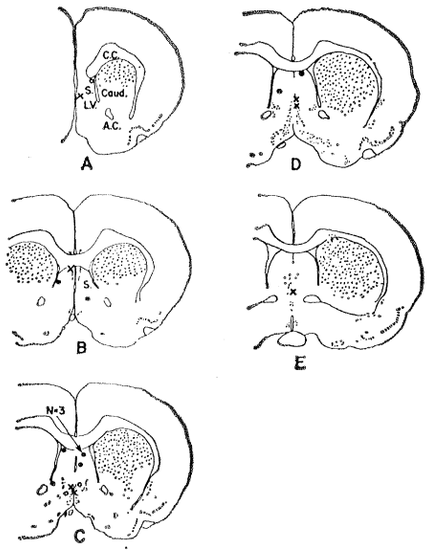

Fig. 2. Electrode placements for all animals whose temporal course of cardiac response to brain stimulation was studied. Stimulation points that produced heart rate acceleration are marked with *X*'s. Stimulation points for which the initial acceleratory phase was absent are marked with circles. Data are from three experiments (*1*, *4*, *5*). Arrow indicates three electrode tips with nearly identical placements. *S*, septal area; *C.C.*, corpus callosum; *Caud.*, caudate nucleus; *A.C.*, anterior limb of anterior commissure; and *L.V.*, lateral ventricle.

ference from laboratory to laboratory in the specific location of stimulation within the septal complex. All of the telencephalic placements of Meyers *et al.* "were found to be in either the medial septal nucleus or the parolfactoria area," whereas mine were located more laterally.

Figure 2 shows electrode placements for 20 animals: the three animals with clear changes in heart rate under the 20-second interval stimulation condition in experiment 3 by Meyers *et al.* (their fourth animal showed only slight heart rate change), 11 animals from an experiment by Kasper (*5*) in our laboratory, and my six animals. For this group of 20 animals the temporal spacing of brain stimulations was sufficient to study the temporal course (see Fig. 1).

In Fig. 2 the stimulation points marked with *X*'s yielded initial heart rate acceleration followed by deceleration. See, for example, the septal-stimulation curve for R49 in Fig. 2 of the paper by Meyers *et al.* (*1*, p. 1234). Stimulation at all other points (open and filled circles in Fig. 2) failed to produce initial acceleration. Closed circles represent cases in which stimulation produced initial slowing followed by compensatory acceleration. See, for example, the curve for subject 17 in my Fig. 1. Open circles represent cases of slowing without any very obvious compensatory acceleration (the curve for subject 1 in my Fig. 1, for example). As might be expected, in almost all these cases the initial slowing was less marked than it was in the animals showing the obvious compensatory acceleration (coming in between the initial and resumed slowing).

In the charts the stimulation points that produced initial heart rate acceleration (the *X*'s) form a cluster near the midline, whereas the stimulation points for which the initial acceleratory phase was absent (the circles) are placed more laterally.

These findings are of considerable interest in relation to Guillery's (*6*) anatomical work. Guillery has divided the ascending fibers in the medial forebrain bundle into two groups, the hypothalamo-septal group ending in the lateral septal nucleus, and the mesencephalo-septal group ending in the medial septal nucleus.

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References and Notes

1. W. J. Meyers, E. S. Valenstein, J. I. Lacey, *Science* **140**, 1233 (1963).
2. J. Perez-Cruet, W. C. Black, J. V. Brady, *ibid.*, p. 1235.
3. R. B. Malmo, *ibid.* **133**, 1128 (1961).
4. ———, *Can. J. Psychol.* **17**, 1 (1963).
5. P. Kasper, thesis, McGill Univ. (1963).
6. R. W. Guillery, *J. Anat.* **91**, 91 (1957).

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Universality in the Genetic Code

Hinegardner and Engelberg (*1*) have presented an argument to reconcile a universal genetic code with the possibility that its codon assignments are the product of "historical accident" (*2*)—that is, that the codon UUU, for example (U-uridylic acid), could very well have been assigned to any of the other amino acids rather than phenylalanine during the course of evolution. The argument used is the customary one—that any mutation which would lead to a change in a codon assignment would have such profoundly deleterious effects upon the cell that it would always be lethal; thus if all cells today are descendants of a single primordial cell line, all cells today should carry the same immutable set of codon assignments (*2*). This argument when made for a nondegenerate code—as it has been in the past—is rather powerful and compelling. However, when made for a degenerate code in the cells we know to exist today, it loses its force and becomes a rationalization. I feel it essential to emphasize here the weakness of this argument.

If it is assumed that a mutation can alter a codon assignment, then the point at issue is whether such a change would persist in nature. Suppose that codon *X* is initially assigned to amino acid *x*, and that a mutation occurs which results in the assignment of *X* to two amino acids, *x* and *y*, in a ratio such that $x/y=b$ [in cases where the parameter b is neither very large nor very small, the codon assignment is properly ambiguous (*2*)]. Concerning such a mutational change in codon assignment Hinegardner and Engelberg state:

> It is hard to imagine any circumstance under which a selective advantage would be gained by the random placement of certain protein amino acids. In fact, a change of this kind would almost certainly have large scale deleterious effects on any organism and therefore the change would not be perpetuated.

On the validity of this statement rests the power of their whole argument. But it is indeed possible to conceive of reasonable circumstances under which such a mutation might well have a selective advantage. For example, suppose that such a mutation, by changing the translation of an existing RNA message, led to the production of a new enzyme function—perhaps one not attainable by an ordinary, one-step mutation. It is reasonable that, in certain environments, the survival of a cell might depend upon this particular enzyme function. In this situation, any inefficiency introduced by an ambiguous codon assignment might subsequently be removed by a series of ordinary, one-step mutations in later cell generations. The end result could be a cell line whose codon assignment had been changed from *X-x* to *X-y*. (It has been tacitly assumed that amino acid *x* had initially at least two related codon assignments.)

Moreover, the occurrence of such an ambiguous codon in the cell does not necessarily have "large scale del-

eterious effects" in all cases. The degree to which a mutation creating an ambiguous codon would affect the cell is obviously a function of the frequency of the relevant codon in the cell's messenger RNA. Although one cannot argue convincingly that particular codons occur only very rarely in higher organisms, a case can be made for this possibility in the microorganisms. Here extreme DNA compositions are known—for example, 20 percent guanine + cytosine (*4*). In such instances it is entirely reasonable that certain codons might occur but very rarely (*3*), so that "large scale" or even appreciable "deleterious effects" would not surely result from mutations involving them. It should also be noted that the UUU codon might well represent an ambiguous assignment, as it directs the incorporation of both phenylalanine and leucine into polypeptides in cell-free extracts derived from many organisms (*5*).

The foregoing is not intended to be a comprehensive treatment of the problem. Much evidence supporting this criticism—involving suppressor mutations—has not been included, and the alternative viewpoints and explanations of universality have not been discussed (see *6* for such a discussion). However, I think that my main thesis has been demonstrated—that it cannot be proved or even made to appear likely that codon assignments (once established by historical accident) are immutable. We simply do not know enough at present to decide this issue.

To conclude, the whole question of *why* the particular codon assignments observed today exist—that is, the question of what mechanism underlies them or whether *any* does—is at present completely open. Now if at this juncture one uncritically accepts the argument reiterated by Hinegardner and Engelberg as valid support for the null hypothesis (that codon assignments are merely the product of "historical accident"), then one is disinclined to think and experiment constructively on the problem of the universality of the genetic code.

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References

1. R. T. Hinegardner and J. Engelberg, *Science* **142**, 1083 (1963).
2. F. H. C. Crick, *Progr. Nucleic Acid Res.* **1**, 163 (1960).
3. C. R. Woese, *Gen. Elec. Res. Lab. Rept. 62-RL-2993G* (1962).
4. C. Schildkraut, M. Mandel, S. Levisohn, J. Smith-Sonneborn, J. Marmur, *Nature* **196**, 795 (1962).
5. R. Sager, I. Weinstein, Y. Ashkenaze, *Science* **140**, 304 (1963); G. von Ehrenstein, *Cold Spring Harbor Symp. Quant. Biol.*, in press.
6. C. R. Woese, *Intern. Cong. Sci. Unions Rev.* **5**, 210 (1963).

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We do not think Woese's criticisms vitiate the arguments presented in our report; nor has he adduced any evidence that contradicts our basic premise.

Nowhere in our paper did we say that our arguments were restricted to a nondegenerate code. Our conclusions and the supporting arguments are essentially independent of the number of codons representing a given amino acid (provided this is a small number). Any mutation in the adaptor-RNA amino acid complex corresponding to a given codon potentially results in the alteration of every protein molecule in which that codon specifies the location of an amino acid. In all but very simple organisms these alterations will have the consequences we have previously discussed.

Woese's postulate, that a beneficial new enzyme can be established after a code mutation leading to the ambiguous placement of two amino acids, is fundamentally unsound. Such a mutation would actually have a variety of consequences. The gene corresponding to one particular enzyme would now synthesize many different protein molecules manifesting various degrees of activity. (For example, if the codon in question appears at five amino acid loci, and if two amino acids are randomly placed with equal probability at these loci, then 32 different structures will result.) These molecules will coexist in the same organism. In addition, comparable changes will take place in other parts of the organism. Because all these changes are random and occur in the same organism, no one enzyme can be selected. Therefore, the permanent establishment of a new enzyme by the mutation of the genetic code, as postulated by Woese, would be an incredible event having a negligible (though not zero) probability of occurring. That changes of reaction rates would occur as a result of such widespread changes is indeed an understatement. The organism would become less efficient, some enzymes would probably not function, and the overall integration of the organism's components would suffer. Again, we cannot say that this would never be beneficial, but it does seem highly unlikely.

In the event that a codon is very infrequently represented in an organism, a change in the genetic code is certainly possible. In fact, the general statement may be made that whenever a concatenation of events makes a code change probable, the change may persist.

Therefore, if it can be shown that some codons are rare in organisms with extreme (adenine + thymine)/(guanine + cytosine) ratios, these organisms might serve as experimental material to test whether the code is chemically determined. If exceptions to universality are found, then the chemical explanation as it is usually presented is ruled out, for the code must be universal if it is chemically determined. Whether or not leucine and phenylalanine code ambiguously *in vitro* (in cell-free extracts) does not bear on the present problem; in organisms this ambiguity is not apparent.

Finally, we should add that a "proof" of the immutability of the genetic code based on biological arguments alone is of course impossible. Our paper only attempted to explain the tremendous stability of the genetic code and did not argue for total immutability.

We share Woese's hope that further investigation on the universality of the genetic code will not suffer as a result of our publication.

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