The correlation between increasing enzymatic activity followed by decreasing amounts of substrate during the first 16 hours after infusion is consistent with the expectation that the infused enzyme would initiate catabolism of the substrate in the plasma. The steadily decreasing concentration of gal-gal-glc-cer in the period after the first day correlates with the presence of low levels of ceramide trihexosidase in the plasma of both recipients throughout this period.

The kinetics of the decay of enzymatic activity suggest that at least two mechanisms are involved. The slow rate of decrease in the period from 1 to 7 days probably represents normal turnover of plasma enzyme, but the fast rate of decrease in the period from 6 to 12 hours is more difficult to explain. Possible incorporation of active enzyme into the tissues or adsorption onto the blood vessel walls has not been ruled out. It has already been reported that prolonged intravenous infusions of arylsulfatase A to patients with metachromatic leukodystrophy and of a crude glucosidase from *Aspergillus niger* to patients with type 2 glycogenosis leads to measurable enzymatic activity in hepatic tissue obtained by biopsy (*13*).

The unexpected enhancement of ceramide trihexosidase activity in the period from 0 to 6 hours is also of interest, and we are unaware of any published mechanisms which would uniquely account for it. However, attention might be directed to an analogous phenomenon in patients with von Willebrand's disease, a syndrome in which there is an inherited deficiency of factor VIII or antihemophilic factor. In these patients, factor VIII activity was enhanced eightfold after infusion with plasma obtained from normal donors or even from patients with factor VIII deficiency. Factor VIII activity reached a maximum about 24 hours after infusion (*14*).

We have sought to determine whether the enhancement of enzymatic activity in patients with Fabry's disease might be an in vitro phenomenon. Prior incubation at 37°C of equal volumes of plasma from a donor and a hemizygote did not increase the total enzymatic activity at *p*H 7.2. This suggests that the enhancement of activity observed in the patients is more likely to be an in vivo effect. A similar conclusion has been reached to account for the enhancement of factor VIII activity after normal plasma infusions in patients with von Willebrand's disease (*14*).

To determine whether normal plasma contained an activator, plasma from blood drawn into acid citrate-dextrose was frozen to inactivate the ceramide trihexosidase (*5*) and was subsequently infused into patient A.G. There was no detectable enzymatic activity in the recipient's plasma during the first 8 hours after infusion. The presence of an activator that is unstable upon freezing was not ruled out. Heparin alone in an amount twice as great as that used in the plasma infusions caused no detectable production of enzymatic activity in the recipient.

These findings provide an experimental basis for the hypothesis that enzyme replacement by plasma infusion will be a means of therapy for this glycosphingolipidosis. The proof of efficacy must rest on the results of clinical tests after a prolonged period of intermittent plasma infusions.

CAROL A. MAPES
RICHARD L. ANDERSON
CHARLES C. SWEELEY
Department of Biochemistry, Michigan State University, East Lansing 48823
ROBERT J. DESNICK, WILLIAM KRIVIT
Dight Institute for Human Genetics and *Department of Pediatrics, University of Minnesota Medical School, Minneapolis*

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Cadmium Toxicity Decreased by Dietary Ascorbic Acid Supplements

Abstract. *Feeding the environmental toxicant cadmium to young Japanese quail for 4 weeks produced growth retardation, severe anemia, low concentrations of iron in the liver, and high concentrations of cadmium in the liver. Dietary ascorbic acid supplements almost completely prevented the anemia and improved the growth rate but did not markedly alter concentrations of iron or cadmium in the liver.*

The hazards of industrial exposure to high concentrations of cadmium have been recognized for many years. More recently, attention has focused on cadmium intake from food, water, air, and cigarettes as a source of risk to the general population, primarily in the production of cardiovascular and respiratory diseases (*1*).

We have investigated the effect of a wide range of dietary components upon the toxic effects of cadmium in the young Japanese quail (*Coturnix coturnix japonica*). This bird has an exceptionally rapid growth rate and is very sensitive to dietary deficits and to toxic materials. We observed that dietary ascorbic acid supplements produce a marked protective effect on the anemia and a lesser protective effect on the depressed growth rate caused by cadmium.

Day-old birds of both sexes were fed an adequate purified soybean protein diet containing a total of 75 mg of zinc per kilogram and approximately 100 mg of iron per kilogram (*2*) for a period of 4 weeks. The basal diet was either fed alone or supplemented with cadmium, ascorbic acid, or cadmium plus ascorbic acid at the concentrations indicated in Table 1. Birds received

Table 1. Protective effects of ascorbic acid against cadmium toxicity in young Japanese quail. Mean values ± standard error at 4 weeks of age.

Dietary cadmium (mg/kg)	Dietary ascorbic acid (% by weight)	Mortality*	Body weight (g)	Hematocrit value (% by volume)	Trace elements in liver (μg/g fresh weight)			
					Zinc	Iron	Copper	Cadmium
				Experiment 1				
0	0	0/8	80 ± 1.6	40 ± 1.2	26 ± 1.4	78 ± 7.8	5.7 ± 0.26	<0.1
75	0	3/9	57 ± 3.1	17 ± 1.0	21 ± 1.1	35 ± 2.9	5.0 ± 0.27	20 ± 2.5
75	1.00	1/7	78 ± 2.2	35 ± 1.4	25 ± 1.7	45 ± 5.8	5.6 ± 0.31	21 ± 0.9
				Experiment 2				
0	0	0/8	77 ± 1.4	40 ± 1.0	21 ± 1.4	89 ± 8.0	4.7 ± 0.20	<0.1
0	1.00	0/8	78 ± 2.4	41 ± 1.2	20 ± 0.5	123 ± 20.9	5.0 ± 0.14	<0.1
75	0	1/6	66 ± 2.5	21 ± 1.0	20 ± 0.9	30 ± 2.1	4.6 ± 0.30	17 ± 2.0
75	0.05	1/7	62 ± 4.3	29 ± 1.4	21 ± 1.0	32 ± 1.6	5.5 ± 0.27	23 ± 1.4
75	0.10	0/7	65 ± 1.8	33 ± 0.5	24 ± 1.5	36 ± 1.5	5.5 ± 0.25	21 ± 1.6
75	0.50	1/7	69 ± 5.0	34 ± 0.7	27 ± 1.2	36 ± 4.4	5.6 ± 0.63	22 ± 2.0
75	1.00	0/8	72 ± 1.6	35 ± 0.8	25 ± 1.9	43 ± 7.4	6.1 ± 0.53	20 ± 1.0

* Number of deaths during period from 1 to 4 weeks of age divided by the total number of birds at 1 week.

diets and demineralized drinking water freely. Rigid procedures were followed to obviate environmental contamination with trace elements (*2*). Eight or nine birds were assigned to each group. Some deaths during the first week were unrelated to the experimental treatment; therefore all deaths during this period were excluded from the mortality values. At 4 weeks of age, birds were weighed, microhematocrits were determined for blood collected from the wing vein, and livers were removed, weighed, wet-ashed, and assayed for zinc, iron, copper, and cadmium by atomic absorption spectrophotometry (*3*).

The data in Table 1 show that anemia was the most marked sign of cadmium toxicity. For every concentration of dietary ascorbic acid supplement used, there was a significant improvement in the hematocrit reading over that of birds receiving cadmium alone. In another experiment (data not shown) identical in design to that of experiment 1, the hematocrit values were very similar to those in experiment 1 and the hemoglobin concentrations, as determined by the cyanmethemoglobin method, were 11.7, 2.7, and 9.2 g of hemoglobin per 100 ml of blood, respectively.

The concentrations of zinc and copper in the liver were little affected by supplemental administration of either cadmium or ascorbic acid. In both experiments the birds receiving 1 percent (by weight) dietary ascorbic acid with cadmium had significantly higher ($P < .05$) concentrations of zinc in the liver than birds receiving cadmium alone. The physiological importance of this effect is questionable because of the variation between the two control groups not receiving supplements. The concentration of iron in the liver was markedly decreased by the administration of cadmium. Birds fed ascorbic acid in combination with cadmium showed a small increase in the concentration of iron in the liver over that in birds fed cadmium alone. The difference was statistically significant ($P < .01$) when data for identical treatments from experiment 2 were combined with those from experiment 1 and compared by Student's *t*-test. High concentrations of cadmium accumulated in the livers of all birds receiving cadmium, irrespective of ascorbic acid supplements.

The depressed growth rate of birds fed cadmium was improved by the administration of ascorbic acid. Some deaths in experiment 1 were produced by cadmium but not in experiment 2. Mortality rates and the severity of the anemia have been somewhat variable during the 2-year period spanned by these and the previously reported experiments (*2*). It seems likely that part of this variation may be related to variability in the soybean protein of inorganic components other than zinc, iron, and cadmium. The concentrations of zinc, iron, and cadmium were monitored throughout.

We reported earlier that the anemia produced by cadmium was accompanied by elevated concentrations of transferrin and low concentrations of iron in blood plasma (*2*). These results and the present data on iron concentrations in the liver support the conclusion that a primary effect of cadmium under these conditions was the production of an iron-deficiency anemia. The ascorbic acid probably improved the utilization of iron, but not sufficiently to correct the anemia completely or to permit storage of iron in the liver. Copper concentrations in the liver were normal throughout; it thus appears that neither cadmium nor ascorbic acid influenced copper metabolism. Hill and Starcher reported that ascorbic acid supplements decreased copper absorption from the intestine and exacerbated symptoms of copper deficiency in chicks fed a diet low in copper (*4*). A concentration of 8 to 9 mg of total copper per kilogram, as present in our diet, was probably in excess of the requirement. It is not known whether the growth depression caused by cadmium was related to antagonism of zinc, iron, or some other factor. The zinc content of the diet was approximately three times the required level (*5*).

On the basis of the amounts of cadmium in the liver, the protective effect of ascorbic acid does not appear to involve prevention of cadmium absorption. It has been shown in in vitro perfusion studies that ascorbic acid depresses the uptake of zinc and cadmium by the isolated small intestine of the rat but that ascorbic acid enhances the subsequent transport (*6*). It is probable that ascorbic acid did not accelerate the loss of cadmium, although Shcherbakova showed that the combination of ascorbic acid and cysteine does accelerate the loss of cadmium from rabbits (*7*).

These data do not preclude the possibility that cadmium interferes with the normal synthesis of ascorbic acid by this bird. The lowest amount of ascorbic acid fed, which appears to be about the minimum protective level, is approximately two and one half times the vitamin C requirement of the guinea pig (*8*).

In this study a very high intake of cadmium, superimposed on an adequate diet, was chosen for the experimental advantage of providing marked effects in a short period of time. On the basis of these results and the well

known cadmium-zinc antagonism, it is possible that dietary zinc, iron, and ascorbic acid are important factors in modifying the adverse effects of cadmium in human beings.

M. R. SPIVEY FOX
BERT E. FRY, JR.

Division of Nutrition,
Food and Drug Administration,
Washington, D.C. 20204

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Homologous Viral Interference: Induction by RNA from Defective Particles of Vesicular Stomatitis Virus

Abstract. *The viral RNA isolated from the defective particles of vesicular stomatitis virus was capable of interfering with the replication of this virus in chick embryo cells. The data indicate that the interfering ability of the defective particles of this virus is due to their nucleic acid component.*

Vesicular stomatitis virus (VSV), a single-stranded RNA-containing virus, replicates in mammalian tissue culture cells and produces not only the bullet shaped and infectious B particles but also defective T particles (*1*). Both the B and T particles possess the same kind and number of polypeptides and antigens (*2*). However, the T particles contain viral RNA which is only one-third the size of the RNA contained in B particles (*3*). Viral RNA's isolated from B and T particles have been shown to sediment on sucrose density gradients at a rate of 40*S* and 19*S* respectively (*3, 4*). Interest in studying the noninfectious T particles arose since it has been shown that T particles can interfere specifically with the infection of cells with B particles (*5, 6*). The interference induced by the T particles in cells depends on the relative input ratio of B and T added to the cells (*6*). Also it has been shown that the interference caused by T particles is an intracellular phenomenon not mediated by interferon (*6*). The interfering constituent of the T particles is probably the viral RNA, since ultraviolet light can abolish the interfering ability of T particles (*6*). This report deals with experiments demonstrating that the viral RNA alone, either isolated from T particles or from VSV-infected cells, can interfere specifically with the replication of VSV virus.

Chick embryo fibroblasts were prepared and grown in Eagle's medium according to procedures described earlier (*7*). Vesicular stomatitis virus stocks (Indiana strain) were prepared according to the method of Huang, Greenawalt, and Wagner (*1*). Concentrated and partially purified suspensions of B and T particles were obtained from chick embryo cultures infected with VSV.

The method used for partially purifying the two types of particles was the same as that used by Huang, Greenawalt, and Wagner (*1*). The particles were purified by two successive centrifugations on sucrose density gradients. The plaque-forming ability of purified T particles was less than 0.5 percent of that of the B particles. Viral RNA was released from the purified virus particles with 0.2 percent sodium dodecyl sulfate and 0.1 percent pronase and sedimented on sucrose density gradient by methods described by Huang and Wagner (*3*). The optical density of the fractions from the sucrose density gradient was measured. The sedimentation coefficient of the RNA from B particles was 40*S* compared with 19*S* for RNA from T particles. The fractions containing the 40*S* VSV RNA or the 19*S* VSV RNA were pooled and precipitated with alcohol. The viral RNA was further purified on a column of cellulose (Whatman CF-11) according to the method described by Franklin (*8*). The single-stranded RNA eluting in the 15 percent ethanol buffer was collected and precipitated with ethanol. Viral RNA purified by the above method was used for the interference experiments. Viral RNA's were also isolated from VSV-infected cells and purified by the above method.

The uptake of single-stranded RNA by mammalian cells is known to be enhanced by diethylaminoethyl dextran (DEAE dextran) (*9*). Therefore DEAE dextran was used for enhancing the uptake of the viral RNA into chick embryo cells. The interference experiments were performed as follows. Monolayer cultures of chick embryo cells containing 3×10^6 cells per culture were washed twice with serum-free medium and then incubated at 37°C for 10 minutes with 1 ml of phosphate-buffered saline containing 80 μg of DEAE dextran (Sigma Chemical Co.).

The DEAE dextran was then removed and 0.5 ml of phosphate-buffered saline containing the viral RNA and 80 μg of DEAE dextran were added to cultures. Control cultures received 0.5 ml of phosphate-buffered saline containing DEAE dextran. After 60 minutes at 37°C with frequent agitation the cultures were washed three times and then infected with VSV or Sindbis virus at an input multiplicity of

Table 1. Effect of preincubation of chick embryo cells with RNA on infection with VSV. PFU, plaque-forming units.

RNA used for incubation*	Incubation with ribonuclease	Virus yield (PFU/cell)†
None	No	650
40*S* RNA from B particles	No	615
40*S* RNA from B particles	Yes	670
19*S* RNA from T particles	No	52
19*S* RNA from T particles	Yes	670
19*S* RNA from VSV-infected cells	No	63
19*S* RNA from VSV-infected cells	Yes	655
Ribosomal RNA from chick embryo cells	No	630

* Ten micrograms of RNA used per 3×10^6 cells. † Average of two separate experiments. Incubation with ribonuclease was done before exposing RNA to the cells. Samples of RNA in phosphate-buffered saline were incubated with ribonuclease (1 μg/ml) for 10 minutes at 37°C.