the extent to which they give rise to an underestimation of the abrupt

Sequence and organization of the human mitochondrial genome

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The complete sequence of the 16,569-base pair human mitochondrial genome is presented. The genes for the 12S and 16S rRNAs, 22 tRNAs, cytochrome c oxidase subunits I, II and III, ATPase subunit 6, cytochrome b and eight other predicted protein coding genes have been located. The sequence shows extreme economy in that the genes have none or only a few noncoding bases between them, and in many cases the termination codons are not coded in the DNA but are created post-transcriptionally by polyadenylation of the mRNAs.

FIG. 3*a*. Title head. Reproduced with permission from *Nature* 290(9 April 1981).

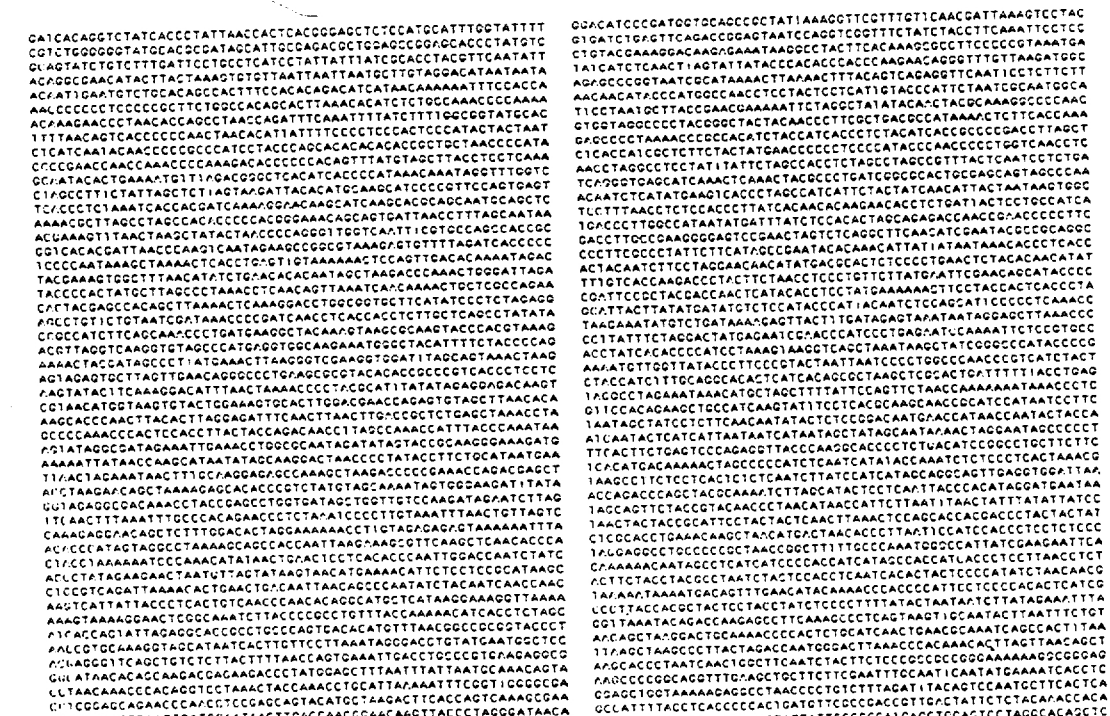

FIG. 3*b*. Sequence and organization of human mitochondrial genome. Reproduced with permission from a far more readable figure in *Nature* 290(9 April 1981).

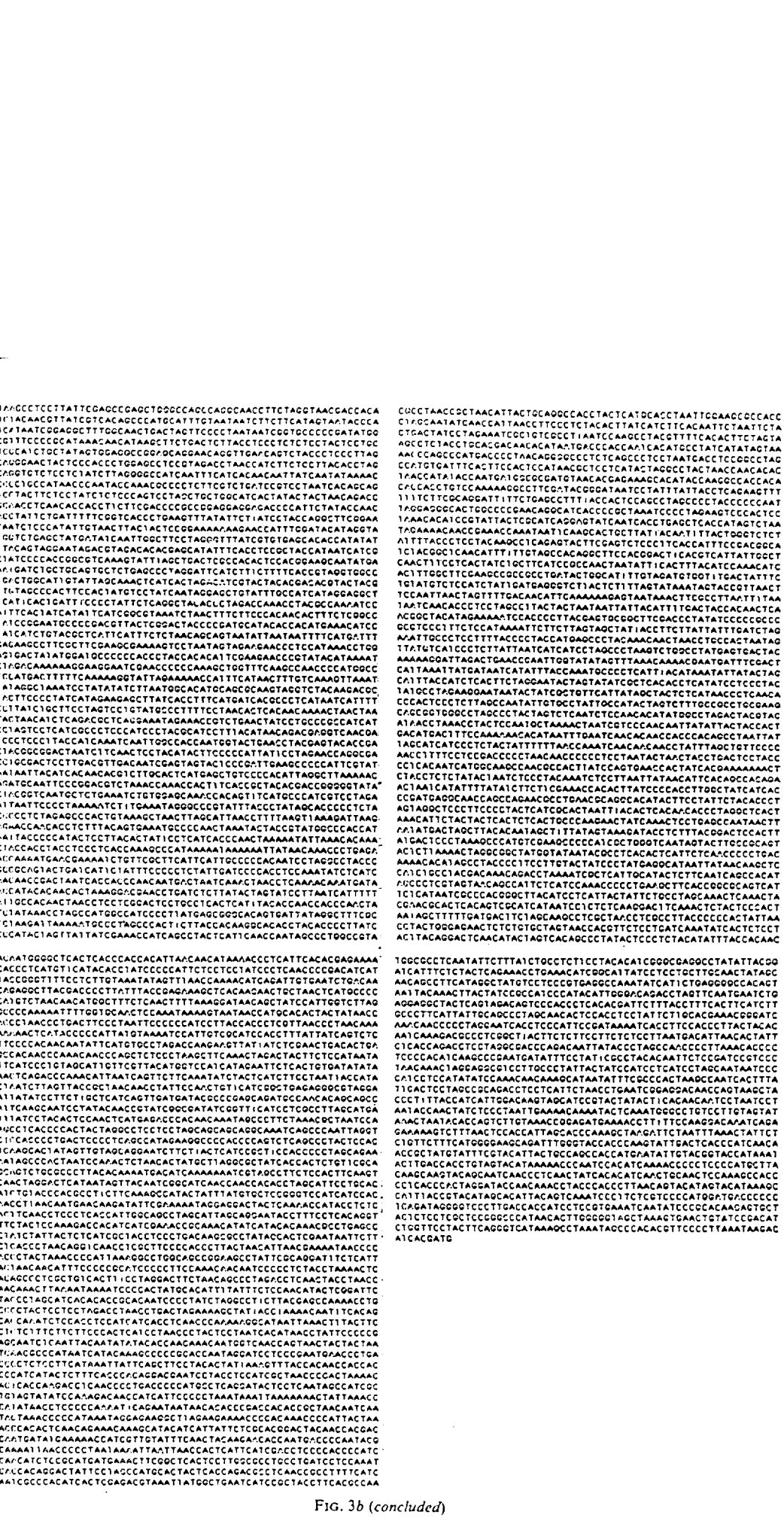

FIG. 3b (*concluded*)

changes in the human condition that will follow from the success of the third cycle in preventing the major threats of heart disease, cancer, and other constitutional diseases. This success is bound to engender many secondary problems: we are already facing an older population—and the dilemmas of work, retirement, and social security policy that then emerge. I have no doubt we prefer these problems to the miseries of premature bad health and disability; but even now they are swept under the rug.

Most of my discussion, and this conference, has centered on the health problems of the United States. Parasitic diseases, whose victims live mostly far away, have had disgracefully low priorities in this country's research efforts. This is the more tragic, for there is no more productive arena for authentic "technological fixes." Yes, that is a problematical phrase, whose problematics come from careless disregard of the social and political obstacles to innovation; but it is hard to see that anything but good would come from a vaccine for malaria or from the control of schistosomiasis or sleeping sickness. The application of reductive molecular biology to the organisms of parasitic disease is a fascinating challenge to a new band of "Microbe Hunters," and there is every prospect of successes to match those of the first wave of microbiology. Similar principles also apply to plant improvement. Despite the complexities that attend farming practices in underdeveloped countries, there will be enormous gains from the development of new crops truly better adapted to the agronomic circumstances of poor countries around the world. Population control technology must be even more sensitive to the human incentives and constraints to its adoption; even so, much fundamental work is needed to offer people better means to implement their intentions, day by day.

Our federal research grants system is supposed to be motivated in the long run by the payoff of the use of scientific advance for health applications. It is a paradox that the frantic hewing to the committed line of a grant, ever since (in the name of accountability!) the project replaced the talented person as the rationale for awards, works to frustrate the broadening of outlook of clinicians and scientists alike. Our research institutions—and these too are given short shrift next to projects in the priorities of funding—in principle could provide both shelter and cement for interlevel and interdisciplinary exploration. Our ability to make these provisions is being seriously eroded both by the general stringency of funding and by the particular ways in which it is administered. There is no easy way to retrench; but if our national aim is to bring our current third cycle to its most fruitful consummation, we will have to reform the ways in which the diverse contributors to creative insight and to practical development are encouraged to cohere.