Increased Incidence of Nontasters of Phenylthiocarbamide among Congenital Athyreotic Cretins

Abstract. The incidence of nontasters for phenylthiocarbamide was found to be significantly higher in 27 athyreotic cretins than in normal adults and children. A significant increase of nontasters among the parents and siblings of these cretins was also found. These findings are discussed in relation to maldevelopment of the fetal thyroid in nontaster genotypes.

The ability to taste phenylthiocarbamide is an example of a human genetic polymorphism (*1*) with most of the observed variations in taste thresholds being accounted for by simple monogenetic recessive inheritance (*2*). Nontasters are homozygous for the recessive allele (tt) and tasters are homozygous (TT) or heterozygous (Tt) for the dominant allele.

In 1942 Richter and Clisby (*3*) showed that phenylthiocarbamide was associated with hypertrophy of the rat thyroid, and a higher than normal incidence of nontasters was later observed in patients with nontoxic goiters (*4*). This relationship of thyroid disease to taste response prompted the following investigation of congenital cretins and their families.

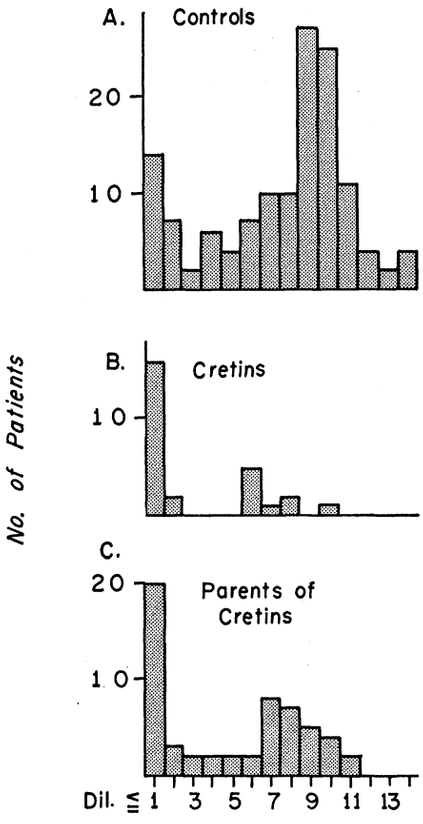

Fig. 1. Threshold dilutions of phenylthiocarbamide for the members of three groups. Dilutions 1 to 4 designate a nontaster; dilutions 5 to 14, a taster.

The patients studied were all thoroughly documented examples of sporadic athyreotic cretinism, which is the most common cause of hypothyroidism in childhood. In 24 of the 31 cases studied, either a serum protein bound iodine or an I^{131} accumulation, or both, supported the diagnosis. Such children have congenital absence of the thyroid gland, and within 4 to 12 weeks after birth they exhibit marked decrease in growth rate and physiological evidence of hypothyroidism. Although rare examples of recurrence of the condition within the same sibship have been reported, there is no evidence that the condition is inherited.

The parents and adult controls were tested with 14 concentrations of phenylthiocarbamide according to the method of Harris and Kalmus (*5*). Their threshold was the weakest concentration at which they were able to differentiate four beakers of water from four beakers of the test concentration. The testing of children and infants was carried out by serially increasing the concentration until the bitterness was detected. This was repeated in most cases until the threshold was established by three responses at the same concentration. Within the childhood controls there was no significant difference in threshold with age, and the distribution was similar to that of the adults. Over 95 percent of the patients and controls were of European ancestry.

The results are shown in Fig. 1 and are expressed by threshold concentration tasted. Dilution 1 is 1300 mg/liter, and each subsequent number is one-half the strength of the preceding concentration. The histogram for the controls is shown in Fig. 1*A* and exhibits bimodality. For purposes of this study, the dividing line between taster and nontaster was placed between concentrations 4 and 5.

By utilizing clinical material from other pediatric endocrine clinics (*6*), 30 families containing 31 cretins were tested by the same observer, and all but four cretins could be tested accurately. It was found that 18 were nontasters and nine were tasters, as compared to the control group of 29 nontasters and 104 tasters. This is a significant difference ($\chi^2 = 22$, D.F. $= 1$, $P = < .001$). Thirty of the mothers were tested and 15 were nontasters, and of the 27 fathers tested, 12 were nontasters. Of the 29 unaffected siblings who were available for study, 18 were nontasters. The incidence of nontasters among parents and siblings of cretins as compared to normal is significantly increased ($\chi^2 = 21.6$, D.F. $= 1$, $P = < .001$).

The demonstration of an association between nontasters for phenylthiocarbamide and athyreotic cretins raises the question as to the nature of the gene-environmental interaction involved in the development of this form of cretinism. It is well known that goitrogenic thiocarbamide substances are found in food (*7*), and it is possible that nontaster fetuses may be susceptible to embryonic thyroidectomy by these chemicals. The time, dosage, and duration of exposure of the fetus to maternally ingested goitrogens would most likely be critical, and variations in these factors could explain the fact that most of the nontasting siblings of cretins are normal.

If the preceding hypothesis is correct, one would expect a lower incidence of athyreotic cretinism among populations (for example, Chinese and Negro) where the incidence of nontasting is low or in countries where the diet contains minimal amounts of thiocarbamides (*8*).

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Rewarding Properties of Intracranial Stimulation

Abstract. Monkeys can be trained to press a lever to obtain intracranial brain stimulation on a large fixed-ratio schedule as well as on a continuous reinforcement schedule. A long extinction curve appears to be indicative of a future high fixed-ratio performance.

Previous reports by Olds and Milner (*1*) and Seward, Uyeda, and Olds (*2*) have indicated that behavior rewarded by intracranial stimulation has a low resistance to extinction. Schedules of

intermittent reinforcement which require a certain resistance to extinction to meet the reinforcement contingency have been reported for low levels of intermittency. Sidman and his co-workers (*3*) showed that cats could be trained on a fixed ratio of 8 (every eighth response was rewarded), but this degree of intermittency is small in comparison to the ratios of 100 or more that can be produced routinely with food reinforcement (Ferster and Skinner, *4*).

In view of the low resistance to extinction and the low levels of intermittency which have been reported when intracranial stimulation was used as a reward, it might appear that intracranial stimulation is a rather weak and ineffective reward. It is the purpose of this study to demonstrate that this is not necessarily the case.

Bipolar stainless steel electrodes, 0.25 mm in diameter, were implanted stereotaxically in 11 monkeys (*Macaca mulatta*). The Horsley-Clark coordinates (Labtronics instrument) were *A*-16, *L*-3, and $H + 3$, and were calculated to place the electrode tip in the vicinity of the medial forebrain bundle. Rectangular pulses, of 0.2 msec duration, 100 pulses per 0.5 second, were obtained from a Tektronix 162 waveform generator and 161 pulse generator which drove a cathode-follower output stage. The output from the cathode follower was fed to the electrodes through a General Radio isolation transformer. The current was monitored on a Tektronix 360 oscilloscope for each train of pulses, and the current usually employed was 3 ma.

After the operation, the monkeys were placed in a primate restraining chair and 2 days after the operation they were trained to press a telegraph key to obtain brain stimulation. After one session on a continuous reinforcement schedule (each lever press produced a brain stimulation), the animals were put onto a fixed ratio schedule, FR-5, which was gradually increased to FR-20. After at least a 1-hour session on FR-20, the current was turned off and the animal was allowed to work until the rate of lever pressing fell below 50 lever presses in a 15-minute period. In succeeding sessions intracranial stimulation reward was reinstated and the fixed ratio was increased to the point where postreinforcement pauses of several minutes were obtained.

Eight of the 11 monkeys learned to press the lever to obtain brain stimulation. All of these animals held a fixed ratio of 10, and seven of them held a ratio of 20. Four of the monkeys worked for brain stimulation on fixed ratios of 50 or greater. The highest ratio obtained was FR-150 in one monkey (Fig. 1*D*) and high rates of responding were maintained on this ratio for seven sessions which lasted a total of 20 hours. The effect on this monkey's behavior of increasing the ratio is illustrated in Fig. 1*A* to 1*D*.

In the extinction test, the four monkeys produced 342, 845, 4316, and 9129 responses before the extinction criterion was met. In subsequent experiments the highest ratios these animals would hold were FR-50, 100, 100, and 150 respectively. The extinction record for the FR-150 monkey is shown in Fig. 1*E*. The other four monkeys stopped responding after less than 100 lever presses when the current was turned off. None of these animals could be trained to hold a fixed ratio greater than 20. It appears that the length of the extinction record is indicative of the future fixed ratio performance of the animal for intracranial stimulation reward.

The results from half of the animals were consistent with previous reports in the literature in showing rather weak reinforcing properties of intracranial stimulation. However, the results from the other animals show that intracranial stimulation can maintain lever-pressing behavior on rather intermittent schedules of reinforcement and can generate a large number of extinction responses. These data are similar to those obtained by Ferster and Skinner (*4*) with food reinforcement. Thus, the data indicate that intracranial stimulation with a particular electrode placement can act as a strong and effective reinforcement.

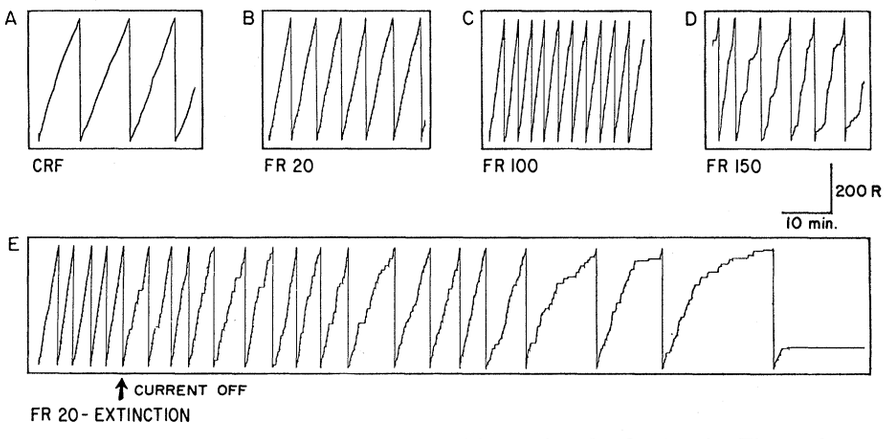

Fig. 1. Panels *A* through *D* show the effect of increasing the fixed ratio. The peak rate of 12,000 an hour was obtained on FR-100. At *D* with FR-150, a "strained ratio" was produced, characterized by long post-reinforcement pauses. Panel *E* illustrates the extinction record after a session on FR-20.

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Heterogeneity of Human Ceruloplasmin

Abstract. Subfractionation of purified human ceruloplasmin, prepared from plasma of 9109 donors, has been carried out by chromatography on columns of diethylaminoethyl cellulose and hydroxylapatite. Electrophoretic analyses of these subfractions on starch gels, at *p*H 8.5 and 5.7, reveal the presence of at least four ceruloplasmins, two of which appear to differ in histidine content.

Hemoglobin (*1*), transferrin (*2*), haptoglobin (*3*), albumin (*4*), and isohemagglutinins of the proteins of human blood have each been shown to exist as two or more genetically determined molecular species. The electrophoretic analyses of Uriel (*5*) and the chromatographic separations of Broman and of Sankar (*6*) have indicated that human ceruloplasmin, the plasma copper protein, is also heterogeneous with respect to its molecular composition. By means of chromatography on calcium phosphate and diethylaminoethyl cellulose columns and by electrophoresis on starch gel we have been able to show that at least four different molecular species of ceruloplasmin are present in a purified preparation of this protein made from pooled plasma of 9109 donors (*7*).

The deep-blue preparation of ceruloplasmin used, CH-181 (*7*), contained 2.2 mg of protein, on the basis of nitrogen analysis, and 58.2 μg of copper per milliliter. To 3.0 ml of this ceruloplasmin solution was added 30.0 ml of a 0.05*M* phosphate buffer of *p*H 6.4.