similarly persistent in anoxic turtles. I now report that turtles are less able to withstand stagnant anoxia.

Snakes (*Natrix fasciata*) and turtles (*Pseudemys concinna*) were collected within an 80-km radius of Gainesville, Florida, during March and April 1963. Crocodiles (*Caiman crocodilus*) and turtles of another species (*Chrysemys picta*) were obtained from animal dealers during November 1966. All animals weighed between 150 and 250 g. All were kept at 22° ± 2°C, given water but no food, and used between 1 and 2 weeks after their arrival in the laboratory. Stagnant anoxia was produced by removing the animals' hearts under local (lidocaine) anesthesia. Incisions through which the hearts could be reached were made under local anesthesia 24 hours before cardiotomy, and were sealed to prevent pneumothorax. Animals which were to be subjected to anoxic anoxia were also incised 24 hours prior to their experiments as described above, but instead of having their hearts removed, they were placed in a continuously flushed, pure N_2 (oxygen tension < 0.4 torr) atmosphere (*1*). All animals breathed this atmosphere freely. Body temperatures of all animals during experiments were 22.0° ± 0.2°C. In experiments where N_2 was breathed, time from the first breath of N_2 to the last breath was measured; in cardiotomy experiments, time from the first breath after removal of the heart to the last breath was measured (Table 1). An electrocardiogram (EKG) was recorded from each N_2-breathing snake or crocodile about 20 minutes after the last breath, and from each N_2-breathing turtle about 120 minutes after the last breath. In all cases these EKC's had characteristics previously correlated with effective cardiac activity, showing that the deterioration of CNS function was not secondary to failure of the heart.

Blood samples taken anaerobically by cardiac puncture from additional N_2-breathing turtles and crocodiles were analyzed for *p*H, lactic acid, and glucose (*2*) (Table 2). These animals received the same treatment as did those in which persistence of breathing was measured. Each was used for a single sample only, in which as much blood as possible (6 to 8 ml) was obtained.

Anaerobic CNS failure is probably due to lack of metabolic energy. In a previous experiment (*3*), turtles (*Sternothaerus minor*) were injected with iodoacetic acid, which diminishes energy production from anaerobic glycolysis. In air, these turtles maintained nearly normal rates of O_2 uptake and survived indefinitely, but in N_2 their survival time averaged less than 20 minutes, compared to more than 12 hours for controls. Anaerobic glycolysis thus seems primarily responsible for maintaining the activity of the CNS under anoxic conditions. The data of Table 1 show that the comparatively long survival of turtles under conditions of anoxic anoxia is dependent on blood circulation. Since CNS failure in turtles occurs so quickly when anoxia is the result of lack of perfusion, and so slowly when O_2-free blood is present, it follows that the long survival of turtles breathing pure N_2 depends on the transfer of substances (presumably glucose and lactic acid) between the blood and the CNS. Snakes and crocodiles are evidently unable to make such significant use of these transfers. Table 2 suggests that the differences between turtles and other reptiles are not related to concentrations of hydrogen ions, glucose, or lactate ions in the blood.

To supply energy at the same rate as aerobic metabolism does, anaerobic glycolysis would require a more than tenfold increase in glucose transport into the cells and in activity of the Embden-Meyerhof pathway. Thus the failure of snakes and crocodiles to maintain CNS function under conditions of anoxic anoxia may be due to insufficient glycolytic capacity of the brain cells, to inability of these cells to take up glucose from the blood at a rate sufficient to meet anaerobic needs, or to buildup of a toxic intracellular concentration of lactic acid.

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Tyrosine Aminotransferase: Enzyme Induction Independent of Adenosine 3′,5′-Monophosphate

Abstract. *The importance of adenyl cyclase and adenosine 3′,5′-monophosphate in the induction of tyrosine aminotransferase by adrenocorticosteroids has been tested in HTC cells derived from a rat hepatoma and grown in tissue culture. Adrenocorticosteroids cause a 10- to 15-fold increase in the rate of synthesis of tyrosine aminotransferase in these cells. Under various experimental conditions, with or without glucocorticoids, neither adenyl cyclase nor cyclic adenosine monophosphate could be detected in HTC cells. In addition, neither the cyclic nucleotide nor* N^6,$O^{2'}$*-dibutyryl cyclic adenosine monophosphate caused increased activity of the transaminase in HTC cells. We conclude that induction of tyrosine aminotransferase by glucocorticoids is not mediated by the adenyl cyclase–cyclic adenosine monophosphate system.*

Increased synthesis of the hepatic enzyme tyrosine aminotransferase (TAT) (E.C. 2.6.1.5) occurs when adrenocortical hormones are administered to intact rats (*1*), perfused through isolated livers (*2*), or added to fetal liver explants in organ culture (*3*). Activity of the enzyme is increased in liver by insulin (*4*) and glucagon (*4*) through increased synthesis of enzyme and in fetal liver explants by epinephrine (*5*), presumably through the same mechanism. The mechanism of induction by these nonsteroid hormones apparently differs from steroid hormones since (i) combinations of a steroid and a nonsteroid hormone cause an additive response but combinations of two different nonsteroid hormones do not (*5*); (ii) the kinetics and magnitude of the responses to the two groups of hormones differ (*4*); and (iii) induction of the enzyme in fetal rats occurs in response to insulin and glucagon but not hydrocortisone (*6*).

Certain physiological actions of chemically diverse hormones, including insulin, glucagon, and epinephrine, appear to be mediated through alterations

in the intracellular concentration of adenosine 3′,5′-monophosphate (cyclic AMP) (*7*). Recently, it was suggested that the steroids estrogen (*8*) and aldosterone (*9*) may also act in this manner. These several findings and the recent report (*5*) that an analog of cyclic AMP, N^6,$O^{2'}$-dibutyryl adenosine 3′,5′-monophosphate (DBC), causes induction of the enzyme in explants of fetal rat liver led us to test for the adenyl cyclase-cyclic AMP system in a line of cells that shows steroid-induced synthesis of TAT in tissue culture. The results of these studies are presented here.

An established tissue culture line, designated HTC, developed from the Morris Buffalo rat hepatoma 7288-c, affords a relatively simple system for studies of hormone-mediated enzyme induction (*10*). The rate of synthesis of TAT is increased 10- to 15-fold on addition of adrenal corticosteroids to cultures of HTC cells (*11*). For these experiments, HTC cells (clone C2) were cultured in modified Swim's medium 77 (*11*). Where indicated, the synthetic glucocorticoid dexamethasone phosphate (Dex) was added to give a final concentration of 10^{-5} mole/liter. Cells in exponential growth were removed from culture, washed by centrifugation, resuspended in about 2 ml of 0.05*M* tris-HCl buffer [tris(hydroxymethyl) aminomethane-hydrochloride, *p*H 7.4] and homogenized in an all-glass Dounce homogenizer. After centrifugation at 2200*g* for 10 minutes, the particulate fraction containing the plasma membranes was resuspended in 2 ml of buffer, centrifuged again, and suspended in 0.5 to 1 ml of the same buffer. In other experiments, liver from Buffalo rats was homogenized in eight volumes of tris-HCl buffer, and the plasma membrane fraction was isolated as described for cells. Adenyl cyclase activity and protein concentration were determined as described (*12*) and tyrosine aminotransferase was assayed by a modification of the method of Diamondstone (*13*).

In none of our experiments, done under a variety of conditions, were we able to detect significant adenyl cyclase activity in HTC cells. The virtual absence of adenyl cyclase is shown in Table 1 (experiment 1), in which membrane fractions incubated with dexamethasone, glucagon, or sodium fluoride were assayed for enzyme activity. Results were no different when the whole homogenate was tested, when cells were disrupted with ultrasound, when the membrane fraction was prepared in 0.25*M* sucrose or 0.05*M* potassium phosphate buffer, *p*H 7.4, or when an adenosine triphosphate-regenerating system was added to the test mixture. On the other hand, in tests on the liver of the Buffalo rat, adenyl cyclase was detected readily, and activity was stimulated by glucagon and sodium fluoride but not by dexamethasone (Table 1, experiment 2). Extracts of HTC cells did not affect the glucagon- or fluoride-stimulated activity of adenyl cyclase from the liver of Buffalo rats; thus, it is unlikely that the tissue culture cells contain an inhibitor of this enzyme.

Table 1. Effect of dexamethasone, glucagon, and sodium fluoride on adenyl cyclase from HTC cells and Buffalo rat liver. The HTC and liver particles were prepared as described. The starting material contained 10^8 HTC cells (10^6 cell/ml) or 500 mg of intact liver. In experiment 1, each test mixture contained 200 μg of protein from HTC particles or 445 μg from the whole homogenate. In experiment 2, 340 and 350 μg of protein were added for HTC and liver particles, respectively. In experiment 2, where HTC and liver particles were combined, results are calculated on the basis of protein from liver particles only. Each test mixture contained 1.2 m*M* ATP-α-^{32}P (25 to 30 count min^{-1} pmole^{-1}), 0.015 percent albumin, 10m*M* theophylline, 5m*M* magnesium chloride, and cell particles in 70 μl of 50 m*M* tris-HCl, *p*H 7.4. Where indicated, glucagon, dexamethasone (Dex), and sodium fluoride (NaF) were added to give a final concentration of 10 μg/ml, 10^{-5} mole/liter, and 10^{-2} mole/liter, respectively. Results shown represent the mean, standard deviation, and number of determinations (in parentheses). Where standard deviations are not listed results represent the mean of triplicate determinations.

Preparation	Addition	Cyclic AMP formed (pmole/mg protein/5 min)
Experiment 1		
HTC particles (7)	None	4.2 ± 1.0
HTC particles (7)	Glucagon	5.2 ± 2.2
HTC particles (5)	Dex	2.1 ± 2.0
HTC particles (7)	NaF	5.6 ± 3.9
HTC whole homog.	NaF	5.7
Experiment 2		
HTC particles	None	6.2
HTC particles	NaF	6.6
Liver particles	None	10.5
Liver particles	Dex	10.2
Liver particles	Glucagon	131.2
Liver particles	NaF	332.2
HTC + liver	Glucagon	132.1
HTC + liver	NaF	389.4

Although it appeared from these results that there was little or no adenyl cyclase in HTC cells, it was still possible that the cells contained low concentrations of cyclic AMP (*14*), and accordingly, a direct analysis (*15*) was carried out on HTC cells and on the livers of Buffalo rats. The concentration of cyclic AMP in the intact liver (0.61 nmole/g, wet weight) was similar to that reported for liver from adult Sprague-Dawley rats, whereas the concentration of the nucleotide in HTC cells (0.06 nmole/g, wet weight) was not significantly greater than the minimal concentration that can be detected by the method used. We conclude, therefore, that activation of adenyl cyclase is not involved in the induction of tyrosine aminotransferase by glucocorticoids in HTC cells.

We next tested directly whether cyclic AMP might induce TAT in HTC cells, as found with fetal liver in organ culture (*5*). Suspensions of HTC cells were incubated with cyclic AMP itself, DBC, DBC plus theophylline, DBC plus theophylline plus dexamethasone (Table 2), or theophylline or glucagon alone (not illustrated). Enzyme induction occurred only in those cultures containing dexamethasone. The steroid-induced enzyme activity was not significantly affected by the other substances.

Table 2. Effect of theophylline, dexamethasone, and N^6,$O^{2'}$-dibutyryl adenosine 3′,5′-monophosphate (DBC) on tyrosine aminotransferase (TAT) in HTC cells. The HTC cells, cultured in Swim's medium 77 to 8 × 10^5 cell/ml, were centrifuged and resuspended at a concentration of 1.0 × 10^6 cell/ml in "induction medium" (*11*) with added dexamethasone, theophylline, or DBC, or in combination, as noted above. After incubation at 37°C for 17 hours, assays were carried out for TAT activity (milliunits per milligram of protein) and for protein concentration. In another experiment, not illustrated, DBC did not cause induction of TAT after incubation for 1, 2, 4, 8, and 23 hours under conditions identical to those described above.

Addition	Final conc. (*M*)	TAT (munit/mg protein)
None	—	9.0 ± 0.5
Dexamethasone	10^{-5}	76.0 ± 6.0
Theophylline	10^{-3}	7.8 ± 0.4
DBC	10^{-4}	8.8 ± 0.3
Theophylline + DBC	10^{-3} 10^{-4}	7.4 ± 0.1
Dexamethasone + Theophylline + DBC	10^{-5} 10^{-3} 10^{-4}	89.0 ± 7.0

Since we have no proof that the cyclic nucleotide or the derivative actually entered the cells, conclusion about the lack of an effect must be tentative. However, when cyclic AMP or DBC was added to intact cells in certain other systems (*5*, *7*, *16*), the results suggested that the cell membrane was penetrated. If we assume that the HTC cells are permeable to DBC, the lack of induction of the transaminase in this system suggests that in adult liver, where both hydrocortisone and cyclic

AMP cause induction of the enzyme, two separate regulatory systems may be involved, possibly dependent on separate structural genes.

Since we detected little or no adenyl cyclase or cyclic AMP in the HTC cells (results obtained were at the limits of sensitivity of the methods used), it appears likely that this line of mammalian cells is deficient in these components. The induction of hepatic TAT by glucagon in vivo is probably mediated by cyclic AMP; thus, the lack of response to glucagon in HTC cells might be attributable to a deficiency of adenyl cyclase, lack of sensitivity of the cells to the cyclic nucleotide, or both. In any event, our data suggest that induction of TAT by glucocorticoids in HTC cells does not involve the adenyl cyclase-cyclic AMP system.

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Pulmonary and Circulatory Adjustments Determining the Limits of Depths in Breathhold Diving

Abstract. *Data on pulmonary gas exchange were collected in breathhold dives to 90 feet in a tank and in open-sea breathhold dives to depths of 217.5 and 225 feet. Thoracic blood volume displacements were measured at depths of 25, 50, 90, and 130 feet, by use of the impedance plethysmograph. The open-sea dives were carried out with an average speed of descent of 3.95 feet per second and an average rate of ascent of 3.50 feet per second. End-dive alveolar oxygen tensions did not fall below 36 millimeters of mercury, while alveolar carbon dioxide tension did not rise above 40 millimeters of mercury except in one case. These findings indicate that for diver Croft, who has unusual lung capacity, neither hypoxia nor hypercapnia determined the depth limits under those conditions. At depths of 90 and 130 feet blood was forced into the thorax, amounting to 1047 and 850 milliliters respectively.*

Recent exploits in breathhold diving have shown that man has the capacity to dive to depths in excess of 200 feet (60 m) (*1*). This raises questions about the validity of the generally held assumption that the depth threshold is determined by a point at which total lung volume (*2*) (the amount of gas contained in the lungs at the end of maximal inspiration) is compressed to the residual volume (volume of gas in lungs at end of maximal expiration). Both recent record-holders in breathhold diving, Robert Croft (217.5 feet) and Jacques Mayol (231 feet), went to considerably greater depths than could be predicted on the basis of their total lung volume/residual volume ratios. Additional factors must therefore be considered. It has been suggested that at greater depths blood is forced into the thorax, replacing air and resulting in a decrease of residual volume and thereby extending the depth limit (*3*, *4*).

This report presents the results of the first measurements of thoracic blood volume made in breathhold dives, demonstrating a shift in blood volume into the thorax at great depths. Moreover, end-dive alveolar gas tensions were obtained in dives up to 225 feet in the open sea, considerably extending the depth range of information in pulmonary gas exchange in breathhold diving.

Studies were carried out on Robert Croft, a U.S. Navy diver, to depths of 90 feet at the Escape Training Tank, Naval Submarine Base, Groton, Connecticut, and extended to open-sea dives off Fort Lauderdale, Florida, culminating in Croft's world-record dives to 217.5 feet and recently to 240 feet. The 240-foot dive was covered by three underwater photographers, who observed a pronounced caving in of the thorax and compression of the abdomen at depth, which may be seen in the cover pictures. They also noticed skinfolds flapping around the chest.

The vital statistics of R. Croft (Table 1) show his unusually large vital capacity and small residual volume. Based on the ratio of total lung volume (total lung capacity) to residual volume his depth threshold would be 197 feet, which is considerably deeper than that of the average person (80 to 100 feet). Moreover, R. Croft is able to exert much larger expiratory pressures and somewhat larger inspiratory pressures than those found in normal healthy subjects. (The expiratory and inspiratory pressures were measured at various lung volumes varying from residual volume to total lung capacity).

Before the dives in the open sea, the subject sat on a platform up to his waist in water. He lowered himself into the water up to the neck and held on to the raft in front of him, which supported two investigators who collected alveolar and expired gas samples. The breathing valve was attached to the raft in a low position. The subject breathed through a tube 1¼ inches (3.2 cm) in diameter into rubber bags. A two-way stop cock allowed switching from one bag to another at the end of collection periods. The filled bags were clamped and stored until the end of the experiment. The