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 differences that exist between "sublethal" and "no-effect" concentrations of pollutants. To the best of our knowledge, other studies in vivo of the 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 (*2*, *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 C57B1/16J, 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 (C57B1/10J-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-

tion by electron microscopy. The tissues were minced in cold 1 percent osmium tetroxide, buffered with 2*M* sodium cacodylate, *p*H 7.4, and fixed in the buffered osmium for 2 hours. Conventional techniques of alcohol dehydration and Epon embedding were used. Five random pieces of tissue were sectioned for each animal. All sections were stained for 30 minutes in an aqueous solution of 1 percent uranyl acetate followed by 10 minutes in concentrated lead citrate. Observations and at least ten photographs were made for each sample on the RCA EMU-2E and RCA EMU-3G electron microscopes (*16*).

Table 1 shows the P:O ratios and rates of oxygen uptake in isolated liver mitochondria prepared from manganese-deficient and control mice, and from pallid and nonpallid mice. The P:O ratios for the manganese-deficient and the pallid mice were not different from those for their respective controls. The absolute rates of oxygen uptake showed considerable variation; however, experiments were performed in pairs (experimental and control animals), and the data are compared on this basis. The ratios of oxygen uptake for each pair clearly indicate that oxidation was decreased in the manganese-deficient animals. Oxygen uptake in mitochondria from manganese-deficient mice was only 66 percent that in controls. No effect was seen in pallid mice.

In electron microscopic studies, liver mitochondria from manganese-deficient mice showed a variety of highly abnormal structures (Fig. 1). Elongated, often branched, forms were found. In all ten deficient mice the majority of mitochondria observed showed an increase in the surface area of cristae. In four animals, apparently early stages of elongation of cristae and reorientation in layers could be seen (Fig. 1B). One of these four, whose littermates showed depressed oxygen uptake, had clumps of mitochondria in which the cristae were parallel to the outer membrane (Fig. 1C). Similar grouping and reorientation of cristae were found in two other animals as well (Fig. 1D). Alterations as severe as these were not seen in all liver cells of one animal or in all hepatic areas. However, when stuctural changes were observed, they were accompanied by a general increase in the surface area of the cris-

Table 1. Oxidative phosphorylation in liver mitochondria from manganese-deficient and pallid mice and their controls. The reaction mixture contained in final concentration: 0.031*M* Na^+; 0.082*M* K^+; 0.006*M* Mg^{2+}; 0.096*M* Cl^-; 0.013*M* HPO_4^{2-}; 0.003*M* $H_2PO_4^-$; and 0.012*M* F^-. Mitochondrial suspension (0.2 ml) was added to 0.8 ml of the reaction medium, with 100 μg of β-hydroxybutyrate as substrate; 20 μg of adenosine diphosphate was added during the assay. Ratio refers to the ratio of O_2 uptake in the experimental animal (either manganese-deficient or pallid) to that in its respective control. The mean P : O ratio (and standard deviation) is given for each group of experiments; O_2 uptake is measured in micromoles per minute per gram of mitochondrial protein.

Experiment (No.)	P : O ratio	O_2 uptake	Ratio
Control mice (diet supplemented with manganese)			
1	3.10	6.29	
2	3.25	10.42	
3	3.35	12.40	
4	3.35	7.92	
	3.28 ± 0.045		
Manganese-deficient mice			
1	3.23	4.15	0.66
2	2.95	7.01	0.67
3	3.35	7.29	0.59
4	3.70	5.61	0.71
	3.31 ± 0.120		0.66 ± 0.019
Nonpallid mice			
5	3.27	11.63	
6	3.27	14.17	
7	3.27	14.02	
8	3.30	6.54	
9	3.10	6.29	
	3.24 ± 0.035		
Pallid mice			
5	3.27	10.24	0.88
6	3.10	12.84	0.91
7	2.98	14.00	1.00
8	3.53	5.40	0.83
9	3.23	6.22	0.99
	3.22 ± 0.092		0.92 ± 0.032

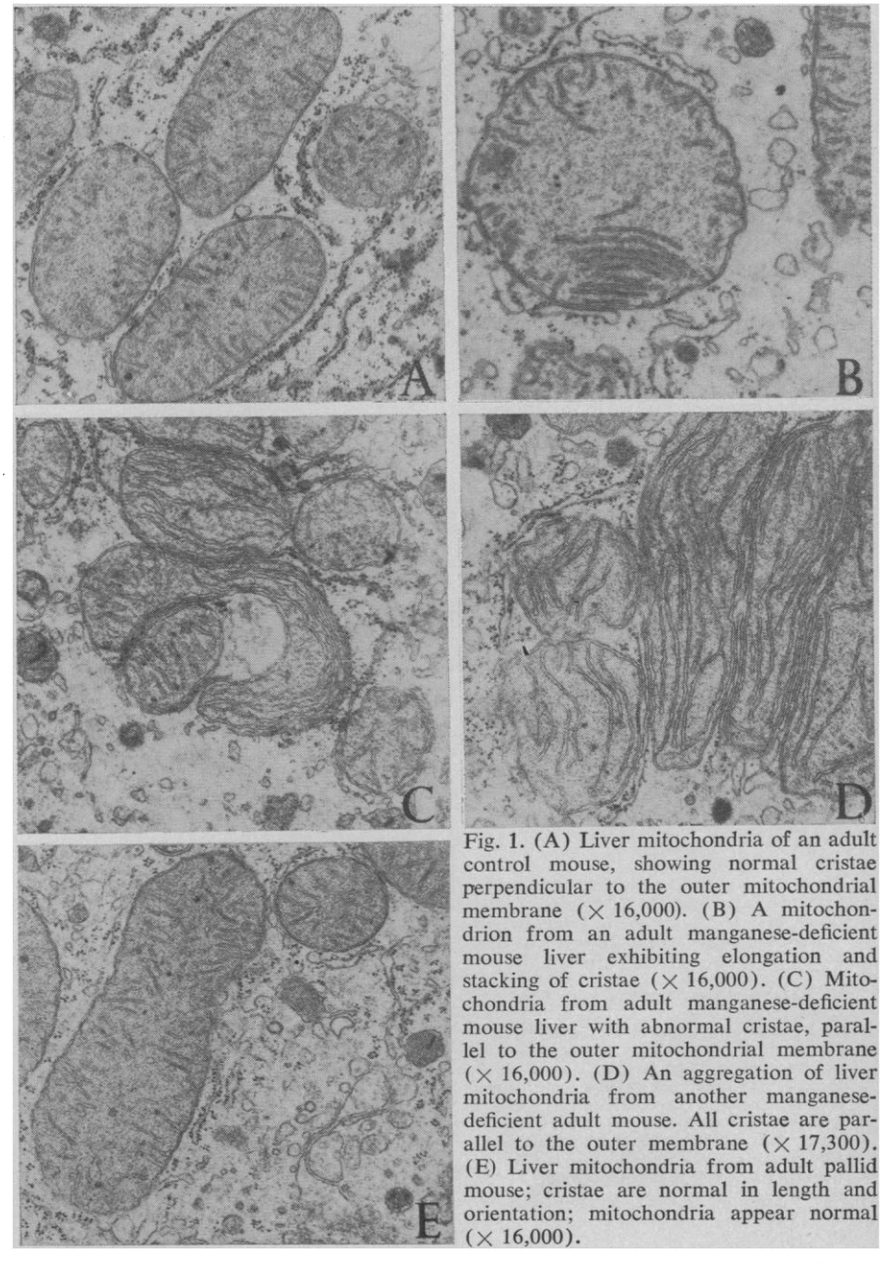

Fig. 1. (A) Liver mitochondria of an adult control mouse, showing normal cristae perpendicular to the outer mitochondrial membrane (× 16,000). (B) A mitochondrion from an adult manganese-deficient mouse liver exhibiting elongation and stacking of cristae (× 16,000). (C) Mitochondria from adult manganese-deficient mouse liver with abnormal cristae, parallel to the outer mitochondrial membrane (× 16,000). (D) An aggregation of liver mitochondria from another manganese-deficient adult mouse. All cristae are parallel to the outer membrane (× 17,300). (E) Liver mitochondria from adult pallid mouse; cristae are normal in length and orientation; mitochondria appear normal (× 16,000).

tae. In liver mitochondria from pallid mice, no distinct ultrastructural changes were seen (Fig. 1E).

The biochemical data presented here support earlier work reporting lowered oxidation in manganese-deficient liver mitochondria (*7*). This and the observation that the ultrastructure of mitochondria in manganese-deficient animals is altered show that manganese is required for normal mitochondrial function and structure in vivo. However, although the mutant gene *pallid* interacts with manganese in the development of otoliths, it does not appear to affect mitochondrial function or structure. This observation is consistent with earlier evidence that *pallid* genes do not act by producing a simple manganese deficiency; instead, metabolic differences between the two conditions were found (*17*).

The morphological findings reported here are consistent with the biochemical observations. In a study on the fine structure of lung tissue, Rosenbaum *et al.* (*18*) found mitochondrial transformations similar to those reported here after the great alveolar cells had been exposed to adaptive quantities of oxygen. These authors suggested that the mitochondrial changes were adaptive rather than degenerative, since they did not occur after toxic amounts of oxygen, and that they were possibly brought about through inhibition of oxidative enzymes (*19*). In manganese deficiency perhaps the activity of such enzymes is affected, causing reduced oxidation and structural change.

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Microcytotoxicity Test: Detection in Sarcoma Patients of Antibody Cytotoxic to Human Sarcoma Cells

Abstract. *A microcytotoxicity test has been used to detect a factor cytotoxic for human sarcoma cells; the factor was found in serums from 70 percent of sarcoma patients, 58 percent of their family members, and 8 percent of serums from normal blood donors. This cytotoxin is an antibody against a common cell surface sarcoma antigen since it is specific for sarcoma cells, is complement dependent, and is extractable with the globulin fraction of serum.*

Tumors induced in animals by a given virus express a common antigen, regardless of their histologic type or anatomic location (*1*). This observation has led to the use of a variety of serological techniques in search of antigenic identity in human tumors, a finding which might implicate a viral agent. A common sarcoma specific antigen has been demonstrated in human sarcomas of different histologic types by immunofluorescence and complement fixation techniques (*2*). It has remained open to question whether this antigen could induce transplantation resistance or was only an intracellular antigen capable of inducing antibody formation, but failing to render the cell vulnerable to immune attack. Transplantation studies, which have resolved similar questions in animal studies, are not appropriate to human investigations. Immune cytotoxicity tests would provide the most direct method in vitro for investigating both surface antigenic expression and immune vulnerability, but classical methods of testing cytotoxicity have not been found applicable to the human sarcoma cells which were resistant to cytotoxic antibody. We now describe a microcytotoxicity test demonstrating that sarcoma antigens are located on the cell surface and render the cell susceptible to immune cytolysis.

Human sarcoma cells were grown at 37°C in 5 percent CO_2 in stationary cultures with RPMI 1640 media containing 20 percent fetal bovine serum, kanamycin, aureomycin, and fungizone (Grand Island Biological). When the cells were more than 50 percent confluent, they were dispersed with 0.25 percent trypsin in Gey's balanced salt solution. After being counted in trypan blue, the cells were diluted to give 10,000 viable cells per milliliter of tissue culture media. These cells were planted in Microtest tissue culture plates (Falcon Plastics) with 0.01 ml per well, and incubated for 3 to 18 hours. Media were then rinsed from the plates and was replaced with 0.01 ml (per well) of the test serums that had previously been inactivated at 56°C in a water bath for 20 minutes. Cells and serum were incubated at 37°C for 1 hour. The serum was removed and 0.01 ml of complement was added to each well. Pooled rabbit serum diluted 1:1 with pooled human umbilical cord serum was used as a source of complement. After incubation with complement for 2 hours, the plates were flooded with 6 ml of RPMI 1640 media and incubated overnight. In the morning each well was examined by phase microscopy, and the remaining viable cells were counted. Dead cells exhibited a retraction of the cell processes and "rounding up"—with loss of the nuclear halo and nucleolus, increased granularity of the cytoplasm, and high refractility (Fig. 1, right). No difficulty was experienced in classifying cells as viable or dead. Six replicate wells were used for each test serum, and each plate contained a positive control and 12 replicates of the negative control. Pooled umbilical cord serum was chosen as a negative control because of its low antibody content and consistent behavior from pool to pool.