Experimental Infections with Coxsackie Viruses

I. Studies on Virulence and Pathogenesis in Cynomolgus Monkeys

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With 4 Figures

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There is accumulating a body of evidence concerning the wide distribution of Coxsackie viruses in many body tissues during human infection. Both Group A and Group B strains have been recovered from such extracellular milieu as blood, cerebrospinal fluid, urine, pericardial fluid, oropharyngeal mucous, and feces (1, 2, 3, 4) and from such tissues as spinal cord, heart and liver (5). The clinical features associated with infection caused by these viruses vary; nevertheless, they very likely have similarities in the infectious cycle. Since the boundaries of infection in human beings can be resolved only slowly, depending on availability of clinical materials, we started a series of studies using cynomolgus monkeys as models in order to obtain a better understanding of the evolution of infection and disease.

Resumé of Literature

Only a few studies have been reported on experimental infection of primate species with Coxsackie viruses. The kind of infection developed by these primates varied; the earliest studies indicated that overt disease seldom occurred. Some species of monkeys were refractory (e.g., M. mulatta and C. aethiops sabaeus) (6, 7); at least, they showed no signs of illness, and failed to develop specific antibodies; however anatomical lesions associated with infection were not described. On the other hand, Melnick and Ledinko (7)

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noted that infection was acquired by cynomolgus monkeys (*M. cynomolgus* or *M. irus*) after oral administration of a Group B, type 2 virus (Ohio type). These monkeys developed fever; B 2 virus was often detected during the first week in the pharynx and in feces. At the end of the second week these monkeys developed specific complement fixing antibodies. Five of 6 cynomolgus monkeys developed signs of generalized weakness; however, sections prepared from brain stem, spinal cord and muscle were uniformly negative. Howitt and Nichols (8) did not obtain any signs of illness in cynomolgus monkeys infected with Group A viruses, types 2, 4 and 5. However, these viruses were recovered from sera, nasal washings and feces after inoculation by intracerebral, intramuscular and intravenous routes. Dalldorf (4, 9) also observed no signs of illness, other than a brief period of fever, following intracerebral and intramuscular injection of Group A, type 14 virus. The A 14 virus was recovered by subinoculation of mice with central nervous system (CNS) extracts obtained from 3 monkeys. Scattered areas of focal lesions similar to those of poliomyelitis were found in the spinal cords of these 3, and in 2 other monkeys. In the same report (9) Dalldorf commented on the occurrence of similar lesions in monkeys infected with A 7 Coxsackie viruses. Chumakov et al. (10) recovered a virus from feces of paralyzed children, which for a period was considered a 4th type of poliovirus; subsequently this agent has been identified as a Group A, type 7 Coxsackie virus (11, 12, 13). After parenteral inoculation of rhesus and cynomolgus monkeys with this A 7 virus, fever, tremors, ataxia and transient paralysis occurred and were associated with lesions quite like those seen in poliomyelitis, except for the presence of lesions in the forebrain.

Chimpanzees have been experimentally infected also with Coxsackie viruses. Infection in the chimpanzee after oral administration of these viruses is similar in many ways to the responses observed in cynomolgus monkeys (6, 14).

The present report summarizes the results obtained in 87 cynomolgus monkeys inoculated with 6 Group B and 1 Group A Coxsackie viruses. The strains were obtained from human beings, and had only a brief history of passage in the laboratory. All strains, except B6, were selected because they were recovered from persons with discrete kinds of illness.

**Materials and Methods**

**Virus Stocks.** The histories of 6 Group B and 1 Group A Coxsackie viruses used in the study are summarized in Table 1. The virus stocks used for inoculation of monkeys were fluids harvested from infected cultures of monkey kidney cells, maintained at 37 ± 0.5°C. Each of the stock viruses was tested for type-specificity, using reference Coxsackie antisera prepared in this laboratory.

**Monkeys.** Healthy cynomolgus (*M. irus*) monkeys, weighing between 2.2 and 3.5 kg. were used. Two monkeys occupied a single cage in a unit designed to contain 46 monkeys. Monkeys were examined daily.

**Inoculation of Monkeys.** Eighty-seven monkeys were inoculated by oral, intramuscular (i. m.), intracardiac (i. card.) or intraspinal (i. spinal) routes. A listing is given in Table 1 of the numbers of monkeys inoculated with each virus stock.