harbors a nitrogen fixer as a pure culture in a specialized organ.

The bacterium from the gland of Deshayes possesses two properties, cellulose digestion and nitrogen fixation, that genetic engineers have been trying (unsuccessfully) to combine in a single bacterium. The combination of these properties in this bacterium makes it a candidate for producing single-cell protein from cellulose without the necessity of adding combined nitrogen.

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11 March 1983; revised 11 July 1983

Cat Scratch Disease: A Bacterial Infection

Abstract. Histopathologic examination of lymph nodes from 39 patients with clinical and pathological criteria for cat scratch disease revealed delicate pleomorphic Gram-negative bacilli in 34 of the 39 nodes. They were within the walls of capillaries in or near areas of follicular hyperplasia and within microabscesses. They were best seen with the Warthin-Starry silver impregnation stain. Organisms in lymph node sections exposed to convalescent serum from three patients and to immunoperoxidase stained equally well with all three samples. The organisms did not react with hyperimmune sera to Legionella pneumophila *nor to several species of* Rickettsia. *These bacilli appear to be the causative agents of cat scratch disease.*

Despite at least 750 reports on cat scratch disease (CSD) over the past 37 years, the etiologic agent of the disease has eluded detection. Clinical, epidemiological, and pathological studies have implicated an infectious agent (*1–5*).

A clinical diagnosis of CSD requires the fulfillment of three of four criteria (*2*): (i) a history of animal (usually a cat or dog) contact with the presence of a scratch or primary dermal or eye lesion (*6*), (ii) a positive CSD skin test, (iii) negative laboratory studies for other causes of lymphadenopathy, and (iv) characteristic histopathology of a biopsied lymph node. A definitive diagnosis will be possible only when the etiologic agent is isolated. Many patients go undiagnosed because the symptoms are atypical. For instance, 10 percent of the patients with otherwise characteristic symptoms report no contact with cats and 35 percent of the patients give no history of a cat scratch.

The Armed Forces Institute of Pathology (AFIP) provides consultation on approximately 50,000 specimens a year, usually to assist when a diagnosis is controversial or unclear. Some of these specimens are from patients suspected of having CSD. In early 1981, we received a lymph node taken from an 11-year-old girl. Although the girl's family had cats, there was no history of a cat scratch. Laboratory findings supported the diagnosis of CSD because organisms failed to grow from this lymph node when cultured on blood agar, chocolate agar, prereduced brucella agar, laked blood agar, phenylethyl alcohol agar, or thioglycollate medium. We stained sections of the node for bacteria, mycobacteria, fungi, and spirochetes. Using a silver impregnation stain we observed many small bacteria that proved to be Gram-negative with a Gram stain modified for tissue. These observations prompted us to search for similar bacteria in lymph nodes from additional patients with CSD.

Between 1 October 1982 and 31 March 1983, 39 lymph nodes with histologic changes of CSD as described by Campbell (*7*) were submitted to the AFIP.

These came from 17 states and Europe. In 28 of 39 specimens the pathologist included CSD in the provisional diagnosis. Twenty-seven of 39 patients were children or young adults. Exposure to cats was not mentioned for 28 patients but six patients had been scratched by cats and five others exposed to cats. In 37 of 39 patients, a single cluster of nodes was enlarged. The location of these clusters was as follows: epitrochlear (four patients), axillary (eight), supraclavicular (one), cervical (six), parotid (two), anterior chest wall (three), inguinal (ten), femoral (one), and thigh (two). After the patients' physicians were contacted, skin tests were done on eight patients and all were positive for CSD. The lymph nodes of seven of these patients contained detectable bacteria.

Bacilli were clearly seen with the Warthin-Starry (WS) silver impregnation stain (*8*) in lymph node sections from 29 of 34 patients and with the modified Brown-Hopp's tissue Gram stain (*9*) in 28 of 34 patients. The bacilli were pleomorphic, ranged from 0.3 to 1.0 μm by 0.6 to 3.0 μm, were Gram-negative, and were not acid-fast. They were in the walls of capillaries (Fig. 1, A and B) and in macrophages lining the sinuses in or near germinal centers. Here the bacilli appeared as single organisms or in chains or clumps. In some cross sections of vessel walls the bacilli encircled the lumen. In longitudinal sections, large numbers of bacilli almost obliterated a short segment of vessel wall. Bacilli were also in thrombosed vessels, and in necrotic foci where they were clustered in histiocytes, giving the appearance of intracellular multiplication (Fig. 1C). In some areas of more extensive necrosis the bacilli appeared as single organisms in vacuoles of activated histiocytes or free in the necrotic debris. When the neutrophils were centered within stellate granulomas, bacilli were only rarely observed within neutrophils or free in the necrotic exudate. The bacteria we observed fulfilled the criteria for a pathogenic organism in these lymph nodes: they were in the tissue, they were limited to the areas of reaction, they were intracellular, and

they increased in number as the lesions developed and decreased as the lesions resolved. The bacteria in the 34 lymph nodes were morphologically identical. On the basis of the clinical evidence and on the histopathologic findings we conclude that these patients had CSD and that the bacilli observed are the cause of the disease.

An immunoperoxidase stain (10) was used to determine whether the bacilli reacted with sera from CSD patients. Serum from a convalescent patient in our study who had a cat scratch, an inoculation papule, a positive cat scratch skin test, and characteristic pathology reacted with the bacteria in the patient's own node and with the bacteria in the nodes of two other patients in the study. Sera from three normal humans served as negative controls. The bacilli stained intensely and there was some light background staining. Serum from two convalescent patients not in our study but used as a source of antigen for CSD skin tests

(11) also reacted intensely with the bacteria in sections of lymphoid tissue from our patient. These data offer further evidence that the patients were infected with the same organisms. We conducted peroxidase-antiperoxidase (PAP) tests on bacteria in lymph nodes from CSD patients using hyperimmune rabbit serum against the Legionnaire's disease bacillus, Rickettsia conorii, and against Rickettsia rickettsii (12). Tissue culture–infected cells or tissue sections known to contain the organisms were used as controls for the stain. Normal rabbit sera were used as negative controls. None of these sera gave a positive reaction.

These bacilli, which we believe are the cause of CSD, are most numerous and most easily seen in damaged vessels and microabscesses. They are rarely seen in the stellate granulomas with central suppuration where infectious agents have traditionally been sought. They are very small—at the limit of the resolving power of the light microscope. The WS silver

impregnation stain, which is not routinely done when searching paraffin sections for infectious agents, coats the organisms making them appear larger and easier to see. The bacilli are rare in suppurative material likely to be aspirated and used for culture. Immunoperoxidase stains with the use of sera from convalescent humans demonstrate a host-immune response to the organism, and the bacteria from different patients are serologically related. The organisms did not react with rabbit antisera to Legionella pneumophilia, R. rickettsii, R. conorii, or R. tsutsugamushi. Even if heavily infected tissue is used as an inoculum, the organisms may prove difficult to grow. Their growth requirements may be difficult to define or they may be obligate intracellular parasites with host specificity.

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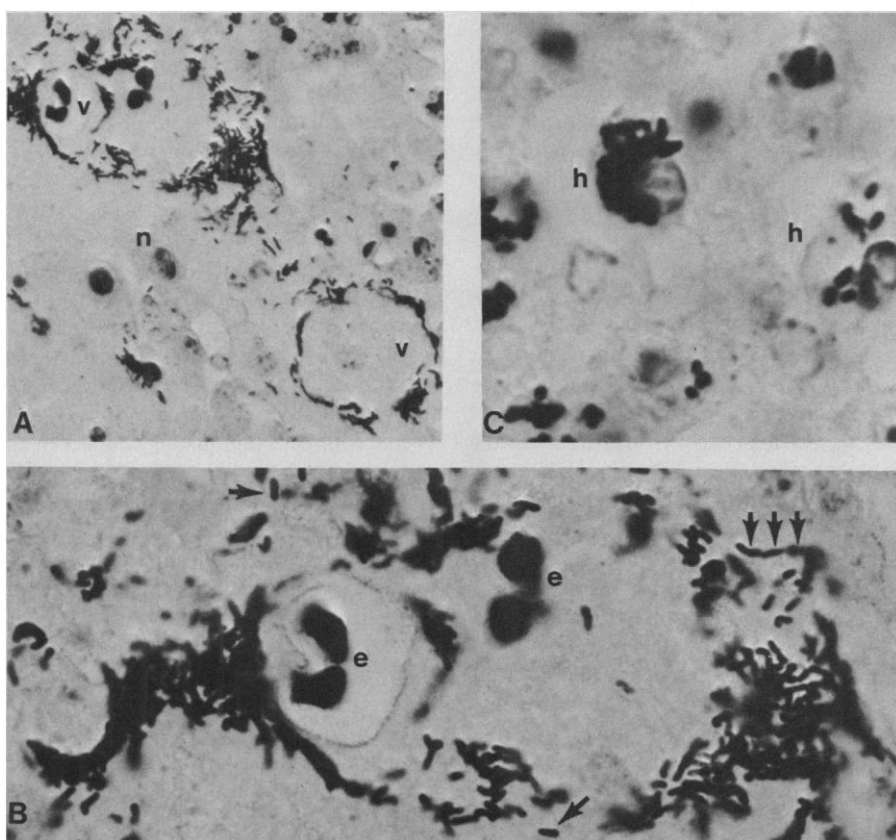
Fig. 1. (A and B) Photomicrographs of WS-stained sections of an inguinal lymph node from a patient with a skin test positive for cat scratch disease. Bacilli at (A) low and (B) high power in the wall of a vessel (v). The WS silver impregnation stain enlarges the bacilli making them easy to see. Cell boundaries are not visible in these photographs. Nuclei (n), erythrocytes (e), and organisms stain black. (A) Parallel tissue sections stained with hematoxylin and eosin revealed a single tortuous blood vessel with cross sections in the upper left and lower right and a tangential cut through the vessel wall between the cross sections (×500). (B) Upper part of (A) demonstrating the bacilli (arrows), singly and in chains, outlining the vessel (×1260). (C) Photomicrograph of axillary lymph node of a patient with a primary cat scratch inoculation papule and a positive cat scratch skin test. Bacilli are present in small histiocytes (h). The bacilli are distinct in one histiocyte but fill the cytoplasm of another (×2000).

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12 April 1983; revised 15 June 1983

Carbocyclic Arabinofuranosyladenine (Cyclaradine): Efficacy Against Genital Herpes in Guinea Pigs

Abstract. *Carbocyclic arabinofuranosyladenine (cyclaradine), a novel nucleoside analog with such desired features as hydrolytic and enzymatic stability, adenosine deaminase resistance, and low systemic toxicity, inhibited the replication of herpes simplex virus types 1 and 2. The 5′-methoxyacetate prodrug form exhibited significant efficacy in the topical treatment of genital infections by herpes simplex virus type 2.*

Until recently it was virtually agreed that viral diseases do not respond to chemotherapy. This belief is vanishing as new agents are developed that are effective against specific viral infections. Among the most promising agents to date are the nucleoside analog 9-β-D-arabinofuranosyladenine (Ara-A) (*1, 2*) and 9-[(2-hydroxyethoxy)methyl]guanine (acyclovir) (*3*). Both drugs have serious drawbacks inherent in their molecular structural design that limit their use in the treatment of herpes simplex virus (HSV) infections, especially HSV-2 genital infections. Thus Ara-A therapy has not been successful in the topical treatment of experimental mucocutaneous HSV infections in animals (*4*) or against oral or genital HSV infections in humans (*5*). The molecular features of the Ara-A molecule are compatible with the substrate requirements of the ubiquitous enzyme adenosine deaminase, and it is generally accepted that the major liability in its effectiveness is rapid deamination to the much less active arabinosyl hypoxanthine (*2*). Clinical studies have demonstrated that topical acyclovir is of minimal benefit in the treatment of recurrent genital herpes (*6*). HSV strains that are deficient in their ability to code for thymidine kinase are resistant to the drug (*7*).

We describe here a novel nucleoside analog that is highly efficacious in the topical treatment of HSV-2 genital infections. Carbocyclic Ara-A (cyclaradine), an adenosine deaminase–resistant analog of Ara-A, inhibited the replication of HSV-1 (strain HF) and HSV-2 (strain MS) in tissue culture at noncytotoxic concentrations (*8*). Preliminary detection of antiviral activity was determined by measuring its ability to inhibit virus-induced cytopathogenic effects in infected cultures (*9*). Cyclaradine was active against acyclovir-resistant HSV variants deficient in DNA polymerase or thymi-
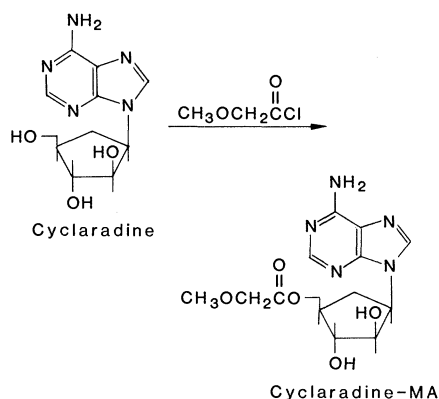

Cyclaradine

Cyclaradine-MA

Fig. 1. Preparation of cyclaradine-MA. To a solution of cyclaradine (0.10 mole) in dimethylformamide (750 ml) was added methoxyacetyl chloride (0.11 mole) in dimethylformamide (140 ml). The reaction mixture was stirred at 4°C overnight and then water (50 ml) and sodium bicarbonate (0.27 mole) were added. The volatile materials were removed in vacuo and the residue was applied to a silica-gel column. Elution of the major fraction with methanol-chloroform (0.15 per liter) gave the pure product (melting point 172° to 174°C). Crystallization from water and subsequent drying in vacuo at room temperature gave the dihydrate (melting point 78° to 80°C).

dine kinase activities (*10*). This finding indicates that the carbocyclic nucleoside is not activated by virus-induced thymidine kinase in infected cells. Optimum derivatization and formulation of cyclaradine to the methoxyacetate ester prodrug form (cyclaradine-MA) yielded a new topically active antiherpes agent (Fig. 1).

Cyclaradine belongs to a class of compounds known as carbocyclic nucleosides, in which a methylene group replaces the oxygen atom of the carbohydrate ring (*11*). Earlier studies showed that these analogs, which lack the labile glycosidic bond, are stable to cleavage by phosphorylases or hydrolases while retaining the potential for therapeutic useful interaction with other enzymes involved in nucleotide metabolism (*8, 12*). In the case of cyclaradine, the exchange of methylene for oxygen also renders the compound inert to adenosine deaminase. Thus, under conditions in which Ara-A is completely deaminated (1 μmole/min per unit of enzyme) by calf intestinal adenosine deaminase (type III, Sigma), no detectable deamination of cyclaradine was observed. As expected, the addition of the adenosine deaminase inhibitor, 2′-deoxycoformycin, to growing P-388 mouse lymphoid leukemia cells increased the cytotoxicity of Ara-A 20-fold, while no increase in toxicity was observed with cyclaradine.

The effect of the compounds on genital herpes infections was studied in guinea pigs, an animal model with many human-like parameters. Beginning 2 to 3 days after the intravaginal inoculation of guinea pigs with 10^5 median cell culture infectious doses per 0.1 ml of HSV-2 [strain MS (*13*)], typical discrete vesicular lesions appeared on the external genitalia in 98 percent of the animals (Fig. 2). Peak mean lesion scores (*14*) 7 days after infection were 3.53 and 3.35 in HSV-infected untreated and placebo-treated control groups, respectively.

Initial examination of the parent compound, cyclaradine, indicated that the unaltered nucleoside analog is not suitable for topical treatment of genital HSV-2 infections. It was subsequently observed that the low solubility of the agent caused precipitation from the gel, and the crystalline material appeared to remain at the surface of the vaginal mucosa. Unlike cyclaradine and its simple alkyl ester derivatives, certain alkoxyalkanoate esters were easily formulated into more amorphous-like materials that could be easily applied topically to the guinea pig. The 5′-methoxyacetate (cyclaradine-MA) was selected for subsequent studies.