thoroughly with ether and 95 percent ethanol, and dissolved in pyridine. The digitonin was precipitated with ether and removed by centrifugation. The cholesterol was reduced to dryness in an air stream and dissolved in ether. The lower limits for activity were not investigated, but the digitonin-precipitated cholesterol induced oospore or sporangium formation at 0.2 mg/liter of medium. Digitonin alone was not active.

Leonian and Lilly (*9*) reported on a fat-soluble substance which induced oospore formation by species of *Pythium* and *Phytophthora*. They stated that the active substance was strongly adsorbed onto sterols, which, when crystallized repeatedly from hot ethanol, were inactive. The active substance distilled at 110° to 135°C at 0.1 mm-Hg and was active at 0.1 to 1 part per million. The digitonin-precipitated cholesterol was active in the same concentration range; thus its reproduction-inducing activity is probably not due to an impurity. It would be of interest to determine whether Leonian and Lilly's substance was a sterol derivative or a different type of compound.

Exogenous sterols are required for the growth of certain protozoa, a slime mold, and pleuropneumonia-like organisms (*10*). A sterol was found to initiate the aggregation of amoebae of the slime mold *Dictyostelium discordeum* Raper to form a multicellular unit, the first step in reproduction. It appears likely that sterols participate directly or indirectly in reproduction of microorganisms, and that those organisms unable to synthesize sterols require an exogenous source.

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References and Notes

1. J. W. Hendrix and J. L. Apple, *Phytopathology*, in press.
2. J. W. Hendrix, *Dissertation Abstr.* **24**, 1783 (1963).
3. R. Sakai, *Rept. Hokkaido Agr. Expt. Sta.* **57**, 1 (1961).
4. I am indebted to J. L. Apple, R. D. Lumsden, and F. A. Haasis for cultures.
5. Ether obtained from Mallinckrodt, St. Louis, Mo.
6. Nutritional Biochemicals Corp.
7. V. W. Cochrane, *Physiology of Fungi* (Wiley, New York, 1958), p. 372.
8. Sigma Chemical Co.
9. L. H. Leonian and V. G. Lilly, *Am. J. Botany* **24**, 700 (1937).
10. E. Heftmann, *Ann. Rev. Plant Physiol.* **14**, 225 (1963).
11. The author was supported by a National Science Foundation Postdoctoral Fellowship. Paper No. 1487, Journal Series, Nebraska Agricultural Experiment Station.

31 January 1964

Heart Rate Reactions and Locus of Stimulation within the Septal Area of the Rat

In the 14 June 1963 issue of *Science* there were two reports (*1*, *2*) dealing with heart rate changes accompanying brain stimulation in the rat. Both papers referred to my earlier report (*3*) of heart rate slowing associated with intracranial self-stimulation of the septal area in rats.

Perez-Cruet *et al.* (*2*) make the following statement. "Our findings on the slowing of heart rate during septal self-stimulation are in substantial agreement with Malmo's results despite the difference in methods of measurement in the two experiments." These workers determined mean heart rates for 10 to 14 periods of 5 minutes each, with brain stimulation and no brain stimulation alternating from one 5-minute period to the next; and they compared the mean heart rate for combined stimulation periods with the mean heart rate for combined nonstimulation periods. With my data, on the other hand, differences for single bar-presses were obtained by subtracting poststimulation heart rates from the prestimulation heart rates, and from these single difference values a mean difference was computed for each animal. Meyers *et al.* (*1*) in summarizing the results of their third study say, "the early effect of septal ICS is accelerative, but the late effect is pronouncedly decelerative." This conclusion was based on results from beat-by-beat measurements.

Although earlier I had not actually measured heart rate during the first second after stimulation, careful inspection of my tracings had not revealed this initial accelerative phase observed by Meyers *et al.*, and this impression gained from inspection was later confirmed by careful beat-by-beat analyses on six animals in my most recent ICSS experiment (*4*, p. 8). These animals were selected for measurement because they were the only ones with satisfactory respiration tracings recorded along with heart rate during septal self-stimulation.

The results of the beat-by-beat measurements for these six subjects are shown in Fig. 1. In no case did the heart rate for any of the first three poststimulation points on the curves exceed the modal level of the prestimulation curve. All curves show an initial drop in heart rate followed by what appears to be a compensatory rise (roughly in proportion to the degree of initial fall) with another fall, more prolonged than the initial one, coming afterward. Only in one case (subject 16) did the rising phase reach the level of the prestimulation curve. In this subject, the rising phase actually exceeded the prestimulation level at two points; but the falling phase after this rise was extremely marked, and the overall main effect was clearly one of heart rate slowing. In short, in no case was initial heart rate acceleration observed.

Clearly, the problem was to discover the reason for the different findings in the two laboratories; and after an exchange of detailed histological findings, it now appears very probable that the different results were obtained because there was a consistent dif-

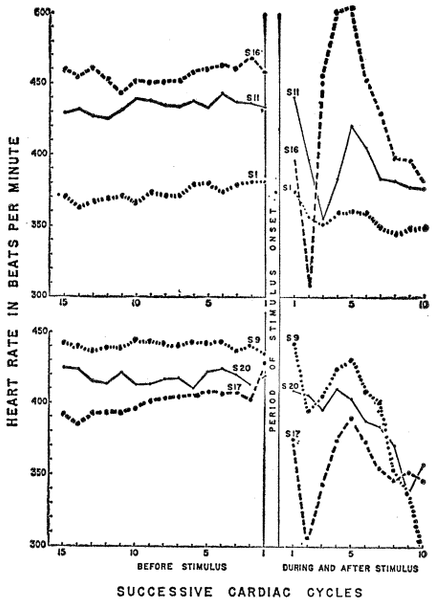

Fig. 1. Temporal course of cardiac response. Data are from six animals in experiment 2 (*4*, p. 8). Averages are based on all available data for these animals (that is, all artifact-free tracings for bar-presses during five 10-minute sessions and one 5-minute session, in which there had been at least 8 seconds between the beginning of the stimulation in question and the termination of the immediately preceding stimulation). Period of stimulus onset: the one cardiac cycle (that is, the distance between the peaks of two successive QRS waves) in which onset of 0.5-second septal stimulation occurred. Since this cycle was a mixture of pre- and poststimulation, it was eliminated from measurement.

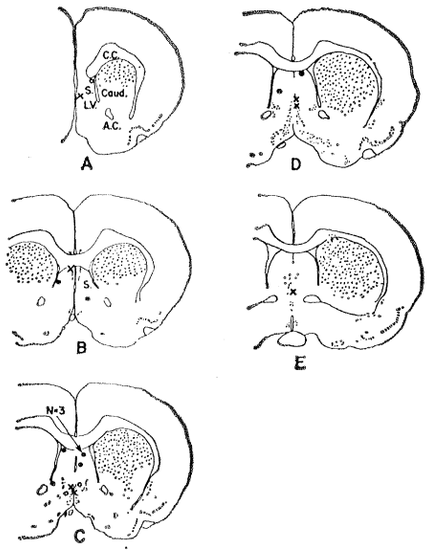

Fig. 2. Electrode placements for all animals whose temporal course of cardiac response to brain stimulation was studied. Stimulation points that produced heart rate acceleration are marked with *X*'s. Stimulation points for which the initial acceleratory phase was absent are marked with circles. Data 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).

15 November 1963

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-