

*Crematogaster emeryana*, and to later emerge carrying pupae which were then transported back to their own nests. The formation of the raid was similar to that observed in the Mojave Desert, except fewer workers were involved (around 70 per colony). At the peak of the raids, I estimated about 22 pupae were returned to each *C. insana* nest every 10 min. The raids began at 07.00 h and continued until around 09.15 h. Soil surface temperatures during this period ranged from 21.5 to 32.5 °C.

The foragers of both *C. bicolor* and *C. insana* normally search independently for food items. Formation of columns therefore involved a considerable departure from their normal foraging behavior. In both species, the raiding column appeared to move directly to the nest which was raided. The mechanism by which this was accomplished (e.g., memory from previous raids, chemical trails, or scouts) was not discovered.

**Ecological characteristics.** *C. bicolor* and *M. kennedyi* were found to be quite similar in nest site location. Nests of both species were abundant in sunny areas where the soil was composed of fine-grained sand. Daily foraging activities of 4 colonies of *C. bicolor*, in which slaves were present, were observed and the number of foragers of each species leaving the nest recorded. Although individuals of the 2 species closely resemble each other in size and color, they can easily be distinguished by their movement patterns while foraging.

Dietary estimates, based on the types of food collected by 80 foragers of each species, indicate that the 2 species collect similar foods. Nectar was collected by 95% of the *C. bicolor* foragers and by 96% of the *M. kennedyi* foragers. The remainder of the diet, for both species, consisted of seeds and insects.

The soil surface temperatures, and therefore times of day<sup>1</sup>, at which foragers of these 2 species were active were so different they scarcely overlapped (figure). By having *M. kennedyi* foragers in the colony, *C. bicolor* increased its foraging activity from a temperature range covering 14° (26–40 °C) to a range covering 31° (26–57 °C). With a greater range of temperatures utilized, colonies with slaves are able to forage for longer periods each day during the spring and summer months when nectar is abundant.

Dietary estimates for the 2 species without slaves and for *C. insana* colonies with *C. emeryana* slaves present

Species	Seeds (%)	Insects (%)	Nectar (%)
<i>C. emeryana</i>	10	0	90
<i>C. insana</i> (without slaves)	55	45	0
(with slaves)	44	36	20

The 2 species at the Arizona site were ecologically quite different. *C. insana* nests were located in open areas, away from shrubs, whereas *C. emeryana* nests were located in the base of cholla cactus plants. Foragers of *C. insana* actively collected insects and seeds at soil surface temperatures between 20 and 41 °C, whereas foragers of *C. emeryana* collected seeds on the ground at temperatures between 14.5 and 49 °C, and gathered nectar from cholla cactus buds and flowers when soil surface temperatures ranged between 14.5 and 62 °C.

The presence of *C. emeryana* slaves within the nests of *C. insana* produced a considerable increase in the breadth of diet (table). Without slaves, *C. insana* foragers collected seeds and insects; with slaves, nectar was added to the diet. The position of the nests of *C. insana* limited the potential gain in foraging times by having *C. emeryana* as slaves. *C. emeryana* foraged at soil surface temperatures up to 62 °C by moving directly from their nest in the base of the cholla cactus up to the top of the plant. Thus, although the soil surface temperatures were often as high as 62 °C, foragers on the cactus seldom experienced temperatures greater than 40 °C. The *C. emeryana* foragers present in the *C. insana* nests, however, could not avoid the direct heat by moving directly up a cholla cactus, thus ceased foraging when temperatures reached 49 °C. Nevertheless, the presence of *C. emeryana* slaves increased the foraging temperature range of *C. insana* from 21° (20–41 °C) without slaves to 34.5° (14.5–49 °C) with slaves. Further studies are required in order to establish with certainty the existence of slave-making in these 2 species of *Conomyrma*.

1 R. A. Bernstein, Am. Nat. 108, 490 (1974).

## Effect of dibutyryl cyclic AMP and analogs on the rate of contractions of myocytes in culture

K. Nath<sup>1</sup> and A. P. Bollon

Department of Biochemistry, University of Texas, Health Science Center Dallas (Texas 75235, USA), 14 March 1978

**Summary.** <sup>2</sup>O, <sup>6</sup>N-butyryl, 3', 5'-cyclic monophosphate (dibu cAMP) when added to fetal rat heart cells in culture inhibits myocyte contraction. This inhibition is 100, 84 and 51% complete when the dibu cAMP concentration used is 2, 0.2 and 0.02 mM, respectively. The potency of dibu cAMP derivatives in myocyte contraction inhibition follows the order, dibu cAMP > <sup>6</sup>N-bu cAMP > <sup>2</sup>O-bu cAMP = AMP > butyrate. The inhibition caused by the first three chemicals is greater than 70%.

The dissociation of heart cells (for example, by degradative enzymes) causes the disintegration of myofibrils with a concomitant appearance of contractile activity<sup>2-4</sup>. We have shown that this contractile activity correlates well with the intracellular microtubule organization<sup>5</sup>. High concentrations of <sup>2</sup>O, <sup>6</sup>N-butyryl, 3', 5'-cyclic monophosphate (dibu cAMP) inhibits myocyte contractions with a concomitant alignment of microtubules in a longitudinal array<sup>5</sup>. The process is reversed by colchicine. Here we report that the dibu cAMP effect is concentration-dependent and that of the various derivatives tested only <sup>6</sup>N-bu cAMP replaces dibu cAMP.

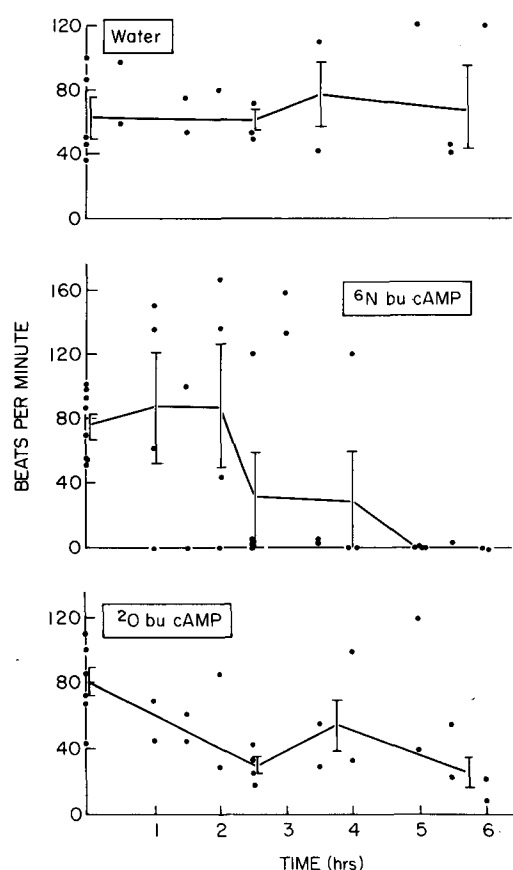
**Methods.** Primary myocyte cultures from 19-day-old rat embryos were grown on glass cover slips<sup>6</sup>. The treatment with various chemicals and the monitoring of the rate of myocyte contractions were performed as described previously<sup>5</sup>.

**Results and discussion.** As shown in the table, dibu cAMP at 2 mM concentration inhibits myocyte contraction almost totally. At 1/10 this concentration of dibu cAMP, the contraction of myocytes is reduced by about 80%. 3 of the 8 cultures treated with 0.2 mM dibu cAMP stopped beating altogether. A further reduction in the concentration of dibu

## Effect of various concentration of dibu cAMP on the rate of myocyte contraction

dibu cAMP concentration	Interval after dibu cAMP addition		p-value	Inhibition	5-6 h beats/min	p-value	Inhibition
	0 h beats/min	3-4 h beats/min					
2 mM	69	0.4	<0.001	%	0	<0.001	%
0.2 mM	45	8	<0.001	99	7	<0.01	100
0.02 mM	59	25	<0.2	82	29	<0.2	84
				58			51

For 2, 0.2 and 0.02 mM dibu cAMP 19, 8 and 6 cultures were used and the beats/min reported above is the average value of about 70, 35 and 30 observations for the 3 dibu cAMP concentrations used, respectively.



Effect of the 2 monobu cAMP on the rate of myocyte contraction. The control experiment with 200  $\mu$ l of water was performed with 5 cultures. The effect of 2 mM  $^6$ N-bu or  $^2$ O-bu cAMP was tested on 8 and 6 cultures, respectively. Each point represents the average value of at least 4 observations made on a single culture (single coverslip). The bar represents the standard error of such points.

cAMP by 10fold caused a reduction of myocyte contraction only by about 50%. None of the 6 cultures treated with 0.02 mM dibu cAMP stopped beating. Thus, a reduction in the rate of myocyte contraction is dependent on the concentration of dibu cAMP used.

We have reported previously that colchicine restores the 2 mM dibu cAMP arrested myocyte contractions<sup>5</sup>. The contraction was restored to about 90% its original value with 100 or 10  $\mu$ M colchicine while the restoration was about 50% when 1  $\mu$ M colchicine was used. This suggested that the simultaneous addition of both colchicine (100  $\mu$ M) and 2 mM dibu cAMP should prevent the arrest of myocyte

contraction. Indeed, when dibu cAMP was added along with colchicine, none of the cultures tested stopped beating. Instead, an average contraction rate of 45 beats/min was reduced by about 50% (30 observations). Colchicine, thus, counters the dibu cAMP effect on myocyte contractions in culture.

Of the 2 monobu cAMP, tested only 2 mM  $^6$ N-bu cAMP, as shown in the figure, appeared as effective as 2 mM dibu cAMP. However, the required duration for the arrest in myocyte contraction was twice as long (about 5.5 h for  $^6$ N-bu as against 3 h for dibu cAMP). 2 mM  $^2$ O-bu cAMP on the other hand could not bring about an arrest in myocyte contraction even though it caused a reduction of about 30 to 60% (24 observations). When 2 mM cAMP was added to 7 cultures there was a reduction of myocyte contraction rate by about 80% but none of the cultures stopped beating (30 observations). The effect of 2 mM cAMP was more like that of  $^2$ O-bu cAMP while 2 mM sodium butyrate hardly affected the rate of myocyte contractions (40 and 30 observations, respectively).

Thus, the most potent inhibitors of myocyte contraction when arranged in the order of their potency follow the order: dibu cAMP >  $^6$ N-bu cAMP > cAMP. The delayed action of  $^6$ N-bu cAMP and the lesser effect of cAMP could relate to the slow absorption rate of these 2 chemicals as well as their differential susceptibility to the action of intracellular phosphodiesterases. The difference in the effectiveness of  $^6$ N-bu and  $^2$ O-bu could be due to the fact that  $^6$ N-bu cAMP and not  $^2$ O-bu cAMP is an intermediate metabolite of dibu cAMP *in vivo*<sup>7</sup>. We conclude that for heart cells in culture the arrest in the rate of myocyte contraction as against a mere reduction in the rate is specific for conditions that favor longitudinal orientation of microtubules<sup>5,8,9</sup>.

- 1 Present address: Merck Institute for Therapeutic Research P.O.B. 2000, Rahway, New Jersey 07065 USA. The author was a Moss Heart Fellow and acknowledges the technical assistance of Ms Martha Peet. This work was supported in part by the American Heart Association, National Science Foundation and American Cancer Society.
- 2 A. Wollenberger, *Circulation Res.*, suppl. II vol. XIV and XV, 184 (1964).
- 3 M. W. Cavanaugh, *J. exptl Zool.* 128, 573 (1955).
- 4 I. Harary and B. Farley, *Exptl Cell Res.* 29, 466 (1963).
- 5 K. Nath, J. W. Shay and A. P. Bollon, *Proc. natl Acad. Sci., USA* 75, 319 (1978).
- 6 A. P. Bollon, K. Nath and J. W. Shay, *Tissue Cult. Ass. Manual* 3, 637 (1977).
- 7 G. I. Drummond and S. J. Hemmings, *Adv. cycl. Nucl. Res.* 1, 307 (1972).
- 8 A. W. Hsie and T. T. Puck, *Proc. natl Acad. Sci., USA* 68, 358 (1971).
- 9 K. R. Porter, T. T. Puck, A. W. Hsie and D. Kelley, *Cell* 2, 145 (1974).