the size and orientation of the proteins involved, the matrix element is $T = 9 \pm 3 \times 10^{-4}$ eV. Using an approximate estimate (*1*)

$$T = \frac{2.7}{(N_d N_a)^{1/2}} \exp\left(-\frac{R}{1.4}\right) \quad (5)$$

T can be related to $N_d(N_a)$, the number of atoms over which the electron is delocalized on the donor (acceptor) molecule, and R, the distance between the heme groups on CP and C. For electronic wave functions delocalized over the heme groups, $N_d = N_a = 20$ and Eq. 5 gives the value $R \simeq 7$ Å. This is consistent with values predicted from the theory for other biological systems and similar to the value $R \simeq 7$ to 10 Å for C-$Fe(CN)_6$ (*3*). The distance between iron atoms is ~15 to 20 Å, similar to distances measured by fluorescence techniques (*8*).

The values of T and Δ obtained in these experiments are similar to those inferred in electron transfer processes in bacterial photosynthesis. Depending on which molecules are involved, the theory predicts values of T between 2×10^{-4} and 3×10^{-3} eV. Values of Δ between 0.4 and 1.6 eV are predicted for a series of electron transfers in bacterial photosynthesis. The CP-C value of $\Delta = 1.07$ eV is within this range.

The electron transfer rate, k, can be directly calculated from the parameters Δ, σ, and T (*1*). A value of $k \simeq 1 \times 10^{7 \pm 3}$ sec^{-1} is calculated for the measured parameters $E_d - E_a - \Delta = 0.70 \pm 0.22$ eV, $\sigma = 0.21 \pm 0.04$ eV, and $T = 9 \pm 3 \times 10^{-4}$ eV. The large error is due to the exponential behavior of the rate expression.

These results show the existence of a new charge-transfer band in the bound complex CP-C which is not present in the individual components. This new band and the parameters obtained are significant demonstrations of the validity of nonadiabatic electron tunneling.

M. J. Potasek

Department of Physics,
Princeton University,
Princeton, New Jersey 08540

References and Notes

1. J. J. Hopfield, *Proc. Natl. Acad. Sci. U.S.A.* **71**, 3640 (1974); J. Jortner, *J. Chem. Phys.* **64**, 4860 (1976); L. N. Gigorov and D. S. Chernavskii, *Biofizika* **17**, 195 (1972).
2. J. J. Hopfield, *Biophys. J.* **18**, 311 (1977).
3. M. J. Potasek and J. J. Hopfield, *Proc. Natl. Acad. Sci. U.S.A.* **74**, 3817 (1977).
4. ———, *ibid.*, p. 229.
5. M. J. Potasek, *Biophys. J.*, in press.
6. C. H. Kang, S. Ferguson-Miller, E. Margoliash, *J. Biol. Chem.* **252**, 919 (1977).
7. M. J. Potasek, in preparation.
8. J. J. Leonard and T. Yonetoni, *Biochemistry* **13**, 1465 (1974).
9. E. Stellwagen, University of Iowa, graciously provided the yeast cytochrome c peroxidase. Significant encouragement and discussions were provided by J. J. Hopfield and K. W. Beeson. The author holds a National Science Foundation postdoctoral fellowship. Research was supported in part by grant DMR 75-14264 from the National Science Foundation.

15 February 1978; revised 28 April 1978

Monensin and the Prevention of Tryptophan-Induced Acute Bovine Pulmonary Edema and Emphysema

Abstract. *3-Methylindole, a ruminal fermentation product of tryptophan, induces acute pulmonary edema and emphysema in cattle, and 3-methylindole is present in the ruminal fluid and blood of cows with a natually occurring form of this disease. Monensin, a polyether antibiotic and widely used feed additive for beef cattle, prevented tryptophan-induced acute bovine pulmonary edema and emphysema. Monensin acted by reducing the ruminal conversion of* L-*tryptophan to 3-methylindole both in vitro and in vivo. Lasalocid, also a polyether antibiotic, showed similar effects in vitro. These results provide a promising approach to prevention of this major respiratory disease of cattle.*

Acute bovine pulmonary edema and emphysema (ABPE) or fog fever is a naturally occurring disease of adult cattle characterized by sudden onset of acute respiratory distress soon after a change to lush forage, usually in the fall (*1*). The disease occurs in many parts of the world including the United States, Canada, and Europe (*1, 2*), and it is the most prevalent respiratory disease associated with groups of pastured beef cattle (*3*). The incidence of ABPE appears to be increasing in parallel with changes to more intensive range and pasture management. This and the absence of proven methods of treatment or prophylaxis results in a significant economic loss to beef cattle producers. Effective prevention would allow more efficient utilization of improved pastures and provide economic benefits contributing toward more profitable red meat production.

Previously (*4*) we demonstrated that 3-methylindole, a ruminal fermentation product of tryptophan, would cause acute pulmonary lesions in cattle, similar to those of ABPE. Additional work has shown that the pathogenesis of the disease probably involves ruminal conversion of L-tryptophan, a naturally occurring amino acid and constituent of forage, to indoleacetic acid which is then converted to 3-methylindole (*5, 6*). We have also found that ruminal microorganisms convert L-tryptophan to indoleacetic acid (*7*) and that a *Lactobacillus* sp. isolated from the rumen metabolizes indoleacetic acid to 3-methylindole (*8*). This process occurs in vivo, as indicated by the presence of 3-methylindole in ruminal fluid and plasma of cows given tryptophan to induce ABPE (*6*). In contrast to tryptophan or indoleacetic acid, both intravenous and oral administration of 3-methylindole results in pulmonary lesions typical of ABPE (*4, 9*). In addition to the pathological similarity between the experimentally induced and naturally occurring diseases, the presence of 3-methylindole in ruminal fluid (*10, 11*) and peripheral blood (*10*) has been demonstrated in cows that developed ABPE after a move from relatively dry to lush, green pasture. These observations lend support to the view that naturally occurring ABPE results from ruminal metabolism of tryptophan. In this report we describe experiments that demonstrate that monensin, a widely used feed additive, can inhibit the production of 3-methylindole and prevent experimentally induced ABPE. These results provide a promising approach to possible prevention of naturally occurring ABPE.

Previous work in our laboratory demonstrated that 3-methylindole production in ruminal fluid could be decreased in vitro and in vivo by the use of antibiotics (*7*), but the effectiveness of these antibiotics at concentrations low enough for practical application in live animals was not investigated. Therefore, we undertook experiments to screen several compounds for their ability to reduce 3-methylindole production. By means of in vitro techniques (*7*) and analytical methods devised in our laboratory (*12*), several antimetabolites were screened for their ability to reduce the conversion of L-tryptophan to 3-methylindole in vitro. Mixtures of 23 ml of ruminal fluid, 10 mg of L-tryptophan (10 mg of L-tryptophan per milliliter of H_2O), and 0.625 mg or 0.125 mg of test compound (25 or 5 μg per milliliter, final concentration) were incubated in triplicate in 50-ml Erlenmeyer flasks fitted with rubber caps. The test compounds were dissolved in 1 ml of H_2O, 0.1*N* aqueous NaOH, or absolute methanol. Each flask was flushed with CO_2 for 1 hour, then incubated at 37°C for another 23 hours. The results obtained were compared to those for con-

0036-8075/78/0714-0153$00.50/0

trol mixtures in which only solvent, substrate, and ruminal fluid were incubated. For these experiments we used ruminal fluid collected from one animal on several different days (at the same time each day), but comparisons between individual treatments and controls were always from the same ruminal fluid samples. Conversion of L-tryptophan to 3-methylindole in control mixtures averaged 43.7 percent. The results for mixtures incubated in vitro are shown in Table 1. The polyether antibiotics monensin and lasalocid (*13*) were the most effective compounds tested. At concentrations of 5 μg/ml, these compounds reduced 3-methylindole formation by 87 to 90 percent. The positive results with monensin were of particular interest because a commercial preparation of monensin is a widely used feed additive for beef cattle and might be a promising preventative for ABPE. Monensin increases production efficiency of cattle on pasture and reduces feed intake (*14*) by increasing the relative proportion of ruminal propionic acid production and possibly by decreasing methanogenesis and protein degradation (*15*). These effects may be complementary to those of reducing 3-methylindole formation and may allow a smoother transition from dry forage to lush pasture.

In subsequent studies we determined the ability of monensin to decrease ruminal production of 3-methylindole in vivo. Eight mature Hereford cows were divided into two groups and given 0.35 g of L-tryptophan per kilogram of body weight to induce ABPE. Each of four cows was given 100 mg of monensin (*16*) orally in gelatin capsules twice daily starting 1 day before and ending 4 days after the tryptophan dose. Four control cows were given L-tryptophan without further treatment. Ruminal fluid samples

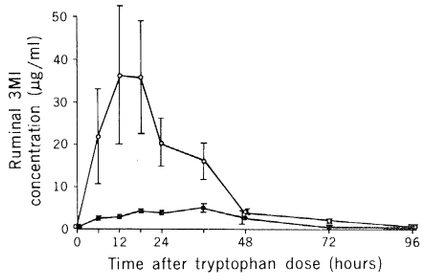

Fig. 1. Mean concentrations of 3-methylindole (*3MI*) in the rumens of four cows each given 200 mg of monensin per day, from −1 to 4 days after an oral dose of 0.35 g of L-tryptophan per kilogram of body weight (●), and in four cows given L-tryptophan without further treatment (○). Bars indicate ± standard error of the mean.

were taken at 0, 6, 12, 18, 24, 36, 48, 72, and 96 hours after tryptophan administration and analyzed for 3-methylindole (*12*). The cows were observed at least daily for clinical signs of respiratory disease, then examined at necropsy 6 days after the oral dose of tryptophan had been given.

The mean concentrations of 3-methylindole in the rumen for each group of four cows are shown in Fig. 1. Mean ruminal concentrations of 3-methylindole in control cows reached a peak of 36.4 μg/ml at 12 hours and persisted at levels above 15 μg/ml from 6 to 36 hours after the cows received tryptophan. Mean concentrations in the rumen of monensin-treated cows never exceeded 5.0 μg/ml.

All control cows responded to tryptophan by developing clinical signs of respiratory disease, and three of the four cows were severely affected. One control cow died of ABPE approximately 132 hours after receiving tryptophan; two other control cows developed severe clinical signs of ABPE and were slaughtered in extremis, and the fourth control had mild clinical signs which had subsided by the time of slaughter. No clinical signs of respiratory disease were observed in any of the monensin-treated cows.

At necropsy, none of the monensin-treated animals had pulmonary lesions of tryptophan-induced ABPE. In contrast, diffuse lesions of congestion, edema, interstitial emphysema, alveolar epithelial hyperplasia of type 2 pneumonocytes, interstitial eosinophilia, and bronchiolar epithelial necrosis and hyperplasia were found in the lungs of the three cows with severe clinical signs. Similar focal lesions were detected microscopically in the fourth cow.

These experiments demonstrate that reduction in ruminal 3-methylindole formation prevents tryptophan-induced ABPE. Monensin and lasalocid, both polyether antibiotics, reduced 3-methylindole formation in vitro. Monensin was further shown to reduce 3-methylindole formation in vivo. These results provide a promising approach to a prevention for naturally occurring ABPE.

Table 1. The production of 3-methylindole in vitro expressed as a percentage of control (± standard error of the mean) in mixtures of ruminal fluid incubated in triplicate with L-tryptophan and various compounds.

Compound	Concentration	
	25 μg/ml	5 μg/ml
Monensin*	7.8 ± 0.4	13.3 ± 2.3
4,4-Dimethyldiphenyl iodonium chloride†	0.8 ± 0.1	105.9 ± 11.9
Diphenyl iodonium chloride†	0.6 ± 0.1	106.1 ± 13.2
1,1,1-Trichloro-2-hydroxy-4-pentanone†	70.0 ± 5.7	87.6 ± 3.7
Dicloralurea†	44.2 ± 5.6	104.0 ± 12.2
Amicloral†	51.0 ± 12.0	104.9 ± 15.8
Dithiooxamide†	38.0 ± 4.5	101.9 ± 12.7
Lasalocid‡	13.4 ± 0.7	9.9 ± 0.7
5-Chloro-8-hydroxyquinoline§	112.1 ± 6.0	107.7 ± 9.2
15-Crown-5§	104.8 ± 4.4	94.1 ± 3.7
DL-α-Methyldopa‖	102.3 ± 7.9	110.2 ± 1.9

*A gift of Elanco Products Company. †A gift of Smith Kline Animal Health Products. ‡A gift of Hoffmann-La Roche, Inc. §Aldrich. ‖Nutritional Biochemicals.

A. C. HAMMOND
J. R. CARLSON
Department of Animal Sciences, Washington State University, Pullman 99164
R. G. BREEZE
Department of Veterinary Microbiology and Pathology, Washington State University

References and Notes

1. J. T. Blake and D. W. Thomas, *J. Am. Vet. Med. Assoc.* **158**, 2047 (1971); I. E. Selman, A. Wiseman, H. M. Pirie, R. G. Breeze, *Vet. Rec.* **95**, 139 (1974); R. G. Breeze, H. M. Pirie, I. E. Selman, A. Wiseman, *Vet. Bull.* **46**, 243 (1976).
2. F. W. Schofield, *J. Am. Vet. Med. Assoc.* **112**, 254 (1948); N. St. G. Hyslop, *Can. Vet. J.* **10**, 251 (1969); E. O. Dickinson, thesis, Washington State University (1970).
3. I. E. Selman, A. Wiseman, R. G. Breeze, H. M. Pirie, *Bovine Practitioner* **12**, 63 (1977).
4. J. R. Carlson, M. T. Yokoyama, E. O. Dickinson, *Science* **176**, 298 (1972).
5. J. R. Carlson and E. O. Dickinson, in *Effects of Poisonous Plants on Livestock*, R. F. Keeler, K. R. Van Kampen, L. F. James, Eds. (Academic Press, New York, 1978), p. 261; E. O. Dickinson, G. R. Spencer, J. R. Gorham, *Vet. Rec.* **80**, 487 (1967); J. R. Carlson, I. A. Dyer, R. J. Johnson, *Am. J. Vet. Res.* **29**, 1983 (1968).
6. M. T. Yokoyama, J. R. Carlson, E. O. Dickinson, *Am. J. Vet. Res.* **36**, 1349 (1975).
7. M. T. Yokoyama and J. R. Carlson, *Appl. Microbiol.* **27**, 540 (1974); A. C. Hammond, T. M. Bray, K. A. Cummins, J. R. Carlson, B. J. Bradley, *Am. J. Vet. Res.*, in press.
8. M. T. Yokoyama, J. R. Carlson, L. V. Holdeman, *Appl. Environ. Microbiol.* **34**, 837 (1977).
9. J. R. Carlson, E. O. Dickinson, M. T. Yokoyama, B. Bradley, *Am. J. Vet. Res.* **36**, 1341 (1975); H. M. Pirie, R. G. Breeze, I. E. Selman, A. Wiseman, *Vet. Rec.* **98**, 259 (1976).
10. M. L. Terry, B. J. Bradley, A. C. Hammond, K. A. Cummins, J. R. Carlson, E. O. Dickinson, *Proc. West. Sec. Am. Soc. Anim. Sci.* **27**, 330 (1976).
11. I. E. Selman, R. G. Breeze, J. A. Bogan, A. Wiseman, H. M. Pirie, *Vet. Rec.* **101**, 278 (1977).
12. B. J. Bradley and J. R. Carlson, *Anal. Biochem.* **59**, 214 (1974).

13. M. E. Haney and M. M. Hoeny, *Antimicr. Agents Chemother.* **1967**, 349 (1968); J. Berger, A. I. Rachlin, W. E. Scott, L. H. Sternbach, M. W. Goldberg, *J. Am. Chem. Soc.* **73**, 5295 (1951).
14. H. A. Turner, R. J. Raleigh, D. C. Young, *J. Anim. Sci.* **44**, 338 (1977).
15. C. J. Van Nevel and D. I. Demeyer, *Appl. Environ. Microbiol.* **34**, 251 (1977).
16. For testing in vivo, monensin was administered as Rumensin (60 g of monensin sodium per pound of Rumensin).
17. Scientific paper No. 5013, College of Agriculture Research Center, Washington State University. Supported in part by NIH grant HL-13645 and Department of Animal Sciences Project 1893. We thank T. M. Bray, M. J. Potchoiba, and L. Wong for technical assistance.

31 January 1978; revised 5 April 1978

Rise and Fall of Cyclic AMP Required for Onset of Lymphocyte DNA Synthesis

Abstract. *The adenosine 3′,5′-monophosphate (cyclic AMP) levels of mouse lymphocytes rose and fell sharply 10 hours after stimulation with concanavalin A. Treatment of the cells with indomethacin reversibly prevented the increase in cyclic AMP and the subsequent onset of DNA synthesis. When the heightened cyclic AMP before S phase was maintained by either inhibiting phosphodiesterase or by adding the 8-bromo derivative of cyclic AMP, DNA synthesis was also blocked. Both the increase and decrease in cyclic AMP appear to be required for progression of lymphocytes into the S phase of growth.*

Numerous studies have attempted to define the relation between adenosine 3′,5′-monophosphate (cyclic AMP) metabolism and cellular proliferation. Results from several experimental systems have led to the widely held concept that cyclic AMP acts primarily as a negative regulator of cell division (*1, 2*). Cyclic AMP analogs or agents that elevate intracellular cyclic AMP inhibit the multiplication of a variety of cultured cells (*3*). Direct measurement of intracellular cyclic AMP has also shown that proliferating, growth-regulated fibroblasts have low amounts of cyclic AMP that increase as the cells establish contact and cease dividing (*4*). Finally, transformed cells, exhibiting unregulated growth, have relatively low concentrations of cyclic AMP that are independent of cell density (*4, 5*). Because of these studies, little attention has been paid to evidence that indicates cyclic AMP plays a positive role in cellular proliferation (*6*).

An increase in cyclic AMP before the S phase of growth in certain cell types has indicated a positive role for cyclic AMP in cell proliferation and led to the idea that a brief rise in cyclic AMP is part of the series of events leading to DNA synthesis (*7*). Investigation of the relation between the cyclic AMP increase and DNA synthesis has been confined to regenerating liver cells, and the results have not been consistent; preventing the rise in cyclic AMP before S phase did not always inhibit DNA synthesis (*8, 9*) and, in one study, the drug indomethacin prevented S phase, but did not block the increase in cyclic AMP (*10*). Additional supportive evidence has come from cell cycle analyses. In several systems including regenerating liver cells in vivo, elevated cyclic AMP in the G_1 phase of growth preceded low levels found in S phase, again suggesting a positive role for the cyclic nucleotide in cell proliferation (*11, 12*).

Mouse spleen lymphocytes remain quiescent (G_0 phase) until triggered by a mitogen such as concanavalin A (con A) to enter a proliferative cycle. We now report systematic measurements of cyclic AMP in such mitogen-stimulated lymphocytes and have investigated the significance of any observed changes. Our results indicate that cyclic AMP has a complex role in lymphocyte proliferation; we found that a sharp rise and fall in cyclic AMP preceded DNA synthesis, and we provide evidence that both the increase and decrease are required for the progression of these cells into S phase.

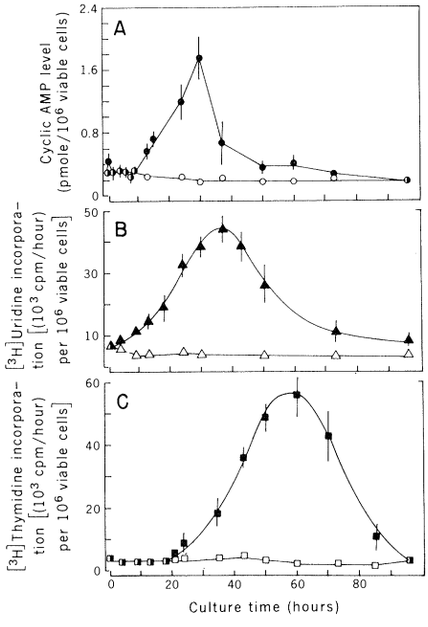

Fig. 1. Change in (A) intracellular cyclic AMP (○, ●) and (B) rates of [³H]uridine (△, ▲) and (C) [³H]thymidine incorporation (□, ■) during proliferative response of con A–stimulated lymphocytes (*13*). Open symbols represent unstimulated control cultures and closed symbols represent con A–stimulated cultures. Each time point signifies the means (± standard error of the mean) from four experiments.

Intracellular cyclic AMP levels were determined at various intervals after con A stimulation (*13, 14*) (Fig. 1). Similar results were obtained whether the cyclic AMP levels were calculated as a function of cell number (Fig. 1) or cell protein content (data not shown). Within the first 30 minutes after addition of mitogen, intracellular cyclic AMP increased by a factor of 1.5 compared to control cells. This rise was transient; the concentration returned to the control value within 1 hour and remained constant until 10 hours after the addition of con A; at that time, cyclic AMP again increased and continued to rise until approximately 30 hours after stimulation, when a sixfold increase over control was reached. Thereafter, the concentration of cyclic AMP fell rapidly and returned to near baseline by 50 hours. The cyclic AMP increase at 10 hours preceded the beginning of S phase, and its decline occurred before the onset of DNA synthesis; however, the cells were not well synchronized, causing some overlap between the two curves. Thus, both a rise and fall in cyclic AMP seemed to occur before the onset of S phase. The cyclic AMP concentration did not change in any obvious way later in the proliferative cycle, but synchrony was lost by 60 hours and later changes may have been masked.

In view of the complexity of RNA metabolism, only a very general comparison can be made between uridine incorporation and cellular cyclic AMP levels (Fig. 1). RNA synthesis began to increase between 2 and 4 hours after exposure to con A and increased over a long time period. This increase occurred before the major rise in the cellular cyclic AMP. Thus, the major cyclic AMP peak did not seem to be related to changes in RNA metabolism; in subsequent experiments, manipulation of the cyclic AMP level did not affect the rate of uridine incorporation into RNA.

Although intracellular cyclic AMP levels reflect the balance between its synthesis, degradation, and export, it is likely that the increase we observed was

0036-8075/78/0714-0155$00.50/0