Table 1. Frequency of *N*, *R*, *C* responses, mean frequency and intensity scores, median latencies of first running, and epileptoid attacks in tamed and nontamed rats.

Group	No.	*N*	*R*	*C*	*F*	*I*	Latencies trial 1st running attack	Latencies trial epileptoid phase
Nonhandled	16	48	59	53	0.70	0.65	26.5	84.5
Handled	8	36	41	3	0.55	0.55	40	Indeterminate
Handled prior to test	7	39	22	9	0.44	0.48	62	Indeterminate

more active and less fearful, and they are poorer hoarders but better learners (*1–6*).

The purpose of the present experiment was to reevaluate the relationship between taming and audiogeic seizures. Previously reported differences are possibly confounded by differences in strain, and endocrine or nutritional status or both (*7*). In addition, currently available data are in conflict. One study (*8*) associates taming with low susceptibility, while a second claims tamed animals to be more sensitive to convulsion (*9*).

The experimental subjects were 31 albino rats of a strain inbred by the department of physiology, Emory University Schools of Medicine and Dentistry, for about 25 years, 47 days of age at the time of the first test. Sixteen, the untamed group, were whelped in a lightproof, extremely quiet room and separated into individual cages at weaning. They were touched only when they were transferred once a week to clean cages and when they were placed in and removed from the test chamber. This involved briskly picking up each animal by the tail. They were exposed to light and noise only during the test period. In contrast, the 15 tamed animals (originally there were 16 but one died) were reared in the regular colony room. At weaning, they were segregated three to a cage and subjected to taming. This consisted of 5 minutes of handling each day, the animal being petted, fondled, and allowed to crawl about in the experimenter's lap. Taming was continued throughout the experiment. In addition, seven were given 2 more minutes of petting just prior to being tested to determine whether a drastic change in treatment enhanced sensitivity. All were allowed free access to Purina Lab Chow and water from the time of weaning.

The animals were tested by being placed in a metal container inside a sound-resistant box and exposed to 2 minutes of noise (average level, 101 db; range, 90 to 104 db) generated by a 4-inch doorbell. Each animal received ten tests on alternate days. During each test the latencies of the first running attack and of the epileptoid seizure were recorded when these responses occurred. After each test the subject's test behavior was rated on a three-point scale from *N* (nonreaction) through *R* (running attack) to *C* (epileptoid convulsion). Frequency (*F*) and intensity (*I*) scores were derived from the data of the ten tests according to the following equations:

$$F = \frac{R + C}{N}\,;\; I = \frac{R(0.50) + C(1.00)}{R + C}.$$

The rationale of these scores and the criteria defining response categories is presented in an earlier paper (*10*). Table 1 presents a summary of these data.

Since the frequencies of *N*, *R*, and *C* ratings were derived from ten tests on 31 animals, the statistical criterion of independence is violated, making a chi-square evaluation impossible. The mean *F* and *I* scores were compared by means of Student's *t* test for small independent samples. Because there were animals in both tamed and untamed groups that did not react on any test, certain of the latencies were indeterminate. Median latencies are therefore reported, and statistical evaluation was accomplished by the Mann-Whitney *U* test for independent groups of unequal *N*.

Inspection of Table 1 suggests that taming has a protective effect directly proportional to its magnitude or immediacy or both. This conclusion, however, is not borne out by the statistical evaluation of the data. There is no reliable difference between the tamed group and the tamed group that was given further handling just before tests (*F* scores: $t = 0.65$, $P > 0.50$; *I* scores: $t = 0.44$, $P > 0.60$). When the scores of these two groups are pooled and compared with those for the unhandled group, differences in all but one case again lack significance. (*F* scores, $t = 1.58$, $P > 0.10$; *I* scores, $t = 1.39$, $P > 0.10$; latency per trial per running attack, $U = 161$, $P < 0.10$; latency per trial per epileptoid phase, $U = 168.5$, $P < 0.04$). Thus taming can be said to have a protective effect only in the sense that the tamed animals require a longer time to have a full-blown epileptic fit.

The basis of these negative results is a matter for speculation. They are probably not due to insufficient handling, since the amount given the present animals compares favorably with that reported in previous studies (*2, 8*). One possibility lies in the strain used. Our rats, derived from Wistar stock, are extremely docile, gentle, and easily handled. It is possible that the petting given the tamed groups could add only an insignificant increment to already maximally tame subjects. Inspection of the wide variability in responsiveness in both handled and nonhandled groups meanwhile suggests that much larger samples would be required to demonstrate the suggested beneficial effects of handling on seizures. Should this be the case, its importance to an understanding of the seizure mechanism is probably limited.

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Protoplasmic Streaming in Plants Sensitive and Insensitive to Chilling Temperatures

Many plant species of tropical and subtropical origin are subject to chilling injury when exposed to temperatures below about 10°C but above their freezing points. Severity of injury generally increases as chilling temperature decreases and as duration of exposure increases. Symptoms may develop during or after exposure.

In a study of physiological responses associated with chilling (*1*), I made some observations on protoplasmic streaming. Effects of temperature on streaming were observed by Sachs (*2*), who noted that streaming ceased at about 11°C in *Cucurbita pepo* and *Lycopersicon esculentum*, both of which are chilling-sensitive plants. In contrast, Sachs cited observations by previous workers that in several lower plants streaming ceased at or near 0°C. This suggests differential responses by chilling-sensitive and chilling-insensitive plants.

In the present work, the influence of chilling temperatures on streaming was observed in cells of young petiole trichomes of several species. Observation of individual cells was possible by moving the microscope, mount, and light, as a unit from one constant-temperature room to another. A fan blew air across the stage to insure rapid temperature equilibrium and to reduce heating of the subject from the illumination. In all the chilling-sensitive plants observed—tomato (*Lycopersicon esculentum*), watermelon (*Citrullus vulgaris*), honeydew melon (*Cucumis melo*), a tobacco (*Nicotiana glutinosa*), and sweetpotato (*Ipomoea batatas*)—streaming ceased or was just perceptible after 1 or 2 minutes at 10°C. Invariably it ceased promptly at 5° or 0°C. In contrast, streaming in the chilling-insensitive plants observed—radish (*Raphanus sativus*), carrot (*Daucus carota*), and filaree (*Erodium cicutarium*)—proceeded at 0° or 2.5°C.

For tomato trichomes, increasing the exposure at 0°C delayed increasingly the resumption of streaming on transfer to 20°C. If exposure to 0°C exceeded about 24 hours, streaming failed to resume in most trichomes. In contrast, streaming in filaree proceeded slowly but steadily at 0°C for up to 3 days. On transfer back to 20°C, rapid streaming was resumed in 1 or 2 minutes.

In addition to these effects on streaming, chilling caused other responses. After 2 days of chilling at 0°C, some trichome cells of tomato were plasmolyzed. In others, the protoplasts formed into aggregated clumps, and small particles executed Brownian movement in the cell sap. Such movement was never observed in unchilled trichomes. This structural disorganization of the protoplast by chilling may result from syneresis of the protoplasmic gel, as has been suggested for a slime mold (*3*).

The striking difference described here between chilling-sensitive and chilling-insensitive plants suggests an association between susceptibility to chilling injury and cessation of streaming. Chilling injury and cessation of streaming may be separate symptoms of some more fundamental disorder that is induced at 10°C or below in chilling-sensitive plants. Alternatively, the visible symptoms of chilling injury could be effects secondary to cessation of streaming.

The contrasting effect of chilling temperatures on streaming in cells of sensitive and insensitive plants may reside in a differential effect of temperature on one or more of the following: cell lipids (*4*) and their role in structure and action of the protoplasm; energy supply from respiration to maintain protoplasmic streaming; energy utilization for streaming; or protoplasmic viscosity. If the mechanism of streaming is related to an adequate supply of adenosine triphosphate (*5*), some interesting comparative biochemistry for chilling-insensitive and chilling-sensitive plants is suggested.

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Direct-Reacting Bilirubin, Bilirubin Glucuronide, in Serum, Bile, and Urine

Since the introduction into clinical chemistry of diazotized sulfanilic acid for the determination of serum bilirubin, it has been recognized that bilirubin occurs in two different forms, one giving a so-called "direct" reaction, the other exhibiting a so-called "indirect" reaction in which coupling takes place only after the addition of alcohol (*1*). In general, when the jaundice is due to excessive destruction of hemoglobin, as it is in hemolytic anemia, most of the bilirubin in serum is of the indirect-reacting form. If the jaundice results from intrahepatic or extrahepatic obstruction to the passage of bile into the intestine, most of the bilirubin in serum is of the direct-reacting form.

Various explanations have been offered to account for this clinically important difference, but the experimental evidence in support of these theories has been controversial (*2*). Recent studies have shown that direct-reacting bilirubin obtained from serum or bile is water-soluble over a wide *p*H range, whereas the indirect-reacting pigment is practically insoluble in water below a pH of 8 (*3*). It has been suggested that the solubility in water of the direct-reacting fraction may be the result of a conjugation of bilirubin with a polar substance (*1, 4*). Because of their instability, satisfactory chromatographic purification of these pigments, particularly the direct-reacting fraction, has not been achieved (*4*).

This difficulty has now been overcome by studying (*5*) the more stable dipyrromethene diazonium pigments, which were obtained by treating serum and urine from jaundiced patients or normal bile with an excess of diazotized sulfanilic acid that was dissolved in dilute hydrochloric acid. In this reaction 1 mole of bilirubin reacts with 2 moles of the reagent, yielding 2 moles of hydroxypyrromethene diazonium salt (*6*).

After preliminary purification and butanol extraction, the azo-pigments obtained from serum, bile, and urine were separated and purified by ascending paper chromatography, employing a solvent system that consisted of 75 parts ethyl methyl ketone, 25 parts *n*-propionic acid, and 30 parts water. By using this system, it was found that direct-reacting bilirubin in the serum of jaundiced patients and almost all of the bilirubin in fresh bile and in the urine of jaundiced patients gave rise to an azo-pigment (B) exhibiting an R_f value of 0.25 to 0.30. On the other hand, the azo-pigment (A) obtained from indirect-reacting serum bilirubin, crystalline bilirubin, and heated bile exhibited an R_f value of 0.45 to 0.50. Diazonium salts of synthetic neoxanthobilirubinic acid and isoneoxanthobilirubinic acid (*7*) were found to have an R_f value that is identical with that of indirect-reacting serum bilirubin and that of crystalline bilirubin. Absorption spectra in the visible range appeared to be identical for azo-pigments A and B.

Hydrolysis of repeatedly chromatographed azo-pigment B in 1*N* hydro-

Table 1. Acid and enzymatic hydrolysis of conjugated hydroxypyrromethene diazonium salt (azo-pigment B). (Values are given in micromoles.)

Substrate azo-pigment	Type of hydrolysis	Product	
		Azo-pigment	Glucuronic acid
B, 0.41	1*N* HCl, 1 hr at 100°C	A, 0.41	0.39
B, 0.10	β-glucuronidase*	A, 0.083	0.09
B, 0.70	β-glucuronidase†	A, 0.61	0.58
B, 0.23	β-glucuronidase† (heat-inactivated)	B	< 0.01
B, 0.36	Concentrated sulfuric acid	Destroyed	0.38
A, 0.55	1*N* HCl, 1 hr at 100°C	A	< 0.01

* Beef liver β-glucuronidase (Warner-Chilcott), 24 hr at 37°C and *p*H 4.6.
† Bacterial β-glucuronidase (Sigma, Lot No. 125–63), 24 hr at 37°C and *p*H 6.2.