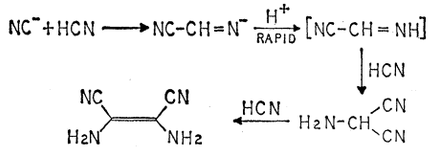

Fig. 2. Steps in the formation of aminomalononitrile and HCN-tetramer from hydogen cyanide.

yield provided they do not postulate unreasonable prebiotic conditions. We are well aware of the dangers implicit in this argument.

Under many conditions hydrogen cyanide is a major product formed in electric discharges through ammonia-methane mixtures. However, it is still difficult to see how 0.1*M* solutions of HCN could arise in large amounts. Such a high steady-state concentration in an extended water mass does not seem likely since the hydrolysis to formic acid requires at most a very few years at reasonable *p*H's and temperature. Perhaps, in a reducing atmosphere, raindrops in the neighborhood of an electrical storm might accumulate sufficient hydrogen cyanide, but we doubt whether this could account for the synthesis of large amounts of adenine. We were thus led to consider possible mechanisms for concentrating hydrogen cyanide from more dilute aqueous solutions.

The eutectic in the HCN–water system occurs at −23.4°C and contains 74.5 (moles) percent of HCN (*6*). Extrapolation of our data to these conditions suggested that tetramer formation should still proceed at an appreciable rate at the eutectic temperature in a solution having the composition of the eutectic mixture. Thus at about −22°C we might expect the proportion of cyanide transformed in a fixed time to be independent of the initial concentration of the HCN solution. We have now shown that tetramer formation in 0.01*M* HCN is accelerated by lowering the temperature from 25°C to −22°C and that at −22°C the rate of tetramer formation is roughly first-order in the initial cyanide concentration at least over the range 0.1*M* to 0.001*M*. The optimum rate of tetramer formation is attained at about −10°C.

In a typical experiment, solutions 0.01*M* in HCN and 0.005*M* in ammonia were made up at room temperature and left at 25°C, 0°C and −22°C for 3 days. Tetramer was clearly detectable only in the solution standing at −22°C; the yield was about 0.1 percent, five times the minimum amount which we could detect. In a series of control experiments sufficient methanol (40 percent by volume) was added to prevent the separation of ice; no tetramer was then detected. In an independent series of experiments with 1.0*M* HCN at 25°C we found that methanol accelerates tetramer formation slightly. These experiments prove that the increased rate of tetramer formation is indeed due to the effect of concentration during freezing. In certain of our experiments in which freezing occurred from below, the eutectic mixture separated as a droplet on the top of the ice, and it was then possible to follow the slow discoloration of the concentrated HCN solution at −22°C.

Recently, reports including some which may have relevance to prebiotic syntheses have dealt with reactions in eutectic (*7*) and solid ice phases (*7, 8*). We believe that these reports and our own experiments raise very directly the question: Did prebiotic synthesis occur at low temperatures? Many of the simple molecules, such as hydrogen cyanide, cyanic acid, cyanamide, formamide, hydrogen peroxide, and many ammonium salts, supposedly important in prebiotic synthesis, are extremely soluble in water and form low temperature eutectics. It seems quite likely that the Strecker synthesis of amino acids, the condensation of formaldehyde to sugars, and many similar reactions might proceed at a measurable rate in near-eutectic solutions at temperatures well below 0°C. At a later stage in biochemical evolution nucleotides and certain amino acids might have become concentrated in a similar way in a strong salt eutectic at low temperature, so favoring the operation of the template principle during polynucleotide formation and other condensations. If there is any basis to these speculations we may have to replace the usual picture of a warm, dilute, prebiotic medium with one more cold and much more concentrated, at least for some syntheses.

Thus concentration by freezing in raindrops and in, or above, lakes and other water masses must be added to evaporation and adsorption in the list of concentration mechanisms which may have been important on the primitive earth. Which played important roles in the evolution of life remains to be determined.

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References and Notes

1. J. Oro and A. P. Kimball, *Arch. Biochem. Biophys.* **94**, 217 (1961). A fairly complete bibliography of work on prebiotic adenine synthesis is given in reference (*2*).
2. J. P. Ferris and L. E. Orgel, *J. Amer. Chem. Soc.* **87**, 4976 (1965).
3. ———, *ibid.* **88**, 1074 (1966).
4. J. P. Ferris, R. Sanchez, L. E. Orgel, unpublished results.
5. J. D. F. Marsh and M. J. Martin, *J. Appl. Chem.* **7**, 205 (1957).
6. J. E. Coates and N. H. Hartshorne, *J. Chem. Soc.* **1931**, 657 (1931).
7. N. H. Grant and H. E. Alburn, *Science* **150**, 1589 (1965).
8. J. Oro, *Nature* **197**, 971 (1963).

23 March 1966

Circadian Rhythm in Pineal Tyrosine Hydroxylase

Abstract. *Tyrosine hydroxylase is the rate-limiting enzyme in catecholamine synthesis. The rat pineal gland is richly innervated by sympathetic nerves from the superior cervical ganglia. The activity of tyrosine hydroxylase was measured in rat pineal gland at 4-hour intervals over a daily cycle of 12 hours of light (7 a.m. to 7 p.m.) and 12 hours of darkness. The results indicate a circadian rhythm with the maximum activity, at 11 p.m. to 3 a.m., about triple the low values observed at 3 p.m. The pattern is similar in phase to that previously reported for melatonin and hydroxyindole-O-methyl transferase activity.*

The level of hydroxyindole-*O*-methyl transferase (HIOMT) in rat pineal gland varies with environmental lighting, being high during hours of darkness and low during hours of light (*1*). Endogenous melatonin levels follow a circadian rhythm similar to that of HIOMT (*2*), while the endogenous serotonin content of the pineal shows a rhythm opposite in phase (*2, 3*). The serotonin rhythm also differs in persisting unchanged during continuous darkness (*3, 4*).

The HIOMT and serotonin rhythms are interrupted by removal of the superior cervical ganglia which inner-

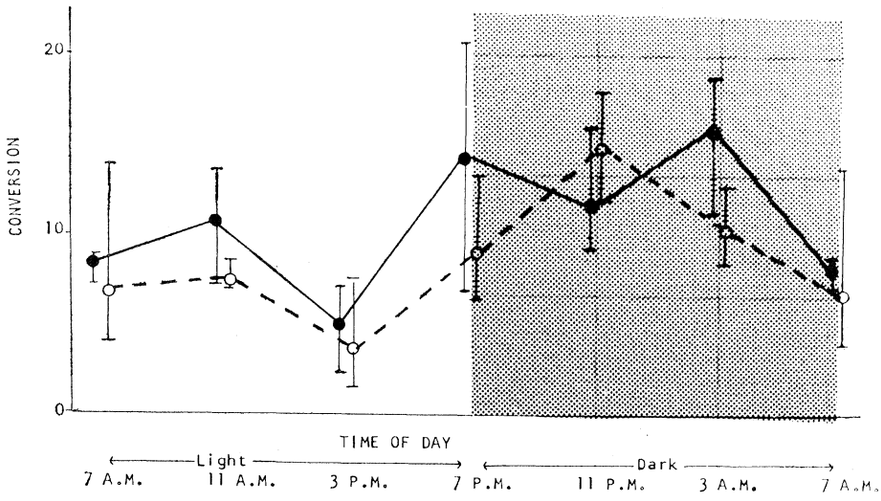

Fig. 1. Tyrosine hydroxylase activity in rat pineals. Solid line, female Wistar rats, incubations with NSD-1034, ratio 3 a.m./3 p.m. = 3.2. Dashed line, male and female hooded rats, incubations with NSD-1034, ratio 3 a.m./3 p.m. = 2.7. Ordinate ("Conversion"): micromicromoles of catechols formed per hour per pineal.

vate the gland (*3, 5*). The nerve pathways are probably noradrenergic (*6*), so that a rhythm in the noradrenaline-forming capacity of the nerve endings in the pineal gland might be expected. The experiments described here indicate that there is a circadian rhythm in rat pineal activity of tyrosine hydroxylase, the rate-controlling enzyme in noradrenaline synthesis (*7, 8*), and that this rhythm is closely parallel to that reported for HIOMT activity and for melatonin.

Groups of rats were kept on a cycle of 12 hours of light (7 a.m. to 7 p.m.) and 12 hours of darkness. Four to six rats were killed at each selected time, the pineals rapidly dissected and homogenized in 0.3 ml of a 1 : 1 mixture of 0.25*M* sucrose and 0.28*M* phosphate buffer, *p*H 6.2. A 0.2-ml portion of the homogenate was incubated for 30 minutes with 0.1 ml of water containing L-tyrosine-C^{14} (uniformly labeled) (specific activity 330 mc/m*M*). In two of the three series done, the radioactive tyrosine solution was made $3 \times 10^{-3}M$ in NSD-1034 (*9*) so that the final concentration in the incubate of this compound, used as a 3,4-dihydroxyphenylalanine (DOPA) decarboxylase inhibitor, was $10^{-3}M$. Where NSD-1034 was used in the incubation, 0.17 μc of L-tyrosine-C^{14} was added, and where NSD-1034 was omitted, 0.25 μc was added. Blanks were run each time with brain tissue heated for 12 to 15 minutes to 80° to 90°C. The incubations were stopped by the addition of acid, and the formed catecholamines were isolated by absorption on alumina. The acetic acid eluants of the alumina columns were counted in a liquid scintillation spectrometer with 10 ml of Bray's mixture (*10*). The blank with heated tissue was reproducible within a few percent and was only slightly higher than that with no tissue at all. All figures given are corrected for the blank.

The results obtained in incubations using NSD-1034, expressed as micromicromoles of catechols formed per hour per pineal, are shown in Fig. 1. Despite the considerable variation from pineal to pineal, which may reflect technical difficulties in handling these small bits of tissue, a circadian rhythm is clearly indicated with high values during the hours of darkness and low values during the hours of light. The apparent conversion was only about one-tenth as great in the absence of NSD-1034, presumably because the reaction would then proceed beyond DOPA, and the formed catecholamines would be more vulnerable to destruction by monoamine oxidase. Monoamine oxidase is highly concentrated in the pineal (*11*). However, the pattern obtained is similar, and the activity at 3 a.m. is about triple that at 3 p.m. whether or not NSD-1034 is used in the incubation.

With NSD-1034, the blank ranged from 845 to 920 count/min. The 3 p.m. values ranged from 163 to 696 count/min above the blank, and the 3 a.m. values, from 920 to 2047 count/min above the blank. In the experiments without NSD-1034, a different batch of tyrosine-C^{14} was used. The blank ranged from 412 to 485 count/min. The 3 p.m. values ranged from 16 to 271 count/min above the blank, and the 3 a.m. values, from 350 to 745 count/min above the blank.

The difference between the high and low values in each of the three series is highly significant ($p < 0.001$). The various times in each of the series were done in different random order, so that the rhythm noted is presumably not due to changing rat age or a similar factor. The individual rat weights varied from 140 to 215 g for the Wistar rats used and 190 to 370 g for the hooded rats, but there was no significant overall correlation in any series between pineal activity and rat weight.

The incubations were run without adding the tetrahydropteridine cofactor found essential for purified beef tyrosine hydroxylase (*7*). In many experiments in this laboratory using crude homogenates, the addition of pteridine cofactors has not improved conversion. However, the possibility that apparent shifts in the pineal tyrosine hydroxylase activity reflect changes in the availability of some cofactor rather than changes in the enzyme level itself cannot be excluded.

The close relation between the rhythm found here for tyrosine hydroxylase and those previously reported for melatonin and HIOMT activity lends some support to the hypothesis that release of a catecholamine transmitter may be involved in the control of HIOMT activity in the pineal.

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References and Notes

1. J. Axelrod, R. J. Wurtman, S. H. Snyder, *J. Biol. Chem.* **240**, 949 (1965).
2. W. B. Quay, *Proc. Soc. Exp. Biol. Med.* **115**, 710 (1964).
3. S. H. Snyder, M. Zweig, J. Axelrod, J. E. Fischer, *Proc. Nat. Acad. Sci. U.S.* **53**, 301 (1965); V. M. Fiske, *Science* **146**, 253 (1964).
4. S. H. Snyder and J. Axelrod, *Science* **149**, 542 (1965).
5. R. J. Wurtman, J. Axelrod, J. E. Fischer, *ibid.* **143**, 1328 (1964).
6. A. Pellegrino de Iraldi and L. M. Zieher, *Life Sciences* **5**, 149 (1966).
7. S. Udenfriend, *Pharmacol. Rev.* **18**, 43 (1966).
8. S. P. Bagchi and P. L. McGeer, *Life Sciences* **3**, 1195 (1964).
9. NSD-1034 (*N*-methyl-*N*,3-hydroxyphenylhydrazine) kindly supplied by Dr. D. J. Drain of Smith and Nephew Research, Ltd., Ware, Herts., England.
10. G. A. Bray, *Anal. Biochem.* **1**, 279 (1960).
11. B. Smith, *J. Anat.* **97**, 81 (1963).
12. Supported by NSF (GB-827), Medical Research Council of Canada (MA-1421), and Federal-Provincial Public Health grant 609-7-108.

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