References and Notes

1. G. L. Nicolson and G. Poste, *N. Engl. J. Med.* **295**, 253 (1976).
2. J. C. Aub, G. Tieslau, A. Lankester, *Proc. Natl. Acad. Sci. U.S.A.* **50**, 613 (1963).
3. M. M. Burger and A. R. Goldberg, *ibid.* **57**, 359 (1967).
4. R. J. Mannino and M. M. Burger, *Nature (London)* **256**, 19 (1975).
5. M. M. Burger, *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **32**, 91 (1973).
6. T. O. Fox, J. R. Sheppard, M. M. Burger, *Proc. Natl. Acad. Sci. U.S.A.* **68**, 244 (1971).
7. S. Shin, V. H. Freedman, R. Risser, R. Pollack, *ibid.* **72**, 4435 (1975).
8. J. C. Bartholomew, H. Jokota, P. Ross, *J. Cell. Physiol.* **88**, 277 (1976).
9. I. Trowbridge and D. A. Hilborn, *Nature (London)* **250**, 304 (1974).
10. M. M. Burger and K. D. Noonan, *ibid.* **228**, 512 (1970).
11. R. J. Mannino and W. A. Strohl, unpublished data.
12. M. E. Baker, *Nature (London)* **262**, 785 (1976).
13. A. D. Glinos and R. J. Weerlein, *J. Cell. Physiol.* **79**, 79 (1972).
14. R. W. Holley, J. H. Baldwin, J. A. Kiernan, *Proc. Natl. Acad. Sci. U.S.A.* **71**, 3976 (1974).
15. D. Paul, M. Henahan, S. Walter, *J. Natl. Cancer Inst.* **53**, 1499 (1974).
16. A. Pardee, *Proc. Natl. Acad. Sci. U.S.A.* **71**, 1286 (1974).
17. R. Keller, A. Bregnard, W. J. Gehring, H. E. Schroeder, *Exp. Cell Res.* **44**, 108 (1976).

18. Supported by the Swiss National Foundation, grant 3.720-76.

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21 February 1978; revised 10 May 1978

Cytidine 3′,5′-Monophosphate Phosphodiesterase: Decreased Activity in the Regenerating and Developing Liver

Abstract. *A decrease in the activity of the enzyme cytidine 3′,5′-monophosphate (cyclic CMP) phosphodiesterase was noted in the regenerating liver of young rats as early as 8 hours after partial hepatectomy, with a maximum decrease occurring 12 hours after the surgery. In comparison, in old rats which showed a slower liver growth, the maximum decrease in the activity of cyclic CMP phosphodiesterase was smaller and occurred at a much later time (2 days after surgery). A similar decrease in the enzyme activity was observed in the fetal liver of guinea pigs. These findings suggest that regulation of tissue concentration of cyclic CMP may be crucial for the regeneration and development of the liver.*

Experimental evidence, although often contradictory, has linked adenosine 3′,5′-monophosphate (cyclic AMP) and guanosine 3′,5′-monophosphate (cyclic GMP) to liver regeneration in rats subjected to partial hepatectomy (*1*, *2*). However, many of the pathophysiologic processes of tissues, including regeneration of the liver, cannot be satisfactorily explained solely on the basis of the presumed actions of these two purine cyclic nucleotides. It has been suspected that other cyclic nucleotides may serve to mediate biological processes that are different from or complementary to those mediated by cyclic AMP and cyclic GMP. Recently, Bloch and co-workers (*3*) identified cytidine 3′,5′-monophosphate (cyclic CMP), a pyrimidine cyclic nucleotide, from leukemia L-1210 cells, and demonstrated that addition of exogenous cyclic CMP, but not cyclic AMP, cyclic GMP, or uridine 3′,5′-monophosphate (cyclic UMP), stimulates the growth of the leukemic cells in culture. These workers also reported that L-1210 cells and regenerating liver have increased concentrations of cyclic CMP compared to their respective controls. Subsequently, Cech and Ignarro (*4*) reported the existence of cytidylate cyclase, the enzyme that catalyzes the formation of cyclic CMP from cytidine triphosphate (CTP), and showed that its activity is higher in regenerating liver and myeloid leukemic tumors than in normal tissue.

We have reported (*5*) the occurrence of cyclic CMP phosphodiesterase in all of many rat tissues examined and have noted that its activity is decreased in tissues undergoing rapid cell proliferation and growth (*5*). In the experiments described herein we compared the changes in the activity of cyclic CMP phosphodiesterase in regenerating and developing liver with changes in the activities of the phosphodiesterases (E.C. 3.1.4) of cyclic AMP and cyclic GMP.

In young male rats (weighing 120 to 150 g) we noted that the activity of cyclic CMP phosphodiesterase decreased as early as 8 hours after partial hepatectomy (Fig. 1) in which about 75 percent of the total liver mass was removed (*6*). The enzyme, shown to be the species with a high Michaelis constant (K_m) (in the millimolar range of substrate), was assayed with both 1 μM and 1 mM concentrations of cyclic CMP in the presence of 10 mM Fe^{2+} (*5*, *7*). The activity was lowest 12 hours after the operation, then gradually recovered thereafter but remained depressed during the entire experimental period; at day 5, when the liver had grown back to nearly its original weight, the enzyme activity was still about 20 percent lower than in the control rats that received sham operations. Increased cytidylate cyclase activity was reported earlier in regenerating liver by Cech and Ignarro (*4*). This, coupled with the depressed cyclic CMP phosphodiesterase activity found in the present study, may account for the higher cyclic CMP content in regenerating liver shown by Bloch (*3*).

In the same animals we also studied changes in the activities of the phosphodiesterases of cyclic AMP and cyclic GMP. Both enzymes were shown to be predominantly the low K_m species (in the micromolar range of substrate). For these assays we used a 1 μM concentration of the respective cyclic nucleotides as substrates in the presence of 20 mM Mg^{2+} (*8*, *9*). Although the changes in activities of these enzymes were less pronounced than the changes that occurred in cyclic CMP phosphodiesterase, their activities were similarly depressed during the earlier phase of the liver regeneration (Fig. 1). However, their activities returned to the control values during days 2 and 3 after partial hepatectomy, at which time the liver was still undergoing rapid growth. We also noted the patterns of changes in cyclic AMP and cyclic GMP phosphodiesterase activity using 1 mM concentrations of substrate. These data (not shown) were qualitatively similar to those obtained with the 1 μM substrate concentrations. Thus decreased hydrolytic destruction of cyclic AMP and cyclic GMP may also be crucial in the earlier phase of liver regeneration, a con-

Table 1. Comparison of the activities of phosphodiesterases for cyclic CMP, cyclic AMP, and cyclic GMP in the liver of the guinea pig fetus (20 days before birth) and adult (over 200 days old). The enzyme activities in the whole liver homogenates were assayed with 1 μM and 1 mM concentrations of the individual cyclic nucleotides as substrates as indicated. Activities are expressed as nanomoles of substrate hydrolyzed per minute per gram of tissue, and the data are presented as means ± standard errors of the means from five fetuses and three adults.

Developmental stage	Phosphodiesterase activity assayed with					
	Cyclic CMP		Cyclic AMP		Cyclic GMP	
	1 μM	1 mM	1 μM	1 mM	1 μM	1 mM
Fetus	0.14 ± 0.01*	90 ± 6*	9.1 ± 0.6†	235 ± 43	9.3 ± 0.9	504 ± 46†
Adult	0.25 ± 0.02	127 ± 6	12.1 ± 0.9	298 ± 43	9.9 ± 1.4	744 ± 90

* $P < .005$. † $P < .05$.

0036-8075/78/0901-0826$00.50/0

tention that supports the observations (*1, 2*) that cyclic AMP and cyclic GMP are elevated during the first 24 hours after hepatectomy.

In old male rats (weighing 350 to 400 g) the rate of liver regeneration after hepatectomy was slower (Fig. 2) than in young rats (Fig. 1), and in old rats the maximum decrease in the activity of cyclic CMP phosphodiesterase was smaller (25 as opposed to 40 percent) and occurred at a much later time (2 days as opposed to 12 hours) after the surgery. These findings suggest that there is an inverse relation between cyclic CMP phosphodiesterase activity and the rate of liver growth.

Assays in which we used both 1 μM and 1 mM cyclic CMP from the developing liver of fetal guinea pigs showed that the activity of cyclic CMP phosphodiesterase was lower than in the adult guinea pig liver (Table 1). In the fetal liver, however, the activity of cyclic AMP phosphodiesterase was lower only when the enzyme was assayed with 1 μM cyclic AMP, whereas the activity of cyclic GMP phosphodiesterase was lower only when the enzyme was assayed with 1 mM cyclic GMP (see Table 1).

The phosphodiesterases of both cyclic AMP and GMP are present in both the low and high K_m forms and are distributed in both the soluble and the particulate fractions (*8*), whereas cyclic CMP phosphodiesterase, which is the high K_m species, is present largely in the cytosol (*5*). The precise effects of changes in the total activities of these three phosphodiesterases on the tissue concentrations of the three cyclic nucleotides, let alone their influences on tissue growth, are still unclear. Nevertheless, the depression of cyclic CMP phosphodiesterase in the regenerating and developing liver appears to be a general phenomenon in cell proliferation. The activity of this enzyme is invariably depressed in the fetal heart and lung (*5*) and in the fetal kidney, intestine, cerebral cortex, and spleen (*10*) of the guinea pig, compared to the corresponding adult tissues. The patterns of changes in the activities of cyclic AMP and GMP phosphodiesterases in the same tissues, however, appear to be variable and tissue specific (*10, 11*). Furthermore, we noted that in rats with isoproterenol-induced cardiac hypertrophy (about 80 percent increase in myocardial weight and protein without a significant increase in cell number), the activity of cyclic AMP phosphodiesterase increased, whereas the activities of cyclic CMP and GMP phosphodiesterases remained unaltered (*5, 12*).

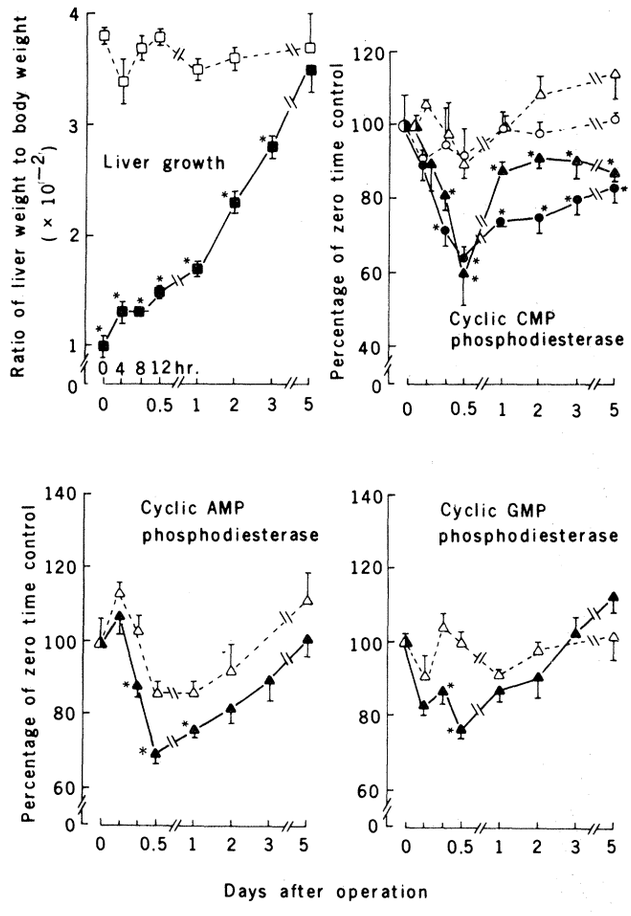

Fig. 1. Liver growth (□,■) and changes in phosphodiesterase activities after partial hepatectomy on young rats. Open symbols indicate the control animals (with sham operations) and filled symbols indicate the hepatectomized animals. The enzyme activities were assayed with either 1 μM (△, ▲) or 1 mM (○, ●) concentrations of the respective cyclic nucleotides as substrates. The data shown are expressed as percentages of the respective enzyme activities (picomoles of cyclic nucleotide hydrolyzed per minute per gram of tissue) seen at day zero, which were taken as 100 percent. The individual enzyme activities for 1 μM substrate at day zero were: cyclic CMP phosphodiesterase, 152; cyclic AMP phosphodiesterase, 11,300; and cyclic GMP phosphodiesterase, 6,600. The cyclic CMP phosphodiesterase activity for 1 mM cyclic CMP at day zero was 99,900. The data presented are the means ± standard errors of the means from three or four rats. Asterisks indicate results significantly different from the control animals (with sham operations) of the same or the closest time points ($P < .05$ to $P < .0005$).

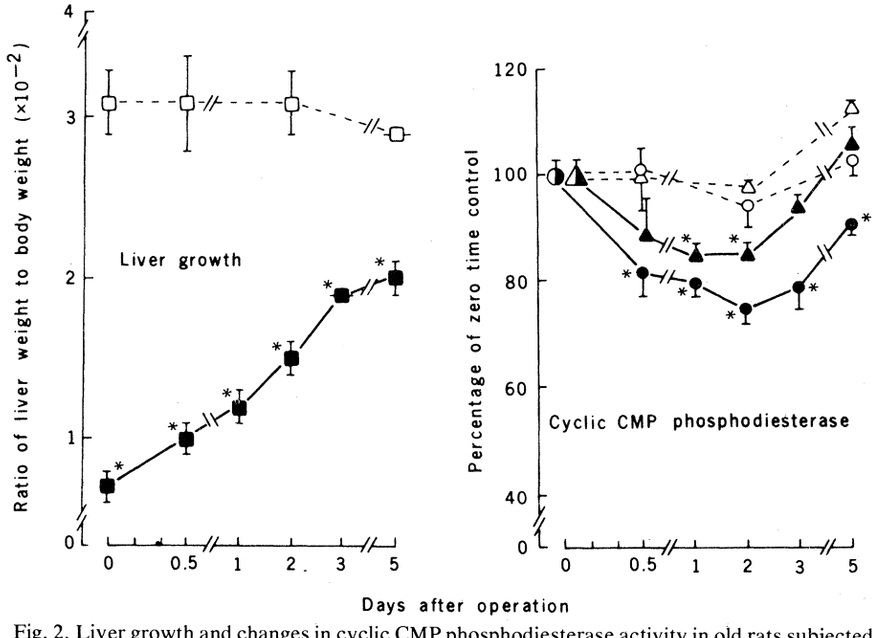

Fig. 2. Liver growth and changes in cyclic CMP phosphodiesterase activity in old rats subjected to partial hepatectomy. The symbols and presentation of the data are the same as in Fig. 1. The activities of the enzyme at day zero for 1 μM and 1 mM concentrations of cyclic CMP were 173 and 107,000, respectively. The number of rats used was three or four per group. Asterisks indicate results significantly different from the control animals (with sham operations) of the same or the closest time points ($P < .05$ to $P < .01$).

Whether the activity of cyclic CMP phosphodiesterase is also depressed in certain malignant growths, such as in the fast growing Morris hepatoma 3924A, in which we observed depressed activity of cyclic GMP phosphodiesterase and, conversely, increased activity of cyclic AMP phosphodiesterase (*13*), and in leukemia L-1210 cells and myeloid leukemic tumors, remains to be seen. If such is the case, then cyclic CMP phosphodiesterase would represent a new site of bioregulation in the pathologic processes. Substances that inhibit and stimulate the activity of this enzyme would probably be potential carcinogens and anticancer agents, respectively.

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References and Notes

1. J. P. MacManus, B. M. Braceland, T. Youdale, J. F. Whitefield, *J. Cell. Physiol.* **82**, 157 (1973); D. J. Gaza, J. Short, I. Lieberman, *FEBS Lett.* **32**, 251 (1973); J. Short, K. Tsukada, W. A. Rubert, I. Lieberman, *J. Biol. Chem.* **250**, 3602 (1975); S. Thrower and M. Ord, *Biochem. J.* **144**, 361 (1974); C. Goridis, *Nature (London)* **257**, 698 (1975); H. Kimura and F. Murad, *Proc. Natl. Acad. Sci. U.S.A.* **72**, 1965 (1975).
2. Y. Miura, H. Iwai, R. Sakata, H. Ohtsuka, E. Elhanan, K. Kubota, N. Fukui, *J. Biochem. (Tokyo)* **80**, 291 (1976).
3. A. Bloch, *Biochem. Biophys. Res. Commun.* **58**, 652 (1974); ———, G. Dutchman, M. Maue, *ibid.* **59**, 955 (1974); A. Bloch, *Adv. Cyclic Nucleotide Res.* **5**, 331 (1975).
4. S. Y. Cech and L. J. Ignarro, *Science* **198**, 1063 (1977); ———, *Biochem. Biophys. Res. Commun.* **80**, 119 (1978).
5. J. F. Kuo, N. L. Brackett, M. Shoji, J. Tse, *J. Biol. Chem.* **253**, 2518 (1978).
6. G. M. Higgins and R. M. Anderson, *Arch. Pathol.* **12**, 186 (1931).
7. The liver was homogenized in three volumes of ice-cold 50 m*M* tris-HCl buffer, *p*H 7.5, containing 3 m*M* 2-mercaptoethanol with the use of glass-Teflon homogenizers. The whole homogenates were used directly as the sources of phosphodiesterases. The cyclic CMP phosphodiesterase activity was assayed in a reaction mixture, in a final volume of 0.1 ml, containing 5 μmole of tris-HCl buffer, *p*H 7.5; 1 μmole of $FeSO_4 \cdot 7H_2O$; 20 μg of snake venom (Sigma); and 0.1 nmole or 0.1 μmole of cyclic [5-^{3}H]CMP containing 82,000 count/min. The reaction was carried out at 37°C for 10 minutes and was terminated by heating the incubation mixture at 90°C for 1 minute. Water (0.4 ml) was added to the incubation tubes, and the entire contents of the tubes were applied onto AG1-X8 columns (Cl^- form, 0.5 by 1.0 cm); 1 ml of water was then applied to recover the reaction product, [5-^{3}H]cytidine.
8. M. M. Appleman, W. J. Thompson, T. R. Russell, *Adv. Cyclic Nucleotide Res.* **3**, 65 (1973).
9. W. J. Thompson and M. M. Appleman, *Biochemistry* **10**, 311 (1971); C. W. Davis and J. F. Kuo, *J. Biol. Chem.* **252**, 4078 (1977).
10. D. Helfman, N. L. Brackett, J. F. Kuo, *Proc. Natl. Acad. Sci. U.S.A.*, in press.
11. C. W. Davis and J. F. Kuo, *Biochim. Biophys. Acta* **444**, 554 (1976).
12. J. Tse, N. L. Brackett, J. F. Kuo, *ibid.*, in press.
13. M. Shoji, H. P. Morris, C. W. Davis, N. L. Brackett, J. F. Kuo, *ibid.* **500**, 419 (1977).
14. This work was supported by research grants HL-15696 and CA-23391 from the Public Health Service.

30 March 1978; revised 8 May 1978

P-State Pairing and the Ferromagnetism of $ZrZn_2$

Abstract. *It is ascertained that, within the range of stability, the transition temperature of the superconducting solutions between Ti and Zr and between Zr and Hf, and the Curie point of the corresponding ferromagnetic solutions between $TiZn_2$ and $ZrZn_2$ and between $ZrZn_2$ and $HfZn_2$, follow a parallel behavior. From this correlation it is concluded that the weak itinerant ferromagnetism of $ZrZn_2$ must be due to electron-phonon interaction. Theoretical arguments are advanced to show that the underlying mechanism is hindered* p*-state pairing, due to a strongly localized repulsive part of the pair-potential that acts as a Hubbard interaction and gives rise to a Stoner instability.*

There can be no doubt that, in metallic ferromagnetic compounds in which the individual elements do not show any magnetic ordering of their own, the moment must be due to itinerant electrons. At present only two such compounds are known, $ZrZn_2$ and Sc_3In.

We want to show that, at least in $ZrZn_2$, the ferromagnetism is due to electron-phonon interaction. This conclusion is a consequence of two seemingly uncorrelated experimental facts, never considered together before.

1) The solid solutions between Ti and Zr and between Zr and Hf show one distinct maximum in their superconducting transition temperature T_s, which occurs between Ti and Zr and is accompanied by some softening of the lattice. The maximum T_s exceeds the T_s of pure Ti and Zr by at least a factor of 2, while from Zr to Hf the solution just shows a monotonic decrease of T_s (*1*).

2) In contrast, the solid solutions between $TiZn_2$ and $ZrZn_2$ and between $ZrZn_2$ and $HfZn_2$ show the parallel behavior for their Curie points T_m (*2–4*). The maximum T_m between $ZrZn_2$ and $TiZn_2$ unfortunately coincides roughly with the limit of stability for the cubic Laves phase, *C* 15 (*3*); $TiZn_2$, the hexagonal Laves phase *C* 14, is no longer ferromagnetic. Obviously, mass variation and mode softening are the only crucial factors.

The following mechanism seems to be the only one to account for the seemingly disconnected facts; that is, the dominant part of the Cooper-pair potential is attractive in the elements, leading to superconductivity, while it becomes repulsive in their *C* 15 compounds leading to ferromagnetism. We wish first to give a naive BCS (Bardeen, Cooper, Schrieffer) argument for this hypothesis and its consequences, and come back later to more sophisticated considerations related to strong-coupling superconductivity theory.

In BCS theory, the change of sign of the pair potential can be obtained by assuming the predominance in the elements of a "hard mode" with frequency $\omega_q \gtrsim \omega_0$, ω_0 being the BCS cutoff (*5, 6*), and of a "soft mode" $\omega_q \ll \omega_0$ in the corresponding compounds. The maximum of T_s in the elements then is a consequence of some softening of the hard mode toward ω_0, while the maximum of T_m in the compounds follows from the soft-mode frequency going to zero at the limit of phase stability. These properties are immediate consequences of the form of the Cooper-pair potential (*5*)

$$V_{pp'} = |g_{pp'}|^2 D(p - p', \epsilon_p - \epsilon_{p'})$$

where

$$D(q, \omega) = 2\omega_q/[\omega^2 - (\omega_q - i\delta)^2]$$

is the phonon propagator and $g_{pp'}$ the electron-phonon coupling constant.

In the "soft mode" case of the compounds, the repulsive part of $V_{pp'}$ increasingly dominates as $\omega_{p-p'} \to 0$. And since for a repulsive pair-interaction the cutoff ω_0 becomes unimportant, $V_{pp'}$ actually extends up to the Fermi wave number, so that its Fourier transform is strongly localized in physical space. For superconductivity this means that *s*-state pairing is strongly inhibited so that *p*-(or higher) state pairing would become essential (*6*). However, the strongly localized Cooper-pair repulsion now acts like a Hubbard interaction, giving rise to a Stoner instability (*7*) and hence to ferromagnetic ordering induced by the electron-phonon interaction and, at the same time, suppressing *p*-state pairing superconductivity. Beyond the structural transformation from *C* 15 to *C* 14 the above effect vanishes. *C* 14, after all, must be considered the link between the cubic *C* 15 phase and the hexagonal close-packed (hcp) elements.

The mass variation going from $TiZn_2$ to $HfZn_2$ (and also from the element Ti to Hf) is another indication of the electron-phonon origin of the magnetic ordering since it is easily explained by the mass-dependence of the mode frequencies and of the electron-phonon coupling constant,

$$|g_{p,p'}|^2 \omega_{p-p'} \propto M^{-1}$$

The above naive explanation is actually confirmed by a more elaborate analysis in terms of strong coupling su-

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