Table 1. Responses of CBHA-C mice to sound stress 23 days after birth following a primary sound stimulus 21 days after birth. Entries are percentages ± standard error of the means. A clonic seizure is mild or incomplete, and a tonic seizure is severe or one in which the animal becomes cataleptic following a series of convulsive movements. Frequencies are calculated as the number in the class divided by N, times 100. Frequencies are not substantially different if calculated as mean litter percentages. Chi-square of homogeneity results, with no response, wild running, clonic seizure, and tonic seizure incidence as columns, and varying combinations of thienylalanine-treated (Th), solvent-treated (So), sham-treated (Sh), and unhandled (Un) groups as rows, were as follows. Overall: χ^2 (Th versus So versus Sh versus Un) = 29.1, d.f. = 9, $P < .001$. All treatments combined: χ^2 (Th + So + Sh versus Un) = 24.48, d.f. = 3, $P < .001$. Each treated contrasted with control: χ^2 (Th versus Un) = 11.4, d.f. = 3, $P < .01$; χ^2 (So versus Un) = 22.3, d.f. = 3, $P < .001$; χ^2 (Sh versus Un) = 14.3, d.f. = 3, $P < .006$. Each treatment contrasted with each other: χ^2 (Th versus So) = 4.5, d.f. = 3, $.50 > P > .20$; χ^2 (Th versus Sh) = 0.7, d.f. = 3, $P > .50$; χ^2 (So versus Sh) = 2.3, d.f. = 3, $P = .50$.

Treatment group	Individuals (No.)	Litters (No.)	Frequency of response						Percent of tonic seizures that are fatal
			No response	Wild running	Clonic seizure	Tonic seizure			
						Recovered	Fatal	Total	
Thienylalanine	96	17	21.9 ± 4.2	7.3 ± 2.7	25.0 ± 4.4	17.7 ± 3.9	28.1 ± 4.6	45.8 ± 5.1	61.4 ± 7.3
Solvent	103	17	14.6 ± 3.5	13.6 ± 3.4	19.4 ± 3.9	15.5 ± 3.6	36.9 ± 4.8	52.4 ± 4.9	70.4 ± 6.2
Sham	104	19	22.1 ± 4.1	9.6 ± 2.9	22.1 ± 4.1	15.4 ± 3.5	30.8 ± 4.5	46.2 ± 4.9	66.7 ± 6.8
Unhandled	120	20	39.2 ± 4.5	10.8 ± 2.8	23.3 ± 3.9	6.7 ± 2.3	20.0 ± 3.7	26.7 ± 4.0	75.0 ± 7.7

the abdomen and held in place with nothing injected. A fourth group served as unhandled control subjects.

Litters were weaned 21 days after birth, at which time mice were weighed and placed, individually or in groups of three, into a galvanized wash tub to which was attached a 4-inch bell (1 inch = 2.54 cm); the tub was suspended in an illuminated sound deadening chamber with a plexiglass lid to permit observation (*2, 6*). After the mice were acclimatized to the chamber for 30 seconds, the bell was rung for 60 seconds; during this time the mice were observed and their seizure responses (*7*) were scored. On day 23, the mice were subjected to the sound stress in the same order and according to the same procedure. Almost no responses to sound stress occurred at 21 days. Responses at 23 days lay on a continuum from wild running through clonic (spasmodic and partial) seizures followed by recovery, or to tonic (rigid and complete) seizures. All seizures were preceded by wild running. Fully two-thirds of the tonic seizures resulted in death of the animal (Table 1).

Chi-square analysis for homogeneity (*8*) revealed that each group under treatment differed significantly from controls, and the three groups under treatment were identical to one another. The same situation obtains when one compares the elicitation of any response (wild running, clonic and tonic seizures combined) with the failure of the sound stress to elicit any response at all. Thus any of the three treatments applied to pregnant mice increased audiogenic seizure susceptibility to the same extent relative to control treatment. It thus seems that the act of manipulating the pregnant mouse, rather than the test substance, produced sufficient stress to cause the behavioral differences in the progeny.

Whether an animal had been subjected to the sound stress individually or as part of a group of three made no difference in any experiment, although this parameter does affect other lines of mice (*9*). Similarly, the proportion of tonic seizures from which animals failed to recover was the same in all experiments. In all but the sham-treated group, chi-square analysis suggested differences in animals from brother-sister matings contrasted with those from continuously outbred populations. But the directions of the differences varied, and an overall test of heterogeneity of pooled data from all four experimental groups revealed no significance ($\chi^2 = 7.71$, d.f. = 3, $P < .06$). There was considerable variation in response between litters in all experiments. Analyses of variance (*10*) in which responses were scaled from 1 (no response) to 4 (tonic seizure) revealed significant litter effects among all four groups. Mortality of pups before weaning was approximately 4 percent in all groups.

Reports of postnatal effects on progeny of handling or otherwise mildly stressing the dam during gestation are frequent (*11*). The subjects have usually been rats, and the effects observed have been on normal behaviors. There are comparable reports in which sham injection of dams (or injection of distilled water, saline, or other control fluids) influences behavior, body weight, or adrenal function of their progeny (*12*). Similar postnatal effects of handling during gestation are known also in mice (*13*). Handling of dams during gestation may, in fact, result in increased embryo mortality (*14*). So profound an influence of a mild maternal stress on susceptibility to audiogenic seizures serves as a caution to investigators using audiogenic seizures as a measure and to those attempting to assess postnatal effects of prenatal treatments.

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Reticulocyte Transfer RNA and Hemoglobin Synthesis

Smith (*1*) has suggested that transfer RNA (tRNA) availability may regulate hemoglobin synthesis in reticulocytes. Central to this argument is the finding that the level of leucine tRNA ($tRNA^{Leu}$) in rabbit reticulocytes is relatively low: only 34 pmole per A_{260} unit, or 220 molecules of $tRNA^{Leu}$ per leucine residue in

rabbit hemoglobin (*2*). In contrast, Smith reports that other tRNA species are present to the extent of at least 500 to 1000 molecules per amino acid residue in hemoglobin. Thus, Smith argues that the availability of $tRNA^{Leu}$ is "marginal at best and may limit the rate of hemoglobin synthesis."

Unfortunately for this argument, reports from other laboratories show much higher values than those cited by Smith for the leucine acceptance of reticulocyte tRNA preparations. Thus, Gilbert and Anderson (*3*) obtained values of 95 pmole per A_{260} unit for rabbit reticulocyte tRNA, which would correspond to 625 molecules of $tRNA^{Leu}$ per leucine residue in hemoglobin, well within the normal range. Yang (*4*) reported a value of 123 pmole per A_{260} unit for leucine acceptance of mouse reticulocyte tRNA, and we found (unpublished work) a value of 92 pmole per A_{260} unit for sheep reticulocyte tRNA.

As a second piece of evidence for the idea that $tRNA^{Leu}$ limits the rate of hemoglobin synthesis, Smith claims that 65 percent of total reticulocyte $tRNA^{Leu}$ is attached to ribosomes. This is much greater than the 20 percent degree of ribosomal attachment he estimates for the average tRNA. However, as Smith himself points out, tRNA associated with supramolecular complexes of aminoacyl-tRNA synthetases and not participating directly in hemoglobin synthesis would be expected to contaminate the ribosome fraction and would therefore give falsely high values for ribosome-bound tRNA. Hence, in the absence of evidence showing what proportion of the "ribosome-associated tRNA's" are in fact bound to polysomal ribosomes, data describing the degree of ribosomal association of various tRNA species are difficult to interpret.

I conclude that although the evidence for functional adaptation of tRNA populations in many cell types is quite strong (*5*), Smith's argument that tRNA availability may regulate hemoglobin synthesis in reticulocytes is not adequately supported by the available data.

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The value for leucine acceptance by reticulocyte tRNA that we published several years ago (*1*) has been reproduced many times in our laboratory, and its use (*2*) is based on current data and on much confidence. Determinations of leucine acceptance of 20 different reticulocyte tRNA preparations give a mean value of 34.56 pmole with a standard deviation of 2.87 pmole per A_{260} unit.

In an effort to reconcile our apparently low value for leucine acceptance with the higher value published by Gilbert and Anderson (*3*), the following information should be considered.

1) Gilbert and Anderson's total value for acceptance of all amino acids was considerably greater than ours, suggesting that their tRNA preparation contained less contaminating material absorbing at 260 nm, but incapable of amino acid acceptance. Actually leucine accounts for about 6 percent of total amino acid acceptance in their study compared to 3.75 percent in ours. These values differ by a factor of 1.6 rather than 3 as suggested by the actual picomole data. Since tRNA preparations vary in the extent of contamination, we believe that the relative acceptances of different amino acids rather than absolute amounts of acceptance per A_{260} unit are more valid for comparing tRNA preparations. Moreover, our enumeration of reticulocyte tRNA molecules (*4*) is based on such relative values.

2) Gilbert and Anderson prepared their tRNA from postribosomal supernatants rather than from whole washed reticulocytes as we have always done. While such preparations may be less contaminated, they are obviously less representative of the tRNA content of the whole cell.

In response to the comment that some of the tRNA we prepare from reticulocyte ribosomes may not be involved in hemoglobin synthesis, a study by Culp, Morrisey, and Hardesty (*5*) showed [as we did also (*6*)] that leucine tRNA is relatively enriched on reticulocyte ribosomes. Incubation of reticulocytes with NaF, an inhibitor of protein synthesis initiation, resulted in depletion of reticulocyte ribosomes of tRNA for leucine and all other amino acids except methionine. Sodium fluoride should specifically deplete ribosomes of tRNA involved in translation but not tRNA attached to ribosomes or other subcellular particles for other reasons.

Although Litt raises some worthwhile points, there is evidence that hemoglobin synthesis in reticulocytes may be restricted by the availability of some tRNA species.

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