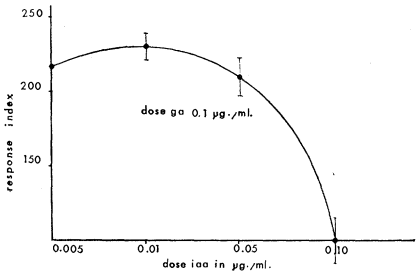

Fig. 1. Effect of different concentrations of indoleacetic acid, applied as sprays on alternate days, on plant height of *Impatiens* treated simultaneously with sprays of gibberellic acid (10^{-4} g/1000 ml), measured after 3 weeks of treatment. Height is expressed as percentage of that of untreated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

J. WEIJER

Department of Plant Science, University of Alberta, Edmonton

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-

tween the adult and the embryonic structures from which the microsomes are derived (*4*).

In order to gain a more general picture of the development of drug enzyme systems, various pathways were studied. These included the side-chain oxidation of hexobarbital, the N-dealkylation of Pyramidon, the deamination of amphetamine, the hydroxylation of the aromatic ring of acetanilid, the oxidation of the ring sulfur of chlorpromazine, and the reduction of the aromatic nitro- group of *p*-nitrobenzoic acid. In addition, both the homogenate, containing all the particulate matter of the cell, and the supernatant (9000*g*), containing only the microsome and soluble fractions, were used in this investigation. Table 1 presents our findings relative to six of these pathways.

Table 1. Development of drug-metabolizing enzymes in rabbit liver (*7*). For methods used in determination of metabolism, see Brodie (*2*) and others (*8*). The type of metabolism represented by each substrate is described in the text.

Preparation	Drug metabolism (μmole/g) * at age		
	2 wk	3 wk	Adult
Hexobarbital			
Homogenate	0.96	2.8	8.4
Supernatant	4.2	10.3	16.6
Aminopyrine			
Homogenate	0.15	0.30	3.25
Supernatant	0.34	1.41	5.2
l-*Amphetamine*			
Homogenate	1.3	1.9	8.8
Supernatant	1.9	15.1	21.1
Acetanilid			
Homogenate	1.0	1.9	4.5
Supernatant	2.9	5.2	7.8
Chlorpromazine†			
Supernatant	11.4	31.5	32.3
p-*Nitrobenzoic acid*			
Homogenate	1.72	5.16	9.9
Supernatant	1.76	5.28	8.1

* Micromoles of drug metabolized, or of metabolite formed, per gram of protein of liver fraction used. The protein was determined spectrophotometrically (*9*). Values given for the metabolism of drugs are those of typical experiments. Enzyme activities for each metabolism at each age were determined at least twice.
† In these studies chlorpromazine metabolism could not be followed satisfactorily in homogenates, due to strong binding of the drug by nuclei or mitochondria.

Table 2. Effect of cell fractions from baby rabbit liver on the metabolism of amphetamine by adult rabbit liver.

Description	Metabolism of *l*-amphetamine	
	μmole*	Inhibition (%)
Baby (homogenate)	0	
Baby (supernatant)	0	
Adult (supernatant)	3.25	
Adult (supernatant) plus baby (homogenate)	0.59	82
Adult (supernatant) plus baby (supernatant)	3.34	0

* Micromoles of amphetamine metabolized by homogenates or supernatants from 1 g of liver.

The newborn rabbit is essentially unable to metabolize any of the drugs used. When it reaches the age of 2 weeks we see an appearance of activity with respect to all pathways, though the extent of this activity is not the same in every instance; it ranges from 5 to 37 percent of the enzyme activity of adults. The 3-week-old animals seem to have even more enzyme activity, and the activity in the liver of the 4-week-old rabbit is in most cases approximately equal to that of the adult.

The apparent lack of enzyme activity in the young animal could be due to (i) an actual absence of enzyme protein; (ii) a deficiency of cofactors—that is, reduced coenzyme II [reduced triphosphopyridine nucleotide (TPNH)]; (iii) the presence of inhibitors of the drug-metabolizing enzymes; or (iv) differences in the nature of the enzymes in the livers of baby and adult rabbits—for example, differences in optimal *p*H or substrate concentrations.

We tried to eliminate the second possibility by adding a TPNH-generating system of glucose-6-phosphate, glucose-6-phosphate dehydrogenase, triphosphopyridine nucleotide (*5*), and nicotinamide in all our determinations.

The presence, in the liver of the baby rabbit of inhibitors of drug metabolism was suggested by differences in the rate of appearance of enzyme activity in homogenates as compared with the rate of appearance in the supernatant (9000*g*). For several of the pathways studied, relatively more enzyme activity seemed to be present in this latter fraction (see Table 1).

Table 2 shows that homogenates of baby rabbit liver do indeed contain some material which inhibits the metabolism of amphetamine. Inhibitors of hexobarbital, acetanilid, and Pyramidon metabolism have also been demonstrated in this type of preparation.

This inhibition is usually not seen when the supernatant (9000*g*) fraction of baby rabbit liver is used. Thus, we seem to be dealing with inhibitors present in the nuclear or mitochondrial fractions.

Also of interest is the fact that this material seems to disappear as the animal matures, at a rate inversely proportional to drug-metabolizing enzyme activity. However, since no inhibitors of *p*-nitrobenzoic acid or chlorpromazine metabolism seem to be present in baby rabbit liver, it is probable that other factors are also responsible for the observed lack of enzyme activity.

To date we have been unable to obtain liver samples having one or more metabolic pathways while lacking others. All such pathways seem to appear at about the same time, though the rate of development is different. It may be, then, that a single event triggers the synthesis or unmasking of all the enzymes (*6*).

JAMES R. FOUTS
RICHARD H. ADAMSON
Department of Pharmacology, College of Medicine, State University of Iowa, Iowa City

References and Notes

1. This research was supported in part by grant B-615 (C2) from the U.S. Public Health Service.
2. B. B. Brodie, *J. Pharm. and Pharmacol.* 8, 1 (1956).
3. V. R. Potter, W. C. Schneider, G. J. Liebl, *Cancer Research* 5, 21 (1945).
4. A. W. Ham, *Annual Report, Ontario Cancer Treatment and Research Foundation* (1956–1957), p. 18.
5. The glucose-6-phosphate, glucose-6-phosphate dehydrogenase, and triphosphopyridine nucleotide used in this study were purchased from Sigma Chemical Company, St. Louis, Mo.
6. Further studies in progress include investigation of the nature of the inhibitors described in this report; study of factors which may stimulate enzyme synthesis; changes in ultrastructure at the time of appearance of these systems; and correlation of in vitro with in vivo results.
7. The *l*-amphetamine and the chlorpromazine used in this study were kindly furnished by Dr. Glenn Ullyot of Smith, Kline & French Laboratories, Philadelphia.
8. C. Mitoma, H. S. Posner, H. C. Reitz, S. Udenfriend, *Arch. Biochem. Biophys.* 61, 431 (1956); N. P. Salzman and B. B. Brodie, *J. Pharmacol. Exptl. Therap.* 118, 46 (1956); J. R. Fouts and B. B. Brodie, *ibid.* 119, 197 (1957).
9. O. Warburg and W. Christian, *Biochem. Z.* 310, 384 (1942).

20 October 1958

Resistance to Sevin by DDT- and Parathion-Resistant Houseflies and Sesoxane as Sevin Synergist

Abstract. The new carbamate insecticide, Sevin, has a low toxicity against houseflies. However, it is activated effectively by low concentrations of Sesoxane, a pyrethrum synergist. This occurs with normal nonselected flies and also with strains selected for resistance to DDT and Parathion, which have considerable cross resistance to Sevin.

1-Naphthyl N-methyl carbamate (Sevin), a new insecticide introduced by Union Carbide Chemicals Company, shows great promise in the field because of the success it has already had in the control of many agricultural pests in the field and because of its low toxicity to mammals.

Recent investigations (*1*) on the house-