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*).

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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-

fly, *Musca domestica,* have shown that Sevin is only about one-tenth as toxic as DDT (the LD_{50} values, for topical application, are 0.2 μg per fly for DDT and 2.3 μg per fly for Sevin). In an attempt to increase the effectiveness of Sevin, several pyrethrin synergists were investigated. Of those investigated, 2-(3,4-methylenedioxyphenoxy)-3,6,9-trioxaudecane (Sesoxane), a pyrethrin synergist discovered by Beroza (*2*) and manufactured by Shulton, Inc., was most effective. This compound has been reported as having some synergistic action for methoxychlor (*3*) and was found in our laboratories to be a synergist for DDT on German roaches. When tried with DDT on houseflies, it showed little synergism. However, Sesoxane in conjunction with Sevin gave a greatly increased toxicity for houseflies. Figure 1 shows the log dose–probit lines for DDT and Sevin, with and without the synergist, for the susceptible houseflies; dosage is expressed as the logarithm of the number of micrograms applied, and mortality is expressed in probits.

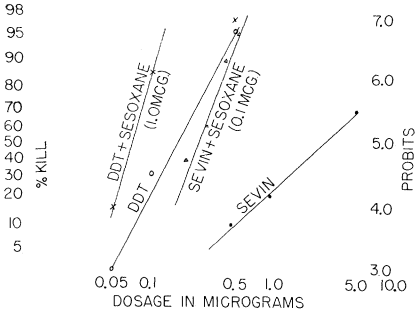

Fig. 1. Dosage mortality curves for susceptible houseflies treated with Sevin and DDT with and without Sesoxane.

Table 1. Mortality, after 24 hours, of susceptible and resistant houseflies topically treated with Sevin and Sevin-Sesoxane combinations. Twenty-five flies were treated at each point. The percentages represent the combined results of duplicate tests.

Sevin (μg/fly)	Sesoxane (μg/fly)	Kill (%)
	Susceptible	
0.5		10
1.0		20
2.0		55
5.0		67
	Parathion-resistant	
5		10
10		10
30		10
	DDT-resistant	
5		20
10		40
30		60
	Susceptible	
0.1	0.00	0
0.1	0.10	40
0.1	0.30	80
0.1	0.50	95
0.5	0.10	95
	Parathion-resistant	
1.0	0.00	0
1.0	1.00	60
1.0	5.00	100
1.0	10.00	100
	DDT-resistant	
1.0	0.00	10
1.0	0.50	100
1.0	1.00	100
1.0	5.00	100
0.1	5.00	100

Since both Sevin and Parathion are strong cholinesterase inhibitors, it may be expected that Parathion-resistant insects will also be resistant to Sevin, and this was found to be the case, as shown in Table 1. However, only a comparatively low resistance, commonly called vigor tolerance, was to be expected from DDT-resistant flies (*4*). Table 1 shows that DDT-resistant flies have about a sevenfold resistance to Sevin—that is, the LD_{50} for susceptible flies was 2.6 μg; for DDT-resistant flies, 18 μg.

Since the combination of Sevin and Sesoxane was effective on the DDT-susceptible flies, it was applied to DDT-resistant flies. This combination proved to be very effective even at small dosages. All mortalities were obtained by applying the chemicals together in acetone solutions topically to adult females. Table 1 shows the results.

The outstanding feature is the high effectiveness of the combination containing a low ratio of synergist to toxicant in contrast to the usual situation, in which large amounts of synergist are necessary.

Comparison of the rates of penetration between Sevin alone and Sevin-Sesoxane combinations shows very little difference between the two; this indicates that the effectiveness of the Sevin-Sesoxane combination is not due to increased penetration.

The effective synergistic action of Sesoxane with Sevin gives hope for control of DDT- and phosphate-resistant insects.

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References and Notes

1. This project was financed in part by funds obtained through contract No. DA-49-007-MD-304 between the University of California and the Office of the Surgeon General, U.S. Army.
2. M. J. Beroza, *J. Agr. Food Chem.* **4**, 49 (1956).
3. *Chem. Eng. News* 36, No. 7, 36 (1958).
4. W. M. Hoskins and H. T. Gordon, *Ann. Rev. Entomol.* 1, 89 (1956).

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Multiple Hemoglobins in Fishes

Abstract. Paper electrophoregrams of the hemoglobins from 14 species of fresh- and salt-water fish demonstrated from one to three components. The relative distribution of these fractions was determined for all species. Mobilities of the three hemoglobins of rainbow trout were calculated from moving-boundary patterns. Low anodal mobility at *p*H 8.8 was characteristic of many species examined.

Since the demonstration of abnormal hemoglobin in man by Pauling *et al.* (*1*), electrophoretic studies have been carried out on many other species. The occurrence of multiple hemoglobins has been reported in other mammals (*2*), in birds (*3*), and in amphibians and reptiles (*4*). Manwell (*5*) has recently reported a difference in alkaline denaturation between adult and postlarval hemoglobins from a teleost fish (*Scorpaenichthys marmoratus*). Examination of fish hemoglobins should therefore reveal some differences which might be species characteristic and perhaps even race dependent.

Samples of fish blood were collected in heparinized tubes, either by cardiac puncture of the larger fish or by amputating the peduncle of smaller fish and collecting the droplets of blood from the severed caudal vein and artery. The red cells were separated from the plasma by centrifugation and washed three times with 0.9-percent sodium chloride. The hemoglobin solutions used for analysis (*6*) were prepared by adding 2 volumes of distilled water to 1 volume of packed red cells. After standing overnight in the cold, the hemolyzates were centrifuged at 25,000*g* for 1 hour. The supernatant

Table 1. Relative distribution of fish hemoglobin components. Values are averages from at least two fish of each species.

Electrophoregram	Percentage of total hemoglobin (%)		
	Highest mobility	Medium mobility	Lowest mobility
	Steelhead trout		
E	54–56	22–23	22–23
	Rainbow trout		
F	54–58	21–23	21–23
	Blueback salmon		
G	51–53		47–49
	Shad		
H	66–70		30–34
	Largemouthed bass		
I	60–64		36–40
	Brook trout		
J	56–64	18–22	18–22
	Silver salmon		
K	50–60		40–50
	Chinook salmon		
L	50–60		40–50