not occur until about 6 to 9 seconds after the commencement of sound stimulation. The D2 mice usually experience wild running within 1 second after sound stimulation. It appears, therefore, that the T_4-treated B6 mice still possess a factor that temporarily offers resistance to AS.

In conclusion, we have demonstrated a close relationship between T_4 levels and the susceptibility of mice to AS. The functional state of the thyroid gland is also believed to exert an important influence on the manifestation of AS in rats (*20*). Furthermore, the strain difference for T_4 levels may serve as a useful natural model for studying the influence of thyroid hormone on various aspects of brain maturation. It would be important to determine whether thyroid hormone acts alone or in conjunction with other hormones to influence AS susceptibility. Further studies are needed to elucidate the role of thyroid hormone on the manifestation of AS in mice.

THOMAS N. SEYFRIED
GILBERT H. GLASER
ROBERT K. YU

Department of Neurology,
Yale University School of Medicine,
New Haven, Connecticut 06510

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Combination Tones at Frequencies Greater Than the Primary Tones

Abstract. *The existence of audible combination tones at frequencies greater than the primary tones that generate them has long been problematic. With primary tones at frequencies* f_1 *and* f_2, *combination tones at* $f_1 + f_2$, $2f_2 - f_1$, *and other frequencies can be demonstrated and measured by using a contralateral probe tone to establish a binaural interaction with a given combination tone. The estimated amplitudes of these higher-frequency combination tones are generally 20 to 40 decibels below the amplitude of the primary tones.*

The auditory sensations produced by an acoustic stimulus composed of two sinusoidal primary tones are augmented with audible tones at frequencies other than those of the primaries. With the primary frequencies labeled f_1 and f_2 ($f_1 < f_2$), the frequencies at which combination tones may be generated are given by $(nf_1 \pm mf_2)$ (n and m are integers). Helmholtz (*1*) proposed that the eardrum and ossicles of the middle ear displaced nonlinearly, thereby distorting the signal and introducing combination tones to the signal transmitted to the inner ear. Recent experimental evidence (*2, 3*) shows that the generation of combination tones, at least those of the particular form $(n + 1) f_1 - nf_2$, involves a high degree of frequency selectivity. Thus the cochlea, wherein the initial auditory frequency analysis is performed, is now implicated as the locus of the distortion mechanism.

Those combination tones thus far noted and measured, with $2f_1 - f_2$ and $f_2 - f_1$ receiving the greatest attention, have all been lower in frequency than the primary tones that produce them. Noteworthy exceptions to this rule have been provided by Helmholtz (*1*), who claimed to hear a pitch at $f_1 + f_2$, and one of Plomp's (*4*) listeners, who was able to identify a tone at $2f_2 - f_1$. Others who have attempted to hear these higher-frequency combination tones have been unsuccessful (*2, 5*), although some evidence suggests that the tones exist, but at very low relative levels (*6, 7*).

The most frequently advanced reason for the inaudibility of combination tones above the primary frequencies is that, owing to the asymmetry of masking—low-frequency tones mask high tones more than the reverse—the higher-frequency combination tones may be masked by the primary tones (*4, 6, 8, 9*). However, determining whether higher-frequency combination tones are not present or are present but inaudible is difficult. Another possible factor contributing to the inaudibility of higher-frequency combination tones is that, if they are introduced by nonlinear motion of the basilar membrane in the cochlear region characteristic of the primary tones, the higher-frequency tones would be at a disadvantage as a result of a directional-coupling effect that favors wave transmission to places of lower characteristic frequency in the cochlea (*10*).

We have found that, with a suitable psychophysical measurement procedure, higher-frequency combination tones can be not only demonstrated to exist (*11*) but also measured. The measurement procedure exploits the fact that a binaural tone seems to be centered intracranially when it is equal both in amplitude and in phase at the ears. To measure the amplitude and phase of a given combination tone generated by low-frequency primary tones presented to the right ear, we present to the left ear a probe tone at the frequency of the combination tone. The subject's task was to adjust the amplitude and phase of this

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probe tone to intracranially center the fused image formed by the binaural interaction of the probe tone and the combination tone. This procedure has previously been applied successfully to the measurement of the combination tones $2f_1 - f_2$ and $f_2 - f_1$ (*12*).

The stimuli used in this study were generated digitally. The primary-tone stimulus, which was always presented to the right ear, comprised two phase-locked sinusoids of frequencies f_1 and f_2 and sound pressure levels (SPL) L_1 and L_2, respectively. The adjusted sound pressure level of the contralateral probe tone was termed L_{probe}. All stimuli were pulsed on for 400 msec with a rise and fall of 50 msec. After digital-to-analog conversion, the electrical signals were filtered, attenuated, and monitored with standard equipment before being presented to TDH-49 earphones. Measurements of the acoustic output of the right earphone revealed that distortion components in the stimulus at frequencies of interest were, in the worst measurable case, −56 dB relative to the primary tones.

The stimuli were presented once per second while the observer adjusted the phase and amplitude of the probe tone, both of which were continuously variable. In addition, the phase was variable in steps of ± 45° and 180°. These fixed increments in probe phase eased the observer's task considerably in achieving a balance in the amplitude adjustment. After making an adjustment the observer was free to remove the headset.

Individual adjustments of probe amplitude with five different combination tones, obtained from two listeners (the authors), are presented in Fig. 1. Of the five types of combination tones represented in Fig. 1, four are higher in frequency than f_2. In Fig. 1A results with the combination tone $2f_1 - f_2$, which has been well studied (*2, 3*), are presented for purposes of comparison. The dashed line in each panel represents the masked threshold of a pure tone at the frequency of the combination tone in question, in the presence of the nearer primary tone as masker.

In Fig. 1B measurements are made at $2f_2 - f_1$. Probe levels in this case are 20 to 30 dB lower than that of the primaries (*13*). The growth in L_{probe} with primary-tone level is approximately 1 dB/dB for subject PMZ, who was able to perform the task over a larger range of primary levels than subject RMS.

At the summation frequency, $f_1 + f_2$, L_{probe} grew rapidly initially and leveled off with increasing primary amplitude (Fig. 1C). Of the higher-frequency combination tones we have encountered thus far, the summation tone at certain primary levels is one of the most easily measured, apparently because of its higher level relative to masked threshold.

With different frequencies for f_1 and f_2, we attempted to measure the combination tones $2f_1 + f_2$ (Fig. 1D) and $2f_2 + f_1$ (Fig. 1E). The results from the one subject who could perform the task confidently show L_{probe} to be on the order of 30 to 40 dB below the primary tones.

Accompanying each adjustment of probe level was an adjustment of probe phase. These are not shown in this report but can be described as an increasing phase lag of the probe with primary level; similar to the functions relating phase and primary level obtained with lower-frequency combination tones (*12*).

In an attempt to measure higher-frequency combination tones with a different procedure, we tried the cancellation method (*8, 9*). With this procedure a cancellation tone was added to the primary stimulus at the right ear. Subjects adjusted the amplitude and phase of the cancellation tone to remove the sensation of the combination tone. We found this task very difficult because of either one or both of two factors: (i) the low levels (relative to masked threshold) of the combination tones and (ii) the relatively narrow frequency separation between primary and combination-tone components. The cancellation procedure works best with a combination tone that can be clearly perceptually isolated to begin with, since the task is to remove it. The few results we were able to obtain with the cancellation method are consist-

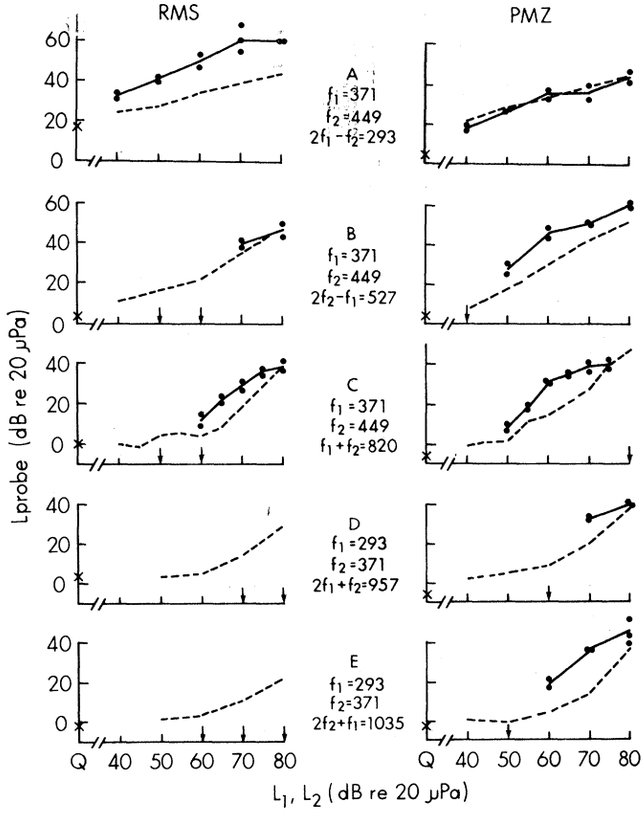

Fig. 1. Filled circles represent subjects' individual adjustments of the level of a contralateral probe tone, L_{probe}, to center the perceived tone intracranially. The independent variable is the (equal) SPL of the primary tones, L_1 and L_2. The solid lines connect the means of the adjustments. The downward-pointing arrows indicate primary levels (not exceeding 80 dB SPL) at which the centering task was attempted but could not be performed. The dashed lines represent the monaural masked threshold of a pure tone, at the frequency of the combination tone, in the presence of one of the primary tones as masker. In (A) this masker was f_1 at SPL L_1; in the other conditions (B to E) the masker was f_2 at L_2. The X on the ordinate denotes threshold in quiet for the probe tone measured at the left ear. The frequencies (in hertz) of the primary tones, f_1 and f_2, and the probe tone at a given combination-tone frequency, are indicated for each pair of panels.

ent with the binaural estimates for the same stimulus conditions.

Although our measurements represent only a limited investigation of higher-frequency combination tones, we feel confident in concluding that, at least for low primary frequencies, such combination tones exist 20 to 40 dB below the primary levels. As others have suggested (*2, 8*), the difficulty in identifying their presence appears to be largely due to the upward masking by the primary tones. Our observations indicate that the relative level of higher-frequency combination tones can be quite high. Thus, even though they are not clearly audible, they may be of some importance in identifying the nonlinear mechanism from which they arise. In this respect, the presence of higher-frequency combination tones brings the psychoacoustic results into closer agreement with physiological investigations of cochlear microphonic in which distortion components above and below the primary frequencies are routinely measured (*14*).

P. M. ZUREK
R. M. SACHS

Central Institute for the Deaf,
818 South Euclid,
St. Louis, Missouri 63110

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11. The existence of higher-frequency combination tones can be demonstrated through forced-choice detection of an interaural phase shift between a probe tone and a combination tone. To do this it is necessary to present the probe at the approximately "correct" amplitude and phase. We have used the estimates of amplitude and phase and required subjects to detect a change of 45° in the phase of the contralateral tone at the frequency of a given combination tone. This could be done quite easily.
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Human Growth Hormone: Complementary DNA Cloning and Expression in Bacteria

Abstract. *The nucleotide sequence of a DNA complementary to human growth hormone messenger RNA was cloned; it contains 29 nucleotides in its 5′ untranslated region, the 651 nucleotides coding for the prehormone, and the entire 3′ untranslated region (108 nucleotides). The data reported predict the previously unknown sequence of the signal peptide of human growth hormone and, by comparison with the previously determined sequences of rat growth hormone and human chorionic somatomammotropin, strengthens the hypothesis that these genes evolved by gene duplication from a common ancestral sequence. The human growth hormone gene sequences have been linked in phase to a fragment of the* trp *D gene of* Escherichia coli *in a plasmid vehicle, and a fusion protein is synthesized at high level (approximately 3 percent of bacterial protein) under the control of the regulatory region of the* trp *operon. This fusion protein (70 percent of whose amino acids are coded for by the human growth hormone gene) reacts specifically with antibodies to human growth hormone and is stable in* E. coli.

Growth hormone, along with at least two other polypeptide hormones, chorionic somatomammotropin (placental lactogen) and prolactin, forms a set of proteins with amino acid sequence homology and to some extent overlapping biological activities (*1, 2*). Since the genes of this set of proteins probably have a common ancestral origin (*1*), they constitute an excellent model to study the evolution, structure, and differential regulation of related genes. In addition, since human growth hormone is of considerable medical importance and its supply is limited, the synthesis of growth hormone in bacteria might provide the required alternate source of this critical hormone.

We have previously isolated and analyzed bacterial clones containing copies of complementary DNA (cDNA) transcripts of messenger RNA's (mRNA's) for these hormones. The complete sequence of rat pregrowth hormone mRNA (*3*) has been reported; in addition, sequence data have been presented for fragments of about 550 bases complementary to part of the coding (amino acid residues 24 to 191) and 3′ untranslated portions of human chorionic somatomammotropin (hCS) (*3, 4*) and human growth hormone (hGH) mRNA's (*5*). A partial sequence of rat prolactin has been determined by Gubbins *et al.* (*6*). These sequence data showed that, whereas the growth hormone genes of the rat and man had significant homology, they also had diverged substantially, such that they differed more than the genes for the functionally distinct human hormones hCS and hGH.

We now report the synthesis, cloning, and sequence analysis of cDNA containing the entire coding and most of the noncoding portions of hGH mRNA. We also describe the insertion of these sequences into an "expression plasmid" containing part of the *Escherichia coli* tryptophan (*trp*) operon whose construction has been realized by Hallewell and Emtage (*7*). We describe the use of this plasmid to promote the inducible bacterial synthesis of high levels of a hybrid protein, 70 percent of which is composed of amino acids coded for by the hGH gene.

Human growth hormone mRNA isolation. Polyadenylated RNA was isolated (*8*) from human pituitary tumors removed by transphenoidal hypophysectomy. To obtain an indication of the integrity and the relative abundance of growth hormone mRNA in each sample, the individual mRNA preparations were translated in the wheat germ cell-free system, and the products were analyzed by electrophoresis on sodium dodecyl sulfate-polyacrylamide gels (Fig. 1). Among the translation products of the five acromegalic tumor RNA's (Fig. 1, lanes 1 to 5), the most prominent band corresponds to a protein of approximate-

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