Prophylactic Therapy of Japanese Encephalitis Passive Immunization Combined with CO_2 Inhalation.* (28012)

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The high mortality and lack of effective methods of treatment for Japanese encephalitis (JE) led us to reconsider possible experimental approaches to the therapy of this disease.

Although the administration of specific antibody (AB) is highly effective in preventing arbor virus infection when given before exposure or early in the incubation period, this procedure has been largely ineffective when the AB is given late in the incubation period or after onset of clinical disease(1,2). Major factors preventing successful serum therapy are: first, the intracellular site of virus growth and second, the failure of AB to pass readily from the blood vascular system into extravascular nervous tissue—*i.e.*, to pass the blood brain barrier. A unique approach to the latter problem was suggested by the observation of Sellers and Lavender(3) that inhalation of increased concentrations of CO_2 greatly facilitates entry of poliovirus into the central nervous system (CNS) from the blood, but had no effect on spread of virus within the CNS. CO_2 inhalation also facilitates passage of trypan blue(4) and certain drugs into the brain(5). The present article describes the effect of hyperimmune serum combined with CO_2 inhalation on the course of experimental JE in Swiss mice.

Materials and methods. Experimental infection. The HV-1 strain of JE virus (JEV), isolated from a fatal case of JE in Taiwan in 1958(6) was used. Mice were from a colony derived from the Naval Medical Research Institute, NAMRI, strain. Weanling, 10 g mice were inoculated intranasally, by instilling dropwise 0.05 ml of a 1:100 dilution containing approximately 150 intranasal LD_{50} of virus. Mice infected in this way uniformly develop typical experimental encephalitis, with weakness, ataxia and paralysis, in 6-7 days and die by the 6th to 9th day. Recoveries have not been observed after encephalitis develops.

Antiserum. Munoz(7) has shown that mice injected intraperitoneally with antigens mixed with Freund's adjuvant develop large amounts of peritoneal fluid, containing specific antibody in high concentration. White adult mice (10-12 g) from NAMRU-2 animal-room were used. For the immunizations a line of HV-1 passed 4 times in suckling mice with an intracerebral LD_{50} of just over 10^8 in adult mice was used in order to minimize sensitization to myelin and possible demyelinizing encephalitis(8). Equal amounts of 10% suckling mouse brain antigen and Freund's adjuvant were mixed and emulsified in a syringe by pumping through a 19 gauge needle in a 37°C water bath. Each mouse received two to three 0.5 ml injections of antigen-adjuvant mixture given 2 weeks apart. Ascites was then allowed to develop for at least 3 weeks. The peritoneal cavity was tapped with a 21 gauge multiple-hole needle and the fluids were aspirated, pooled, centrifuged and finally passed through a Seitz Ek filter. Toxicity of peritoneal fluids was tested by injecting 0.5 ml intravenously into adult mice. The neutralization index (N.I.) of the antibody was measured by mixing undiluted fluid with serial dilutions of virus and injecting into adult mice intracerebrally(9).

Exposure to CO_2. Swiss mice weighing 8-10 g were exposed to CO_2 by placing them in a covered glass jar through which CO_2 from a cylinder was allowed to flow at a rate of 2 liters per minute and which was mixed with air flowing at a rate of 12 liters per minute thus producing a concentration of ap-

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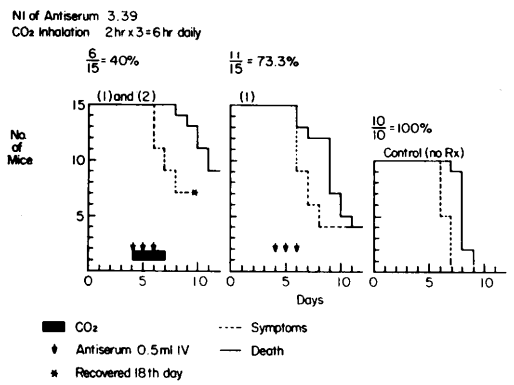

FIG. 1. Effect of treatment with antibody and with antibody combined with inhalation of increased concentration of CO_2 in Japanese encephalitis in mice.

proximately 14% CO_2 in air. Normal mice tolerated continuous exposure to this concentration of CO_2 for as long as 24 hours with no untoward effect except rapid, shallow breathing.

Experimental. Fig. 1 summarizes the first experiment in which 2 groups of 15 and 1 group of 10 mice, were inoculated intranasally. Four, 5 and 6 days later the 2 groups of 15 animals received 0.5 ml of AB, NI 3.4, intravenously. Immediately after each injection of AB, one group of 15 AB treated animals together with the 10 untreated controls were exposed to CO_2 in the manner described for periods of 2 hours, repeated 3 times daily on the 3 successive days of treatment with AB. A reduction in mortality occurred from 10/10 in untreated controls to 11/15 in AB treated mice and a further reduction to 6/15 in mice receiving AB combined with exposure to CO_2. Incubation periods were longer in the AB and especially the AB-CO_2 groups. A second experiment with a lot of AB having an NI of 5.1 gave reductions from 10/10 in controls to 13/15 in the AB treated group and to 8/15 in the combined AB-CO_2 group. Incubation periods in the AB treated groups were also prolonged in this experiment, particularly in mice exposed to CO_2.

Treatment begun on the fourth day precedes the onset of detectable encephalitis by 1-3 days. To determine whether treatment would be effective if begun after the onset of apparent disease, 3 pairs of 2 groups of 15 mice each were infected intranasally and treatment with AB or with AB followed by exposure to CO_2 was begun on the 4th, 5th or 6th days and was continued for 3 days following the first treatment for each of the 3 pairs. A group of 10 mice served as untreated, infected controls. In groups in which treatment was started on the fourth day, 8/15 receiving the combined treatment and 12/15 receiving AB alone survived. When treatment was started on the fifth and sixth days, only 3 animals receiving AB alone survived. None of the untreated controls and none of the animals receiving combined treatment on the fifth and sixth days survived.

Six mice receiving the combined treatment beginning on the fourth day, two in the first experiment, one in the second and three in the third, survived after having developed definite symptoms of encephalitis. One mouse receiving AB alone beginning on the fourth day also recovered from obvious encephalitis.

Discussion and summary. Combined treatment with AB followed by exposure to CO_2 protected a significant proportion of mice against lethal infection with JEV when treatment was started on the 4th day after inoculation, before disease was evident. (Variance ratio by analysis of variance for AB-CO_2 treated mice *vs* untreated controls greater than 0.01%.) AB alone was much less effective. We have not shown that the passage of AB into CNS tissue was increased by CO_2 treatment but this is the most reasonable explanation of our results in view of the adverse effect of CO_2, and the limited protection by AB alone. When the first treatment was delayed until the fifth or sixth day, there was no protection and most patients with JE are not seen by physicians until one or more days after onset of encephalitis. Thus effective therapy of human JE by this method is not promising.

On the other hand the experimental test was very rigorous. Intranasal inoculation of JEV is probably followed by invasion of the CNS *via* the olfactory nerve producing a generalized encephalitis before an effective AB

response occurs. In human infection which is usually inapparent, the introduction of virus into the skin is followed by proliferation of virus outside of the CNS but is rarely followed by CNS disease(10). More than half of those who do develop encephalitis recover. A significant factor in resistance to CNS invasion and in recovery after encephalitis is thought to be specific AB response. If so, administration of AB followed by exposure to CO_2 would augment the natural immune response and might be more effective in the human than in the experimental disease. That some AB treated mice did recover after development of CNS symptoms indicates that the precarious balance between host and parasite can be influenced favorably even when the CNS is invaded. Further experimental study of combined CO_2 AB therapy with an experimental disease more like human arborvirus encephalitis is desirable.

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Effect of *Salmonella enteritidis* Endotoxin Preparations and Lipid A on Dermal Reactivity in Rabbits to Epinephrine.* (28013)

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Although the biologic effects of bacterial endotoxins have been studied extensively, information on chemical and/or physical determinants of the endotoxin complex remains limited. Westphal and associates(1) have suggested that the lipid A portion of the complex may be responsible for some or all of the biologic effects (*e.g.,* fever, enhancement of resistance to experimental infection) other than antigenicity. The lipid A component of phenol-extracted *Escherichia coli* endotoxin also produces necrosis of sarcoma 180 in mice and alters dermal reactivity in rabbits to epinephrine(2,3). However, the lipid A was only approximately 1/10 as active as the original lipopolysaccharide from which it was isolated. The aqueous ether extraction method to prepare endotoxin from *Salmonella enteritidis* has yielded products of high activity and it has been shown that the biologic activity of these products does not bear a direct relationship to their lipid A content(4-6). It was suggested that the configuration of the entire complex plays an important role in the elicitation of host reactivity. The present study was undertaken to determine on a comparative basis the po-

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