tially (interval 1) the mean weight of golds in both groups was slightly smaller than that of the normals, although the differences were not significant. After 4 weeks, the golds in the group of larger fish had attained and exceeded the mean weight of the normals, but the difference only approached significance ($P = .12$). However, when the individual weight gains were compared (difference in weight for each fish between the start and the end of the 4-week interval) the golds had grown faster than the normals ($P < .001$).

In the same comparison for the group of smaller experimental fish, golds caught up with normals after 4 weeks, and their mean weight was not significantly different ($P = .48$). Again, the gains in weight were greater in golds than in the normals ($P = .032$).

After an additional 4 weeks, the differences within the experimental groups were yet larger. The mean weight of the golds in the group of larger fish was now significantly greater than that of the normals ($P = .027$), and the gain in weights of the individuals was again significantly higher for the golds ($P = .014$). In the group of smaller fish, the difference in mean weight between golds and normals increased further in the golds' favor, but the difference was still not significant ($P = .36$). The gain in weights of the individuals, however, was again significantly greater among the golds ($P = .032$).

The experiment confirmed the general observation that, when Midas cichlids are kept in groups having both normals and golds, the golds grow faster than the normals. The behavioral mechanism responsible for this difference is the advantage golds have in aggressive behavior over that of normals (*6*). This aggressive behavior is translated into improved access to food (*8*), particularly right after the initial rush to obtain food (*9*).

One curious result of this experiment was that the controls were in such remarkable agreement. Fighting involves the expenditure of energy and potentially results in injury and disturbance of the endocrine physiology (*10*). If gold and normal fish differ in aggressiveness, then the more aggressive golds would be expected to fight more among themselves, and consequently their growth should be slower (*11*). This problem will be treated elsewhere (*8, 9*).

This work on the Midas cichlid suggests that colorful gold morphs have an advantage over the normal, cryptically colored individuals in direct intraspecific competition for food. The advantage, however, disappears when all the fish are of the same color. Since there must be counterselective forces, perhaps predation, working against the brilliantly colored morphs, one would anticipate the continuance of polychromatism at a level appropriate to the ecological setting of each population. If the competition were interspecific, with a closely related species, one could predict the evolution of one species in which all individuals are brightly colored.

GEORGE W. BARLOW
Department of Zoology and *Museum of Vertebrate Zoology, University of California, Berkeley 94720*

References and Notes

1. G. Fryer, *Proc. Zool. Soc. London* **132**, 153 (1959); W. Hovanitz, *Symp. Soc. Exp. Biol.* **7**, 238 (1953); J. Huxley, *Acta Congr. Int. Ornithol.* **11**, 309 (1955); C. Kosswig, *Copeia* **1964**, 65 (1964); G. B. Moment, *Science* **136**, 262 (1962).
2. J. A. Allen, *Nature* **237**, 348 (1972); B. Clarke, *Bull. Syst. Assoc.* **4**, 47 (1962); H. B. D. Kettlewell, *Heredity* **10**, 287 (1956).
3. C. H. F. Rowell, *Advan. Insect Physiol.* **8**, 145 (1971); R. K. Selander, *Avian Biol.* **1**, 57 (1971).
4. A. J. Hogan-Warburg, *Ardea* **54**, 8 (1966).
5. R. K. Murton, *Ibis* **113**, 97 (1971).
6. G. W. Barlow and P. Ballin, in preparation.
7. The aquariums were 60 by 60 cm and 28 cm deep (101 liters); temperature 26° to 28°C; water continuously aerated and filtered, and changed weekly; 13-hour photoperiod.
8. G. W. Barlow, D. H. Bauer, K. R. McKaye, in preparation.
9. G. W. Barlow and K. R. McKaye, in preparation.
10. B. E. Eleftheriou and J. P. Scott, Eds. *The Physiology of Aggression and Defeat* (Plenum, New York, 1971).
11. One could assume that golds have a faster intrinsic rate of growth than normals, but that this advantage is canceled by the effects of fighting. However, other work (G. W. Barlow and S. J. Wallach, in preparation) has shown that groups of golds and of normals are about equally aggressive after they have been together for a few days.
12. I am grateful to L. Machlis and R. F. Green for their help, and to A. Bond and C. J. Berg for commenting on the manuscript. Supported by NSF grants GB 13426 and GB 32192X.

30 August 1972; revised 20 October 1972

Cyclic Adenosine Monophosphate and Hypertension in Rats

Abstract. *Aortas from spontaneously hypertensive and stress hypertensive rats contained significantly lower intracellular concentrations of cyclic adenosine monophosphate than did their respective controls. Adenylate cyclase activity was normal but was less responsive to stimulation, while phosphodiesterase activity (especially the low Michaelis-Menten constant form) was significantly elevated. Human aortas contained two forms of phosphodiesterase that were similar to those in rat aortas.*

The elevation of blood pressure in essential hypertension is due, for the most part, to a general increase in resistance in peripheral vessels, which could not be convincingly related to metabolism or to the concentration of circulating catecholamines (*1*). There is also no evidence in hypertension of increased sympathetic activity or of increased sensitivity of vascular smooth muscles to normal concentrations of circulating amines or to normal sympathetic tone (*2*). The defect seems to lie in the vascular smooth muscles themselves, as suggested by changes in their ionic constitution (*3*). Since adenosine 3′,5′-monophosphate (cyclic AMP) seems to control the tone of vascular smooth muscles (*4*), we studied cyclic AMP metabolism in vessels from hypertensive animals and in those from normotensive animals.

Two groups of rats with characteristics that closely approximate human essential hypertension were investigated: (i) spontaneous hypertensive rats (SHR) (*5*) which are produced by selective breeding of spontaneously hypertensive animals and continuous inbreeding with hypertensive mates—they might be described as genetically hypertensive (*6*); and (ii) stress hypertensive rats (StHR) (*7*) that are made hypertensive by exposing them to intermittent neurogenic stimulation stress (*8*)—hypertension appears in 4 to 6 weeks, and the animal remains hypertensive for up to 20 weeks. Tissues from both normal and hypertensive rats were quickly excised after the rats were decapitated, and then were frozen in liquid nitrogen, and treated in identical manners. We used the entire length of the aorta, from the heart to the bifurcation. The animals used as controls for the SHR and StHR rats were of the same strain, age, and sex as the hypertensive animals. Adenylate cyclase and phosphodiesterase activities in the frozen tissues were compared to those in fresh tissue preparations, and no deterioration of the activity was observed. Cyclic AMP content was determined by the protein-binding method described by Gilman

(*9*). The radioactivity readings of phosphodiesterase-treated blanks were subtracted from the sample reading. The concentrations of cyclic AMP obtained were also similar to those in aortas of animals killed by microwave irradiation, a procedure which destroyed both adenylate cyclase and phosphodiesterase in 30 seconds (*10*). Although the small peripheral vessels would be the ideal tissue to use, they are almost impossible to obtain free from connective tissue in the quantities needed for this type of experiment. Aortas were thus used since they at least reflect the changes occurring at the resistance vessels, and are generally used in metabolic studies on hypertension.

There are significant differences in the metabolism of cyclic AMP in hypertensive rats as compared to that in their respective controls (Table 1). Although some question can always be raised concerning the proper controls to use for the SHR animals (*11*) there is no question about the controls of the StHR rats, which were from the same group of animals, but were not subjected to the stress. The controls for the SHR animals were of the same age, strain, and sex, but with normal blood pressure.

As adenylate cyclase activities were normal or even slightly elevated in the aortas of both SHR and StHR rats, the intracellular concentrations of cyclic AMP seem to be controlled in each case by the higher phosphodiesterase activities and, even more critically, by the higher proportion of the high-affinity form of phosphodiesterase (PDE-II) (Table 1). Phosphodiesterase was shown to exist in a number of tissues in two forms that varied in their Michaelis-Menten constants (K_m) (*12*). The form that has the lower K_m (PDE-II) seems to be more important in in vivo metabolism of cyclic AMP because of the low concentrations of cyclic AMP present in vivo. A higher proportion of PDE-II and a lower concentration of cyclic AMP were also found in the kidneys and hearts of SHR rats. The importance of PDE-II in the control of cyclic AMP concentrations is particularly evident in the kidneys of SHR rats. While the maximum velocity (V_{max}) of the total phosphodiesterase activity in the kidneys of SHR rats was identical to the control level, the proportion due to PDE-II was significantly higher. The concentration of cyclic AMP in the kidneys of SHR rats was significantly lower than that in kidneys of control rats.

Studies with human aortas (*13*) revealed the presence of two forms of phosphodiesterase with similar kinetic properties to those from rat aortas. Human aorta preparations also hydrolyzed guanosine 3′,5′-monophosphate (cyclic GMP) at ten times the rate that they hydrolyzed cyclic AMP, and they had a higher proportion of low-K_m form of the enzyme. It seems therefore that the cyclic AMP system in human aortas is basically similar to that in rats. This may increase the value of studies with rat tissues.

A decreased concentration of intracellular cyclic AMP in the aortas of both SHR and StHR rats provides an explanation for the increased tone of these vessels in both hypertensive models, as cyclic AMP concentrations are inversely correlated with vascular smooth muscle tone (*14*). This decreased concentration may also be the reason for the observed pathology in the aortas of hypertensive rats and in particular the elevated water and ion contents (*3*). The decreased sensitivity of the adenylate cyclase, in each type of hypertension, to β-adrenergic stimulation (isoproterenol) (Table 1) points to a significant property of the aortas. The difference in adenylate cyclase activity in the two control groups probably reflects the varying β-adrenergic sensitivity of the aortas of different types of rats (*15*). Decreased sensitivity of adenylate cyclase to stimulation in both types of hypertensive rats may reflect the biochemical mechanism underlying the resistance of these vessels to relaxation. The decreased sensitivity of adenylate cyclase from the aortas of SHR rats, and the complete absence of response of adenylate cyclase from the aortas of StHR rats to sodium fluoride,

Table 1. Cyclic AMP metabolism in the aortas of normal and hypertensive rats. Cyclic AMP content was determined as described by Gilman (*9*). The phosphodiesterase activity was determined as described by Thompson and Appleman (*16*). The K_m and the percent of V_{max} due to PDE-II were determined with a computer program based on the calculations of Cleland (*17*). The presence of two forms of PDE was confirmed by chromatography on diethylaminoethylcellulose (DEAE) (*18*). The total V_{max} is expressed for a 10-minute interval as micromoles per 5 mg of tissue (wet weight). Adenylate cyclase activity is expressed as picomoles of cyclic AMP formed per milligram of tissue (wet weight). The incubation mixture for this determination contained 0.5 μmole of adenosine triphosphate, 1 μmole of theophylline, 32.5 μg of pyruvate kinase, 10 μmole of phosphoenolpyruvate, 20 μmole of tris(hydroxymethyl)aminomethane (*p*H 7.4), 2.75 μmole of KCl, and 7.5 μmole of $MgSO_4$ in a total volume of 0.5 ml. The incubation was carried out for 10 minutes at 37°C. Cyclic AMP produced was assayed by the procedure described by Gilman (*9*), after the mixture was purified on Dowex-formate columns (*19*). All results are expressed as the average ± standard error. Numbers in parentheses indicate the number of animals used for each determination.

Parameter	SHR Rats		StHR Rats	
	Control	Hypertensive	Control	Hypertensive
		Cyclic AMP		
Concentration [nanomoles per gram of tissue (wet weight)]*	0.98 ± 0.23 (3)	0.45 ± 0.16 (3)†	0.37 ± 0.07 (4)	0.20 ± 0.02 (4)†
		Phosphodiesterase activity		
K_m, PDE-I (mM)	1.09 ± 0.28 (6)	0.42 ± 0.08 (6)†	0.48 ± 0.04 (6)	5.42 ± 1.02 (6)‡
K_m, PDE-II (μM)	1.02 ± 0.01 (6)	0.91 ± 0.01 (6)	0.73 ± 0.05 (6)	6.05 ± 0.07 (6)†
V_{max}	0.06 ± 0.01 (6)	0.10 ± 0.01 (6)†	0.04 ± 0.003 (6)	0.20 ± 0.02 (6)‡
Percent of total V_{max} as PDE-II	4.64 ± 1.56 (6)	12.92 ± 2.55 (6)†	1.95 ± 0.35 (6)	7.33 ± 2.31 (6)†
		*Adenylate cyclase activity**		
Unstimulated	7.6 ± 3.0 (3)	9.3 ± 1.8 (3)	2.9 ± 0.3 (4)	6.2 ± 1.9 (4)
Stimulated with isoproterenol, $10^{-5}M$	37.1 ± 4.6 (3)†	6.0 ± 0.5 (3)	6.9 ± 0.7 (4)†	4.3 ± 1.1 (4)
Stimulated with sodium fluoride, $8 \times 10^{-3}M$	30.4 ± 5.6 (3)†	19.8 ± 3.7 (3)	44.4 ± 5.3 (4)†	7.1 ± 1.5 (4)

* Similar results were obtained when the results were expressed per milligram of protein. † Significantly different from controls, $P < .05$. ‡ Significantly different from controls, $P < .01$.

probably indicates structural damage to the enzyme itself. This again may represent a significant aspect of the biochemical aberration in hypertension.

M. SAMIR AMER

Department of Pharmacology, Mead Johnson Research Center, Evansville, Indiana 47721

References and Notes

1. W. S. Peart, *Pharmacol. Rev.* **18**, 667 (1966); A. Sjoerdsma, *Circ. Res.* **9**, 734 (1961).
2. P. Sleight, *Brit. Heart J.* **33**, 109 (1971); J. L. Hirschman and E. T. Herjindal, *J. Amer. Pharmaceut. Ass.*, **NS11**, 555 (1971); R. B. Hickler and L. D. Vandam, *Anesthesiology* **33**, 214 (1970).
3. A. P. Somlyo and A. W. Somlyo, *Pharmacol. Rev.* **20**, 197 (1968); *ibid.* **22**, 249 (1970).
4. G. A. Robison, R. W. Butcher, E. W. Sutherland, *Cyclic AMP* (Academic Press, New York, 1971); C. Lugnier, Y. Bertrand, J. C. Stoclet, *Eur. J. Pharmacol.* **19**, 134 (1972); L. Triner, Y. Vulliemoz, M. Verosky, G. G. Nahas, *Cardiovasc. Res.* **316**, 310 (1970); L. Volicer and S. Hynie, *Eur. J. Pharmacol.* **15**, 214 (1971).
5. K. Okamoto, *Int. Rev. Exp. Pathol.* **7**, 227 (1969).
6. The SHR rats (Wistar) had a mean blood pressure of 224.0 ± 4.6 mm-Hg, which was significantly different ($P < .01$) from their controls, which had a mean blood pressure of 137.3 ± 1.5 mm-Hg.
7. The StHR rats (Sprague-Dawley) had a mean blood pressure of 167.0 ± 1.7 mm-Hg, which was significantly different ($P < .01$) from their controls, which had a mean blood pressure of 111.3 ± 2.3 mm-Hg.
8. J. P. Buckley and H. H. Smookler, in *Physiological Effects of Noise* (Plenum, New York, 1970), p. 75.
9. A. G. Gilman, *Proc. Nat. Acad. Sci. U.S.A.* **67**, 305 (1970).
10. M. J. Schmidt, D. E. Schmidt, G. A. Robison, *Science* **173**, 1142 (1971).
11. B. V. Clineschmidt, R. G. Geller, W. M. Govier, A. Sjoerdsma, *Eur. J. Pharmacol.* **10**, 45 (1970).
12. W. J. Thompson and M. M. Appleman, *J. Biol. Chem.* **246**, 3145 (1971).
13. The results obtained with the human aortas should be viewed cautiously since they were collected sometime after death, and the patients had received a variety of drugs before. No adenylate cyclase activity could be demonstrated in any of the six samples examined.
14. L. Triner *et al.*, *Ann. N.Y. Acad. Sci.* **185**, 458 (1971).
15. R. A. Bernstein, L. Linarelli, M. A. Facktor, G. A. Friday, A. Drash, P. Fireman, *J. Allergy* **49**, 86 (1972); R. G. Goffey, P. J. Logsdon, E. Middleton, *ibid.* **49**, 87 (1972).
16. W. J. Thompson and M. M. Appleman, *Biochemistry* **10**, 311 (1971).
17. W. W. Cleland, in *The Enzymes*, P. D. Boyer, Ed. (Academic Press, New York, ed. 3, 1970), p. 1.
18. M. S. Amer and G. R. McKinney, *J. Pharmacol. Exp. Ther.* **183**, 535 (1972).
19. F. Murad, V. Manganiello, M. Vaughan, *Proc. Nat. Acad. Sci. U.S.A.* **68**, 736 (1971).
20. I acknowledge the assistance of R. Bressler, A. W. Gomoll, G. R. McKinney, and J. L. Perhach and the technical assistance of C. F. Gatewood and C. A. Wolf.

26 September 1972

Cerebral Hydroxylases: Stimulation by a New Factor

Abstract. *Cerebral tryptophan 5-hydroxylase and tyrosine hydroxylase are stimulated by a solution of lyophilized, protein-free 30,000g supernatant of brain homogenates (concentrate 1). This preparation can be further purified by passage through Sephadex G-75 and phosphocellulose columns to yield concentrate 2. Unlike concentrate 1, concentrate 2 is unstable after several days of storage even at −27°C. Neither preparation is active without the presence of a reduced pteridine cofactor. The stimulating factor appears to be thermostable, alkali-labile, dialyzable, and light-sensitive.*

Efforts to purify cerebral tryptophan 5-hydroxylase (E.C. 1.99.1.4) led us to the observation that protein-free supernatants or filtrates of cerebral homogenates contain a factor that, in nanogram amounts, stimulates hydroxylation of tyrosine or tryptophan in vitro (*1, 2*). This factor was obtained from the 30,000*g* supernatant of brain homogenates after precipitation of protein with 60 percent ethyl alcohol. Unlike the protein factor (*3*) that stimulates hepatic phenylalanine 4-hydroxylase (E.C. 1.14.3.1), this factor appears to be specific for cerebral hydroxylases of L-tryptophan and L-tyrosine. The existence of this factor has been confirmed (*4*). In view of the difficulties attendant to purification of cerebral hydroxylases (*2, 5, 6*), the knowledge of stimulatory factor, even before its ultimate characterization, will be of benefit to others.

Rats were killed by decapitation, and their brains were removed immediately in a cold room (4°C). Brains were homogenized in three volumes of 0.01*M* tris(hydroxymethyl)aminomethane (tris) acetate buffer (*p*H 7.6) containing $10^{-3}M$ 2-mercaptoethanol (or dithiothreitol), and the homogenate was centrifuged at 30,000*g* for 30 minutes at 2°C. The supernatant was cooled to −2°C, and an equal volume of cold absolute ethanol was added. The suspension was centrifuged, and the supernatant was flushed with nitrogen gas (pumped over water) for 2 minutes. It was then immersed in boiling water long enough to bring it to 70° to 80°C and diluted with an equal volume of oxygen-free boiling water. After the suspension was rapidly cooled in ice, the denatured protein was removed by centrifugation. The supernatant was concentrated under vacuum at low temperature to one-fifth of its original volume and extracted three times with an equal volume of peroxide-free ethyl ether. The aqueous solution, under nitrogen, was passed through a nitrocellulose filter [80 or 100 mμ (Millipore)]; Amicon filters inactivated the factor. The filtrate was lyophilized and the residue taken up in water. To standardize the assay conditions, the stock solution was diluted such that 50 μl diluted to 3.0 ml had an absorbance of 0.550 at 255 nm (concentrate 1). At this stage, portions of this solution stimulated partially purified cerebral tryptophan 5-hydroxylase by 40 to 50 percent. This activity remained for several weeks if the solution was sealed under nitrogen and stored at −27°C. The solution was purified further by passage through a 1 by 25 cm column of Sephadex G-25 (*7*) or G-75 equilibrated with 0.1 percent mercaptoethanol. The factor, in a manner similar to pteridines, was eluted with 0.1 percent mercaptoethanol. The fractions corresponding to pteridines, assessed by fluorescence at 450 nm, were lyophilized and applied at *p*H 7.0 on a 1 by 10 cm column of phosphocellulose (H^+) (1.76 meq/g, Schleicher and Schuell) and eluted again with 2-mercaptoethanol (*8*), yielding concentrate 2. All the above procedures must be done in the absence of light.

Tryptophan 5-hydroxylase was purified by two methods (*2, 5*), and tyrosine hydroxylase was prepared from rat brain (*6*). The activity of these enzymes was measured by separation of 5-hydroxy[^{14}C]tryptamine or [^{14}C]dopamine, respectively (*9*). Pteridine content of concentrates 1 and 2 was measured with the pteridine-dependent phenylalanine 4-hydroxylase of *Pseudomonas* (*10*). The stimulating factor

Table 1. Reactivation of tryptophan 5-hydroxylase by concentrate 1 (C1). All assays contained 3 mg of protein; tris-acetate buffer (*p*H 7.6), 50 m*M*; 2-mercaptoethanol, 1 m*M*; pargyline, 0.2 m*M*; L-tryptophan (including L-[2-^{14}C]tryptophan, 1.1×10^6 disintegrations per minute), 0.088 m*M*; and $DMPH_4$, 0.46 m*M*. Formation of 5-hydroxy[^{14}C]tryptamine was measured. Conditions of dialysis are given in the text.

Enzyme preparation	Specific activity (nmole mg^{-1} hr^{-1})
30,000*g* supernatant	0.50
30,000*g* dialyzed	.12
30,000*g* dialyzed + C1	.78
30,000*g* supernatant	.30
30,000*g* from Sephadex G-75	.16
30,000*g* from Sephadex G-75 + C1	.28