tic pulse was emitted from their equipment at the instant that the microwave pulse was emitted, which is not necessarily the case.

Many materials, including such screening as that in the circular waveguide used by Chou *et al.*, "sing." In early rf hearing work, A.H.F. had difficulty carrying out tests in which the subject's head was to be shielded, because so many materials and material junctions "sing" acoustically in response to and in correlation with high peak pulsed microwave energy. The inside of horn antennas also often "sing" very softly. Chou *et al.* (*6*) report that when using a horn applicator "it was necessary to place a cat's head in close proximity [to the horn aperture] in order to record a detectable CM [cochlear microphonic]." When we consider that microphonics are found only after averaging from selected subjects, and then only in a waveguide or with a horn applicator when it is very close to the head, it is difficult to dismiss the possibility of response to extraneous acoustic energy in the experiments of Chou *et al.*

We also note that Chou and Guy (*21*) show a picture of an animal with its head in the circular waveguide. The animal's head is lying on what they state is a slab of polystyrene foam. They also report that they used a carbon electrode placed against the round window to record the microphonics. In our experiment 2, we had to replace the microwave absorber (polystyrene foam block with carbon deposited in cone-shaped depressions on the underside) because the holographic technique showed that the polystyrene foam block vibrated in response to high peak power microwave pulses.

The evidence discussed herein suggests that the locus of the rf hearing effect is in the cochlea. Among the many mechanisms in the cochlea that might account for the perception of microwave energy, consideration should be given to the possibility of thermoacoustic expansion within the cochlea. White (*22*) suggested that transient elastic waves may be generated by thermoacoustic expansion in cochlear structures and thus induce the rf hearing effect.

If the locus of the effect is within the cochlea, as it appears, then defining the mechanism will be difficult. But the microwave-induced hearing phenomenon may prove useful in the analysis of the function of a portion of the auditory system that is poorly understood.

ALLAN H. FREY
ELAINE COREN

Randomline, Inc.,
Huntingdon Valley, Pennsylvania 19006

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Human Chorionic Gonadotropin: Induction of Ovulation in the Squirrel Monkey

Abstract. *The minimum dose of human chorionic gonadotropin that would cause ovulation in the squirrel monkey (*Saimiri sciureus*) was found to be between 100 and 250 international units.*

For many years human chorionic gonadotropin (hCG) has been the drug of choice for inducing ovulation in humans and in domestic and laboratory animals. Dosages used for humans have been reported to be as high as 20,000 I.U. of hCG administered as a single dose, but more generally doses of 2000 to 8000 I.U. are used (*1*). For ovulation induction in domestic animals the dosage range is 500 to 5000 I.U. of hCG. The minimum effective dose (MED) that causes ovulation in primates is not known. In the rabbit, the MED is 25 I.U. (*2*), and administration of only 100 I.U. of hCG has been reported to cause chromosome abnormalities in 6-day blastocysts (*3*).

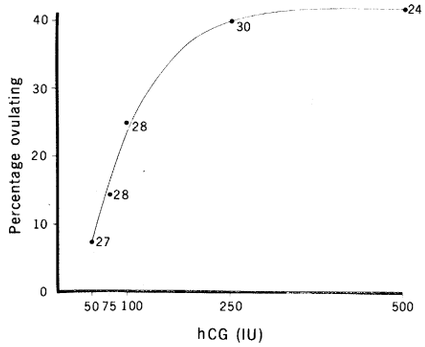

Fig. 1. Percentage of squirrel monkeys ovulating in response to a given dose of hCG after treatment with FSH for 4 days. Values at each point indicate numbers of animals tested.

Human chorionic gonadotropin is commonly used to treat human obesity (in doses of 1500 to 2000 I.U. administered daily over extended periods) despite warnings from the Food and Drug Administration that hCG has not been demonstrated to be effective adjunctive therapy in weight control programs (*4*). In view of the concern about excessive administration of hCG to humans, the present study was designed to determine the minimum dose of hCG that would induce ovulation in a nonhuman primate, the squirrel monkey (*Saimiri sciureus*).

Squirrel monkeys (N = 137) of Bolivian and Guyanan origin (*5*) were subjected to a standard regimen of follicle-stimulating hormone (FSH) administration (*6*) for 4 days (1 mg of FSH per day). On the afternoon of the fourth day a single injection of 50, 75, 100, 250, or 500 I.U. of hCG (A.P.L.® Ayerst, Montreal) was given intramuscularly. The ovaries were examined by means of a laparoscope (*7*) 36 hours later to determine if ovulation had occurred. Because there is a seasonal response to ovulation in squirrel monkeys (*8*), all trials were conducted during April, May, and June of 1978 and 1979.

The responses of these nonhuman primates to the different doses of hCG are shown in Fig. 1. For the groups receiving 50, 75, 100, 250, and 500 I.U., the mean number of ovulations per female were,

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respectively, 1.0 (2 ovulations among 2 animals), 1.0 (4 among 4), 1.1 (6 among 5), 1.3 (15 among 12), and 1.5 (15 among 10). With the exception of one animal in the group receiving 500 I.U. of hCG that had four ovulations (2.9 percent of the animals ovulating), all animals showed only single (76.5 percent) or double (20.6 percent) ovulations.

The 41.7 percent ovulation rate with 500 I.U. of hCG is comparable to the 42.9 percent rate reported earlier (*8*) for animals that received the same amount of hCG with the dose being administered after 5 days of treatment with 5 mg of progesterone per day to mimic the luteal phase of the reproductive cycle.

The results indicate that a dose-response curve can be drawn for the ovulatory response of the squirrel monkey to hCG injection. Full responsiveness is reached by a dose of 250 I.U. of hCG, and the MED is between 100 and 250 I.U. This dose is considerably less than that commonly used with other nonhuman primates and suggests that the MED in humans might also be less than is commonly used clinically.

W. RICHARD DUKELOW
Endocrine Research Unit, Michigan State University, East Lansing 48824

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β-Galactosidase and Selective Neutrality

A recent report (*1*) concluded that the observed amino acid composition of β-galactosidase from *Escherichia coli* is not ($P \simeq 10^{-24}$) the sole result of a selectively neutral evolutionary process with respect to (i) equal interchangeability among amino acids, (ii) base replacement at the gene level, and (iii) replacement drawn from a pool of amino acids at their natural abundances in proteins. Any one of these three hypotheses was by itself an adequate explanation of the amino acid composition of about 60 percent of the β-galactosidase molecule, but none was capable of explaining the composition of the remaining molecular structure.

Although individually each of the above hypotheses fails to explain fully the experimentally observed amino acid composition of β-galactosidase, together they, in principle, might. To illustrate, let us consider that an urn contains 1000 balls. Three investigators have three different hypotheses about them: all balls are white; all are red; and all are black. On emptying the urn it is found 200 are white, 300 are red, and 500 are black. All three hypotheses are strongly rejected, but it is obvious that a linear combination of the three explains the observations perfectly. The purpose of this note is to show that an explanation of this sort does not suffice for β-galactosidase.

All possible linear combinations of hypotheses (i) to (iii) were tested to see if any combination of the three would better explain the observed amino acid composition of β-galactosidase than each individually. Let n_{1i}, n_{2i}, and n_{3i} be the expected number of residues of the i^{th} (i = 1 to 20) amino acid type under each of the three hypotheses. Because there are 1021 residues in the protein, n_{1i} = 51.05 for all 20 amino acids, and the numerical values for n_{2i} and n_{3i} are in table 1 of (*1*). Denoting by f_1, f_2, and f_3 the fraction by which each hypothesis ($f_1 + f_2 + f_3 = 1$) contributes toward the expected composition, under the composite hypotheses this expected composition is given by

$$n_i = f_1 n_{1i} + f_2 n_{2i} + f_3 n_{3i}$$

The values of f_1, f_2, and f_3 which best explain the observed amino acid composition of β-galactosidase were determined by minimizing

$$\chi^2 = \sum_{i=1}^{20} \frac{(n_i - n_0)^2}{n_i},$$

n_0 being the observed number of residues of each amino acid. The best f_i were $f_1 = 0.34$, $f_2 = 0.34$, and $f_3 = 0.32$. The expected mole fraction of each amino acid under this best linear composite hypothesis was then calculated from the first equation above. The minimal value of χ^2 was 71.87, which, for 17 degrees of freedom, has an associated probability of 1.03×10^{-8}. The composite hypothesis is rejected. At the 1 percent level of significance, aspartic acid, glutamine, and leucine were present in excess of expectation, and cysteine and lysine were present in amounts below expectation. These five amino acids represent 237 residues and account for 68 percent of the χ^2 value of 71.87. The remaining 77 percent of the residues of the β-galactosidase molecule were in reasonable agreement with expectation.

The poor agreement with experiment, for a certain portion of the molecular structure of β-galactosidase, under each of the hypotheses individually, thus cannot be much improved by linear combinations of these hypotheses. For this portion they are wrong singly; they are wrong in combination. The biological significance of this result is that protein structure, at least that of β-galactosidase, and probably generally, is not simply the result of compositional optimization with respect to the conflicting requirements of the three hypotheses.

RICHARD HOLMQUIST
Space Sciences Laboratory, University of California, Berkeley 94720
THOMAS CONROY
Department of Physics, University of California at Berkeley

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