To determine whether animals treated with hemicholinium for 11 weeks were permanently poisoned, we amputated the regenerating leg and allowed it to regrow without further administration of a drug. Regeneration was identical to that in controls, indicating that the effect of hemicholinium was revertible. To demonstrate that the initial amputation did not stimulate subsequent regeneration we measured the growth rates 10 weeks after the first amputation and similarly after a reamputation. The first and second rates of regeneration were identical in each of two salamanders studied. We then considered that treatment with hemicholinium might inhibit growth because 25 percent of the body weight was lost during treatment. For that study, the hind limb of an untreated salamander was amputated, but no food was given to the animal during the 10-week regeneration period. Despite a weight loss of 50 percent, the rate of limb growth was not different from that in previous controls.

The diminished growth rate in treated salamanders correlated well with the microscopic findings. Salamanders treated for 5 weeks with hemicholinium showed only about 30 mitotic figures in the median sagittal section of the regenerate. However, more than 400 were seen in similar sections from controls. Colchicine (2 mg/kg) was injected into both groups 12 hours before the salamanders were killed so that mitotic figures would accumulate and could be counted. Evidently hemicholinium did not inhibit regeneration by means of an effect like that of colchicine, for far fewer mitotic figures were seen among treated specimens than among controls. Furthermore, the fact that the mesenchymal cells were not enlarged argues against the possibility that hemicholinium blocks cell division at an earlier stage. The drug also prevented the massive dedifferentiation of muscle tissue normally seen in sections just proximal to the amputation site. In untreated amputees the neurilemmal nuclei increased in number and enlarged with dedifferentiation of the nerve invading more proximal parts, but treated animals showed virtually no nerve changes. Gross and microscopic examinations showed that the vascularity of these regenerating stumps was also strikingly diminished.

Since the salamanders were maintained in a partially paralyzed or paretic state by treatment with hemicholinium, it is possible that disuse of the stump could account for the observed inhibition of growth. However, in one experiment in which triethylcholine (400 mg/kg) was administered, the rate of regeneration was nearly 72 percent of control, despite the fact that this drug paralyzed the animal more effectively than hemicholinium did. Botulinum toxin, which also produces paralysis, has been reported (6) not to delay regeneration of the salamander limb. Furthermore several changes were observed which cannot relate to the paralytic effect of drug treatment. After 5 weeks of treatment with hemicholinium the taste buds of the salamander showed regressive and degenerative changes. After 19 weeks of treatment the taste buds disappeared, but they returned when treatment was discontinued. Children with dysautonomia not only show a parasympathetic insufficiency with a growth disturbance but also a lack of fungal taste buds (7).

There is much evidence supporting the hypothesis that acetylcholine is a neurotrophic factor (3), but there are also compelling reasons against its ready acceptance. Singer (8) reports that microinfusion of atropine into the regenerate halts limb growth. On the other hand, infusion of acetylcholine into a denervated limb does not restore its regenerative capacity. However, an infusion cannot duplicate neural activity. A more cogent reason for excluding acetylcholine as the growth mediator is that the concentration of the substance in sensory nerves is negligible compared to that in motor nerves. Yet the sensory nerve is far more capable of influencing regeneration than is the motor component. On the other hand, regenerating tissue contains concentrations of acetylcholine which are greater than normal but which then return toward normal during the period of early differentiation.

As yet we have no way of deciding whether treatment with hemicholinium retards growth primarily by blocking synthesis of acetylcholine or by functioning like curare (9). We have attempted to rule out the latter effect by simultaneously administering choline (10:1 molar ratio) and hemicholinium. However, the combination proved toxic. A direct inhibition of mitosis by hemicholinium has not been excluded, but in view of the ability of drug treatment to mimic in the salamander some features of dysautonomia, a disease characterized by a cholinergic defect, hemicholinium appears to act as a cholinolytic agent.

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Polychlorinated Biphenyl: Interaction with Duck Hepatitis Virus

Abstract. Ten-day-old mallard ducklings fed a polychlorinated biphenyl at concentrations of 25, 50, and 100 parts per million for 10 days suffered no apparent clinical intoxication. Five days later these birds were challenged with duck hepatitis virus, and they suffered significantly higher mortality than birds which were not exposed to the polychlorinated biphenyl.

Polychlorinated biphenyls (PCB) along with DDE [1,1-dichloro-2,2-bis-(*p*-chlorophenyl)ethene] are reported to be the most abundant of the chlorinated hydrocarbon pollutants in the global ecosystem (1). Despite the fact that PCB's have been in wide use since 1930 (2), they remained ecologically inconspicuous until 1966 when a Swedish chemist reported their presence in the tissues of pike and other wildlife (3). Since then, their presence has been reported in additional wildlife of Europe and North America (1, 4). These compounds are interesting because they are an important source of interference in the chemical detection of DDT and its metabolites (5) and

because they are a potential hazard in the environment; on a weight basis PCB preparations have been shown to have an estradiol-degrading potential about five times that of p,p'-DDE or technical grade DDT [1,1,1-trichloro-2,2-bis-(p-chlorophenyl)ethane] (*1*).

As part of an investigation of possible interactions between organochlorine pollutants and infectious diseases, this study was initiated to determine whether any interaction, antagonistic or synergistic, occurs with PCB and duck hepatitis virus (DHV) in mallards (*Anas platyrhynchos*).

Four separate diets, three with PCB and one without, of a standard duck starter ration were fed to groups of 10-day-old mallard ducklings. The three PCB diets contained Aroclor 1254 (in a corn oil premix) concentrations calculated to be 25, 50, and 100 parts per million (ppm). These diets were fed to five groups of 25 ducklings each, three principal and two control groups (Table 1).

All birds were leg-banded with color-coded, numbered bands, weighed, and fed their respective diets for 10 days. On day 11 each bird was weighed again, and every fifth bird from each treatment group was killed. Tissues were collected for residue analysis, histology, and liver weights. During the 10-day feeding trial, no birds died or became clinically ill, although ducklings in the group given 100 ppm PCB were noticeably hyperexcitable after day 3 as were those in the 50-ppm group 4 days after treatment.

Body weights of birds receiving PCB in their diet were significantly heavier at the end of the 10-day feeding trial than those fed the diet without PCB (Table 1). The heaviest body weights occurred in birds fed the highest concentrations of PCB, and the trend appeared to be linear. Differences in body weights among treatment means were highly significant ($F = 20.30$, d.f. = 4, 119) when tested by analysis of covariance. No statistical comparisons between individual treatment means were made. A similar analysis of liver weights (determined as a percentage of final body weight) disclosed highly significant differences among treatment means ($F = 5.82$, d.f. = 4, 18). These differences appeared to be within groups treated with PCB rather than between treated and untreated groups (Table 1). The small sample sizes ($N = 5$) necessitates caution in interpretation of liver weight data. No interpretations of differences among body and liver weights are offered at this time.

Table 1. Final mean body and liver weights of mallard ducklings after being fed PCB (Aroclor 1254, Monsanto, St. Louis) for 10 days. Numbers in parentheses are the number of birds used. The liver and body weights were adjusted by covariance for a common starting mean; the liver weight was determined as percentage of body weight. The final control did not receive either PCB or DHV.

Treatment	PCB (ppm)	Body weight (g)	Liver weight (g)
PCB + DHV	100	381.6 (25)	0.046 (5)
PCB + DHV	50	368.8 (25)	.037 (5)
PCB + DHV	25	352.8 (25)	.029 (5)
DHV control	0	3[illegible]	.036 (4)
Control	0	369[illegible]	.034 (5)

Twelve days after the experiment began, birds were placed in crates and transported approximately 5 miles to isolation units where they were maintained on PCB-free feed and water for 5 days before being challenged with DHV. No birds died or became clinically ill during the interim period between PCB feeding and virus challenge although two birds were lost from the 100-ppm group due to accidents.

On day 15 of the experiment all but the untreated controls (birds not receiving either PCB or DHV) were inoculated intraperitoneally with 1.5 LD_{50} (50 percent effective lethal dose for ducks) of DHV per bird. The first birds died about 47 hours after inoculation. Mortality was recorded hourly for the remainder of the 80-hour experimental period with one exception; there were no observations between hours 63 through 69. The onset of mortality in all groups given PCB plus DHV began at least 8 to 16 hours before that in the control group treated with the virus only (Fig. 1). In addition, mortality levels occurring among PCB-plus-virus treatment groups at the end of the experiment were significantly higher at the .01 level of probability than for ducklings receiving the virus only (chi square = 7.49, d.f. = 1). There was no significant difference in mortality among ducklings receiving the different concentrations of PCB plus virus (chi square = 3.79, d.f. = 2). No birds in the untreated group died (Table 2).

All birds that died during the experiment had gross pathognomonic liver lesions, indicating typical duck hepatitis infection (*6*). No other gross lesions, with the exception of varying amounts of edema within the pericardium and thoracic cavity of some

Table 2. Mortality among mallard ducklings caused by DHV during an 80-hour experimental period. No observations were made between 31 to 47 and 62 to 70 hours. The final control did not receive either PCB or DHV.

Treatment	PCB (ppm)	Mortality			
		No. of deaths/ total sample	Percentage	Onset (hours)	Cessation (hours)
PCB + DHV	100	8/18	44.4	31–47	75
PCB + DHV	50	13/20	65.0	35	76
PCB + DHV	25	7/20	35.0	31–47	75
DHV control		3/21	14.3	62–70	73
Control		0/20	0.0		

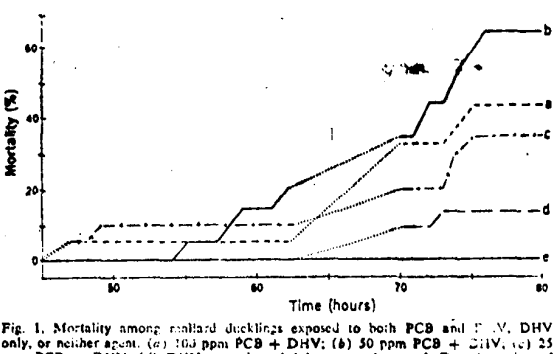

Fig. 1. Mortality among mallard ducklings exposed to both PCB and DHV, DHV only, or neither agent. (*a*) 100 ppm PCB + DHV; (*b*) 50 ppm PCB + DHV; (*c*) 25 ppm PCB + DHV; (*d*) DHV control; and (*e*) untreated control. Dotted portion of lines prior to 47 hours and between 62 and 70 hours represent periods during which no observations were made.

birds, were observed. There did not appear to be any relation between treatment and the presence or absence of edema. Histological and residue analysis have not been completed at this time.

The suggestions that PCB may render a host more susceptible to certain types of infectious agents is the significant finding in this study. None of the concentrations of PCB fed resulted in detectable chemical intoxication even with the physical stresses of weighing, handling, confinement, and crowding during transportation, transportation itself, or relocation in a different environment. However, when the stress of an infectious agent was added, these sublethal concentrations appeared to influence the resulting mortality rates, causing two- to fourfold increases (14 percent among virus controls versus 35 to 65 percent among groups receiving PCB plus DHV) and reduced incubation time (Fig. 1 and Table 2).

Similar increases in mortality were obtained with other organochlorine compounds in our laboratory (*7*). In those studies 30-day-old mallard ducklings which had been fed sublethal concentrations of *p,p'*-DDT or dieldrin exhibited three- to ninefold increases in mortality over that of DHV controls (6 percent among virus controls versus 19 to 59 percent among groups receiving dieldrin plus DHV, and 19 to 40 percent among groups receiving *p,p'*-DDT plus DHV). It is unlikely that in all these instances mortality in the DHV control groups would be less than half that in any interaction group on the basis of chance alone.

This study illustrates one of the potentional effects of sublethal concentrations of chemical pollutants which are often alluded to but rarely documented. It also emphasizes the real difference that exist between "sublethal" and "no-effect" concentrations of pollutants. To the best of our knowledge, other studies in vivo on possible effects of organochlorine pollutants on the susceptibility of a vertebrate host to a viral agent have not been reported.

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Liver Mitochondria from Manganese-Deficient and Pallid Mice: Function and Ultrastructure

Abstract. *Oxidative phosphorylation was studied in isolated liver mitochondria from manganese-deficient mice and in those from a mutant strain, pallid. In mitochondria from manganese-deficient mice, ratios of adenosine triphosphate formed to oxygen consumed were normal, but oxygen uptake was reduced. Electron microscopy of these mitochondria revealed ultrastructural abnormalities including elongation and reorientation of cristae. No biochemical or structural abnormalities were found in mitochondria from pallid mice.*

In a number of animal species a striking effect of dietary deficiency of manganese during pregnancy is an irreversible congenital ataxia in the offspring. The ataxia, characterized by imbalance and loss of body righting reflexes, results from abnormal development of the inner ear, with defective morphogenesis of the otoliths (*1*, *2*). A mutant gene in mice, *pallid* (*pa*), also causes congenital ataxia resulting from impaired development of otoliths (*3*). When the diet of pregnant mutant mice is supplemented with high amounts of manganese the otoliths and postural behavior of the offspring are normal. Thus, there is a relationship between the gene *pallid* and manganese metabolism (*3*, *4*).

Although considerable attention has been paid to the role of manganese in enzyme function, its specific biochemical action in vivo has remained unclear (*5*). A variety of evidence, however, has implicated manganese in mitochondrial function, particularly in oxidative phosphorylation (*5*, *6*). In our laboratory, isolated liver mitochondria from manganese-deficient rats showed abnormal oxidative phosphorylation which appeared to be due to reduced oxidative capacity rather than to lack of coupling (*7*). We now report on P:O ratios (moles of adenosine triphosphate formed relative to gram atoms of oxygen consumed) and rate of oxygen uptake in isolated liver mitochondria from manganese-deficient and from pallid mice, and on the fine structure of liver tissue as studied with the electron microscope.

Female mice maintained in a colony originally derived from a four-way cross of inbred strains C57Bl/10J, C3H/J, ARK/J, and DBA/2J (*8*) were given a purified diet (*9*) containing 1 part manganese per million (deficient diet) during pregnancy. Their progeny were maintained on the same diets and were killed as adults for use in these studies. Hybrid mice of the same strain were fed a similar purified diet except that it contained 45 parts of manganese per million (control diet). Pallid mice (*pa*/*pa*) and their nonpallid littermates (C57Bl/10J-*pa*) were maintained on a stock diet (*10*).

For mitochondrial preparations, four hybrid deficient, four hybrid control, five pallid, and five nonpallid mice were decapitated; their livers were put into cold 0.25*M* sucrose, cut into small pieces, and washed three times. Mitochondria were separated as previously described (*11*). Oxygen uptake was determined by the polarographic assay of oxygen after the method of Chance and Williams (*12*), with β-hydroxybutyrate as the substrate in the reaction medium (Table 1). The oxygen electrode was according to Packer (*13*). We calculated P:O ratios by determining the reduction in the amount of added adenosine diphosphate per unit of oxygen consumed. Adenosine diphosphate was measured by absorbance at 260 nm (*14*). The concentration of protein in the mitochondrial suspension was determined by means of the biuret reaction (*15*).

Median lobular hepatic tissue was excised from ten manganese-deficient hybrid, five control hybrid, and five pallid adult female mice for examina-