terferon caused by a massive infection of cells by the virus, the mechanism underlying this phenomenon is not easy to understand. If such an autointerference should result from a simultaneous infection of adjacent cells, then plaque formation by this virus would have never occurred, because each infected cell releases newly produced virus at the end of the growth cycle which infects many neighboring cells almost at the same time. An explanation of this phenomenon, therefore, may be that one PFU of this virus corresponds to many virus particles, and consequently in an infection of cells at a moi of higher than 0.1 all cells are attacked by many non-infectious virus particles resulting in a von Magnus phenomenon(12). Alternatively, an interferon inducer as detected in the NDV-L(MCN) cell system(10) may be present in the original seed virus suspension or be produced on infection of the cells. What mechanism really operates in the autointerference requires further investigation.

Summary. When HEP Flury strain of rabies virus was inoculated onto chick embryo fibroblasts at a multiplicity of higher than 0.1, no plaques were formed and almost all cells remained stainable with neutral red. Monolayers pretreated with such a concentration of HEP Flury virus showed interference with plaque formation by vaccinia and WEE viruses. This was not due to presence of interferon in the seed virus suspension, nor to the presence of tissue components in the inoculum. In bottle cultures of chick embryo cells, virus growth after infection at higher multiplicities was accompanied by production of a considerable amount of interferon, and the highest virus yield was obtained when the multiplicity of infection was about 0.01.

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Liver Metabolism and Toxicity of Thiophosphate Insecticides in Mammalian, Avian and Piscine Species.* (31497)

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Organic phosphorothionate or phosphorodithioate insecticides are toxic to animals by virtue of their capacity to inhibit acetylcholinesterase. The actual inhibitors of cholinesterase are the oxygen analogues which are metabolically formed from the parent insecticides *in vivo*(1,2). The relative susceptibility of various species to poisoning by these insecticides might, therefore, be determined in whole or in part by the rates at which the oxygen analogues accumulate in the different organisms. This accumulation will be dependent not only upon the rates of formation of the oxygen analogues but also upon the rates at which the parent insecticides or their oxygen analogues are destroyed by tissue

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hydrolases or other mechanisms of inactivation(3,4). There is considerable information concerning the enzymatic metabolism of thiophosphate insecticides by tissues of insects and common laboratory mammals (2,5), and Potter and O'Brien(6) recently reported that liver slices from a variety of aquatic and terrestrial animals "activated" Parathion. It was felt that additional comparative studies of the metabolism of several thiophosphate insecticides would be useful to the understanding and/or prediction of their toxicity to various vertebrate species that might be incidentally exposed to these chemicals. In the present investigation a comparison was made of the net accumulation of active cholinesterase inhibitors when highly purified samples of 3 thiophosphate insecticides were incubated with mammalian, avian and piscine liver slices. Inactivation of the insecticides or their oxygen analogues by liver homogenates was also studied. A limited number of toxicity tests suggested that the value of comparative metabolism studies in predicting the comparative toxicity of thiophosphate insecticides varies with different compounds.

Materials and methods. The insecticides used in this study were 0,0-dimethyl-S-(1,2-dicarbethoxyethyl) phosphorodithioate (Malathion), 0,0-diethyl 0-(p-nitrophenyl) phosphorothioate (Parathion), and 0,0-dimethyl S-(4 oxo-1,2,3-benzotriazin-3 (4H)-ylmethyl) phosphorodithioate (Guthion) and their corresponding oxygen analogues Malaoxon Paraoxon and Gutoxon. All samples were of the highest purity (>99%) that were available from the manufacturers.† Stock solutions (0.01 M) of the compounds in absolute ethanol were stored in dark bottles in the refrigerator and appropriately diluted with water or buffer just before use in the metabolism studies.

Acetylcholinesterase assays were performed by the manometric method of DuBois and Mangun(7), using 50 mg of homogenized rat brain as the enzyme source. Less than 10% inhibition of cholinesterase was produced when as much as 50 millimicromoles (mμm) of the parent insecticides were added to the 3 ml cholinesterase test system. In contrast, 50% inhibition was produced by 0.031, 0.024 or 0.39 mμm of Paraoxon, Gutoxon or Malaoxon, respectively.

Mammals used in this study were male Holtzman rats (200-250 g) and male Swiss-Webster mice (25-30 g). Avian species, obtained from the Massachusetts Audubon Society, were chickens (Leghorn cockerels 300-350 g) and male and female English sparrows (20-30 g). The sparrows were held for 5-10 days after they were captured and were fed a commercial bird seed. Sunfish *(Leopomis gibbosus)* and bullheads *(Ictalurus melas)* weighing 40-80 g were furnished by the Massachusetts Department of Fish and Game and were taken from a local fresh water pond. The salt water fish were winter flounder *(Pseudopleuronectes americanus,* 220-235 g) and sculpin *(Myoxocephalus scorpius,* 260-360 g) taken from Narragansett Bay. All fish were used within 2-3 days of their capture.

The animals were sacrificed and their livers were removed and sliced with a Stadie-Riggs microtome or homogenized in deionized distilled water. All liver samples were kept at 0-4°C until used and all incubations with insecticides were completed within 6 hours after sacrifice. The production and accumulation of active inhibitors of cholinesterase from the parent insecticides or the destruction of the anticholinesterase activity of the oxygen analogues by liver slices or homogenates were measured by bioassaying aliquots of the liver incubation mixtures in the rat-brain-cholinesterase test system. The procedures were similar to those described previously(8,9). Ten to 20 mg (dry weight) of liver slices were shaken for 30 minutes at 38°C under oxygen with 30 mμm of Parathion or Guthion or 300 mμm of Malathion in a total volume of 3 ml of Krebs-Ringer phosphate buffer pH 7.6 containing 0.1 M glucose. Beakers which contained the insecticides, but no liver, were included in each run. At the end of the incubation period the slices were removed, dried and weighed, and the incubate solutions were frozen until they could be assayed in the cho-

† Malathion, Parathion, Malaoxon and Paraoxon were supplied by American Cyanamid Co., Princeton, N. J. Guthion and Gutoxon were furnished by Chemagro Corp., Kansas City, Mo.

TABLE I. Accumulation of Cholinesterase Inhibitors from Thiophosphates Incubated with Liver Slices at 38°C.

Species	No. of animals	P=0 equiv. accumulated/100 mg liver (dry wt)/30 min from: Parathion	Guthion	Malathion
Rat	4	5.80 ± .76*	9.03 ± 1.06	Trace
Mouse	5	7.68 ± .74	14.18 ± 2.04	6.4 ± 1.7
Sparrow	5	10.71 ± 1.80	15.56 ± 2.78	105.2 ± 32.2
Chicken	4	3.43 ± .72	4.85 ± .24	3.01 ± 1.7
Sunfish	4	19.97 ± 7.09	8.74 ± 1.72	161.0 ± 29.5
Bullhead	4	14.52 ± 1.56	3.64 ± .67	11.6 ± 2.0
Flounder	5	5.20 ± .81	11.24 ± 1.60	16.9 ± 3.8
Sculpin	4	.04 ± .02	.03 ± .01	6.1 ± .8

* Figures are means ± standard errors of the means.

linesterase test system. Aliquots of 0.6 ml or less were assayed in preliminary experiments to determine appropriate amounts of liver incubates which would produce between 20% and 70% inhibition of rat brain cholinesterase. The amount of inhibitor that had accumulated was calculated by comparing the cholinesterase inhibition produced by the incubate solutions with the inhibition produced by known quantities of the appropriate oxygen analogues. All values were expressed in terms of oxygen analogue (P = 0) equivalents. This is defined as the quantity of purified oxygen analogue that would have been required (in the original incubation mixture) to produce an equivalent inhibition of cholinesterase.

The capacities of livers from various species to inactivate the oxygen analogues of the insecticides were measured by incubating 0.5 mμm of Paraoxon or Gutoxon or 5 mμm of Malaoxon in test tubes for 10 minutes at 38° with 50 or 100 mg (wet weight) of homogenized livers in a 5 ml volume of 0.02 M phosphate buffer pH 7.2. The reaction was stopped by placing the tubes in a boiling water bath for 90 seconds. Aliquots (0.6 ml) of the incubation mixtures were assayed in the cholinesterase test system and the inhibition produced by aliquots from tubes containing liver were compared with that produced by aliquots from a substrate blank that was carried through the same incubation, heating and storage procedure.

The enzymatic hydrolysis of Paraoxon by 40 or 80 mg (fresh weight) of homogenized liver was determined by measuring the amount of p-nitrophenol formed during 60 minutes incubation with 400 mμm of Paraoxon as described by Neal and DuBois(10).

The hydrolysis of Malathion was measured at 38° by the manometric procedure described by Cook *et al*(11), using 200 mg or 400 mg of homogenized liver and 20 micromoles of the insecticide in 3 ml of 0.026 M bicarbonate buffer.

For toxicity tests on mice and sunfish, corn oil solutions of the insecticides were injected intraperitoneally. This route of administration was chosen over more conventional methods for fish toxicity tests, since the purpose of the test was to attempt to evaluate the correlation between toxicity and metabolism. Intraperitoneal injections seemed to represent the most convenient means of exposing similar absorbing surfaces, in both species, to known quantities of the insecticides. Injections of corn oil only (10 ml/kg, the maximum volumes used for the insecticide solutions) did not produce obvious effects during the two-hour observation period. The water temperature in the fish observation tanks was 18-20°C.

Results. The quantities of active cholinesterase inhibitors that accumulated when the insecticides were incubated, under identical conditions, with liver slices from various species are shown in Table I. Since under natural conditions the body temperatures of fish would be considerably below the 38°C used for these comparative studies, an experiment was conducted in which liver slices from 4 sunfish were incubated at 18°. Under these conditions 5.38 ± .74, 2.58 ± .32, and 59.9 ± 6.7 P = 0 equivalents accumulated from Parathion, Guthion and Malathion re-

TABLE II. Destruction of Anticholinesterase Activity of Oxygen Analogues of Thiophosphates by liver Homogenates.

Species	No. of animals	P=0 equivalents destroyed by 50 mg (wet wt) liver in 10 min		
		Paraoxon	Gutoxon	Malaoxon
Rat	4	.401 ± .002*	.157 ± .031	5.28 ± .03
Mouse	5	.381 ± .003	.275 ± .017	4.00 ± .02
Sparrow	6	.227 ± .010	.131 ± .010	2.83 ± .30
Chicken	4	.331 ± .009	.213 ± .014	3.46 ± .07
Sunfish	4	.120 ± .022	.205 ± .010	1.59 ± .17
Bullhead	5	.122 ± .005	.101 ± .044	.97 ± .28
Flounder	5	.041 ± .006	.039 ± .030	.81 ± .09
Sculpin	5	.076 ± .010	.109 ± .020	1.27 ± .14

* Figures are means ± standard errors of the means.

TABLE III. Hydrolysis of Paraoxon and Malathion by Liver Homogenates.

Species	No. of animals	p-nitrophenol formed from Paraoxon millimicromoles/60 min/40 mg*		Malathion hydrolysis μl CO_2 produced/30 min/g*	
		Mean	Range	Mean	S.E.
Rat	5	71.2	(60.0-80.0)	1589	± 28
Mouse	7	61.6	(41.0-72.0)	1118	± 39
Sparrow	7	.2	(.0- .96)	526	± 28
Chicken	4	5.7	(4.9- 6.7)	399	± 48
Sunfish	6	7.8	(4.8-12.8)	72	± 13
Bullhead	5	.2	(.0- .8)	135	± 9
Flounder	5	Not tested		201	± 58
Sculpin	5	" "		73	± 29

* Wet wt of liver.

spectively. The data in Table I indicate that for a single compound the variation among different species within a given class of animals may be as great as the variation among different classes. Furthermore, the relative quantities of inhibitors that accumulated in the presence of liver slices from various species differed depending upon the insecticide tested.

The differences in the quantities of cholinesterase inhibitors that accumulated during incubation with liver slices from various species might be due to differences in the capacities of the livers to activate the parent insecticides or differences in the rates of hydrolysis or other reactions which lead to inactive compounds. To investigate this further, the relative capacities of liver homogenates to destroy the anticholinesterase activity of the oxygen analogues were tested. The results are shown in Table II. Under the conditions described under *Methods* the amount of substrate was a limiting factor with Malaoxon in the case of mammalian livers; however, it is obvious from these data that livers from all the fish species destroyed considerably less Malaoxon and Paraoxon as compared with mammalian and avian livers. In the case of Gutoxon, there were overlapping values among the 3 classes of animals.

The loss of anticholinesterase activity of the oxygen analogues represents the total destruction of activity by all mechanisms that are operative in the test system. These could include nonspecific tissue binding(10) as well as enzymatic hydrolysis or other reactions leading to inactivation. Therefore, other techniques for measuring the hydrolysis of Paraoxon and Malathion were tested. The results of these measurements are shown in Table III. Comparison of these data with the data in Table I shows that the relative values obtained for rates of detoxication differ depending upon the assay method. It is not known which method would most accurately reflect *in vivo* conditions, but regardless of the method employed for measuring inactivation, livers from all the fish species contained much less activity than livers from mammals when Paraoxon or Malaoxon (or Malathion) were used as substrates.

The data in Table IV show that within the

TABLE IV. Acute Toxicity of Thiophosphate Insecticides and Their Oxygen Analogues to Mice and Sunfish.

Compound	Dose*	Mortality in 2 hr (No. dead/No. injected)	
		Mice	Sunfish
Corn oil	10 ml/kg	0/4	0/4
Parathion	40 mg/kg	4/4	0/4
"	20 "	0/4	–
Paraoxon	16 "	–	2/4
"	8 "	–	0/4
"	2.5 "	3/4	–
Guthion	16 "	–	1/4
"	10 "	4/4	–
"	8 "	1/4	0/4
Gutoxon	1 "	1/4	4/4
"	.125 "	–	3/4
Malathion	1500 "	2/4	–
"	100 "	0/4	2/4
Malaoxon	100 "	4/4	–
"	10 "	0/4	4/4
"	.25 "	–	2/4

* All compounds were given intraperitoneally in corn oil.

limited conditions of the toxicity tests, sunfish were much more susceptible than mice to both Malathion and its oxygen analogue. This is consistent with what would be predicted from the metabolism studies and agrees with the greater toxicity of Malathion to bluegills (relative to mammals) that has been reported for conventional fish toxicity studies(12). Parathion and Paraoxon appeared to be considerably less toxic to sunfish than to mice. It could be argued that the lower body temperature of the fish would reduce the rate of metabolic formation of Paraoxon from Parathion *in vivo*, and so account for the discrepancy between toxicity data and metabolism data. However, incubation of Parathion with sunfish liver slices at 18° resulted in the accumulation of quantities of Paraoxon which were only 30% less than when mouse liver slices were incubated at 38°. Liver metabolism data also failed to explain the relative resistance of sunfish to Paraoxon. Guthion was about twice as toxic to mice as to sunfish. This is consistent with what would be predicted from the liver slice experiments. However, Gutoxon was approximately 10 times as toxic to sunfish as to mice. The destruction of the anticholinesterase activity of Gutoxon by liver homogenates from these 2 species was approximately equal at 38°C. However, if it is assumed that the rate of destruction was decreased by 2- to 4-fold at 18° (the water temperature of the fish toxicity tests) and if adjustment is made for the smaller liver/body weight ratios of the sunfish (approximately one-half that for mice), the greater susceptibility of sunfish to poisoning by Gutoxon might be explained on the basis of smaller total degradation of the chemical.

Discussion. The total capacity of liver slices to *form* cholinesterase inhibitors from organic phosphorothionate or phosphorodithioate insecticides should, theoretically, equal the sum of the quantity of inhibitor accumulated plus the quantity destroyed during the incubation period. Because of differences in conditions for assaying liver activation and degradation of the insecticides, the data obtained in this study do not permit a strictly quantitative analysis. However, the results permit a semiquantitative consideration of the relative capacities of livers of various species to activate phosphorothionate insecticides.

The high rate of degradation of Malathion or its oxygen analogue by mammalian livers as compared to fish livers, can readily explain why the accumulation of a cholinesterase inhibitor from this insecticide was less for mammalian than for fish liver slices. Measurements of the loss of anticholinesterase activity of Paraoxon indicate that sunfish and bullhead livers have only about ¼ the capacity of rat and mouse livers to inactivate Paraoxon. Two to four times as much Paraoxon accumulated when Parathion was incubated with sunfish and bullhead liver slices as compared to rat and mouse livers. Considering these relationships it appears that the capacities of the livers from these 4 species to *form* Paraoxon from Parathion are not greatly different. In the presence of flounder liver slices only about ¼ to⅓ as much Paraoxon accumulated from Parathion when compared with liver slices from the fresh water fish. This could not be explained on the basis of a greater rate of inactivation of the oxygen analogue by flounder liver. It appeared therefore, that flounder livers had less capacity to activate Parathion. On the other hand

flounder livers appeared to have a relatively large capacity to form Gutoxon from Guthion. Sculpin liver slices failed to cause an accumulation of appreciable quantities of oxygen analogues from any of the 3 parent insecticides. This suggests that the liver of this species does not contain the necessary enzyme system(s) to catalyze the oxidative desulfuration of phosphorothionates, since livers from this species also had little or no capacity to destroy the oxygen analogues.

Sparrow liver slices appeared to have a greater capacity to form Malaoxon from Malathion than chicken liver slices since the rates of destruction of either Malaoxon or Malathion by liver homogenates from the two species did not differ appreciably. The destruction of anticholinesterase activity of either Gutoxon or Paraoxon was about 50% greater with chicken livers than with sparrow livers. However, incubation of the parent insecticides with sparrow liver slices resulted in an accumulation of 3 to 4 times as much of the cholinesterate inhibitors as when they were incubated with chicken liver slices. These observations suggest that sparrow livers also had greater capacity to form the oxygen analogues from Parathion and Guthion. Similar attempts could be made to explain other species differences in the accumulation of cholinesterase inhibitors that were observed in this study. Proof of these explanations, however, requires additional studies of activating reactions under conditions which assure their independence from inactivating reactions.

Liver was the only tissue for which metabolism tests were conducted in this investigation. It is possible that metabolism in other tissues might more closely correlate with susceptibility to poisoning. In this connection it should be noted that Neal and DuBois(10) suggest that the formation of Paraoxon from Parathion is the rate limiting reaction leading to the secondary hydrolytic detoxication of this insecticide by mammalian livers. The present investigation shows that avian and piscine livers have relatively much lower capacity than mammalian livers to hydrolyze Paraoxon. It seems possible, therefore, that detoxication of Parathion in birds and fish follows a different pathway or occurs in different tissues than in mammals. Recent studies in this laboratory(13) have shown that, in the presence of appropriate cofactors, homogenates of mammalian, avian and piscine livers are capable of reducing Parathion and Paraoxon to their less active amino derivatives; however, the relative importance of this reaction as a detoxication mechanism *in vivo* has not been determined.

Summary. The quantities of active cholinesterase inhibitors which accumulated when Malathion, Parathion and Guthion were incubated with liver slices from 2 mammalian, 2 avian and 4 piscine species were measured. The capacities of liver homogenates from these 3 classes of animals to inactivate the oxygen analogues of these compounds were also compared. The relative capacities of liver slices from various species to produce and accumulate cholinesterase inhibitors varied depending upon the thiophosphate studied. Fish livers had considerably less capacity than mammalian and avian livers to inactivate the oxygen analogues of Parathion and Malathion, but the destruction of the anticholinesterase activity of Gutoxon by liver homogenates did not differ appreciably among the 3 classes. The greater susceptibility of sunfish than mice to poisoning by Malathion or Malaoxon correlated well with what would be predicted from the liver metabolism studies. Toxicity-metabolism correlations were less obvious for Guthion and Gutoxon, and the lesser susceptibility of sunfish than mice to poisoning by Parathion and Paraoxon was opposite to what would be expected from liver metabolism data.

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Inhibitory Effect of Pyridoxine Deficiency on Growth of a Transplanted Tumor in Rats.* (31498)

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There have been many attempts to analyze differences in requirements of certain nutritional factors between host and tumor tissue (1,2,3,4). Of special interest have been the studies on vitamins. Major chemotherapeutic advances in the treatment of malignancy have resulted from these studies.

The effect of vitamin B_6 as a constituent that may influence tumor growth has had an interesting but rather enigmatic history. Bischoff *et al* reported the retardation of growth of Sarcoma 180 in mice deficient in pyridoxine; addition of vit. B_6 to the deficient diet caused a significant increase in the growth of the tumor(5). Kline *et al*(6) showed that in rats partially depleted of pyridoxine, the percentage of takes of the Flexner-Jobling carcinoma was lower, number of regressions was higher, and the size of the tumor smaller than in control animals receiving a diet containing pyridoxine and the same number of calories. Similar results were obtained by this group for the Yale adenocarcinoma-1 and a mouse fibrosarcoma. Boutwell *et al*(7) reported that they could lower the incidence of the induction of epithelial tumors in mice only by lowering the concentration of all B vitamins in the diet, and that pyridoxine depletion alone did not affect tumor incidence.

A number of other induced and transplantable tumors have since been observed and evaluated for growth characteristics in hosts made deficient in vit. B_6. In several investigations the pyridoxine antagonist, 4-desoxypyridoxine, was utilized to induce the deficiencies. Results varied according to tumor, host, and experimental procedures(8,9,10,11, 12).

The experiment described here was formulated to demonstrate the relationship of tumor growth to vit. B_6 by quantitating the growth of a transplanted tumor in rats that were made deficient in vit. B_6 by a combination of deficient diet and an antimetabolite regimen.

Materials and methods. Exp. 1. The rats used were Marshall 520 strain obtained from the National Cancer Institute, Bethesda, Md. Eighteen young female animals, ranging in weight from 77 to 122 g, were divided into 3 groups, 2 with 7 animals (deficient group and pair-fed group) and one with 4 animals (*ad libitum* group). The groups of 7 were subdivided into 3 and 4 animals and caged in wire bottom cages so that the collective body weights of the animals in each comparable cage were equal.

The complete diet was as shown in Table I

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