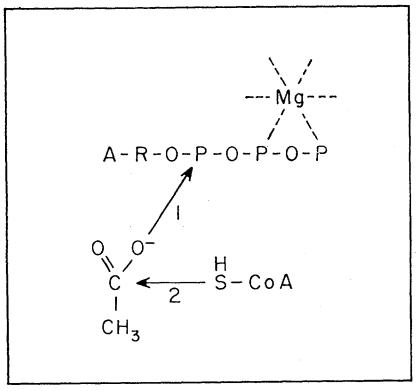

Fig. 1. Schematic representation of acetyl CoA formation on enzyme surface.

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.

17 October 1958

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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References

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).

1 December 1958

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-

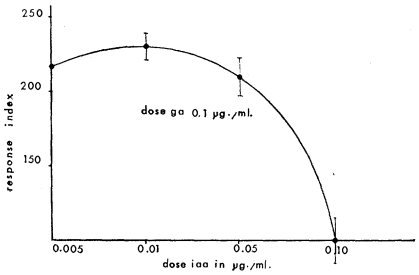

Fig. 1. Effect of different concentrations of indoleacetic acid, applied as sprays on alternate days, on plant height of *Impatiens* treated simultaneously with sprays of gibberellic acid (10^{-4} g/1000 ml), measured after 3 weeks of treatment. Height is expressed as percentage of that of untreated plants.

Table 1. Influence of growth substances on the penetrance of doubling in a progeny of a highly inbred strain of "double" *Impatiens*. Sprays (5 ml) of the growth substances in a concentration of 10^{-4} g/1000 ml were applied on alternate days from the four-leaved seedling stage until flower-bud initiation (approximately 7 weeks). Fifty plants were used per treatment. The figures in parentheses indicate the number of treatments.

Treatment	Flowers	
	Double	Single
Control (37)	32	18
Indolepropionic acid (45)	36	14
Indoleacetic acid (30)	37	13
β-Naphthoxyacetic acid (30)	30	20
α-O-Chlorophenoxy-acetic acid (30)	34	16
α-O-Chlorophenoxy-propionic acid (30)	32	18
Gibberellic acid (30)	50	
α-Naphthaleneacetic acid (30)	35	15

Table 2. Influence of combined sprays of gibberellic acid (GA) (concentration 10^{-4} g/1000 ml) and indoleacetic acid (IAA) (concentrations 5×10^{-6} to 10^{-4} g/1000 ml) on the penetration of flower doubling and flowering time of *Impatiens*.

Concn.		Double-flowering plants (%)	Flowering time* (day)
IAA	GA		
5×10^{-6}	1×10^{-4}	98	+19
1×10^{-5}	1×10^{-4}	100	+19
5×10^{-5}	1×10^{-4}	100	+21
1×10^{-4}	1×10^{-4}	100	+22
		64	

* Days prior to flowering date of control plants.

moting substances might influence this penetrance, several compounds were tested on seedlings of the highly inbred "double" ($p_t p_t$) lines. Gibberellic acid at a concentration of 10^{-4} g/1000 ml, applied as a spray in doses of 5 ml each alternate day until flowering, had the most pronounced effect. The plant treated showed full penetrance of doubling, whereas the control yielded 67 percent double-flowering individuals (33 percent flowered single). From this experiment it became evident that gibberellic acid may play an important role in the physiological requirements necessary for expression of the gene for doubling. The plants that received gibberellic acid up to flowering time flowered 3 weeks earlier than the control. The height of the treated plants was approximately 2½ times that of the average height of the controls.

Of six other growth-substances tested, none had an effectiveness approaching that of gibberellic acid (Table 1).

The seedlings that received indoleacetic acid (IAA) were slightly stunted in stature, and their flowering time was delayed by 2 weeks. Moreover, when IAA and gibberellic acid were combined, at concentrations of 10^{-4} g/1000 ml each, we noticed that the elongating effect of gibberellic acid was completely inhibited and that the treated plants grew to normal stature. These plants flowered 3 weeks ahead of the controls and showed full penetrance of doubling. To confirm this, seedlings of the same strain were treated in the same manner as before with a solution of gibberellic acid (10^{-4} g/1000 ml) plus a series of concentrations of IAA. Figure 1 illustrates the general trend of internode extension in intact plants after 3 weeks of alternate-day treatments. Relatively small amounts of IAA increased the elongating effect of gibberellic acid, but with increasing concentrations of IAA the response to gibberellic acid at a concentration of 10^{-4} g/1000 ml decreased, and at IAA concentrations of 10^{-4} g/1000 ml the elongating effect of gibberellic acid was completely nullified. On the other hand, the secondary effects of gibberellic acid, such as early flowering and increase in penetrance of doubling, were not affected by simultaneous treatment with IAA (Table 2). In general, the fertility of the treated plants was much greater than that of plants treated with gibberellic acid only.

It has been assumed that there is, in the intact plant, an inhibitory system which limits the growth rate (*3*). Growth of intact internodes is considered to be regulated by a three-factor system, consisting of auxin, an auxin inhibitor, and a hormone with physiological properties similar to those of gibberellic acid. The above results indicate that the concentration of the auxin inhibitor depends upon the concentration of auxin present. On the basis of the dose-response curve (shown in Fig. 1) it appears that the relationship between auxin concentration and auxin inhibitor production is not linear. At relative high auxin levels the production of auxin inhibitor is so greatly accelerated that there is more auxin inhibitor present in the plant tissue than there is auxin. Future biochemical studies may throw some light on these highly interesting phenomena (*4*).

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References and Notes

1. P. W. Brian, H. G. Hemming, M. Radley, *Physiol. Plantarum* 8, 899 (1955).
2. J. Weijer, *Genetica*, in press. B. Kanna, *J. Coll. Agr. Tokyo Imp. Univ.* 12, 421 (1934).
3. P. W. Brian and H. G. Hemming, *Ann. Botany (London)* 22, 1 (1958).
4. These investigations were supported by the University of Alberta general research grant No. 178.

4 November 1958

Drug Metabolism in the Newborn Rabbit

Abstract. A number of drugs that are metabolized through the action of enzymes present in liver microsomes in the adult rabbit are not metabolized in livers of newborn rabbits. The development of metabolic pathways during a period of 4 weeks is presented. Evidence is given for the presence in livers of baby rabbits of inhibitors of some of these drug-enzyme systems.

One of the factors that modifies the actions of drugs and that is taken into account in their therapeutic use is the age of the subject. It is known that the young, both of animals and of human beings, are more sensitive to certain drugs than the adults. This greater sensitivity could result from differences in the responsiveness of the receptors or from differences in the fate of the drug —that is, with respect to absorption, distribution, excretion, or metabolism—or from both. We chose to investigate possible differences in drug metabolism (*1*).

A variety of drugs are metabolized through the action of enzymes present in liver microsomes (*2*). The greater sensitivity to drugs of the young animal may be in part due to a deficiency of these enzymes, since other studies have shown that certain tissues, including liver, have enzyme levels which are several times higher in the adult than in the embryo (*3*). Also, electron micrographs show that differences do exist be-