0.5 percent, yeast extract is inhibitory. The isolates grow well on yeast extract, but are unable to grow on the ether-extractable fraction, suggesting that the growth factors are not lipoid in nature. Although glucose was added to the initial isolation medium, glucose, galactose, sucrose, ribose, or glycerol do not stimulate growth. We were unable to grow the isolate anaerobically in the yeast extract–glucose medium in a Brewer jar. Attempts to grow the isolates on solid media have failed, at least partly due to the difficulty of obtaining a good solid medium at 55°C with a *p*H of about 2.

The DNA base composition of isolate 122-1B2 was determined by buoyant density sedimentation in $CsCl_2$ and was 25 percent guanine plus cytosine content.

There seems little doubt that this organism is related to known members of the Mycoplasmatales. The absence of a rigid cell wall (*1*) and the low content of guanine and cytosine (*5*) support this notion. The unusual physiological properties, however, suggest that the relation may be rather distant and seems to warrant classification within a new genus and species. We propose the name *Thermoplasma acidophila.*

The very existence of *T. acidophila* broadens considerably the range of habitats in which "mycoplasma-like" organisms have been found. The only previously described saprophytic species of *Mycoplasma* is *M. laidlawii,* an organism that was originally isolated from sewage (*6*). However, the fact that this same organism has been isolated from a variety of animal hosts, including the oviducts of cattle where accidental contamination seems unlikely (*7*), indicates that the saprophytic nature of this species may be questionable (*1, 8*). Due to the unusual conditions needed for growth of *T. acidophila,* it seems unlikely that it can grow as a commensal or parasite on some host and probably represents a truly saprophytic member of the order Mycoplasmatales. In addition to its inherent interest, the habitat of this organism, a burning coal refuse pile, is of considerable interest. Because of the restricted distribution and unstable nature of such piles, it seems unlikely that they are the primary habitats of these organisms. Similar organisms have been isolated from acid hot springs of Yellowstone National Park (*9*), and it seems possible that such thermal springs are the primary habitat. The structural simplicity of these free-living mycoplasmas suggests that they might be homologs of a primordial organism, and hence the study of these organisms may provide some insight into aspects of the origin of life. A culture of *Thermoplasma acidophila* has been deposited with the American Type Culture Collection as ATCC 25905.

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Tongue Cooling: A New Reward for Thirsty Rodents

Abstract. *Thirsty rodents will persistently lick a stream of dry air pumped through a standard drinking tube. This air-licking is attenuated by experimental manipulations which reduce the evaporative cooling of the tongue and mouth produced by the airstream. This suggests that such cooling is itself an effective reward for thirsty rodents. We tested this hypothesis by presenting thirsty rodents with a piece of cold, dry metal. Different species spent from 9 to 40 percent of their session time licking the cold metal. When deprived of water hamsters reared from birth without access to drinking water licked cold metal in preference to metal maintained at room or body temperature. This preference was approximately equal to that of littermates reared normally. We conclude that tongue cooling is a primary reward for thirsty rodents.*

Rats deprived of water will lick persistently at a stream of air pumped through a standard drinking tube (*1*). This air-licking looks very much like drinking; the the experienced observer has difficulty distinguishing between these activities. Air-licking occurs at stable rates of up to 10,000 licks per hour and shows no tendency to extinguish, althought it fails to restore the animals' body fluid balance. In fact, air-licking intensifies the rats' thirst by causing loss of water through evaporation from the tongue each time it comes into contact with the airstream (*1*).

Air-licking is not restricted to rats. We have found that it can also be demonstrated in water-deprived mice, hamsters, and gerbils (*2*). Thus it seems to be a general characteristic of rodents deprived of water that they will lick a stream of air at least as persistently as they lick water. Furthermore, air-licking occurs not only when thirst is induced by lack of water, but also under other conditions that induce drinking of water. These include subcutaneous injections of hypertonic saline (*3*) and certain schedules of food delivery to animals that have been deprived of food (*4*). Thus, air-licking and drinking seem to be controlled by the same motivational variables. No one has yet reported an experimental manipulation that induces drinking of water but fails to elicit air-licking.

In view of the fact that air-licking

is such a powerful phenomenon and is so closely related to drinking, it is important to discover the basis of its rewarding effects. Since air-licking and drinking share no common postingestional consequences, it is necessary to examine the sensory effects of the airstream's contact with the tongue and mouth to gain insight into the rewarding effects of air-licking. What is it about the contact of a stream of air with the tongue or mouth (or both) that renders it rewarding to the thirsty rodent? The hypothesis that has received the most attention is that the reward consists of the cooling of the tongue by the airstream. There have been a number of attempts to test this hypothesis. For example, it has been found that warming the air does not reduce the rate of air-licking (*1*, *5*). But this is a very inappropriate way to test the "cooling hypothesis"; for, the warmer the air, the more effectively it evaporates fluid from the tongue, thus enhancing the cooling effect, or at least compensating for the direct warming effect of the air. A more valid way of testing this hypothesis is to humidify the air, thus reducing its evaporative capacity, or to dry out the mouth so that there will be very little fluid on the tongue available for evaporation by air-licking. We have conducted such experiments and found that air-licking is attenuated by humidifying warm air or by surgical desalivation (*6*).

It seemed to us that the most direct way to test the hypothesis that tongue cooling is rewarding to thirsty rodents would be to see whether they would lick a piece of cold, dry metal. To this end we constructed a test chamber containing a standard stainless steel drinking tube, the tip of which was sealed off with lead solder. Cold water was circulated through the tube, and the temperature was monitored to the nearest 0.5°C by a thermometer whose sensing head was inserted into the tip of the tube. The chamber consisted of a Plexiglas box mounted on a wire-mesh floor. The tube protruded 1 cm into the box through an opening in one wall 5 cm above the floor. Contact with the tube was detected by a Grason-Stadler drinkometer and recorded with counters and event markers.

In a preliminary experiment two males and two females of each of four species were tested. Rats, mice, guinea pigs, and hamsters were deprived of water until each animal's body weight was 80 percent of that attained when they were given free access to food and water. They were then given a variable number of 30- to 60-minute daily sessions in the test chamber, the tube being maintained at 9° to 16°C. As a control, condensation of moisture was observed on a tube kept at the same temperature as the tube made available to the animal. Before each session the temperature of the tube given to the animal was adjusted to lie above that at which condensation would occur (dew point).

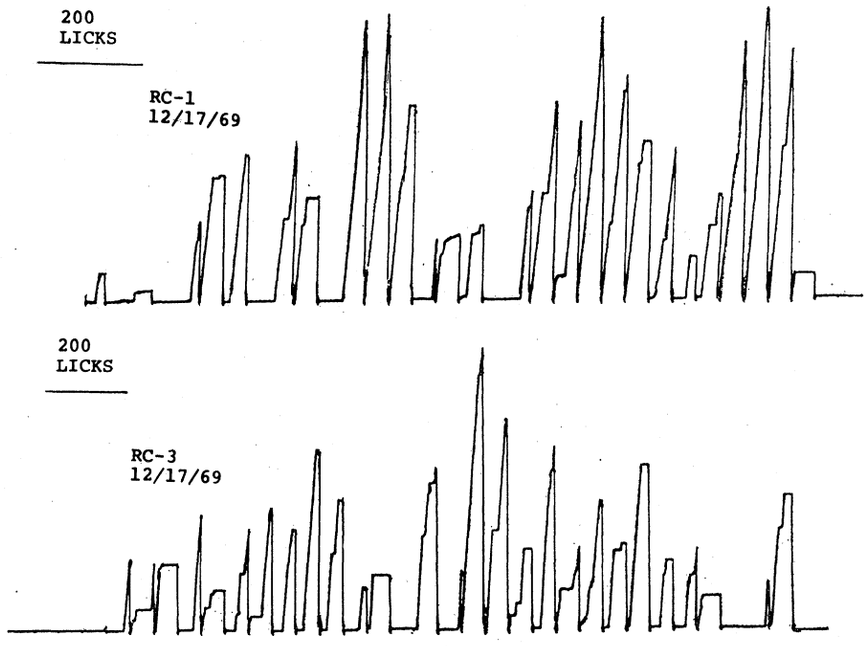

Fig. 1. Records of licking for two rats deprived of water on their first session of exposure to a cold metal tube. The record moves from left to right, each contact with the tube moving the tracing vertically. The height representing 200 contacts is indicated by a horizontal line. The pen automatically reset to zero at the end of each minute. (This type of recording underestimates the number of licks since the animals may maintain their lip or paw in continuous contact with the tube while they are licking it. Under such conditions the pen would not move vertically.)

On the first session the mean percentage of time spent licking the tube varied from 9 for the mice to 40 for the hamsters. Records of licking for two rats are shown in Fig. 1. Variability among animals tended to be high, even among members of the same species and sex. On subsequent sessions this variability persisted and was accompanied by a high degree of variability within subjects (*7*). Because the hamsters generated the least amount of inter- and intrasubject variability, they were given an extended series of tests which incorporated several controls. In addition, six more hamsters, three of each sex, were added to the group. When the tube was cold and the animals were deprived of water, substantial proportions of the hour-long sessions were devoted to licking the tube (Fig. 2). But when the tube was maintained at room temperature (24° to 27°C) or higher [body temperature (38°C) or hotter (45° to 50°C) (*8*)] or when the animals were exposed to the cold tube while fully satiated on water, tube-licking almost completely extinguished.

Since cooling of the tongue is a normal consequence of drinking water at room temperature, it could be that this cooling becomes a secondary reward based on the primary rewarding capacity of water. This seemed unlikely in view of the strength and persistence of air-licking and cold-licking; indeed, Riccio *et al.* (*9*) found that air-licking occurs in 18- and 23-day-old rats reared without water. We undertook an experiment to determine whether this result could be extended to cold-licking in hamsters. A litter of ten hamsters was raised in a cage with a drinking tube high above the floor so that only their mother could reach it. At the age of weaning (15 days) they were split into two groups. Four control animals were given free access to water for 12 hours daily, and the six experimental animals were given frequent stomach intubations of water at body temperature (*10*).

At the age of 28 to 42 days each animal was deprived of water overnight (12 to 14 hours) and given 20-minute sessions with two tubes avail-

able (*11*). In the first session for three experimental animals and one control, one tube was cold (6° to 16°C; different constant temperatures for different animals) and one was at or slightly above body temperature (38° to 40°C); for two animals in each group one tube was cold and the other was at room temperature (24° to 26°C). The experimental animals in these two conditions spent 4 to 39 percent of the session time licking the cold tube, with a mean of 20 percent; the three controls spent 14 to 35 percent of the time licking the cold tube, with a mean of 24 percent. The mean percentages of session time spent on the control tube were 8 and 6 for the experimental and control animals, respectively. With the exception of one experimental animal, all preferred the cold tube to the tube at body or room temperature.

To obtain an estimate of the amount of time which animals deprived to this degree would spend drinking water, we gave one animal in each group its first session with water at room temperature. The experimental animal drank it for 13 percent of the session time, the control for 17 percent. On their second session these animals were tested with a cold tube; they spent, respectively, 28 percent and 13 percent of the time licking it. Thus, for both the experimental and control animals licking the cold tube tended to be at least as persistent as licking water, and there were no significant differences between the groups. This experiment indicates that cold-licking is a primary reward, independent of prior experience with water.

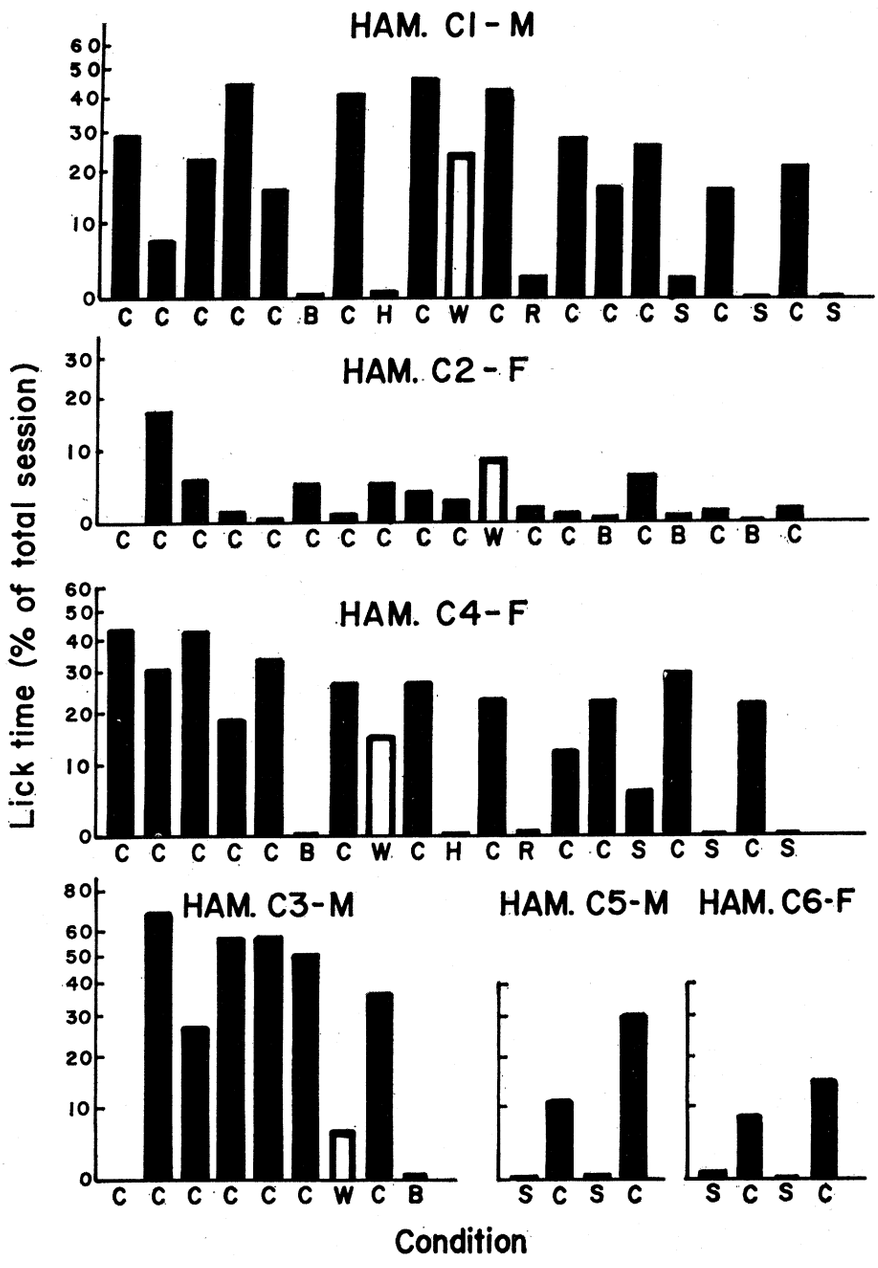

Fig. 2. Percentage of total session time that hamsters spent licking a metal tube as a function of tube temperature and contents. *C*, Cold; *B*, body temperature; *H*, hot; *R*, room temperature; *W*, water at room temperature (under all other conditions the tube was dry); and *S*, satiated animal with cold tube (under all other conditions the animals were deprived of water). On the first session for C2-F and C3-M the recording equipment broke down; both animals were observed to lick well in this session.

It has been known for a long time that there are fibers in the nerves innervating the tongue that are excited by cold stimuli placed on the tongue (*12*). However, no one who has studied the responses of these fibers has ascribed any particular functional significance to them. It seems to have been assumed that since most of the skin is innervated by receptors responsive to cold stimuli, the tongue is similarly innervated. Our results suggest that the cold receptors in rodents' tongues serve two special functions, at least one of which is not shared by cold receptors in other parts of the skin: (i) they help to identify water, which, of all naturally occurring substances to be found in most environments, is the most effective in absorbing heat from the skin; and (ii) feedback from these receptors to the central nervous system is rewarding to animals whose bodies have been depleted of water. Thus, the cold receptors in the tongue help thirsty rodents to detect water, and increase the probability that they will drink it. That other rewards in addition to cooling of the tongue are normally also derived from drinking water is indicated by experiments showing that rats whose tongues have been denervated continue to drink water (*13*) and that rats can learn to successfully regulate their water balance by being trained to inject water directly into their stomachs, thus bypassing the oropharynx (*14*). However, our experiments show that these rewards are not essential for the maintenance of licking in thirsty rodents; tongue cooling constitutes a sufficient reward for maintaining such behavior.

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8. The hot tube was used because many fibers in the lingual nerve which increase their rate of firing when the tongue is cooled respond similarly when the tongue is heated to 45° to 50°C [Y. Zotterman, in *Handbook of Physiology*, J. Field, Ed. (American Physiological Society, Washington, D.C., 1959), vol. 1, section 1, pp. 431–458]. Our failure to demonstrate licking of the hot tube was probably due to the fact that pain fibers in the lingual nerve are also excited by stimuli in this temperature range [E. Dodt, *Acta Physiol. Scand.* **31**, 83 (1954)].
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10. The control animals were also intubated, but no water was injected. The intubation tube was always carefully dried before insertion into the mouth. The drinking water available to the control animals was maintained at room temperature.
11. In this experiment each control animal was paired with an experimental animal and both were tested under the same conditions. The two experimental animals that could not be paired with controls (since there were only four controls) were tested with the cold tubes maintained at the mean temperature used for the other animals.
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Adenyl Cyclase of Cultured Mammalian Cells: Activation by Catecholamines

Abstract. *Chang's liver cells and 3T6 mouse embryo fibroblasts contain high amounts of catecholamine-sensitive adenyl cyclase, whereas HeLa cells contain relatively low amounts of activity. Both epinephrine and fluoride ion stimulate activity of each cell line. In contrast to normal liver, Chang's liver cells show greater response to epinephrine and no detectable stimulation by glucagon.*

Adenyl cyclase responsive to adrenocorticotropin is present in cultured adrenal tumor cells (*1*). However, adenyl cyclase appears to be absent or undetectable in cultured rat hepatoma (HTC) cells (*2*). The studies reported here demonstrate that three cultured mammalian cell lines, including a cell line derived from human liver, contain adenyl cyclase activity that is stimulated by catecholamines. Also the amount of activity in various cell types in culture may differ greatly. The presence of hormone-sensitive adenyl cyclase in several established cell lines should provide a useful tool for the study of cellular control mechanisms which involve adenosine 3′,5′-monophosphate (cyclic AMP).

Chang's liver cells and HeLa cells were grown with constant stirring in suspension culture in minimum essential medium (*3*) with 10 percent horse or calf serum (for Chang and HeLa cells, respectively) under an atmosphere of CO_2 and air (5 : 95). Chang's cells were also grown in monolayer in the same medium as for suspension culture but in stationary glass containers (*4*). Mouse 3T6 fibroblasts were grown in plastic petri dishes (Falcon) in Dulbecco medium with 10 percent calf serum under an atmosphere of CO_2 and air (10 : 90) (*5*).

For assay of adenyl cyclase activity, cells were washed with cold 0.15*M* NaCl and lysed by the addition of approximately 10 volumes (based on packed volume) of a solution containing 5 m*M* $MgSO_4$ and 20 m*M* glycylglycine buffer, *p*H 7.6. Lysates of cells grown in stationary culture and of HeLa cells were homogenized in an all-glass homogenizer. Lysates of Chang's cells in suspension culture were dispersed with use of a vortex mixer, since omission of homogenization did not change yield of enzymic activity. Cat and rat liver were homogenized gently in an all-glass homogenizer. Male rats (Sprague-Dawley) were about 60 days old. The final incubation mixture contained 0.625 m*M* [α-^{32}P]ATP [approximately 1.5×10^6 disintegrations per minute (dpm)], 1.25 m*M* $MgSO_4$, 5 m*M* caffeine, 40 m*M* tris(hydroxymethyl)-aminomethane (*p*H 7.5), in addition to approximately 5 mg (based on wet weight) of whole cell lysate in a volume of 100 μl. Incubations were carried out with or without additions of hormones or NaF as indicated. After incubation for 20 minutes at 30°C, the incubation mixtures were chilled and 25 μl containing 25 μg of unlabeled cyclic AMP was added to each; the mixtures were then heated at 95°C for 3 minutes. After centrifugation, portions of the heated supernatant fractions were subjected to thin-layer chromatography on Mylar sheets of polyethylenimine cellulose (Brinkmann) with a mixture of ethanol and 0.5*M* ammonium acetate (5 : 2, by volume) as developing solvent. The portions of the chromatogram containing cyclic [^{32}P]AMP were located by inspection under ultraviolet light; they were cut out and placed in vials for determination of radioactivity by liquid scintillation spectrometry. For calculation of cyclic AMP formation, corrections were first made by subtracting the small amount of radioactivity recovered after incubations in each assay with heat-inactivated cell lysates in place of active cell lysates. With use of these assay conditions, the accumulation of cyclic AMP was approximately proportional to incubation time and to amount of cell lysate present.

In some instances portions of the adenyl cyclase incubation mixtures were first subjected to chromatography on cellulose thin layers with a mixture of isopropanol, concentrated ammonium hydroxide, and water (70 : 10 : 20, by volume) as solvent; radioactivity in the areas containing cyclic AMP was eluted. Portions of the eluates were then subjected to treatment with partially purified beef heart phosphodiesterase (*6*) together with a known sample of tritiated cyclic AMP and in both the presence and the absence of theophylline. Portions were then chromatographed again on polyethylenimine-cellulose thin layers with ethanol and ammonium acetate (5 : 2, by volume) as solvent. With use of these procedures the ^{32}P-labeled product of the incuba-