Inadequacies of Neo-Darwinian Evolution as a Scientific Theory

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It seems to me worthwhile to begin my talk by offering a summary of the position I wish to present.

In the first place, Darwinism provided the program for a theory which made plausible an explanation of species without recourse to a *deus ex machina*. The notion that speciation is a continuous process governed by natural law was an attractive one to scientists. Certainly the continuity of evolutionary process has been amply demonstrated by the uses made of it in paleontology, taxonomy, in ecology and in natural history generally. However, the continuity of evolution does not demonstrate that natural laws are operative, for the laws are not known. It is as if some pre-Newtonian cosmologist had proposed a theory of planetary motion which supposed that natural force of unknown origin held the planets to their courses. The supposition is right enough and the idea of a force between two celestial bodies is a very useful one, but it is hardly a theory. It became a scientific theory only after Newton made explicit the description of the concepts of force, velocity, angular acceleration and the like and provided a quantitative description of planetary trajectories.

The notion of natural selection depends upon the empirically verifiable observation that offspring on the average resemble their parents more closely than they do the other members of the population, that individuals are not all the same; that all environments are not the same. Concepts such as natural selection by the survival of the fittest are tautologous; that is, they simply restate the fact that only the properties of organisms which survive to produce offspring, or to produce more offspring than their cohorts, will appear in succeeding generations.

The notion that the germ cells prescribe the properties of the phenotype which develop in a given environment would be true both for a Darwinian and a Lamarckian theory. Current knowledge suggests that the germ cell can be modified while still in the phenotype parent but the means of modification are of a special character, and nowadays the effects of the modifications are not predictable. So this modern genetic theory bears somewhat the same relation to the older theories of heritability that modern nuclear theory bears to classical atomic theory; the atom can be decomposed, but atomic theory still has its uses.

Any principal criticism of current thoughts on evolutionary theory is directed to the strong use of the notion of "randomness" in selection. The process of speciation by a mechanism of random variation of properties in offspring is usually too imprecisely defined to be tested. When it is precisely defined it is highly implausible. The issue of plausibility is central to my argument; namely that when reasonable assumptions are made concerning certain natural processes, together with the assumption of certain specific kinds of randomness in the variation of heritable properties, then other phenomena which are empirically observable appear to be highly unlikely events. As the Jansenist logician Arnauld of Porte Royale put it: "In some cases the likelihood of success is so slight that no matter how great the advantage or how small the expense, good sense advises against risking a wager. It would be sheer folly to bet even

ten coppers against 10,000 gold pieces that a child arranging at random a printer's supply of letters would compose the first twenty lines of Virgil's Aeneid."

I shall not dwell on the first two issues. They are hardly controversial.[1] However it may be worth mentioning that the mechanism of heredity by gene action is insufficient to explain observations which can be attributed to cytoplasmic factors; also the experiments of Sonneborn *et al.* (1) on paramecia appear to demonstrate a Lamarckian kind of inheritance.

In addition there is a recent report of work by J. Brun at Lyon (2) who has found that the nematode *Caenorhabditis elegans* can adapt to quite elevated temperatures if the nematode is given about 8 to 10 generations to adapt to each ½° step. Since the nematode is self-fertilizing, selection presumably cannot be invoked to explain a progressive adaptation. The major issue is the randomness of variation in phenotypic properties and in the precise definition of the space of these properties. It is hardly novel to point out that for very many properties precise definitions are exceedingly difficult to make. However modern genetics offers at least a few clues as to the relation of the space of genotypes to that of the phenotypes, and hence it provides a vehicle for making explicit definitions of random variation. Whether the current genetic dogma is correct or not is another matter.

The Chairman, DR. MEDAWAR: Do you mind my interrupting? For the sake of intelligibility, would you explain to the audience the sense in which you are using the term "space"? It is familiar enough to mathematicians but many of us may not understand it.

DR. EDEN: I am using "space" in two somewhat different but related senses. In the first place, I compute the cardinality of a certain set, that is the number of its elements. In the case of proteins, for example, the space of all proteins is used to refer to the totality of different sequences. I can write a chain of 250 amino acid residues in 20 letters, starting, let us say, with a chain of 250 glycine residues and ending with a chain of 250 valine residues. That is one meaning.

In addition, I would like to associate a metric with certain properties relevant to the problem of distinguishing one population of organisms from another. The totality of these measures I call a space. In other words, I can identify some point in this space with some organism according to the values of the properties I have chosen as coordinates in this space. This is the other use I have made of the word "space". When referring to the phenotype space, I believe I am using the term in essentially the same way it is used by population geneticists today.

I would like to say something about the phenotype spaces. Although I do have some biological competence, it is not in those fields which are closest to evolution, so I must tread with caution. It seems quite reasonable today to accept the postulates that there is a gene associated with each enzyme, that the genes are arranged in a linear string (except perhaps for some bacteria in which it is ring-shaped), that the linear string consists of a sequence of nucleotides, that there is a mapping from the nucleotide string to the amino acid string corresponding to some protein. For the sake of definiteness I will also assume that the correct mapping is that described by Nirenberg and his co-workers (3), although that is not essential to the main argument.

I would like first to make a few simple numerical computations which bear on the issue of plausibility that certain events can arise from random variation. I shall define a random variation by prescribing that every possible elementary variation is equally probable. Geneticists may object that the frequency of occurrence of point mutations is by no means uniform over the space of all possible point mutations. However, there is to my knowledge, no way of predicting the distribution of mutation frequency for an arbitrary organism so there is no reason to make any other assumption. Dr. Mayr has given as the definition of randomness of mutation, "It merely means a) that the locus of the next mutation cannot be pre-

[1] This assumption of general agreement was an error on my part as the discussion on this point indicates. However, the notion that neo-Darwinian evolutionary theory is incapable of disproof is not a novel one with me.

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dicted, and b) that there is no known correlation between a particular set of environmental conditions and a given mutation. It does not bring into question the facts that the probability of mutation is much higher at some loci than at others and that the number of possible mutations at any given locus is severely limited by the other mutational sites of the cistron and indeed by the total epigenotype" (4). Of course, the narrower the distribution for the probability of mutation, the less the justification for using the word "random", at least in the first sense, so that one may with equal validity see the process as essentially deterministic with the superposition of some "noise". If, in truth, mutational distributions are of this character, then in a probabilistic sense one can predict the next mutation. I may not understand Dr. Mayr's second point. It seems to me that one of the virtues of modern work in evolution is that it has gotten as far as it has in the face of such difficulties, so that it also seems to me of dubious value to raise the current state of ignorance to the status of a principle. It is my impression that studies on mutagens may well shed some light on this latter point.

Let us consider first the space of polypeptide chains of length 250 or less. We may think of words which are 250 letters long, constructed from an alphabet of 20 different letters. There are about 20^{250} such words or about 10^{325}. Let us compare this with certain other quantities, for example the number of protein molecules that could ever have existed on earth in organisms. Assume a biosphere of cells 1 cm. thick over the surface of the earth, a protein concentration in these cells of 30%, a density of 1, an age for life on earth of 10 billion years and an average lifetime of a protein molecule of 1 second. Of course all these quantities except density err very heavily toward the high side. The number of protein molecules that ever existed is by this computation about 10^{52}. Clearly the number of species of protein molecules is much smaller than this, say 10^{40}, but it would be immaterial to our purposes to try to make such a reduction. It is obvious that 10^{52} is such an infinitesimal number when compared with 10^{325} that we would be understating the case badly to say the space of protein molecules has barely been scratched. Yet this relatively small set of 10^{52} proteins contains within it all the useful proteins which have existed to date.

Two hypotheses suggest themselves. Either functionally useful proteins are very common in this space so that almost any polypeptide one is likely to find has a useful function to perform or else the topology appropriate to this protein space is an important feature of the exploration; that is, there exist certain strong regularities for finding useful paths through this space.

We cannot now discard the first hypothesis but there is certain evidence which seems to be against it. If almost all polypeptide chains were useful proteins we would expect that existing protein would exhibit very different distributions of amino acid residues. It is possible to find pairs of proteins which differ very markedly in distribution, but for the great bulk of known proteins, the assumption that they are samples drawn from the same population, as demonstrated by a simple chi-square test, is very plausible.

More specifically we may consider the α and β chains of human hemoglobin A (5). They contain 140 and 146 residues respectively. When the chains are arranged for optimal homology it is found that they agree in 61 places, there are 9 "gaps" and 76 places in which they differ. It is quite plausible to assume that one was derived from the other or both from a common precursor. If the Nirenberg mapping is accepted as correct, then 42 places required a minimal nucleotide change of one, 33 required two changes and one required three changes. Thus, at the least, the chain of events leading from α to β required a minimum of 111 point mutations, exclusive of deletions and additions, or 120 if we wish to include the "gaps". Yet if we look at the distributions of residues, they are quite similar, with a mean difference of about 1½ per amino acid type.[2] Certainly the

[2] The discussion as to whether point mutation or deletion and insertion was the correct mechanism for change in hemoglobin is irrelevant to the argument given here. In either case one would not anticipate that the distribution for the amino acids occurring in the α but not the β chain is very close to the distribution of residues occur-

coding constraints would not imply so good an agreement. Finally it may be noted that the distribution for those places which the two polypeptide chains have in common is rather different from either of the distributions of places in which the chains differ.

Dr. Wright has implied in his comment on my working paper that he regards the size of the protein space as largely irrelevant. He points out that the game of twenty questions can identify one point in a very large space of answers without bothering to examine most of the space. Of course, he is correct, but I believe he has misunderstood my argument.

Simply stated, there are some paths which lead fairly directly from one point to another in this space but there are many more paths of very much greater length between the same two points. The actual path-lengths traversed are limited by the number of generations in the organism's history so that the long paths are inaccessible, only the short ones can have been taken.[3]

I can illustrate this by another numbers game on the α and β chains of hemoglobin. Assume that 120 point-mutations lead between α and β by a unique path. By the Nirenberg code the average number of amino acids that can be reached in a single step (call this "of distance 1") is between 8 and 9.

Further assume a uniform distribution of point mutations anywhere in the length of 420 nucleotides, a mutation rate of 10^{-6}, and an average population size of 10^6. It is essential to the calculation that each step in this path correspond to a position on the fitness surface higher than all positions of distance 1 from it. Note therefore that we need to compute the expected time for 1 step to be taken and then multiply it by only 120. We must also make an assumption concerning the extent of selection pressure for each step. With the strongest possible selection pressure it would require 20 generations to convert the population from one level to another. On this basis we would expect on the order of 2,700,000 generations to be required for one hemoglobin chain to transform to the other. This is a little large but not implausible. We could reduce the estimate by changing the assumptions somewhat. However, the central point is that it is exceedingly unlikely that the trajectory on the fitness surface followed the shortest path. It is much more plausible to assume that the path meandered over the fitness surface seeking a higher level at every step, following neither the path of steepest ascent nor the shortest path. How long would the path be if we assumed some kind of "randomness" in the fitness surface? The mathematical problem appears to me to be a difficult one and I have no estimate to offer except that it clearly is many powers of 10 greater than the minimal distance of 120.[4]

Much of modern molecular genetics concerns itself with the mapping of phage and bacterial chromosomes. One of the striking results of bacterial genetics is the discovery that genes are organized into larger units under the control of an operator, with the genes linearly arranged in the order in which the enzymes to which they give rise are utilized in a particular metabolic pathway. Such arrangements, for which there is strong evidence, include the lac, his, try and cys operons. The fact that "supergenes" found in the chromosomes of metazoa are very difficult to decompose by recombination suggest that there may be some such order in the more complex forms of life as well.

So far as *E. coli* is concerned we might ask what is the probability that a rearrangement of unordered genes will organize certain sub-groups into operon clusters. Cuzin

[3] Dr. Wright has also taken issue with my related analogy to the writing of a library of books. I note that Dr. John Kendrew in his recent popular account of modern molecular biology, "The Thread of Life", uses the identical model. He writes: "It may be surprising that a random process like this can improve a species or even produce a new species, indeed lead eventually to the whole vast diversity of animal and plant life we see around us. But it must be remembered that these processes have operated over an enormous span of time, more than five hundred million years."

In this instance Dr. Kendrew has been misled by the attractive irrelevancy of the length of time available. Five hundred million years may be long in human terms; it is the blink of an eye in eternity. The length of time is relevant only when the probabilistic structure of events and changes occurring in this time are also known.

[4] If one assumes that insertion and deletion was the principal mechanism rather than point mutation, the computation given here is irrelevant. However, a new set of difficulties is substituted. Since more than one amino acid residue may be altered at each step, the path from one hemoglobin to the other hops over the protein space in wilder jumps, and yet at each successful jump the hemoglobin must have been biologically valuable. If the frequency of genetic modification is high and the density of useful hemoglobins in the protein space is also high, then one must explain why there are so few variants occurring in the blood of the species in question.

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and Jacob in Paris (6) and Beckwith and Signer at Harvard (7) have demonstrated that it is indeed possible to transpose segments of the bacterial chromosomes from one place to another by procedures that might well have occurred in nature.

If I understand the rather complicated procedure correctly, it involves two or more transfers of genetic material, an event of the frequency of a recombination, say 10^{-3} and two events which exhibit the frequency of mutations, say 10^{-6}.

Now in the experimental situation the geneticist can select for each stage in the process and need not wait for the occurrence of very rare events. However, each step in this chain, while it can occur in nature, does not of itself confer any selective advantage to the phenotype so that the individual steps are independent. To make a very rough estimate, a transposition of chromosomal material should occur in an unselected environment with a frequency of 10^{-15} for each sequential pair of genetic transfers.

Now the workers mentioned above have shown that the transposed segments are arbitrary lengths of genetic material. Thus on the assumption that the ring chromosome of *E. coli* opens at the uniquely appropriate place and that the episome provides the precise gene next in metabolic order, the probability of such an event, assuming a uniform distribution on chain segmenting, is 10^{-21}. Then to achieve a single ordered pair of genes on these assumptions would require something like 10^{36} genetic transfers. Sexual genetic transfer in *E. coli* takes about two hours and there are only about 10^{12} such periods dating from the beginning of life to now. Finally, genetic transfer between bacteria is a rare event. I have been unable to find estimates in the literature but I will assume that at any instant in time 10^{-6} of the bacterial populations are "mating". Thus one would need an average population of *E. coli* of 10^{30} (about 10^{13} tons or a layer on the surface of the earth two centimeters thick) if one expected to find a single ordered gene pair in 5 billion years.

I am well aware that such estimates are fraught with danger. A change in one of the biological parameters, a discovery of a new transposition mechanism can make such speculations an exercise in futility, but the point is that such estimates can only be overthrown by the finding of a new *determinate* feature.

As a matter of fact such a mechanism seems to operate in recombination, which after all involves the breakage of two pairs of DNA strands and a transposition of their pieces. Homologous sections obviously "recognize" each other (an essential and surprising fact that would seem to require a physical explanation), but there is no reason to assume that the ends of the recombinant fit. However, there is a way out, and I wish to thank Dr. Maurice Fox for calling this possibility to my attention. Since each recombinant strand is associated with a complementary one, the Kornberg enzyme can repair any gaps by "reading" the complementary strand. Without such a biological and deterministic mechanism the process of recombination would almost always lead to nonsense.

As a last numerical exercise, consider the following: The human genetic complement comprises about 10^9 nucleotides or about one nucleotide for each year since life appeared on earth. Because at some time or other there were no nucleotides, the average rate of accrual is about one nucleotide per year. Dr. Wright has objected that evolution should be reserved for biological phenomena and not for pre-biology, and I certainly agree with him. However, in this example I am not at all referring to pre-biology. It is immaterial whether we start with 1 nucleotide, 100 or indeed the 10^7 nucleotides of a bacterium. To increase from 10^7 to 10^9 instead of from 1 to 10^9 means the addition of 99×10^7 nucleotides instead of $100 \times 10^7 - 1$ nucleotides, not a great difference. If Dr. Wright is proposing that the notions of a naturalistic evolution are further restricted only to genetic events in which the change in total genetic length is negligible and perhaps incidental to the process of speciation, then indeed what I have said is irrelevant. Nevertheless it seems to me that most striking use has been made of evolution in studies of phylogeny; and if the chromosomes of the organisms of 2

and 3 billion years ago were as large and complicated as our own, so that the problem of assembling a meaningful ordered sequence of 10^9 nucleotides is pushed back in time to pre-biology, then the explanation not only of the origin of life, but also of the complexity of life is to be found entirely in physics or chemistry.

It may be that the formation of the nucleotides and amino acids are not biological questions, nor perhaps the formation of the first replicating entities, or the DNA, RNA, -messenger RNA, -amino acid code, but surely it is a biological and presumably evolutionary question to ask how new enzymes are created, new functions developed, how the complex may derive from the simple, or how a line of life may accumulate information.

I would like now to return to the description of the space of phenotypes. First, it is my understanding that the variations in phenotype induced by point mutations or deletions are frequently discrete and not continuous; eye and skin pigments, hair and vein patterns change drastically, enzyme function is markedly diminished or regulation changed. In the phenomenon of polymorphism and speciation generally, it is plausible to assume that recombination is much more important than mutations. Here too, the polymorphic manifestations frequently do not blend into a continuous scale. In other words, some values may simply not be accessible. There are two important consequences. First of all, discreteness drastically decreases the space of phenotypes; random variation takes on a more restricted meaning. Second, it leaves us with the problem of discovering which points are accessible without reference to selection, and why.

Mimetic phenomena have been widely discussed by natural historians. Consider the two-toned pink orchid and the two-toned pink praying mantis which is its mime. Here the mimesis is both in terms of form and of color. I am informed by Dr. Lettvin that the colors are metamers, that is, that the spectral distributions of the two pigments (animal and vegetable) are different but that they have the same hue and saturation to our eyes. It can hardly be that the mimesis was designed for human eyes; nor does this phenomenon seem to be an accident, because we are aware of many color mimetic pairs. It may be that a solution to this anthropomorphic view is that the *perceptual* color space is common to all organisms, as are the visual pigments of light-sensitive sense organs. This does not tell us how to define the two phenotype spaces which so resemble each other along certain coordinates, but it does suggest a reduction in the number of points needed in this space.

Finally, a word should be said about behavior. Much behavior is innate. Even those parts of behavior which are adapted through learning presuppose an innate mechanism for deciding what behavior is "good" and what is "bad" for the organism, as well as a mechanism for making inductive inference by correlating the organism's behavior with the organization of its perceptual world. Behavior is one of the key isolating mechanisms for populations and is modifiable by evolution. The ethologists have begun to provide structural models for certain aspects of behavior; but, in the main, behavioral dimensions are very ill-defined. We can merely say that it seems plausible to believe that very strong constraints exist as to the character and extent of behavioral variation.

Dr. Conant once commented that an incomplete theory is not discarded until a better one has been proposed. I cannot presume to satisfy that prescription. Nevertheless I would suggest that there are principles of organization to look for concerning which we are beginning to accumulate evidence. The helical non-integral screw symmetries in proteins and nucleic acids are repeated in larger structures such as the helical cylinder of tobacco mosaic virus protein, a protein that will organize itself into this structure even in the absence of the RNA core. In appropriate and unexceptionable aqueous media these structures are thermodynamically highly stable. A recent report in *Science* by Morgan and Uzman (8) described the packing of ribosomal particles of about 180 Å° diameter in the chromatoid body of *Entamoeba invadens* into long helical arrays. There is no physical principle known to me that predicts a very

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There is also great thermodynamic stability to be found in the tertiary structure of many enzymes. There is a high likelihood that complementary strands of DNA will "recognize" each other and form a double helix in correct register with no other biological material to mediate this recognition. The long range periodicities in reconstituted collagen and the synthetic formation of myosin fibrils suggest that physical or chemical explanations are appropriate.

I would also like to suggest that an opposite way to look at the genotype is as a generative algorithm and not as a blue-print; a sort of carefully spelled out and foolproof recipe for producing a living organism of the right kind if the environment in which it develops is a proper one. Assuming this to be so, the algorithm *must* be written in some abstract language. Molecular biology may well have provided us with the alphabet of this language, but it is a long step from the alphabet to understanding a language. Nevertheless a language has to have rules, and these are the strongest constraints on the set of possible messages. No currently existing formal language can tolerate random changes in the symbol sequences which express its sentences. Meaning is almost invariably destroyed. Any changes must be syntactically lawful ones. I would conjecture that what one might call "genetic grammaticality" has a deterministic explanation and does not owe its stability to selection pressure acting on random variation.

One concluding comment on randomness: Certainly organisms contain many built-in as well as learned ways for trying to survive. Many also have ways for enhancing the survival of offspring. Both tasks require interpreting information from the environment so that the genotype must specify that the phenotype be motivated to interpret the environment to this purpose.

The question as to whether the genotype receives information about its environment and modifies itself so as to improve the survivorship of the phenotype to which it may give rise, is answered "No" by evolutionists. I do not take issue with the empirical evidence in favor of this position. However a "Yes" answer does not require that one adopt a mystical or teleological principle. After all, computer programs can be written to do precisely this and sometimes so-called adaptive programs work very well.

I might tell you an anecdote in this regard. There is a good deal of work that has been done on game-playing with computers; and perhaps the most successful work has been done by Samuel on playing checkers. In the course of this work, what was required was to play many checker games, a person against the computer, which involved taking up a lot of human time, let alone computer time. Dr. Samuel, at IBM, had at his disposal a large number of computers; so rather than play against the computer himself, he had one computer play against the other computer and accumulated much more experience this way. However, there was a certain problem. For reasons best known to the computers, they made a decision at one time to lose the game rather than to win the game; and this is as difficult a task as winning the game. So the point is that what looks to us as motivation, what looks to us as teleology, need not be. Again, from the point of view of the computer, it certainly is not; the computer has no motivation.

On the other hand, every attempt to provide for "computer" learning by random variation in some aspect of the program and by selection has been spectacularly unsuccessful, even though the number of variants a computer can try can easily run into billions. Of course, the simple explanation may be that the computer programmers weren't smart enough to set up the problem right. It seems to me that an adequate theory of adaptive evolution would supply the computer programmer with the correct set of ground rules and perhaps some day it will.

[5] I was in error here. I should have known better. As Dr. Weisskopf pointed out to me, the occurrence of helices can be predicted on thermodynamic grounds. Briefly stated, a linear array of elements with regular attachment sites and with sufficient degrees of freedom at these sites will under predictable environmental conditions exhibit a surface free energy minimum in a helical configuration.

LITERATURE CITED

1. Sonneborn, T. M. 1963 in "The Nature of Biological Diversity". ed. M. Allen. McGraw Hill, New York, p. 165, Patterns and Stone.
2. Brun, J. 1965 "Genetic Adaptation of *Caenorhabditis elegans* (Nematoda) to High Temperatures". Science, *150*:1467.
3. Brimacombe, R., J. Trupin, M. Nirenberg, P. Leder, M. Bernfield, and T. Jaouni 1965 "RNA Codewords and Protein Synthesis VIII". Proc. Nat. Acad. Sci., *54*:954-960.
4. Mayr, E. 1963 "Animal Species and Evolution". Harvard U. Press, p. 176.
5. Ingram, Vernon M. 1963 "The Hemoglobins in Genetics and Evolution". Columbia U. Press.
6. Cuzin, F. and F. Jacob 1964 "Deletions chromosomiques et integration d'un episome Sexual F–Lac+ Chez *Escherichia coli*, K 12". Compt. Rendu Acad. Sc., Paris *258*:1350-1352.
7. Beckwith, J. R. and E. R. Signer 1966 "Transposition of the Lac Region of Escherichia coli I. Inversion of the Lac operon and transduction of Lac by 80". J. Molecular Biol., *19*:254-265.
8. Morgan, R. S. and B. G. Uzman 1966 "Nature of the Packing of Ribosomes within Chromatoid Bodies". Science, *152*:214-216.

Discussion

PAPER BY DR. EDEN

The Chairman, DR. MEDAWAR: Gentlemen, I would like to throw Dr. Eden's paper open for discussion, and there certainly is a very great deal in it to discuss.

I should like to open the discussion by questioning him on one particular point, namely, his allegation that the principles of evolution by natural selection are tautologous and, therefore, vacuous. I think that is mistaken. I would like to go through the points with Dr. Eden, if he wouldn't mind.

Supposing we start with the assumption, which is certainly true, that all the human beings alive in two hundred years' time will be descendants of human beings alive today. I don't think this can seriously be questioned. As I see it, the theory of evolution by natural selection would make the following two statements, neither of which is tautologous. The first is that human beings alive today will not take an equal share of being counted among the ancestors of the human beings alive in two hundred years' time. That is not a tautologous statement. For we might take a share of the ancestry of future populations that was strictly proportional to our numbers, but we don't. Some people will make a greater contribution than others. That is the first point.

The second point is that these inequalities in the contribution people make to the population of the future will be related to their genetic makeup. That is also not a tautology. That is all, so far as I know, that is contained in the theory of evolution by natural selection.

It is true that expressions like "survival of the fittest," which belong to a very elementary level of discussion, are tautologous; but we aren't really talking at this level. I would like Dr. Eden to say, are those two statements of mine tautologous?

DR. EDEN: No, certainly not. You misunderstood which statements I regard to be tautologous. Perhaps "tautology" is the wrong word. Perhaps I should say "definition." There are two empirically verifiable facts. No. 1, taken in the crudest sense, is that offspring resemble their parents. If you wish, you can put it in somewhat more modern terms in the sense that offspring represent the outcomes of the germ plasm of their parents. That is certainly an empirically verifiable observation.

The other observation is that there are events in life which cause certain organisms to live and certain organisms to die; and which organism dies before it produces offspring is a function of certain parameters of the world in which it lives. To that extent, it is fit or unfit to live in that environment. Of course, only those which survive long enough can produce offspring and some produce more offspring than others.

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, J. R. and E. R. Signer 1966 "Trans-
of the Lac Region of Escherichia coli
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Those are the two empirically verifiable statements and I believe that my second statement corresponds to the two statements that you made. Beyond that, there is nothing to be said about natural selection. There are no other rules; and that is why I used the analogy of the cosmologist, namely, there must be some rule of evolutionary behavior that can be tested. We can make certain observations that are empirical and, hence, we should look for those rules that can in principle be falsified by observation. This essentially what I have been trying to say. I do not believe that simply stating these two empirical events baldly is sufficient to claim that it is a theory. It is simply a redefinition.

The Chairman, DR. MEDAWAR: I think it is a theory. It couldn't be otherwise.

DR. ERNST MAYR: I just want to say that although these two statements are perfectly correct, they are both rather irrelevant to the theory of natural selection. The fact that offspring are similar to the parents in some ways is perfectly true but it has nothing to do with natural selection. The question is, which of the offspring will, in turn, have the greatest probability of having offspring? This is the core of the theory of natural selection, which does not depend on an individual's resemblance to its parents but on its own genotype or phenotype, which controls the probability of this individual leaving offspring. This is what the population geneticists define as fitness.

As to your second theory—that there are factors in the environment which control fitness, which contribute to fitness – this, again, is perfectly true but as a bare fact it is also quite irrelevant. You can imagine a continent without any organic life but with a great deal of variation in the environment; and yet this has, again, nothing to do with fitness. It is the interaction between the genotypes and phenotypes on one hand and the heterogeneity, the changes of the environment, which is the important factor in natural selection. So to summarize once more, the two statements which you made are totally correct but largely irrelevant.

DR. EDEN: At this juncture, I really can't say anything. So far I see no difference between what you said and what I have said. You have explicitly introduced heterogeneity in the environment as well as heterogeneity in the phenotype. Clearly that is an empirical fact. I don't know whether it is worth trying to carry this discussion any further right now. I have to think about what you said and perhaps read what you have written. But, to repeat what I believe I said: There is a certain continuity in the properties of the organisms which exist; the continuity is carried from parent to child, and each successive generation is presented with an environment, including the inanimate world around it, the physical properties of that inanimate world and other organisms. As a consequence certain of them survive and certain of them don't. Those that survive will continue to produce according to their kind, with variation of course. So, I am still puzzled by your statement that we are saying different things.

The Chairman, DR. MEDAWAR: I was puzzled by your saying that these were vacuous statements. They are not vacuous statements. They are so, each one of these statements.

DR. EDEN: No, no, those two statements are by no means vacuous; but they are not a theory.

DR. C. H. WADDINGTON: I am a believer that some of the basic statements of neo-Darwinism are vacuous; and I think there is a confusion here, possibly, about whether we are talking about Darwinism or neo-Darwinism. Dr. Medawar mentioned this phrase, "the survival of the fittest," and it is a very elementary, old-fashioned, long outdated concept; but, of course, this is what Darwin was talking about. By "fittest," he meant best able to carry out the functions of life, best adapted to some environmental situation and some way of life. By a fit horse, he meant a horse that could gallop fastest and escape best from wolves, or whatever it might be. That is a real theory which is perfectly capable of refutation.

What has happened to it since, in the process of turning this into a lot of mathematics, is that "fitness" has been redefined, leaving out anything to do with way of

life, simply in terms of leaving offspring. So the theory of neo-Darwinism is a theory of the evolution of the changing of the population in respect to leaving offspring and not in respect to anything else. Nothing else is mentioned in the mathematical theory of neo-Darwinism. It is smuggled in and everybody has in the back of his mind that the animals that leave the largest number of offspring are going to be those best adapted also for eating peculiar vegetation, or something of this sort; but this is not explicit in the theory. All that is explicit in the theory is that they will leave more offspring.

There, you do come to what is, in effect, a vacuous statement: Natural selection is that some things leave more offspring than others; and you ask, which leave more offspring than others; and it is those that leave more offspring; and there is nothing more to it than that.

The whole real guts of evolution—which is, how do you come to have horses and tigers, and things—is outside the mathematical theory. So when people say that a thing is vacuous, I think they may be thinking of this part of it, this type of statement. The sheer mathematical statement is largely vacuous. The actual way this is applied, not by the mathematical theorists but by the biologists working with the subject, is not vacuous at all.

DR. ALEX FRASER: I think, if I get correctly what you were saying, that there is a genotype space which is of almost infinite size, and that you can imagine restrictions being put on this by the physical universe. I don't think anybody would find this as being a new statement or one that is not perfectly acceptable.

I think what was missed, though, in your genetical argument, is the fact that the genetical system in itself, and in its evolution, is a process of restricting that space; so that the ensuing sub-sample starts taking on much higher probability orders, once the evolutionary process has started.

An illustration, I think, can be given in terms not of nucleotides but of whole chromosomes. There is an evolutionary process in Drosophila involving the order within the chromosomes, namely, different inversions; and as one looks at this it seems a most improbable business in terms of differing inversions being established. J. T. Patterson, I think, made the calculation that in any species of Drosophila over a few hundred generations 500,000 separate inversion events will occur; in which case, what is improbable is not that you have established inversion polymorphism in its various forms, but why hasn't there been a much greater variety of inversion polymorphism established?

The selection system takes sub-samples through this genotypic space easily. I have students working in 2^{30} and 3^{30} genetic spaces on a computer; and they take this as quite a normal process. What is surprising, to me anyway, is how many times you get the same answer coming out when you deliberately specify to the computer that it should not be restricted in its answer, when you have kept your genetic scheme as wide and as unspecified as possible.

The Chairman, DR. MEDAWAR: Would you like to answer that, Dr. Eden?

DR. EDEN: I think I would have to know more of the details. I agree with you fully that the mechanisms which have been proposed, whether they are recombination mechanisms or mutational mechanisms, certainly constrain the space. What I would like to find is the characterization of these constraints. Clearly, we have the evidence available to us, namely, that we are alive, and the evidence that life has developed to this state in a relatively small number of generations; so we have what a mathematician might call an existence theorem. There is some path by which we have arrived at this relatively small corner in this large space, on the basis of a relatively small number of generations. What I am claiming is simply that without some constraint on the notion of random variation, in either the properties of the organism or the sequence of the DNA, there is no particular reason to expect that we could have gotten any kind of viable form other than nonsense. It is the character of the constraint that makes things possible, not the variation. That is the point I have been trying to make.

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With regard to your experiments, I cannot comment without knowledge of your procedures. You are certainly right, you can work in spaces of 2^{30} or 3^{30}. We certainly cannot now, or, as far as I can tell, perhaps never, work with computers in spaces of 2^{300}. So in that sense we can't ever answer the question as to whether there are other domains in this tremendous space which are equally likely to be carriers of life.

What I am struck by is the fact that we seem to occupy a rather small corner of the space, as shown by the rather crude tests that I have been able to put on it, for example, the distribution of amino acids. What the characterization of constraints with regard to the distribution of amino acids is, I am not quite sure. Presumably, there are some that are purely physico-chemical. There usually are large numbers of glutamic and aspartic acids. Usually there are a large number of glycine and alanine residues, and so on. The range of charge variation in a protein is severely restricted, and presumably these are physico-chemical constraints. They should be looked for and taken into account and that is essentially what I am trying to say.

There may be other constraints as I have also tried to say. There may be constraints simply having to do with what I might call the syntax of the DNA chain. There is no question that the frequency with which mutation can occur is a function of the general organization of the gene or the chromosome in which a particular locus occurs. Again, I would presume that there are physical laws which control, but these laws need not necessarily be physical ones. There are biological mechanisms, biological repair mechanisms, biological error-correcting mechanisms, that have been identified and presumably they too act to constrain.

DR. V. F. WEISSKOPF: I am, of course, completely ignorant in this field but I thought we ought to discuss some of the special statements which struck me in Dr. Eden's talk rather than the philosophical statements about vacuousness. Discussions about the latter seem to me to be like medieval tournaments; you begin to offend your opponent before you really start fighting.

For example, there are two points which struck me and which I would like to have the experts explain. One was the business about the development of hemoglobin. It seemed to me to be the straightest way to come to the variations we find now. The straightest way is, of course, a very improbable one. Why did nature take it? The other point is the question of the groupings of genes. Is it not most puzzling that genes that are operationally similar are also located near each other in the DNA molecule?

DR. EDEN: Can I just repeat, both for the benefit of Dr. Weisskopf and the other people in the audience, what I said in both instances, at least as I reflect on my reading in this field. First of all, with regard to the DNA code, as you recall, the theory suggests that there are three consecutive letters in an alphabet of four types and that corresponding to every triplet of the possible 64 there is some amino acid. Suppose we were to change one of these letters in a sequence of three. Whether the currently defined code is correct or not is really immaterial. There are details that undoubtedly will be cleared up, but the code has been completely worked out. I can now make nine distinct substitutions, putting in any one of the other three letter types in any position. I can then ask, How many of the amino acids can arise from any particular triplet? It turns out, empirically, that almost all possible transformations lead to a different amino acid. The maximum number would be nine and, as a matter of fact, the average is about eight. It could be seven and my argument would not change very much; so that is the first point.

DR. WADDINGTON: Surely we can go along with this point.

DR. EDEN: The second point can be illustrated by the lac operon. It is simply saying this: If I intend to go from one protein type to another protein type through a long chain of single changes, as in the two subchains of hemoglobin, I can compute that it would require a minimum number of 120 changes.

DR. MICHAEL LERNER: I think that this is not right at all. I think most of the changes may be reading frame shifts. They are not substitutions; they are insertions or deletions.

DR. EDEN: The evidence seems to be against either insertion or deletion. I am sorry I don't have the illustrations here, but if you look at the two alpha and beta subchains within the hemoglobin, what you observe is that there are long stretches of sequence identity throughout the chain interrupted occasionally by a single amino acid difference. You have to open up the chain in certain places and leave room for one or two deletions or insertions; but there is a point-for-point agreement throughout the whole chain. Roughly speaking, one-half of the chain has not changed. This procedure may have gone by shifting, by misreading, by missing, etc.; but somehow all these insertions and deletions ended up with the two molecules looking very much alike when they are put in register. I find that implausible.

DR. LERNER: There is a very good reason for it; because for every deletion at one place you have to have an insertion somewhere else. Otherwise, you get only nonsense; so there is an automatic restriction.

DR. EDEN: Not necessarily.

DR. LERNER: Usually there is.

DR. EDEN: No, because in this particular case they happen to be of different lengths; so at least six times there must have been an addition or a deletion without getting nonsense.

DR. LERNER: But this is a whole triplet. I am talking about single nucleotides, not a triplet, just a single nucleotide insertion.

DR. EDEN: A single nucleotide insertion will change a single letter.

DR. LERNER: No, it changes the whole reading.

DR. EDEN: Yes, you are right; but I still don't see your argument.

DR. LERNER: You can't compute the number of mutational steps that have occurred on this basis because you don't know exactly what happens.

Question: If such phenomenon would have happened in a frequent manner, the change would be entirely different.

DR. FRASER: Not if there were a restriction in the hemoglobin, a constraint on some part of it but not much on the other part; in which case you would expect one bit of it to have the key function left, and natural selection is holding you to this; but reading changes can shift the other bit around.

How many mutation steps are involved in this at the present moment it is not possible to calculate. You can't make any statement of sets. If you are going to talk about nucleotides, then you should count the nucleotide changes. I think Lerner is quite correct—to make a statement from amino acid changes as to how many mutational steps there have been is not at the present moment possible.

DR. GEORGE WALD: I want to ask Fraser and Lerner why one doesn't find hemoglobin diseases in which this phenomenon you are talking about has occurred? Each hemoglobin mutation involves the replacement of one amino acid in the sequence by another, hence one nucleotide in the DNA sequence of the corresponding gene by another. I don't know of any instance in which one has yet discovered a hemoglobin with a long run of shifts.

DR. MAYR: Because it doesn't survive.

DR. WALD: All right, then it doesn't enter our argument.

DR. EDEN: On the other hand, we know of hemoglobin diseases in which it turns out that a perfectly adequate explanation is made simply by assuming that there was a single nucleotide chain in a single position in approximately half a dozen hemoglobin types. It turns out in this half dozen, or dozen by now, of hemoglobins, which differ in a very few positions, that each one of the transitions can be explained by a single DNA change.

DR. LERNER: I'm sorry, I still consider that irrelevant. You don't know how they arose; you only know that that is what they are. You know that there has been a substitution; but experimental evidence on mutations suggests that tautomerization is a very rare thing, that normally what you do have is insertion or deletion as a mechanism of mutation.

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DR. EDEN: In that case, I think I would conclude from what you are saying that my estimate of the number of changes required is very, very minimal. There is no way in which it can be smaller than the 120 I suggest. To reach the same end product by a sequence of insertions and deletions would require a vastly larger number of steps, which makes it worse.

DR. LERNER: No, the other way around.

DR. WILLIAM BOSSERT: For example, one could imagine a double deletion and a single deletion some distance apart. In fact, then, with two acts or three acts, you have changed quite a range of the chain, quite a range of the amino acids. So, it is not one chain per amino acid, in fact, but two acts, one in each twenty.

DR. EDEN: That is right, but I come back to what I said before: If we look at the two chains, the alpha and beta chains, what we find is that there are long stretches in which they agree and other stretches in which they disagree. Suppose there were changes in several places at a time; we would expect some transposition one way or another. It is an empirical observation that we do not find it.

You may very well be right in that it may not be possible to compute the number of changes necessary to go from one form to the other. I do not claim to have done so; I am computing a lower limit only.

DR. J. L. CROSBY: There is one point which I think one has got to remember. All the intermediate changes—or am I being extra simple—have got to be viable. It seems to me that you may be able to say that if your changes take place by reading frame shifts, we would need fewer of them. But, your reading frame shift seems to decrease enormously the possibility of intermediate stages being viable. This isn't a set of models we have got on a bench or a set of pretty pictures on a blackboard. We are going through a series of stages, each of which has got to be capable of existing viably, if it is going to have any possibility of giving rise to the next. We must bear that in mind.

DR. SIDNEY FOX: We have been vexed by one of the main problems that has apparently concerned Dr. Eden, the fact that such a very minute number of contemporary proteins exist against the background of the theoretically possible number of isomers. We are also provoked by the relatively high proportion of glutamic acid and aspartic acid which he has referred to. Some years ago, as an outgrowth of both of these considerations, we found it possible, under conditions that can be imputed to the geological environment, to combine all of the amino acids of proteins simultaneously in single polymers which have many of the properties of the proteins. The first syntheses occurred when sufficient proportions of aspartic acid and glutamic acid were heated with other amino acids.

At this point, I should comment on Wright's statement, cited by Dr. Eden, that prebiology has no relevance to evolution. If we accept that statement, we are in the position of believing in a discontinuity between pre-life and life. While such an inference may be defensible for prebiology and selection, I believe it is not for prebiology and evolution, unless one equates evolution to selection.

The most astonishing consequence of these studies of heating amino acids under the appropriate conditions is that the polymers produced have a markedly limited heterogeneity. This has been shown in many ways and it is outlined in the second paragraph of my working document. I think we should place this in apposition to another point of view, which is not well known, the observation first reported by Gamow, Rich and Yčas (Adv. Biol. and Med. Phys. *4*:23, 1956) that contemporary proteins, as judged across the entire panel of phylogeny, are close to random. This conclusion has also been drawn by Williams (Williams, Clegg, and Mutch, J. Mol. Biol. *3*:533, 1961), by Šorm (Šorm and Keil, Adv. Protein Chem, *17*:167, 1962) and by Vegotsky and Fox (in Florkin's Treatise on Comparative Biochemistry, Academic Press, New York, IV 1962, p. 185).

On the premise of a logical evolutionary span from pre-life to life and from preprotein to protein, the total picture is one of an evolution from a highly ordered primordial state to a considerably less ordered

state, when looked at purely from the standpoint of the protein.

This progression is in keeping with the second law of thermodynamics and contrasts with what I find often to be a supposition (Oparin, A. I. "Origin of Life on the Earth," Academic Press, New York, 1957 p. 185) that primordial protein was wildly disordered. Many speak also of order in contemporary proteins, when what is often truly meant is a biological repeatability of sequence rather than thermodynamic order.

This realignment of concepts leads us to some interesting new concepts. For example, variation in residue sequences in protein, for the entire gamut of organisms, is a basis for evolution. The necessary variety could conceptually be aided, or fixed, through the coding mechanism by nucleic acids. One role of the nucleic acids, then, would be to contribute to evolutionary changes by aiding randomization of proteins. (Pattee, H. H., Biophys. J., *1*:683, 1961).

DR. A. W. KOZINSKI: I have only one comment: So far as I understood your presentation, you have considered three parameters: first, mutation rate; second, generation time; and third, frequency of recombination. The frequency of recombination is what I want to reconsider.

I believe you have underestimated the frequency of recombination by assuming it to be stochastic, i.e., the successful recombination for different markers resulting from random meeting of two individuals.

It is important to introduce to computations another type of recombinant resulting in the formation of clusters of recombinants. There are numerous examples—transduction is the most obvious. I believe also transduction could play a very important role at early stages of primitive life on earth. In this system (transduction), a single individual might be a donor of perhaps 300 units of genetic markers at once *within one* generation of the organism.

I think that by introducing this into your calculation, one will eliminate the apparent paradox.

DR. EZRA SHAHN: My understanding of enzyme activity is that there are active sites, destruction or alteration of which would impair enzyme efficacy, which may be held in position by nonactive regions in which amino acids may be changed at will. It is conceivable that the protein configurations which are altered in the alpha and beta strands are of this latter type. If this is the case, and if I interpret your X's and dashes correctly, there are strings of such changes. It is precisely these which could be introduced by an addition and a deletion of nucleic acids. That is, two simple changes in a nonactive region of an enzyme could produce a whole string of changes. This would not require that each triplet be independently changed; and this total change within the strand would be able to occur without any impairment of function.

DR. EDEN: May I comment on that last statement? Your hypothesis is certainly an acceptable one; except, again, the evidence does not seem to be the case in the alpha and the beta chains. I have looked at the distribution of chain lengths in which these differ. It turns out that most of the chain lengths in which they differ are one unit long although there are some that are two, three, five and so on. The distribution of chain length differences appears to be random. If I had any way to compute what that distribution should be on the basis of some plausible model I would try to do so. The fact of the matter is, that there are many cases in which there is simply a single amino acid that has been changed with the contiguous sequences (left and right) being unchanged, and the two molecules themselves are very much in register.

DR. WALD: I think it is important to stick with this factual discussion a little longer; because we are likely to be spending a great deal of time with matters that don't have such clear facts associated with them. I want, then, to support what has just been said. We have, by now, a rather large material, involving hemoglobin mutations; none of which as yet exhibits this kind of phase shift that is being talked about. Phase shift is a possibility, but not yet found.

I want to add a further note which has a large bearing, I think, on Eden's discussion. Having been a little challenged by something that Simpson recently wrote, alleging that all changes in proteins are adaptive, I took a little trouble to find

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whether a single amino acid change in a hemoglobin mutation is known that doesn't affect seriously the function of that hemoglobin. One is hard put to find such an instance. Do you understand what I am saying? One talks as though there were at least long runs of the amino acid sequence that one could toy with freely, that don't matter very much. One is hard put to find a single instance in which a change in one amino acid in sequence does not change markedly the properties. The restrictions are enormous.

A third point, please, which is factual. I don't understand the basic argument involving these hemoglobins, or the cytochromes which have been worked out even better; because, in fact, on a very crude basis one finds that as you go back in phylogeny the number of such amino acid changes tends to increase in a quite regular way. If you make a rough estimate (and it is as rough as can be), it looks as if something of the order of 10 million years is needed to establish a mutation. That is, each of these single amino acid changes appears relatively frequently in individuals as pathology; but to establish one such change as a regular characteristic in a species seems to take something of the order of 10 million years. However, we have got the 10 million years; so I don't quite see the problem being raised in this regard.

DR. CONWAY ZIRKLE: Mr. Chairman, I wish merely to indulge in a little improbability, one that is at least as great as that cited by Dr. Eden. If we can assume, I think quite reasonably, that our parents were heterozygous for about 10,000 loci, we can see how slight the chances are that any one of us would have been born instead of some nonexisting brother or sister. The number of our ancestors also increases exponentially per generation back to a point where everyone probably is descended from everyone but, of course, in a different degree.

Now, what is the probability of any one of us being here in this room after the human race has been on earth for about one million years? I am convinced that the chances against any one of us having been born is practically infinite; and this forces me to accept a solipsism and to assume that this room is empty, except for myself, of course, and that the only existence any of you have is in my imagination.

DR. MEDAWAR: That would be a good place at which to end. However, we will continue.

DR. NIELS BARRICELLI: I would like to point out that if a mutation produces an extensive shift of reading frame in one direction or another, it most likely would be a very harmful or lethal mutation; but it would also usually be a recessive mutation which might not appear in any living organism. Still this could be the first step for another mutation which reduces the piece which is changed by the preceding mutation to a very small segment of the protein molecule. The result of these two mutations does not have to be lethal or harmful. It is perfectly consistent with present information to assume that such sequences of two or more mutations are very likely, particularly when we consider the abundance of recessive lethals in many populations.

The Chairman, DR. MEDAWAR: No more comments. Professor Ulam must now really have a turn. You will have an opportunity later perhaps to comment.