cate that 6-MA has two minimum energy conformations corresponding to configurations in which the amino group is coplanar with the purine moiety and the methyl substituent is directed either toward, or away from, the imidazole ring of the base (*4*). When directed toward the imidazole moiety, the methyl group would not prevent Watson-Crick pairing between 6-MA and thymine. However, in the alternate conformation, 6-MA would interfere with normal Watson-Crick base pairing, because the methyl group blocks the N(6)-H site that is used for hydrogen bonding between adenine and thymine. In agreement with our crystallographic results, the molecular orbital calculations predict that the most stable conformation for 6-MA is the one that would disrupt normal Watson-Crick pairing. A Corey-Pauling-Koltun space-filling molecular model of 6-MA indicates that there is steric hindrance between the methyl group and atom N(7) when the N(6)-C(MET) bond is *cis* relative to the C(5)-C(6) bond, whereas no analogous interaction occurs when C(6)-C(MET) is *trans* to C(5)-C(6).

Since the conformation we have observed for 6-MA is sufficiently stable to persist through various crystalline environments, it is possible that 6-MA assumes the same conformation within double-helical DNA. If so, this modified base would probably exert appreciable effects on DNA secondary structure. By interfering with Watson-Crick pairing within modified DNA, 6-MA could effectively denature those double-helical regions which in the unmodified state would be recognized and cleaved by site-specific restriction enzymes. It is reasonable to assume that such effects on the secondary structure could interfere with the binding of restriction enzymes to DNA, which might explain the role 6-MA plays in protecting DNA from scission. However, if 6-MA were incapable of participating in Watson-Crick pairing, then it would be difficult to rationalize how modified DNA's can replicate and be transcribed with high fidelity. Possibly a less stable conformation which permits Watson-Crick pairing is imposed on 6-MA by replication and transcription enzymes, thereby permitting modified DNA's to function adequately. However, it is also quite possible that the preferred conformation of 6-MA within double-helical DNA is different from that of the free base, since the stabilization provided by Watson-Crick pairing between 6-MA and thymine may be sufficient to maintain the orientation in which N(6)-C(MET) is *cis* to C(5)-C(6).

HELENE STERNGLANZ
CHARLES E. BUGG
Institute of Dental Research and
Department of Biochemistry,
University of Alabama in Birmingham,
University Station, Birmingham 35294

References and Notes

1. W. Arber and S. Linn, *Annu. Rev. Biochem.* **36**, 467 (1969); T. J. Kelly and H. O. Smith, *J. Mol. Biol.* **51**, 393 (1970); M. Meselson, R. Yuan, J. Heywood, *Annu. Rev. Biochem.* **41**, 447 (1972); A. Haberman, J. Heywood, M. Meselson, *Proc. Natl. Acad. Sci. U.S.A.* **69**, 3138 (1972); C. Mulder and H. Delius, *ibid.*, p. 3215; J. F. Morrow and P. Berg, *ibid.*, p. 3365; J. D. Smith, W. Arber, U. Kühnlein, *J. Mol. Biol.* **63**, 1 (1972); U. Kühnlein and W. Arber, *ibid.*, p. 9.
2. H. Sternglanz and C. E. Bugg, *Biochim. Biophys. Acta* **308**, 1 (1973).
3. G. Germain, P. Main, M. M. Woolfson, *Acta Crys.* **A27**, 368 (1971).
4. H. Berthod and B. Pullman, *C. R. Hebd. Seances Acad. Sci. Ser. D. Sci. Nat.* **276**, 1767 (1973).
5. C. K. Johnson, "ORTEP, a Fortran thermal-ellipsoid plot program for crystal structure illustrations," *Report ORNL-3794*, revised (Oak Ridge National Laboratory, Oak Ridge, Tennessee, 1965).
6. Supported by NIH grants CA-12159, DE-02670, and RR-145.

11 June 1973; revised 10 August 1973

Psychologic Stress and Threshold for Repetitive Ventricular Response

Abstract. *A psychologically stressful environment reduced the threshold of the dog's ventricle for repetitive response. Elicitation of such a response indicates the presence of electrical instability and a predisposition to ventricular fibrillation, the mechanism of sudden death.*

Sudden death claims over 400,000 lives annually in the United States. While in the majority of victims the underlying basis is coronary heart disease, the immediate mechanism is an electrical derangement in heart rhythm believed to be ventricular fibrillation (VF). If the afflicted patient is promptly resuscitated, survival is possible for many years. Thus, this arrhythmia represents a reversible electrical accident. It has been postulated that the susceptible patient has an electrically unstable heart characterized by a reduced threshold for ventricular fibrillation (*1*). The present report indicates that psychologic factors can predispose to electrical instability of the heart.

In the normal as well as the infarcted heart, markedly suprathreshold pulses are required to trigger VF when they are delivered during the brief interval of the ventricular vulnerable period. However, when three early sequential pulses are administered, the current required for VF is markedly reduced; and in the animal with infarction, currents at threshold level for diastolic depolarization suffice to precipitate VF (*2*). This technique, designated as sequential R/T pulsing (*3*), was employed in dogs to measure the threshold for repetitive ventricular response in stressful and nonstressful environments. A repetitive ventricular response rather than VF was selected as the end point. The animal does not perceive a repetitive response and therefore can be retested frequently, whereas VF with the attendant traumatic resuscitative procedures precludes psychologic studies. We have found that repetitive firing evoked by sequential R/T pulsing consistently anticipates the development of VF (*4*); the repetitive response occurs when 66 ± 4 percent of the VF threshold current is administered.

In these experiments two bipolar catheters, with platinum electrodes having an interelectrode distance of 1.5 cm, were placed at the apex of the right ventricle via a jugular vein. Both catheters were exteriorized at the nape of the neck and the dogs were permitted to recover for 1 week. Electrical pulsing of the awake animal was achieved with square wave cathodal pulses of 2-msec duration. The timing of each pulse could be varied with an accuracy of ± 3 msec. The current intensity ranged from 0 to 100 ma (constant current, accuracy ± 3 percent). The amplitude of the first stimulus (S_1) was set at twice the mid-diastolic threshold for a single propagated response. The pulse was discharged progressively earlier in the cycle, in 10-msec steps, until a response no longer occurred. This defined the boundary of the effective refractory period for a stimulus of twice threshold intensity (*5*). The delay of S_1 was

then set at 10 msec beyond the effective refractory period boundary. The amplitude and delay of the second (S_2) and third (S_3) pulses were similarly determined. The three pulses resulted in a sequence of three early extrasystoles. The current of S_3 was then increased progressively by 5-ma increments until a repetitive ventricular response resulted. The response to each current level was tested twice. Repetitive ventricular response threshold was defined as the S_3 current necessary to induce more than three propagated responses in two out of three trials. While sequential R/T pulsing was carried out the heart rate was paced at 200 beats per minute.

Five dogs were tested in two different environments. The first was designed to minimize discomfort and disturbance to the animal and consisted of a cage in a sound-attenuated room with a one-way mirror. Cables connecting to the cardiac catheters, secured at the nape of the neck, were suspended from the ceiling of the cage, thus permitting freedom of movement. The investigators and electronic equipment were situated in an adjacent room. An acclimatization period of at least 1 hour was allowed before testing. When the dog appeared to be in a resting state, as indicated by a low heart rate and absence of activity, testing for a repetitive response was initiated. Pacing was begun at a rate of 200 beats per minute followed by sequential R/T pulsing. Five determinations were made of the S_3 current required for a repetitive ventricular response. A minimum time of 2 minutes was allowed to elapse between trials. After each determination, cardiac pacing was interrupted for 30 seconds in order to indicate the nonpaced heart rate. In this environment, the heart rate was low, 100 ± 5 (standard error of the mean) beats per minute, and usually the dogs were recumbent with eyes closed. Sequential pulsing did not result in any adverse behavioral effects.

Testing was also conducted in a stressful environment. The dog was placed in a Pavlovian sling; cables were connected to the cardiac catheters in precisely the same manner as in the cage, and sequential R/T pulsing was carried out at a paced rate of 200 per minute. The S_3 current for inducing a repetitive ventricular response was determined five times and the nonpaced heart rate was recorded between these tests. The stress consisted of a low energy shock of 5 joules delivered transthoracically and synchronized to the R wave to prevent the occurrence of VF. The shock was applied at the end of R/T testing. This procedure was repeated on three successive days. On subsequent days electrical shock was no longer administered. The data reported were obtained on days 4 and 5 of experimentation. At this time, when the dogs were placed in the sling, they were restless, frequently salivated excessively, exhibited somatic tremor, and had a nonpaced heart rate of 136 ± 7 beats per minute.

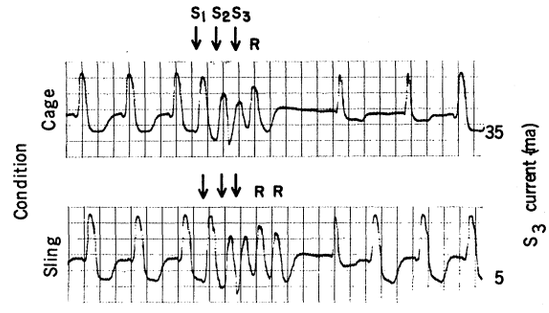

Fig. 1. Sequential R/T pulsing in the conscious dog. In the cage, where dogs were never stressed, 35 ma in S_3 elicits but a single repetitive response indicated by letter *R*. However, in the sling where animals had received an electrical shock on the previous day, the threshold is reduced to 5 ma and now a dual repetitive response is elicited (*RR*). The heart rate was maintained constant at 200 beats per minute by ventricular pacing.

There was a marked difference in threshold of S_3 current for eliciting a repetitive response in the two environments. In the cage, where dogs were never stressed, the mean current in milliamperes for a repetitive response was 43 ± 5 S.E.M. in five animals. However, in the sling, where dogs had experienced discomfort, the mean threshold in the same animals was 14 ± 6 ma. An illustrative example of repetitive ventricular responses to sequential R/T pulsing under the two experimental conditions is illustrated in Fig. 1. There appeared to be a qualitative relation between the S_3 current threshold and the nonpaced heart rate (Table 1). In dogs 2, 3, and 5, heart rate was 30 beats per minute higher in the sling than in the cage, and S_3 threshold was decreased by 25 ma. In dogs 1 and 4, in which the change in heart rate was greater (50 and 40 beats per minute), there was a correspondingly greater reduction in S_3 threshold (30 and 35 ma, respectively).

These findings suggest that psychologic stress can exert a profound effect on the threshold for VF. In the present study, threshold was lowered to only one-third of its control value; reductions of such magnitude are observed during acute myocardial infarction following experimental coronary artery ligation (*6*). The changed threshold while the animal was in the stressful environment probably resulted from increased sympathetic activity as exemplified by the rapid heart rate and somatic tremor. The potency of sympathetic discharge in predisposing to VF has been shown in two recent studies. In the first, stimulation of the posterior hypothalamus, which was without arrhythmogenic effect in the normal animal, precipitated VF in dogs with occlusion of the left anterior descending coronary artery (*7*). In the second study, stimulation of the stellate ganglia markedly increased susceptibility to VF during sequential R/T pulsing (*8*). This response was unre-

Table 1. Comparison of stress (sling) to nonstress (cage) on S_3 current threshold and nonpaced heart rate (HR) during sequential R/T pulsing. Five determinations were carried out in each animal. Abbreviation: bpm, beats per minute. The mean is given ± standard error of the mean.

Dog (No.)	Cage		Sling	
	S_3 (ma)	HR (bpm)	S_3 (ma)	HR (bpm)
1	35	110	5	160
2	30	90	5	120
3	40	90	15	120
4	45	100	10	140
5	60	110	35	140
Mean ± S.E.M.	43 ± 5	100 ± 5	14 ± 6	136 ± 7

lated to acceleration of heart rate or increase in blood pressure.

From ancient to modern times, medical thinking and folklore shared the notion that sudden death may be provoked by psychological factors (*9*). The present study suggests a model for analyzing the neurophysiologic pathways by which stress may alter electrical stability of the heart.

BERNARD LOWN
RICHARD VERRIER
RAMON CORBALAN

Cardiovascular Laboratory,
Department of Nutrition,
Harvard School of Public Health,
Boston, Massachusetts 02115

References and Notes

1. B. Lown and M. Wolf, *Circulation* **44**, 130 (1971).
2. P. Axelrod, R. Verrier, B. Lown, *Am. J. Cardiol.* **31**, 117 (abstr.) (1973).
3. P. L. Thompson and B. Lown, *Clin. Res.* **20**, 401 (abstr.) (1972).
4. R. Verrier, B. Lown, A. Calvert, unpublished observations.
5. B. F. Hoffman and P. F. Cranefield, *Electrophysiology of the Heart* (McGraw-Hill, New York, 1960), p. 253.
6. C. M. Phibles, R. A. van Tyn, L. D. McLean, *J. Thorac. Cardiovasc. Surg.* **42**, 228 (1961).
7. J. Satinsky, B. Kosowsky, B. Lown, J. Kerzner, *Circulation* **24** (Suppl. 2), 60 (abstr.) (Oct. 1971).
8. R. Verrier, P. L. Thompson, B. Lown, *Clin. Res.* **20**, 402 (abstr.) (1972).
9. G. L. Engel, *Ann. Intern. Med.* **74**, 771 (1971).
10. Supported by grants MH-21384 from the National Institute of Mental Health and HL-07776 from the National Heart and Lung Institute of the National Institutes of Health.

25 April 1973; revised 9 July 1973

Pyrimidine Starvation Induced by Adenosine in Fibroblasts and Lymphoid Cells: Role of Adenosine Deaminase

Abstract. *In the presence of 10^{-4} to 10^{-5} molar adenosine, established cell lines of fibroblastic or lymphoid origin die of pyrimidine starvation. Less than lethal concentrations inhibit cell growth. Over a broad concentration range, the effects of adenosine are prevented by providing a suitable pyrimidine source. We suggest that the recently described immune deficiency disease associated with absence of adenosine deaminase may be the result of pyrimidine starvation induced by adenosine nucleotides in cells of the lymphoid system.*

Study of the purine salvage pathways of mammalian cells has shown that the action of the two enzymes adenosine kinase and adenosine deaminase must be precisely balanced. Excessive deamination of adenosine sets in motion the adenosine cycle (*1*), and leads to loss of purines from the cell (*2*). Excessive phosphorylation of adenosine by the kinase results in a lethal interruption of pyrimidine synthesis at a late stage of the biosynthetic pathway (*3*). Ordinarily the adenosine concentration in the cell must be sufficiently low and the activity of each enzyme so regulated as to prevent both of these effects.

The addition of exogenous adenosine to fibroblast cultures leads rapidly to nearly total depletion of the cellular pyrimidine nucleotide pool (Fig. 1). To one of two cultures of 3T6 cells growing exponentially in medium containing 10 percent horse serum [a serum free of adenosine deaminase (*3*)], adenosine was added to $10^{-4}M$. Three hours later, the medium was removed from both cultures, the cell layers were extracted with perchloric acid, and the proteins were removed by centrifugation. The perchlorate was precipitated with dilute KOH, and the supernatant was analyzed quantitatively for nucleotides by high pressure liquid chromatography (Varian Aerograph LCS 1000), and the elution method of Brown (*4*), with minor modifications. Figure 1A shows the nucleotide pattern of the cell extract of control cultures, to which no adenosine was added. All the ribonucleoside diphosphates and triphosphates except cytidine diphosphate (CDP) are readily identified. Figure 1B shows the pattern given by the adenosine-treated cells. The adenosine diphosphate (ADP) and adenosine triphosphate (ATP) peaks are obviously larger, and the uridine diphosphate (UDP), cytidine triphosphate (CTP), and uridine triphosphate (UTP) peaks are drastically reduced.

Table 1 shows the amounts of the nucleoside diphosphates and triphosphates obtained by measurement of the areas under the peaks. For the less abundant nucleotides, larger samples of extract were used so as to increase the accuracy of measurement. The presence of adenosine in the medium led to a 40 to 60 percent expansion of the pools of ADP and ATP; guanosine triphosphate (GTP) was also increased, although guanosine diphosphate (GDP) was not. UDP was reduced by 79 percent, and UTP and CTP were reduced by more than 90 percent. These results are in accord with the conclusion reached earlier that the addition of exogenous adenosine results in an interruption of pyrimidine synthesis in the fibroblast; at adenosine concentrations below $2 \times 10^{-4}M$ this was the only cause of cell death, since the lethality was prevented by the addition of a suitable pyrimidine source (*3*).

The toxicity of adenosine depends on its direct conversion to adenosine monophosphate (AMP) by adenosine kinase (*3*). Adenosine deaminase should have a protective effect, since the deamination reaction is not reversible, and the products—inosine and its free base, hypoxanthine—do not affect cell growth even when present in high concentration (*3*). The recently reported association between absence of adenosine deaminase and a human disease of the lymphoid system manifested by a greatly reduced number of lymphocytes and impaired immunity (*5*) naturally suggested to us that the absence of deaminase in cells of the lymphoid system

Table 1. Nucleoside diphosphate and triphosphate pool in cells exposed to adenosine.

Nucleotide	3T6			MGL-5		
	Absorbance at 254 nm			Absorbance at 254 nm		
	Control	Ar ($10^{-4}M$)	Change (%)	Control	Ar ($2 \times 10^{-5}M$)	Change (%)
ADP	57.9	93.5	+ 61	5.6	8.5	+ 52
GDP	16.1	15.3	− 5	3.7	4.0	+ 8
UDP	32.2	6.8	− 79	5.3	0.9	− 83
ATP	94.8	131.5	+ 39	26.1	53.0	+ 103
GTP	18.5	26.0	+ 40	5.3	8.0	+ 51
UTP	22.2	2.0	− 91	9.0	0.9	− 90
CTP	18.4	1.5	− 92	3.0	0.8	− 73