Development of an Experimental Animal Model for Reactive Arthritis Induced by *Yersinia enterocolitica* Infection

**JERI L. HILL** and **DAVID T. Y. YU**

Department of Medicine, University of California Los Angeles Center for Health Sciences, Los Angeles, California 90024

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We attempted to induce experimental arthritis in rats by systematically testing the effect of *Yersinia* infections in five strains of rats, using the intragastric, intraperitoneal, and intravenous routes of inoculation. We observed that Lewis rats which were given $10^4$ to $10^5 *Yersinia enterocolitica* WA organisms via the intravenous route consistently developed arthritis. The arthritis was most severe at 3 weeks and subsided at 6 weeks. No arthritis was observed when this bacterial strain was administered to Buffalo, Fisher, DA, and LDA rats. No replicable bacteria were detected in the joints. This aseptic characteristic parallels that seen in the human condition and establishes this as an animal model of *Yersinia*-induced arthritis. The probable reason for arthritis development in only the Lewis rats became apparent when we analyzed the numbers of live bacteria in the spleens and livers of these infected animals. The arthritis-susceptible Lewis rats harbored 10-fold more bacteria than the arthritis-resistant rat strains, and this systemic infection also persisted for a significantly longer period. Speculations as to why human subjects who develop *Yersinia*-induced arthritis are genetically predisposed have been centered principally around the role of the HLA-B27 histocompatibility antigens. The present study reveals a heretofore unrecognized critical factor: the susceptibility of the hosts to the virulence of the infectious organisms. In addition, the present animal model will provide the necessary tool for the investigation of this and other important host and bacterial factors.

Reactive arthritis in humans is characterized by two major features. First, in each patient, the development of arthritis is preceded by an episode of infection by one of several organisms. These include *Shigella flexneri, Salmonella typhimurium, Campylobacter jejuni, and Yersinia enterocolitica* (1, 2, 8, 10, 11). The arthritis is termed reactive rather than infective because the infectious organisms cannot be recovered from the arthritis tissues. The second feature is that the majority of the patients have the HLA typing of HLA-B27, indicating a very strong genetic predisposition to the development of arthritis (reviewed in reference 7).

Despite this information, it is not clear how the genetic factors affect the host-bacterial reactions to promote the development of arthritis. As in the investigation of many other diseases, the availability of an animal model might be crucial. Our study was prompted by personal communication from Philip B. Carter (Chapel Hills, Raleigh, N.C.) that some rats in his laboratory were fortuitously observed to develop arthritis after infection with the *Y. enterocolitica* strain designated as WA. *Y. enterocolitica* organisms having the same serotype as this WA strain have been reported to cause arthritis in humans (6). This particular strain of *Y. enterocolitica*, the WA strain, can also cause potentially lethal infections in rats and mice (3). In our initial experiments, graded doses of the bacteria were given to five individual strains of rats to determine the sublethal bacterial doses they could tolerate as well as the frequency of arthritis. To ensure adequate access of the organisms into the bloodstream, the intravenous route was selected. We found that a severe arthritis consistently appeared in one of the five strains of rats tested. Subsequent experiments were directed at the following two questions: (i) whether this was a reactive or an infective arthritis, and (ii) whether there was a measurable parameter of host-bacterial interaction which could distinguish the arthritis-susceptible from the arthritis-resistant strains of rats.


**MATERIALS AND METHODS**

**Experimental animals.** Male rats weighing 325 to 375 g (12 to 16 weeks old) were used in all experiments. Lewis rats were obtained from Charles River Breeding Laboratory, Kingston, N.Y.; Buffalo and Fisher rats were obtained from Simonsen Laboratories, Gilroy, Calif., and DA and (Lewis × DA)F1 (also known as LDA) rats were obtained from the Trudeau Institute, Senalac, N.Y. We also purchased Lewis rats which were certified by the purchasing source (Charles River Breeding Laboratories) as being free of specific pathogens. Such pathogen-free rats were housed under a laminar-flow hood until bacterial inoculation.

All animals received antibiotic-free food and water ad libitum and were maintained at an ambient temperature of 20 to 25°C in level 3 biosafety containment in the University of California Los Angeles animal facility.

**Bacterial cultivation and enumeration.** Four *Yersinia* strains were used in this study. *Y. enterocolitica* WA, which is serotype O:8, was the kind gift of P. B. Carter. *Y. enterocolitica* 8501, which is serotype O:3, was the kind gift of H. H. Mollaret, Pasteur Institute, Paris, France. This strain has the same serotype and biotype as the arthritis-causing *Y. enterocolitica* strain in Finland. *Y. pseudotuberculosis* 269 was the kind gift of Auli Toivanen and Kaisa Granfors, University of Turku, Turku, Finland. This strain was isolated in Finland, where reactive arthritis caused by *Y. pseudotuberculosis* is not uncommon. *Y. enterocolitica* 1223-75, which is serotype O:20, was the kind gift of Kaye Wachsmuth, Centers for Disease Control, Atlanta, Ga. This strain was isolated in the United States, where *Yersinia*...
induced arthritis is relatively rare. It was chosen for our study because it is virulent when tested by the in vivo lethality test in mice.

All four strains of bacteria were passaged in our laboratory in Swiss Webster mice (Jackson Laboratory, Bar Harbor, Maine) and preserved at -70°C in 50% glycerol. Before an experiment, a sample of each bacterial strain was recovered by being cultured at room temperature on tryptic soy agar plates (Difco Laboratories, Detroit, Mich.). Plasmid-positive colonies of these *Yersinia* bacteria were selected both by their colony appearance on agar plates and by their auto-agglutinating property (9). Further cultures were carried out in tryptic soy broth to late-log phase at room temperature. After the cultures were washed with phosphate-buffered saline, 0.2-ml aliquots were injected intravenously into the rats. Bacterial concentrations were estimated by spectrophotometry prior to injection into the rats. A colony count of the sample was carried out the next day when the colonies first became visible. These counts were within the range of 0.5 to 5.0 times the values estimated by spectrophotometry. For simplicity, only the values estimated by spectrophotometry are shown in this paper.

Irradiation of bacteria. *Y. enterocolitica* WA organisms were cultured and harvested as described above. The sample was then irradiated with 7,000 rad (model Gammator M; Gammatron, N.Y.). This irradiation dose was decided from the results of an experiment in which the bacteria were treated with graded doses of irradiation. A dose of 7,000 rad was the minimum necessary to completely inhibit the growth of this bacterial strain on agar plates.

Measurement of the number of bacteria in rat tissues. After the rats were sacrificed, the spleens and livers were dissected. Cell suspensions of each organ were prepared. Serial dilutions of the preparations were plated on tryptic soy agar plates. The number of bacterial colonies was counted after 48 h of incubation at room temperature. Results are expressed as CFU per organ or CFU per gram of tissue.

The method of assessing the presence of viable bacteria in the joint tissues is described in Results.

Assessment of the severity of arthritis. The volumes of the two hind paws of each rat were measured with a plethysmograph (Ugo Basile, Milan, Italy). This apparatus quantitates the volume of each paw by measuring the amount of mercury which is displaced when a paw is immersed into its mercury chamber. The measurements appear as readings over a range of 0 to 50 U. The sum of the values from the two hind paws was taken as an index of the severity of the swelling from arthritis.

To minimize pain and suffering of animals, especially during the arthritic phase, all animals were housed on shavings in large animal pans. This practice eliminated the pain and nonspecific injury encountered by animals in cages with wire grid bottoms. Shavings were changed every 2 to 3 days after inoculation of bacteria, and daily during the diarrhea phase, to minimize possibility of re-infection by bacteria in excreted feces. Animals were given inhalant anesthetic at time of sacrifice.

**Histological sections.** Histological sections of the knee and ankle areas were prepared at the Pathology Department, Hollywood Presbyterian Hospital, Los Angeles, Calif. Sections were stained by the Gram stain as well as by the hematoxylin and eosin stains (13).

**Statistical calculations.** The values for the individuals in each experimental group were computed into the means and the standard errors of the means. Comparisons between groups were made by the Student *t* test.

**RESULTS**

**Effect of dose of Y. enterocolitica WA on survival of rats.** *Y. enterocolitica* WA has been reported to be potentially lethal to rats (3). In our first experiment, graded doses of bacteria were injected intravenously into five strains of rats. A dose of 10⁸ *Yersinia* organisms proved to be lethal for all five strains of rats, with no survival 5 days after inoculation and no apparent differences among the strains. The rates of survival on day 14 after inoculation with lower doses are compared in Table 1. The Lewis rats appeared to be the most susceptible. None of the six Lewis rats given 10⁶ bacteria survived at day 14. They died at day 10.5 ± 2.2. The other four strains of rats, on the other hand, survived for this period when given the same number of bacteria. The results presented in Table 1 are pooled from two separate experiments. In each experiment, all five strains of rats were tested simultaneously. Although data are presented in Table 1 as survival through day 14, all surviving rats were observed further and were found to survive until sacrifice at 4 to 6 weeks. The sublethal doses tolerated by each individual rat strain were used for the remainder of the experiments.

**Incidence of arthritis.** Rats which were given the sublethal doses shown in Table 1 were observed for a total of 6 weeks for signs of arthritis. Arthritis was regarded as being present when the hind-paw volumes exceeded 3 plethysmograph units. The incidence of arthritis in each rat strain is shown in Table 2. The most striking finding was that arthritis was observed only in the Lewis strain. Arthritis occurred at a dose of 10⁴ to 10⁵ bacteria per rat but not at a dose of 10³.
bacteria per rat. A dose of $10^6$ bacteria per rat was lethal. While Lewis rats showed a 100% incidence of arthritis, Buffalo, Fisher, DA, and LDA rats were negative at doses of $10^4$ to $10^6$ bacteria per rat. All four paws of some of the Lewis rats appeared swollen. However, in all the Lewis rats, the most severe arthritis was generally at the hind paws. Soft-tissue swelling and pain were most obvious, as was erythema. No consistent lesions were observed in the eyes, genitals, skin, or tails. Raising the Lewis rats pathogen free did not affect their susceptibility to arthritis.

**Course of arthritis.** The course of arthritis was closely monitored in 12 Lewis rats. Plethysmograph measurements of hind paws were recorded at weekly intervals for a total of 6 weeks. A mild arthritis was observed 1 week after injection; the mean plethysmograph value was $10.4 \pm 3.2$. Maximum arthritis was observed at week 3; the mean plethysmograph value was $78.2 \pm 7.8$ ($P < 0.005$ compared with value at week 1). At week 6, the arthritis had regressed and the plethysmograph value became $6.7 \pm 2.5$. Eight rats which were given only intravenous phosphate-buffered saline were observed in parallel. No arthritis appeared in these control rats (Fig. 1 and 2).

Histological sections were prepared from the knee and ankle areas from both control rats and rats at the height of arthritis. The appearance of a normal knee joint is compared with that of an arthritic knee joint and an arthritic ankle joint in Fig. 3. Sections of the arthritic joints reveal moderate to marked inflammation of the soft tissue and synovial tissue with infiltration by many mononuclear cells and a few polymorphonuclear cells. Especially in some ankle sections, there are uncharacterized multinucleated giant cells and some chronic granulomatous reaction.

**Assessment of the presence of viable bacteria at the joints.** Eighteen Lewis rats were each given intravenous injections of $10^4$ *Y. enterocolitica* WA cells. Groups of three were then sacrificed and studied at days 3, 7, 14, 28, 35, and 42.

Two procedures were carried out under sterile conditions to test whether viable bacteria were present in the joints. In the first, the knee and ankle joints were opened and the synovial fluids were transferred by sterile loops (Fisher Scientific, Tustin, Calif.) onto tryptic soy agar plates. In the second, a piece of tissue of about 0.5 cm$^3$ was excised from each joint with a bone cutter and cultured in tryptic soy broth.

For all of the rats examined by the above procedures, no

![FIG. 1. Photographs of the hind paws of one Lewis rat 3 weeks after injection with phosphate-buffered saline (left) and another Lewis rat 3 weeks after injection with *Y. enterocolitica* WA (right). Arthritis was observed in the rat injected with bacteria.](image)

![FIG. 2. Course of arthritis in Lewis rats after injection with *Y. enterocolitica* WA. Twelve rats were injected with bacteria. At weekly intervals, the volumes of the hind paws were measured with a plethysmograph. Each point in the figure represents the sum of plethysmograph values in both hind paws of a rat. The bar lines represent the mean values of the measurements from the 12 rats.](image)
bacteria were detected on the agar or in the broth after 48 h of culture. About one-half of the cultures were maintained for 2 weeks, with no bacterial growth detected.

For one rat, histological sections were made of the swollen ankle area 2 weeks after intravenous inoculation with bacteria. The sections were stained with Gram stain. No bacterium-like structures were observed.

Enumeration of the absolute bacterial CFU in the organs of infected rats. Lewis rats were given intravenous doses of 10⁴ *Y. enterocolitica* WA organisms. Non-Lewis rats were given 10⁵ organisms. With the exception of the Lewis rats at day 7, groups of three were sacrificed at weekly intervals to assess the numbers of bacterial CFU per spleen and liver. For the Lewis rats at day 7, five rats were examined instead of three. The findings in the spleens were striking. Non-Lewis rats showed 8.5 × 10⁴ to 4.7 × 10⁶ CFU per spleen at day 7 after injection. They became almost completely cleared 1 week later. Lewis rats, on the other hand, harbored a mean value of 7.4 × 10⁷ CFU per spleen at day 7 after injection and still showed 9.4 × 10⁷ and 7.5 × 10⁷ CFU per spleen 1 and 2 weeks later, respectively. These values were statistically significant when compared with the corresponding values for each group of the non-Lewis rats (P < 0.005 for day 7 values, P < 0.0005 for day 14 values, and P < 0.025 for day 21 values). Findings in the livers parallel those in the spleens (Fig. 4). The values in the Lewis rats at days 7 and 21 were significantly higher than those in each group of the non-Lewis rats (P < 0.0005 and P < 0.05, respectively).

Enumeration of bacterial CFU per gram of spleen or liver of the infected rats. For each uninfected rat, the spleen weighed 1.0 to 1.4 g, while the liver weighed 7.0 to 12.0 g. For the non-Lewis rats, these weights remained unchanged during the course of infection. For Lewis rats, the weights of the livers also remained unchanged. In contrast, gross splenomegaly was obvious at day 14, and the weight of each spleen became almost double.

At day 7 after inoculation of bacteria, the average CFU per gram of spleen tissue were 1.1 × 10⁶, 2 × 10⁶, 3.6 × 10⁶,
TABLE 3. Incidence of arthritis in Lewis rats after inoculation with irradiated *Y. enterocolitica* WA

<table>
<thead>
<tr>
<th>Dose (CFU)</th>
<th>Irradiation</th>
<th>No. with arthritis/total no.</th>
</tr>
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<tbody>
<tr>
<td>10⁴</td>
<td>No</td>
<td>6/6</td>
</tr>
<tr>
<td>10⁵</td>
<td>Yes</td>
<td>0/4</td>
</tr>
<tr>
<td>10⁶</td>
<td>Yes</td>
<td>0/4</td>
</tr>
<tr>
<td>10⁷</td>
<td>Yes</td>
<td>0/4</td>
</tr>
</tbody>
</table>

8.3 × 10³ and 7 × 10⁷ for the Fisher, DA, LDA, Buffalo, and Lewis rats, respectively. The corresponding values for the livers were 4.1 × 10⁶, 7 × 10⁵, 5.5 × 10⁷, 9 × 10⁶, and 2.2 × 10⁶. Negligible CFU were detected in the spleens and livers of the Fisher, Buffalo, and DA rats at days 14 and 21 after injection of bacteria. For the Lewis rats, the average CFU per gram of spleen tissue at days 14 and 21 were 5 × 10⁷ and 7.5 × 10⁷, which were very similar to the values at day 7. In contrast, the CFU per gram of liver tissues were 10⁴ at day 14 and 10⁷ at day 21; these values were lower than the values at day 7. For all but one of the above values, the standard errors of the means are less than 50% of the corresponding mean values.

Effect of irradiation on the ability of *Y. enterocolitica* to induce arthritis. A sample of *Y. enterocolitica* WA was irradiated with 7,000 rad and injected intravenously into 12 Lewis rats. The doses used are shown in Table 3. The rats were observed for 4 weeks. No arthritis developed in any Lewis rats inoculated with these nonreplicating bacteria. Six rats simultaneously inoculated with unirradiated WA organisms developed arthritis of the same severity and time course as those shown in Fig. 2. In addition, groups of two rats, each given 10⁸ or 10⁷ irradiated WA organisms were sacrificed at days 7 and 30. Preparations of their livers and spleens cultured on agar plates showed no bacterial growth.

Effect of infecting Lewis rats through intragastric and intraperitoneal routes. No arthritis was observed in groups of three Lewis rats given the bacteria intragastrically at doses of 10⁴, 10⁵, and 10⁶ organisms per rat. However, at an intraperitoneal dose of 10⁰ to 10⁶ organisms per rat, three of six animals developed arthritis.

The numbers of viable bacteria recovered at day 21 from the group given intragastric and intraperitoneal inoculation were drastically different. No bacteria were recovered from the spleens and livers of the group given intragastric inoculation. The numbers of CFU recovered from the spleens of the six rats given intraperitoneal inoculation were: 0, 1.7 × 10⁷, 2.5 × 10⁸, 1.2 × 10⁹, 6.9 × 10⁹, and 1.4 × 10¹⁰. The corresponding values for the livers were: 2.8 × 10⁷, 8.1 × 10⁷, 6.6 × 10⁸, 5.4 × 10⁹ and 3.2 × 10ⁱ⁰.

Effect of infection with other *Yersinia* organisms. We also examined the arthritis-causing potential of three other *Yersinia* bacteria: *Y. enterocolitica* 8501, *Y. enterocolitica* 1223-75, and *Y. pseudotuberculosis* 269. Simultaneously, five Lewis rats were inoculated with the arthritis-inducing *Y. enterocolitica* WA organisms. Each bacterial strain was injected into a group of six Lewis rats (Table 4). These rats were then observed for 6 weeks. None of them developed arthritis.

In a parallel experiment, an additional three rats inoculated with the non-WA bacterial strains were sacrificed at day 7 after injection to enumerate the bacterial CFU in their spleens. Their values are almost 100- to 1,000-fold less than those of a group of five WA-inoculated Lewis rats assayed in parallel (*P* < 0.0025 when the WA-inoculated Lewis group is compared with each of the non-WA-inoculated Lewis group).

**DISCUSSION**

The mechanism of *Yersinia*-induced arthritis is an enigma. This is so, although multiple strains of arthritis-causing bacteria have been isolated (1, 2, 8, 10, 11), large numbers of patients have been recruited for study, and the major genetic factor causing predisposition to arthritis has been identified (7). Some critical questions remain unclear, such as whether the development of arthritis requires prolonged persistence of the bacteria in the hosts and whether any discrete bacterial components home to the joint tissues. It would be almost impossible to answer such questions concerning in vivo events unless an experimental animal model were available.

The major objective of the project described in this paper was to establish such a model.

Through a systematic analysis of several variables, we have verified the personal communication from P. B. Carter that such a model is indeed possible. Three experimental factors are critical. They include the *Yersinia* strain, the type of rats, and the route of bacterial administration. The route has to be intravenous or intraperitoneal but not peroral. This was unexpected, because the human condition is induced by gastroenteritis. Perhaps the mucosal defense in the rat species is more effective in preventing access of the *Yersinia* organisms from the bowel to the general circulation.

Our arthritis model would be quite meaningless if viability of the *Yersinia* strains was not necessary, if coinfection with other organisms was needed, or if, unlike the situation with patients, viable bacteria could have been recovered from the joints. However, irradiation of the bacteria eliminated both their ability to replicate and their ability to induce arthritis. Lewis rats which were apparently pathogen free could also develop *Yersinia*-induced arthritis. Lastly, when the joints from 15 infected animals were examined, no colony-forming bacteria could be cultured.

The clinical value of our findings depends largely on whether the mechanisms of *Yersinia*-induced arthritis in the Lewis rats are the same as those in humans. This remains to be proven. If such proves to be the case, our results are potentially interesting in that they allow us to assess the role contributed by the virulence of the bacteria and also the susceptibility of the hosts to this virulence. These two factors have not heretofore been suspected to be important when the human condition was being investigated. The interpretation of our results becomes quite straightforward if one assumes that *Y. enterocolitica* WA can consistently induce arthritis in the Lewis rats because they carry discrete arthritis-causing components. There can then be two possible explanations of why arthritis did not develop in our Buffalo, Fisher, DA, and LDA strains. The first possibility is that in these arthritis-resistant rats, the putative arthritis-causing *Yersinia* components cannot initiate the cellular or

**TABLE 4.** Ability of four different *Yersinia* strains to induce arthritis in Lewis rats

<table>
<thead>
<tr>
<th>Strain</th>
<th>Dose (CFU)</th>
<th>No. with arthritis/total no.</th>
<th>Bacterial count in spleen (10⁶) (±)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WA</td>
<td>10⁴</td>
<td>5/5</td>
<td>7,400 ± 1,900 (5)</td>
</tr>
<tr>
<td>8501</td>
<td>10⁴</td>
<td>0/6</td>
<td>92 ± 84 (3)</td>
</tr>
<tr>
<td>1223-75</td>
<td>10⁴</td>
<td>0/6</td>
<td>1.4 ± 0.9 (3)</td>
</tr>
<tr>
<td>269</td>
<td>10⁴</td>
<td>0/6</td>
<td>33 ± 31 (3)</td>
</tr>
</tbody>
</table>

* The number of rats in each group examined for bacterial colony counts is enclosed in parentheses.
humoral processes which might induce arthritis. The second possibility is that although these rats do have the cellular or humoral capacity to develop arthritis, a sufficient amount of the arthritis-causing bacterial components is unavailable. The reason we failed to induce arthritis in the non-Lewis rats, then, is that they are more resistant to the infection and do not allow the bacteria to multiply to a sufficient degree to release the required amount of components. This possibility is supported by results shown in Fig. 4 and might be tested by attempting to alter, for instance, macrophage or T-cell function in arthritis-resistant rat strains to see whether these procedures can convert them to arthritis-susceptible animals.

The hypothesis that the relative virulence of the bacteria to the hosts is important would also explain the results of the experiment in which we compared the arthritis-causing Y. enterocolitica WA with the other three Yersinia strains. Y. pseudotuberculosis and serotype O:3 Y. enterocolitica are well known to cause arthritis in humans. The O:3 Y. enterocolitica strain we examined belongs to the same serotype, biotype, and phage type of the arthritis-causing strains of Y. enterocolitica isolated in Finland. Thus, this strain should, theoretically, carry the putative arthritis-causing bacterial components. One explanation for this disparity is that rats might respond differently to these non-WA bacteria than do humans. However, the most likely reason for their negative effect in the rats is probably related to results shown in Table 4. When we administered $10^6$ of each of these bacterial strains to the Lewis rats, the numbers of bacteria which were recovered in the spleens 1 week afterwards were at least 100-fold lower than those in the arthritis-causing Y. enterocolitica WA. This again indicates that a relative lack of virulence is associated with failure to induce arthritis.

In summary, we postulate from our findings that two aspects are important in reactive arthritis: whether the bacteria carry potentially arthritis-causing components and whether the infected hosts are highly susceptible to the infection. The latter, in turn, depends on both the inherent virulence of the bacteria and on the genetic susceptibility of the hosts. The availability of this animal model will allow investigators to examine the host genetic factors in detail.

The host immune response to the potentially arthritis-causing bacterial component(s) could prove to be an area of extreme interest in this animal model. In other animal models of arthritis, experiments involving transfer of serum (12) or cells (14) of arthritic rats has established a role for both the humoral and cellular compartments of the immune system. In the clinically relevant animal model described herein, the sera and cells of rats with Yersinia-induced arthritis could prove to be most valuable reagents in the investigation of pathogenetic mechanisms.

The major weakness of our hypothesis is that none of our results directly supports the idea that the putative arthritis-causing components can induce arthritis in the non-Lewis rats, even when given in large quantities. One can argue, perhaps, that this postulate would explain why considerable numbers of patients with reactive arthritis are HLA-B27 negative and do not have any apparent genetic predisposition (7). However, the actual testing of this hypothesis must await the identification of those particular bacterial components. Very importantly, the animal model provides a valuable tool for investigators to identify these arthritis-causing components and perhaps even their structural prerequisites. This has been the case in peptidoglycan-induced arthritis (5) as well as adjuvant-induced arthritis (4), two models in which the clinical relevance to human disease is much less certain than the Yersinia-induced arthritis model.

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LITERATURE CITED