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7. This variability was not attributable to the different temperatures used for different animals. Variation of temperature from 9° to 16°C failed to produce any systematic differences in the rate of cold-licking.
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.
12. For data on the rat and hamster, see B. Appleberg, *Amer. J. Physiol.* **44**, 129 (1968); and I. Y. Fishman, *J. Cell. Comp. Physiol.* **49**, 319 (1957). For a review of older work on the cat, see Y. Zotterman (*8*). The discharge pattern in single fibers produced by sudden cooling of the tongue has two components. The immediate response is a short-lived phasic discharge at a very high frequency. This rapidly declines to a sustained, low tonic frequency which may be as little as 7 percent of the initial frequency. The fact that the phasic discharge tends to be so much greater than the tonic discharge may account for the tendency of our animals to continuously move their tongues over the cold tube, rather than to lay their tongues motionless against it, and to lick an airstream at a high rate instead of merely positioning their tongues so as to keep them in constant contact with the airstream.
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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-

Table 1. Adenyl cyclase activity of cultured cells and normal liver. Enzyme activity is expressed as the number of nanomoles of cyclic AMP formed per 100 mg of protein per 20-minute incubation. Values given are the means ± standard error of values assayed on separate cultures harvested on different days. Each individual assay involved at least duplicate (usually triplicate) incubations for control, fluoride ion, and hormones at one or more concentrations. The numbers in parentheses are the number of cultures assayed separately for each condition studied.

Additions to assay	Adenyl cyclase activity					
	3T6 fibroblast	HeLa cell	Chang's liver cell		Rat liver	Cat liver
			Monolayer	Suspension		
Control	13.8 ± 4.4 (10)	3.0 ± 0.3 (3)	16.0 ± 1.0 (3)	21.5 ± 1.1 (32)	1.6 ± 0.2 (3)	0 ± 0.2 (3)
l-Epinephrine						
$1.6 \times 10^{-7}M$	35.8 ± 3.0 (3)	3.3 ± 0.2 (2)		33.8 ± 2.4 (4)		
$1.6 \times 10^{-6}M$	78.1 ± 3.1 (3)	5.3 ± 0.5 (2)	29.1 ± 4.4 (2)	48.1 ± 3.1 (5)		
$1.6 \times 10^{-5}M$	86.0 ± 11.8 (10)	5.5 ± 0.5 (3)	41.8 ± 3.2 (2)	74.5 ± 9.1 (14)		
$1.6 \times 10^{-4}M$	73.0 ± 8.2 (6)		47.1 ± 3.5 (3)	77.0 ± 5.0 (32)		3.6 ± 0.6 (3)
NaF (8 m*M*)	236.4 ± 16.2 (10)	19.8 ± 2.8 (3)	140.0 ± 1.4 (3)	95.5 ± 8.7 (27)	8.0 ± 1.0 (3)	29.1 ± 2.1 (3)
Glucagon						
0.2 μg/ml			16.5 ± 1.1 (3)	21.4 ± 1.0 (3)	3.9 ± 0.3 (3)	9.0 ± 0.6 (3)
2.0 μg/ml			18.6 ± 1.8 (3)	21.2 ± 0.7 (5)		10.1 ± 0.9 (3)

tions with Chang's liver, HeLa, and 3T6 fibroblast cell culture lysates was identical to tritiated cyclic AMP with respect to rate of degradation by phosphodiesterase and extent of inhibition of degradation by phosphodiesterase by the presence of theophylline (*7*).

The results of assays of adenyl cyclase activity for three cultured cell lines are summarized in Table 1. The relative amounts of activity are the same whether values are calculated on the basis of cyclic AMP formed per biuret protein as reported here, per protein using the method of Lowry (*8*), per tissue or cell wet weight, or per cell number (for the suspension cultures). Individual assays were generally carried out with triplicate determinations for each condition studied. Significant stimulation over control activity by 1.6×10^{-6} to $1.6 \times 10^{-4}M$ epinephrine as well as by NaF ($P < .01$) occurred consistently with each enzyme preparation from each of the cell lines. Epinephrine at $1.6 \times 10^{-7}M$ did not stimulate HeLa cell adenyl cyclase but did significantly increase the activity of the 3T6 fibroblast and Chang's liver cell enzymes. The 3T6 fibroblast adenyl cyclase showed high activity in the presence of epinephrine and even greater activity with NaF, an activity much greater than that found in many normal tissues—for example, the liver (Table 1) (*9*). For propagation of the 3T6 fibroblasts in these studies, cells were trypsinized, diluted for subculture, and replated generally at intervals of about 10 to 14 days (*5*). Adenyl cyclase assays were usually carried out from 2 to 10 days after subculture. Although serotonin has been reported to stimulate growth of 3T6 fibroblasts (*10*), this effect may be independent of adenyl cyclase activation since in our studies serotonin over a wide concentration range did not activate the cyclase of 3T6 cultures harvested either early or late in the rapid growth phase. In other studies 3T3 mouse fibroblasts, cultured under conditions similar to those used for 3T6 cells, also had high amounts of catecholamine-sensitive cyclase, whereas several primary cultures of human fibroblasts had very low adenyl cyclase activity with little or no stimulation by epinephrine (*7*).

Chang's liver cells, a line of cells derived from human liver, contained very much higher amounts of catecholamine-sensitive adenyl cyclase than did normal liver (Table 1). However, the Chang cell enzyme is not activated at all by glucagon, whereas glucagon is a much more potent activator of enzyme in normal liver than is epinephrine (*9*). Although it has been suggested that liver may contain separate adenyl cyclases responsive to only glucagon or to epinephrine (*11*) and that these enzymes may be in separate types of cells, it appears that a single cat liver adenyl cyclase is responsive to both glucagon and epinephrine (*9*). It should be noted also that either glucagon or epinephrine can totally deplete liver glycogen in vivo. Chang cell adenyl cyclase was not activated by serotonin, histamine, triiodothyronine, vasopressin, or adrenocorticotropin, but was activated by isopropylnorepinephrine and norepinephrine (the relative potencies are about 2.0 and 0.1, respectively, that of epinephrine). Also, stimulation by catecholamines was blocked by the β-blocking agents propanolol and dichloroisopropylarterenol but not by the α-blocking agent dibenzylene (*7*). In these respects the tissue culture enzyme resembles the enzyme of normal liver (*9*, *12*).

The adenyl cyclase of HeLa cells was also significantly stimulated by epinephrine (Table 1). The total activity of adenyl cyclase in HeLa cells was relatively low, particularly in comparison with the activities in the other two cell lines. This difference was not due to differences in cyclic nucleotide phosphodiesterase activity (studied directly by addition of tritiated cyclic AMP to the assay system) or ATP breakdown (ATP determined chromatographically after assay incubation) under the assay conditions used (*13*). Although the adenyl cyclase activity of HeLa cells is low, it may be of regulatory significance, however, since HeLa cell growth has been reported to be inhibited by cyclic AMP (*14*). The lower activity of HeLa cells may be related to its derivation from tumor tissue, since hepatoma cells, for example, either in culture (*2*) or derived from an animal (*15*) contain either very low or no adenyl cyclase activity.

It appears that different cultured cells, such as HeLa and Chang's liver, which in many respects show rather similar enzymatic activities, may have very different amounts of hormone-sensitive as well as basal amounts of adenyl cyclase present. This finding may be of significance for our understanding of factors necessary for or involved in the maintenance of a cell or tissue in a differentiated state. These studies represent the first demonstration of hormone-sensitive adenyl cyclase in established cell lines other than in those of endocrine origin (*1*, *16*). Also, the presence of hormone-sensitive adenyl cyclase in several fairly well characterized and widely available tissue culture systems provides a new and potentially useful tool for study of the relation of hormonal control to the cell cycle as well as to other aspects of cell regulation.

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Visual Adaptation in Monkey Cones: Recordings of Late Receptor Potentials

Abstract. *The retinal cones of cynomolgus macaque monkeys show marked amounts of adaptation when the receptor potential is elicited by brief incremental stimuli presented against steady backgrounds of increasing intensity. The results can be accounted for by mechanisms of response compression, modified by the effects of photopigment bleaching, which together set the gain of the system at each background level, while also making the response nearly linear over a significant range of intensities above and below that of each adapting stimulus.*

Discriminations of light intensity can be made from starlight to sunlight, over an intensity range of ten billion to one. The term adaptation describes the ability of the eye to adjust its sensitivity as conditions of external illumination vary over this enormous range.

Some of the mechanisms of visual adaptation are well understood. There is a shift from highly sensitive scotopic (rod) vision to less sensitive photopic (cone) vision that begins about 10,000 times above the rod absolute threshold. Rod signals become saturated at intensities at which photopic vision functions and make no further contribution to intensity discrimination. The pupillary reflex tends to keep retinal illumination constant as more light strikes the eye, but it does not succeed in doing so, being sufficient to account for only a very small fraction of the total photopic adaptation that occurs (*1*).

Adaptation that takes place for fixed pupil size, within the remaining millionfold range of photopic vision, has proved particularly difficult to explain. An early idea was that the bleaching of photopigments was the only additional adaptive mechanism (*2*). According to the reflection densitometry of Rushton and Henry (*3*), bleaching by steady lights reaches about 10 percent at 3.4 log photopic trolands, rises to about 90 percent at 5.3 log trolands, and thus definitely occurs within the normal range of outdoor photopic vision. The primary effect of bleaching is simply to decrease system sensitivity in proportion to the fraction of pigment bleached. For example, if half the photopigment were bleached away, then twice as much light would be required to elicit the same response as before bleaching.

This mechanism is not nearly sufficient, however, to explain photopic adaptation in its entirety, because very large changes in sensitivity are associated with trivial percentages of bleaching (*4*). Thus additional adaptive mechanisms must exist. Some recent theories have proposed that there may be a form of automatic gain control in the retina, with an inhibitory feedback signal being used to control sensitivity (*5*). Although difficult to imagine within receptors, such a system would be plausible if adaptation took place mainly downstream, for example, in the layer of the retina where the bipolar cells are located, as proposed by Dowling (*6*). But this type of proposal is hard to accept as defining the only site of neural adaptation, because it would leave the receptors with an enormous range of intensities to handle, and an explanation of how they do this would still be required. Hence, it seemed likely to us that important adaptive mechanisms, in addition to bleaching, exist within the receptors.

We find that substantial adaptation does occur in photoreceptors and that our results are reasonably well fit by a simple model. The basic mechanism of adaptation in the receptor is the response compression which results from a nonlinear relationship between intensity of stimulation and amplitude of the receptor potential. This is modified in important ways by the effects of bleaching in the case of cones, but probably not for rods.

Using the technique of K. T. Brown (*7*), we have isolated and recorded the late receptor potential (late RP) from cynomolgus macaque monkeys, which with respect to vision are near relatives to man. There is good evidence that the late RP is the signal that is transmitted along the receptor, playing a crucial role in the activation of second-order cells (*8*). In our work this potential was recorded extracellularly with a microelectrode whose tip was located in the outer-segment layer of the receptors by methods described previously; the tip was directed toward the macular region where cones predominate. The position of the electrode tip in the macula was confirmed by direct observation. Also, we have accumulated more than 40 spectral sensitivity functions (*9*) which indicate that under the conditions of stimulation in this work the responses come only from the three types of cone receptors that have been identified in the primate retina. The many cones in the immediate region of the electrode generate tiny potentials that summate to produce a recordable response, whose size varies from about 2000 μv at high intensities down to 10 μv or less at the lowest intensities that we have used, where the signal is lost in noise despite the response averaging procedures that we used throughout.

The basic experiment consisted of presenting steady light fields to the eye, allowing sufficient time for a stable response voltage to be reached. Incremental flashes 150 msec long were then added to the same area of the retina. When presented against any