A mouse model of coxsackievirus-induced myocarditis is being used to investigate nutritional determinants of viral virulence. This approach was suggested by research carried out in China which showed that mice fed diets composed of low selenium ingredients from a Keshan disease area suffered more extensive heart damage when infected with a coxsackie B₄ virus than infected mice fed the same diet but supplemented with selenium by esophageal intubation. Selenium deficiency in our mice increased the virulence of an already virulent strain of coxsackievirus B₃ (CVB3/20) and also allowed conversion of a non-virulent strain (CVB3/0) to virulence. Such conversion of CVB3/0 was accompanied by a change in the viral genome to more closely match that of the virulent virus, CVB3/20. As far as the authors are aware, this is the first report of host nutrition influencing the genetic make-up of an invading pathogen. Nutritionists may need to consider this mechanism of increased viral virulence in order to gain a better understanding of diet/infection relationships.

Our interest in the connection between nutritional deficiency of selenium and increased viral virulence had its origin with research carried out by Chinese scientists into the epidemiological characteristics of Keshan disease, the selenium-responsive cardiomyopathy that affects infants, children, and women of child-bearing age in selenium-poor regions of China. Many investigators in the West, particularly trace-element researchers, are by now familiar with the geographic distribution of Keshan disease. The cardiomyopathy occurs only in those areas of China in which the soils are so low in selenium that locally-produced food is unable to furnish at least 19.1 μg of dietary selenium daily to adult males. Less widely appreciated perhaps is the fact that in addition to its well-documented geographical distribution, Keshan disease also exhibits a pronounced temporal variation, both seasonal and annual. The seasonal fluctuation of the disease especially is clearly delineated with peak incidence in southern China in summer and peak incidence in the north in winter. Such seasonal swings in the incidence of a disease suggest the role of an infectious agent and the
Chinese were able to isolate a number of different viruses from Keshan disease victims. One such virus, a coxsackie B\textsubscript{4} virus, was tested by the Chinese for its ability to cause heart damage in mice of differing selenium status. It was found that selenium decreased the cardiotoxicity of this virus to selenium-deficient mice. The results of these studies were published originally in 1980\textsuperscript{4} and in review form in 1987\textsuperscript{3}.

**Selenium and viral virulence**

In 1992, the authors initiated a collaboration to investigate further the role of host selenium nutriture as a determinant of viral virulence. Our first experiment sought to confirm the results of the earlier Chinese study which showed that selenium deficiency in mice could increase the heart damage caused by a cardiovirulent strain of coxsackievirus. For this purpose, we used coxsackievirus B\textsubscript{3}/20 (CVB\textsubscript{3}/20), a strain of CVB\textsubscript{3} that causes heart damage in normal animals. Feeding a selenium-deficient diet to weanling mice for 4 weeks prior to inoculation with CVB\textsubscript{3}/20 resulted in more than a 2-fold increase in the histopathology score (thereby indicating increased heart damage) when compared to infected mice fed the same diet supplemented with 0.2 ppm Se as sodium selenite\textsuperscript{5}. Similar differences in heart damage were obtained when CVB\textsubscript{3}/20-infected mice fed a vitamin E-deficient diet containing menhaden oil were compared to infected mice fed the same diet supplemented with 38.4 mg of RRR-\textalpha-tocopheryl acetate/kg\textsuperscript{6}. Thus, the Chinese results showing that selenium deficiency increased the virulence of a cardiovirulent virus in mice were verified and extended to include similar effects of vitamin E deficiency.

The next series of experiments examined the influence of selenium and vitamin E deficiency on the pathogenicity of a non-virulent strain, coxsackievirus B\textsubscript{3}/0 (CVB\textsubscript{3}/0), i.e. a strain that causes no apparent heart damage when administered to normal (non-deficient) animals. When this avirulent strain was inoculated into mice deficient in either selenium or vitamin E, it caused a moderate amount of heart damage, whereas CVB\textsubscript{3}/0 given to mice fed nutritionally adequate diets caused no apparent damage\textsuperscript{6,7}. That is, the phenotypic expression of a benign virus was altered to virulence by changing the type of diet fed to the host.

But how did a diet deficient in either of two antioxidant nutrients allow a benign virus to convert to virulence? The mechanism presumably involved oxidative stress since antioxidant activity is what these two nutrients have in common. Moreover, feeding fish oil, a potent dietary pro-oxidant, exacerbated the viral-induced heart damage in vitamin E-deficient mice\textsuperscript{6}, whereas feeding DPPD, a synthetic antioxidant, decreased viral-induced cardiopathy in vitamin E-deficient mice\textsuperscript{8}. One could
presume that oxidative stress in the host ‘weakened’ cardiomyocytes so that they were less resistant to invasion by the virus. An impaired immune response by the nutritionally compromised host might also lead to the same end result (more heart damage).

On the other hand, could replication of the benign virus in a host cell with altered redox milieu (such as might be expected in a selenium or vitamin E-deficient mouse) allow it to convert to virulence? To answer this question, we carried out a passage experiment in which the benign CVB3/0 strain was inoculated into two groups of mice fed a diet either adequate or deficient in selenium⁹. After 10 days, virus was harvested from the hearts of the mice, passed through HeLa cells in culture, and then re-inoculated separately into a second group of mice, all of which were fed the selenium-supplemented diet. In the normal mice that were infected with virus that had been allowed to replicate initially in selenium-adequate mice, there was no evidence of any heart damage. However, in mice that were infected with virus that had been allowed to replicate initially in selenium-deficient mice, a moderate amount of cardiopathology occurred. In other words, passage of CVB3/0, the benign avirulent strain, through a selenium-deficient mouse had somehow altered the virus such that it was then able to cause heart damage even in a selenium-supplemented mouse.

Selenium and viral genetics

The passage experiment described above gave a strong indication that conversion of the benign strain, CVB3/0, to a cardiovirulent strain, designated CVB3/0Se⁻, by allowing the former to replicate in a selenium-deficient mouse, occurred as a result of genomic changes in CVB3/0. However, to prove the point unequivocally, it was necessary to compare the nucleotide sequence of the genome of CVB3/0, the original benign input strain, with that of CVB3/0Se⁻, the newly virulent output strain obtained after passaging CVB3/0 through a selenium-deficient mouse. There are 7 nucleotide differences between the avirulent CVB3/0 and two myocarditic strains, CVB3/20 and CVB3/M. Sequencing results revealed that of these 7 differences, 6 were changed in the CVB3/0Se⁻ to be identical to that of the virulent genotype. One additional nucleotide position, at 2690, which differs between virulent and avirulent CVB3 strains, was not altered in the CVB3/0Se⁻ (Table 1). Exactly identical shifts in genomic base composition were observed when the CVB3/0 was passed through mice fed diets deficient in vitamin E¹⁰. Thus, by changing the nature of the diet fed to the host, we were able to alter the genetic make-up of an infecting virus so that the virus converted from avirulence to virulence.
Table 1 Base composition of virulence-determining genomic sites of the coxsackievirus

<table>
<thead>
<tr>
<th>Genomic Position</th>
<th>CVB3/0 (avirulent)</th>
<th>CVB3/OSe- (virulent)</th>
<th>CVB3/20 (virulent)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>234</td>
<td>C</td>
<td>T</td>
<td>T</td>
</tr>
<tr>
<td>788</td>
<td>G</td>
<td>A</td>
<td>A</td>
</tr>
<tr>
<td>2271</td>
<td>A</td>
<td>T</td>
<td>T</td>
</tr>
<tr>
<td>2438</td>
<td>G</td>
<td>C</td>
<td>C</td>
</tr>
<tr>
<td>2690</td>
<td>G</td>
<td>G</td>
<td>A</td>
</tr>
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<td>C</td>
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<td>T</td>
</tr>
<tr>
<td>7334</td>
<td>C</td>
<td>T</td>
<td>T</td>
</tr>
</tbody>
</table>

Adapted from Beck et al.

What is the mechanism by which replication in an oxidatively compromised host cell allows a virus to convert to virulence? The genome of RNA viruses, like coxsackievirus, does not exist as a collection of precisely uniform nucleotide sequences. Rather, these viruses are constantly changing and mutating into different forms to produce a 'swarm' of closely-related species (so-called 'quasispecies' concept) \( \text{11} \). The genomic sequence of such a viral swarm clearly does not mean to imply that each and every sequence in the swarm has exactly the same polynucleotide composition. Instead, the sequence is really a consensus sequence, representing the average base composition of all the different RNA viruses present in the swarm. Given this state of affairs, a number of possible mechanisms could lead to the emergence of a virulent strain of coxsackievirus \( \text{12} \). First, an impaired host immune response could allow a microheterogenic viral component to mutate and eventually dominate the input virus. Indeed, in our model a deficiency in either selenium or vitamin E led to decreased T cell activity \( \text{5,7} \). Alternatively, a particular viral sub-strain in the swarm might, for whatever reason, be favored for growth under conditions of intracellular oxidative stress. Finally, it seems possible that an increased load of intracellular oxidizing species could attack the RNA of the genome directly thus increasing the rate of mutation. Additional research is needed to distinguish among these mechanistic possibilities.

Selenium and viral disease

Although our results raise intriguing mechanistic molecular biology questions, our findings have significant public health implications as well. The study of diet/infection interrelationships has been an important component of nutritional investigation for many years. Scrimshaw and colleagues \( \text{13} \) published an extensive monograph on this topic in

British Medical Bulletin 1999;55 (No. 3)
Micronutrients in health and disease

![Diagram](image)

**Fig. 1** Epidemiological interactions among diet, agent, and host. In the past, nutritionists have considered only effects of host nutritional status on the host itself (left panel). Now nutritionists must also be alert to possible effects of host nutritional status on the pathogen (right panel). Figure reproduced from Levander\textsuperscript{14} with permission of the *Journal of Nutrition*.

1969 which summarized much of the work that had been done up until that time.

This research area has continued at a brisk pace. For example, an April 12, 1999 search of PubMed, the service that allows access to the roughly 9 million citations in Medline, under the term ‘Nutrition and Infection’ yielded 6299 articles, almost half of which have appeared since 1990. And yet despite this high level of scholarly activity, it appears that no one ever thought to consider effects of host nutritional status on the genetic make-up of an infecting pathogen until the report of Beck et al\textsuperscript{10} (Fig. 1). It seems that all thinking was directed only toward effects of host diet on the host, particularly the immune system of the host. This paradigm shift might require another look at long-standing nutrition/infection observations to determine whether changes in interpretation are required.

**Work for the future**

At this time, we simply do not know the scope of this phenomenon. That is, we don’t know how many pathogens might be affected by selenium or vitamin E deficiency in the same way that coxsackievirus appears to be. Nor do we know how many different nutritional deficiencies might affect the virulence of the coxsackievirus (or other viruses) by altering its genetic composition. Moreover, recent results showing effects of gold, arsenic, and mercury on coxsackievirus virulence\textsuperscript{15,16} indicate that environmental toxicology must also be considered in order to gain a more complete understanding of the determinants of viral virulence. At this stage of knowledge, we must keep our minds open to all possible influences on the course of viral disease.

**References**

1 Levander OA, Beck MA. Interacting nutritional and infectious etiologies of Keshan disease. Insights from coxsackie virus B-induced myocarditis in mice deficient in selenium or vitamin E. *Biol Trace Elem Res* 1997; 56: 5–21
Selenium and viral virulence


8 Levander OA. The selenium-coxsackievirus connection: chronicle of a collaboration. *J Nutr* 1999; In press


15 Smith AD, Guidry CA, South PK et al. Aurothiomalate (ATM) and aurothioglucose (ATG) causes a non-virulent strain of coxsackievirus B3 (CVB3/0) to exhibit virulence. *FASEB J* 1999; 13: LB233

16 South PK, Smith AD, Morris VC et al. An avirulent strain of coxsackievirus B3 causes death in mice pretreated with mercury or arsenic. *FASEB J* 1999 13: LB234