GPS-1 cultures. Table 1 shows the results of these titrations. The results obtained demonstrate that pools of poliovirus grown in trypsinized monkey kidney cultures attained $TCID_{50}$ titers, as calculated by the method of Reed and Muench (*8*), of 0.5 log less in GPS-1 cultures than they do in trypsinized monkey kidney cultures.

A pool of type I poliovirus, Mahoney strain, was prepared from GPS-1 cultures. The cytopathogenic effect for this culture was complete in less than 24 hours. This pool was titrated three times in both trypsinized monkey kidney and GPS-1 cells. On each occasion the titer was higher in the former than in the latter. The titers obtained in trypsinized monkey kidney cell assays were of the order of those obtained when poliovirus produced in trypsinized monkey kidney is titrated in the same cells. This indicates that infective virus particle concentrations are the same when pools are grown in either culture.

The cell strain derived from embryonic guinea pig liver was found to be susceptible to infection by poliovirus. This cell line, as well as GPS-2, is being investigated for virus sensitivity. Preliminary results show that polioviruses, measles, and vaccinia grow and produce cytopathogenic effects in GPS-2 which are identical with those in GPS-1.

Heavy suspensions of GPS-1 cells in complete medium have been inoculated with and without cortisone into adult and suckling guinea pigs by several routes to determine whether or not these cells have undergone transformation to a malignant state. No gross evidence of malignancy has been observed.

Since the first report in 1957 (*2*) of the susceptibility of the nonprimate cell line ERK-1 to poliovirus, there has been considerable controversy. Some critics of the work doubt that the cell is actually of rabbit origin. It is considered a primate cell line contaminant. Some support for this stand can be drawn from the work of Melnick and Habel (*9*), who found by complement-fixation techniques that the ERK-1 cell line possessed an antigen in common with HeLa and other primate cell lines. These latter findings, however, can also be used to support the theory that many cell lines possess a common antigen which was not present in primary cells.

Though primary cell cultures may be refractory to infection by poliovirus, it is possible for cell lines when transformed to become susceptible to infection by this virus. Since primary guinea pig tissues have been found to be refractory to infection and cell lines susceptible, it would seem that some change or transformation has occurred.

In our laboratories a guinea pig embryo liver and two guinea pig spleen cell lines, all susceptible to poliovirus, have been established by the techniques described. It seems very unlikely that all three cell lines are in reality primate cell line contaminants.

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### References and Notes

1. Guinea pig spleen cell lines (Stanfield #1 and #2), referred to as GPS-1 and GPS-2.
2. F. W. Sheffield and G. Churcher, *Brit. J. Exptl. Pathol.* 38, 155 (1957).
3. Poliovirus types I, II, and III used in this study were Mahoney, MEF-1, and Saukett, respectively. Stock pools of virus were prepared in trypsinized monkey kidney cultures.
4. J. C. N. Westwood, I. A. MacPherson, D. H. J. Titmuss, *Brit. J. Extpl. Pathol.* 38, 138 (1957).
5. W. Cherry and R. N. Hull, *Anat. Record* 124, 483 (1956).
6. I. S. Johnson, *Ann. N.Y. Acad. Sci.*, in press.
7. C. Waymouth, *J. Natl. Cancer Inst.* 17, 305 (1956).
8. L. J. Reed and H. Muench, *Am. J. Hyg.* 27, 493 (1938).
9. J. L. Melnick and K. Habel, *Federation Proc.* 17, 526 (1958).

17 December 1958

## Role of Magnesium in Acetyl Coenzyme A Formation by Acetothiokinase

In a recent discussion (*1*) of the role of magnesium in enzyme-catalyzed reactions involving adenosine triphosphate, Ingraham and Green postulated that magnesium acts by chelating with the substrates and the enzyme in such a way that the net effect is to alter the "entropy of activation" and thereby promote the reaction. Without considering the implications of this hypothesis in the general problem of metal ion catalysis in enzymic reactions, I should like to point out that the specific hypothesis concerning the role of magnesium in acetyl coenzyme A (CoA) (*2*) formation advanced by Ingraham and Green (*1*) is in contradiction to published experimental findings.

In analyzing the sequence of events leading to the formation of acetyl CoA from ATP, acetate, and CoA (see *1*, Fig. 2), Ingraham and Green proposed that a complex is first formed between CoA, $Mg^{++}$, and the enzyme and that this is followed by reactions with ATP and acetate. This results in the liberation of free inorganic pyrophosphate and in the formation of acetyl AMP, linked through magnesium to the enzyme. It was proposed that in the final step of the reaction there is a transfer of the acetyl group from the magnesium-bound acetyl AMP to the sulfhydryl group of CoA.

While it is clear that $Mg^{++}$ is required for the over-all reaction, the series of reactions proposed by Ingraham and Green (*1*) leads to certain verifiable predictions. These are (i) that CoA is required for the isotope-exchange reaction between $P^{32}$-labeled inorganic pyrophosphate and ATP and (ii) that $Mg^{++}$ is required for the formation of acetyl CoA from acetyl AMP and CoA. It has already been demonstrated that the formation of acetyl CoA from ATP, acetate, and CoA (reaction 3, below) actually occurs in at least two discrete steps (reactions 1 and 2).

$$\text{ATP} + \text{acetate} \overset{Mg^{++}}{\rightleftarrows} \text{acetyl AMP} + \text{PP} \quad (1)$$

$$\text{Acetyl AMP} + \text{CoA} \rightleftarrows \text{acetyl CoA} + \text{AMP} \quad (2)$$

$$\text{ATP} + \text{acetate} + \text{CoA} \overset{Mg^{++}}{\rightleftarrows} \text{acetyl CoA} + \text{AMP} + \text{PP} \quad (3)$$

Reaction 1, which is easily measured by the acetate-dependent exchange of $PP^{32}$ and ATP or by the conversion of acetyl AMP to ATP, requires the presence of $Mg^{++}$. Not only is CoA not required for this reaction but if it is added the rate of exchange is decreased (*3*). Reaction 2, which is measured by the conversion of acetyl AMP to acetyl CoA, occurs in the absence of $Mg^{++}$. Neither the rate nor the extent of the reaction is affected by the addition of $Mg^{++}$.

That this is not due to the presence of $Mg^{++}$ in the enzyme or reactants can be inferred from the requirement for added $Mg^{++}$ when the over-all reaction (reaction 3) is studied (*3*). The most direct interpretation of these data is that $Mg^{++}$ is involved only in the initial step of the reaction—namely, the reaction sequence involving ATP.

It is conceivable that these conclusions can be interpreted by proposing that exogenously supplied acetyl AMP is utilized by the enzyme at some unrelated transacylating site which does not require $Mg^{++}$ (*4*). I feel that this is probably unlikely, since the presence of acetyl AMP excludes ATP from reacting with the enzyme. This is shown by the fact that if acetyl AMP is added to the complete system (ATP, acetate, CoA, $Mg^{++}$, and the enzyme), acetyl CoA synthesis continues but pyrophosphate formation is decreased to approximately one-tenth the rate found in the absence of acetyl AMP. When the added acetyl AMP is exhausted, the rate of PP formation is restored to the control level (*3*). This provides evidence that acetyl AMP competes for the site occupied by ATP. Were the acetyl AMP to be used at a second site on the enzyme, one would not expect an inhibition of the reaction in which ATP is utilized.

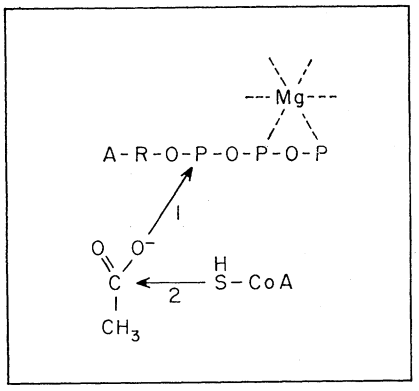


Fig. 1. Schematic representation of acetyl CoA formation on enzyme surface.

In my view the simplest hypothesis consistent with the experimental findings is that the sites necessary for enzymatic activity are specific for ATP, acetate, and CoA (Fig. 1). Magnesium may be a functional part of the specific site for ATP or, alternatively, the site may be specific for a particular ATP-Mg complex. Thus, the presence of ATP, $Mg^{++}$, and acetate would lead to an elimination of PP (or a dissociable Mg–PP complex) and the formation of acetyl AMP (bound to the enzyme). In the presence of CoA this would be followed by a magnesium-independent cleavage of the acetyl AMP to form acetyl CoA. On the basis of this hypothesis acetyl AMP would be expected to share, at least in part, the site with ATP.

The only enzymatic system which to my knowledge is analogous to the acetate-activating system and which has been studied in sufficient detail with respect to the magnesium requirements involves luciferin. Airth, Rhodes, and McElroy (*5*) have shown that the activation of luciferin by crystalline luciferase is markedly stimulated by $Mg^{++}$ but that no such requirement is evident when luciferyl adenylate is the substrate either for the light-producing reaction or for the formation of luciferyl CoA (*6*).

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### References and Notes

1. L. L. Ingraham and D. E. Green, *Science* 128, 310 (1958).
2. The following abbreviations have been used in this report: AMP, adenosine 5′-monophosphate; ATP, adenosine triphosphate; CoA, coenzyme A; PP, inorganic pyrophosphate.
3. P. Berg, *J. Biol. Chem.* 222, 991 (1956).
4. D. E. Green, personal communication.
5. R. L. Airth, W. C. Rhodes, W. D. McElroy, *Biochim. et Biophys. Acta* 27, 519 (1958); W. D. McElroy, personal communication.
6. The studies reported here were carried out while I was a scholar in cancer research of the American Cancer Society; they were supported by a research grant from the U.S. Public Health Service.

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In the report to which Berg refers we proposed a general mechanism for ATP-catalyzed enzymatic syntheses, the essence of which involves the gathering of reactants within a single enzyme-Mg complex and then a sequence of displacement reactions. By this device the term for entropy of activation becomes relatively small, and thus the principal barrier to reaction is overcome.

As a specific example of this mechanism we considered the enzyme acetokinase, which catalyzes the synthesis of acetyl CoA from acetate, ATP, and CoA only in the presence of $Mg^{++}$, and postulated that the Mg enzyme forms a complex with ATP and CoA and that two consecutive displacement reactions then ensue—the displacement, first, of a pyrophosphate group by acetate and second, of adenosine monophosphate by CoA. After these displacement reactions the products—acetyl CoA, adenosine monophosphate, and inorganic pyrophosphate —dissociate from the Mg-enzyme complex.

In the necessarily brief development of this hypothesis we omitted to stress (i) that the order of complex formation is not critical and (ii) that any exchange or partial reaction catalyzed by the Mg-enzyme complex does not require the presence of a ligand which does not participate in this reaction. Thus, the exchange of ATP with PP requires only acetate and not CoA, while the displacement of adenosine monophosphate in enzyme-bound acetyl AMP by CoA does not require the presence of ATP or its products of cleavage.

It appears to us that the behavior of the acetokinase system is in full accord with this hypothesis. Only a single enzyme is required for the synthesis. No intermediates formed in the reaction have been found to dissociate from the enzyme (*1, 2*). The only recognizable components are the initial and final products. Berg (*1*) has demonstrated that synthetic acetyl AMP can give rise to acetate and ATP in the presence of inorganic pyrophosphate and $Mg^{++}$, and to acetyl CoA and inorganic pyrophosphate in the presence of CoA. He has not, however, been able to demonstrate that acetyl AMP is formed in any measurable amount during the over-all reaction in the presence of $Mg^{++}$. We take this negative result to mean that the intermediate is not acetyl AMP but the corresponding magnesium chelate, which has no measurable dissociation. We assume further that the enzyme-bound magnesium chelate of acetyl AMP can interact with CoA with the same facility as acetyl AMP added to the $Mg^{++}$-free enzyme. Thus, the lack of a $Mg^{++}$ requirement for the formation of acetyl CoA from acetyl AMP has no bearing on the question whether the intermediate is free acetyl AMP or its Mg chelate. The problem is whether or not CoA can react with the Mg-chelated and enzyme-bound intermediate. There is no evidence yet available bearing on this point.

Berg raises the further objection to our hypothesis that CoASH is not required for the acetate-dependent exchange of $PP^{32}$ and ATP, and that if added it decreases the rate of exchange. As stated above, the order of complex formation is of no importance, so the inhibition of $PP^{32}$ exchange by CoASH is not a criterion of the validity of the proposed mechanism.

Until the ambiguities and uncertainties in the observations cited by Berg have been resolved by further decisive experiments, we find no compelling reason to revise the mechanism we have proposed for the acetokinase system.

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1. P. J. Berg, *J. Biol. Chem.* 222, 991 (1956).
2. H. Beinert, D. E. Green, P. Hele, H. Hift, R. W. Von Korff, C. V. Ramakrishnan, *ibid* 203, 35 (1953).

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## Interaction of Gibberellic Acid and Indoleacetic Acid in Impatiens

*Abstract.* Gibberellic acid promotes doubling, early flowering, and fruiting directly in *Impatiens*. It also lengthens the stem, probably through a neutralization of auxin inhibitors. Such a mechanism would leave the growth-promoting auxins unchecked to produce the increased elongation characteristic of gibberellin application. It seems that application of additional auxin produces a rapid build-up of the auxin inhibitor(s) in the plant.

Though gibberellic acid has auxin-like activity in the sense that it induces cellular elongation in stem tissue, it is difficult to decide whether it should be regarded as an auxin (*1*). While working with *Impatiens balsamina* L. on the inheritance of flower doubling, I noticed that the "double" variety showed incomplete penetrance of the recessive gene $p_t$ which is responsible for the production of double flowers (*2*). Outbreeding of highly inbred "double" strains of *Impatiens* restored full penetrance of $p_t$ for one generation only; the $F_3$ generation of such an outcross again showed considerable reduction in penetrance.

To determine whether growth-pro-