supply of pathologically controlled, Walker 256 rat tumors at suitable stages of development.

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## The Effect of Unrelated Vaccines on the Localization of Paralysis in Mouse Encephalomyelitis. (18940)

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Recent studies of poliomyelitis suggest that the inoculation of unrelated prophylactic and therapeutic agents increases the risk of paralysis in the inoculated limb(1-4). The importance of the observation to immunization programs and its implications regarding the pathogenesis of poliomyelitis prompted us to seek an experimental counterpart so that the phenomenon might be studied in the laboratory. The results of mouse tests in which the Lansing strain of poliomyelitis virus was used were irregular but suggested that the injection of vaccine may hasten the onset of paralysis, as has recently been reported(5,6). It was thought that mouse encephalomyelitis might prove more suitable since it is a natural disease of mice and is in so many respects similar to poliomyelitis. The present report describes experiments in which the introduction of vaccines apparently did increase the frequency of paralysis in the inoculated extremity following the intracerebral injection of mouse encephalomyelitis virus.

*Methods.* The mice used in the present experiments were of the Albany Swiss and Standard strains. Groups of 20 to 60 animals, 3 to 5 weeks old, were assembled by stratified random sampling.

The prophylactic agents tested, fluid-medium pertussis vaccine(7), precipitated diphtheria-tetanus toxoid, precipitated diphtheria-tetanus toxoid with pertussis vaccine, and typhoid-paratyphoid vaccine, were prepared in this laboratory(8). The bacterial count of the pertussis vaccine and of the triple combined product was 20,000 million per ml; that of the typhoid-paratyphoid vaccine 1,500 million per ml. The vaccines and other test substances were injected subcutaneously in the left front leg in 2 doses of 0.1 ml amounts a week apart at varying intervals in relation to the virus inoculation. A front leg was chosen for the site of vaccine injection because the virus strain employed induces paralysis in the front legs less frequently than in the hind legs(9).

A TO strain of mouse encephalomyelitis (Theiler) virus, No. 4727, was used throughout. Stock 5% mouse brain suspensions were stored until use in a dry-ice cabinet. The virus was injected into the left cerebral hemisphere in doses of 0.03 ml of $10^{-2}$ or $10^{-5}$ dilutions. Control groups received virus or vaccine only or saline in place of vaccine followed by virus. The mice were observed daily for at least 21 days following the inoculation of the virus. Mice were destroyed as soon as paralysis appeared.

During the interval between the inoculation

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TABLE I. Local Reactions in Forelegs of Mice Following the Subcutaneous Injection of Various Vaccines.

| Vaccine | No. of mice | Degree of reaction* None | Slight to moderate | Severe | Impaired function |
|---|---|---|---|---|---|
| Pertussis | 74 | 7 | 44 | 20 | 3 |
| Pptd. diph.-tet. toxoid and pertussis | 80 | 0 | 17 | 43 | 20 |
| Pptd. diph.-tet. toxoid | 78 | 10 | 63 | 5 | 0 |
| Typhoid-paratyphoid | 78 | 75 | 3 | 0 | 0 |
| Pertussis bacilli of vaccine | 108 | 0 | 42 | 63 | 3 |
| Supernate of vaccine | 104 | 102 | 2 | 0 | 0 |
| Saline | 76 | 75 | 1 | 0 | 0 |

* The mice with *slight to moderate* reactions were those with the least degree of swelling that could be palpated or seen. Those with *severe* reactions had more extensive and marked induration but no impairment of gait. Mice with *impaired function* were excluded from the tests.

of the mouse encephalomyelitis virus and the predicted appearance of paralysis the animals were carefully examined for local response to the vaccines. The degree of reaction was graded and all mice in which the local reaction might obscure or resemble paralysis were excluded from the experiments. As may be noted in Table I, inflammation was most frequently found following the injection of pertussis vaccine alone or in combination with precipitated diphtheria and tetanus toxoids. The reactions induced by the toxoids were less severe and typhoid-paratyphoid vaccine, saline, and the cell-free supernate from fluid pertussis vaccine caused little or no reaction.

*Results.* Of the prophylactic preparations tested, pertussis vaccine either alone or combined with toxoids yielded the most striking effects. Nine experiments involving more than 800 mice have been performed in which pertussis vaccine alone was used. In each case the frequency of paralysis in the inoculated limb was greater than in the control groups. The total score showed the frequency to be approximately 7 or more times greater than expected (Table II, Group 1). In each experiment the mean interval between the inoculation of virus and the onset of paralysis was shorter in the vaccine-treated groups. The differences were not statistically significant but the consistency in the 9 experiments proved to have a probability factor of 1:512. The frequency of paralysis *irrespective of location* was no greater in the vaccine-treated than in the control groups. The inoculation of saline did not have a measurable effect.

Experiments have been performed in which pertussis vaccine was given at various times in relation to the inoculation of virus. These also are summarized (Table II, Group 5). The localization of paralysis in the injected extremity was commonest in those mice which had received vaccine prior to or on the same day as the virus. When both doses of vaccine were delayed until several days after the virus, there was no effect.

Tests were made separately of the bacterial elements and the supernatant fluid of pertussis vaccine. The sedimented cells were washed once and resuspended in saline. The supernatant fluid was filtered through a Berkefeld candle. The results indicate that the effect is due to the cellular rather than the fluid component (Table II, Group 2).

The experiments in which diphtheria-tetanus toxoid and typhoid-paratyphoid vaccine were used are also summarized in Table II, Groups 3 and 4. The first yielded results similar to but less marked than those of pertussis vaccine. The inoculation of typhoid-paratyphoid vaccine had no effect.

It is interesting to note that the inoculation of vaccine alone has never been followed by paralysis although more than 600 mice so treated have been observed for 35 days or more, and the majority of these were presumably latently infected with mouse encephalomyelitis virus(10,11). Future experiments of

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TABLE II. Frequency of Foreleg Paralysis Following Injection of Vaccines.

| Group No. | Vaccine injected | Time in relation to virus | No. of mice | Mean incubation period, days | Paralyzed No. | Paralyzed in injected foreleg* No. | % |
|---|---|---|---|---|---|---|---|
| 1 | Pertussis | | 188 | 11.7 | 146 | 49 | 34 |
| | Saline | | 193 | 12.9 | 151 | 7 | 5 |
| | Pertussis | 7 days before | 224 | 12.7 | 155 | 52 | 33 |
| | None | and on same day | 239 | 14.4 | 164 | 4 | 2 |
| | Pertussis† | | 60 | 5 | 53 | 40 | 75 |
| | None† | | 62 | 8.3 | 58 | 4 | 7 |
| 2 | Pertussis | " | 100 | 13.9 | 63 | 22 | 35 |
| | Cellular component | | 105 | 14.2 | 66 | 17 | 26 |
| | Fluid component | | 104 | 14.5 | 69 | 1 | 1 |
| | None | | 98 | 14.3 | 57 | 1 | 2 |
| 3 | Pertussis | " | 138 | 11.7 | 106 | 34 | 32 |
| | Pptd. diph.-tet. toxoid | | 142 | 13 | 111 | 19 | 17 |
| | Pptd. diph.-tet. toxoid and pertussis | | 131 | 12.6 | 104 | 34 | 33 |
| | Saline | | 144 | 12.9 | 118 | 6 | 5 |
| 4 | Typhoid-paratyphoid | " | 78 | 13.8 | 64 | 1 | 2 |
| | Saline | | 76 | 13.3 | 58 | 1 | 2 |
| | None | | 79 | 13.7 | 69 | 2 | 3 |
| 5 | Pertussis | 14 and 7 days before | 29 | | 21 | 8 | 38 |
| | " | 7 days before and on same day | 28 | | 24 | 10 | 42 |
| | " | 4 days before and 3 days after | 30 | | 21 | 9 | 43 |
| | " | Same day and 7 days after | 31 | | 22 | 8 | 36 |
| | " | 4 and 11 days after | 30 | | 23 | 2 | 9 |
| | " | 7 days after (1 dose only) | 29 | | 21 | 3 | 14 |
| | None | | 30 | | 23 | 1 | 4 |

* Does not include mice paralyzed in both front legs.

† Virus inoculated intracerebrally as 0.03 ml of a $10^{-2}$ dilution of infected mouse brain; in all other experiments a $10^{-5}$ dilution was used.

this kind will be undertaken. The association of recent immunization and poliomyelitis in man could be due to the activation of a latent infection, the localization of active infection occurring during a critical period following vaccination, or to the introduction of virus with the vaccine. Nothing in the present experiments lends support to either the first or third hypothesis but neither should be dismissed because of mouse experiments.

Whether the paralysis-localizing and the inflammation-producing activities of the vaccine are identical remains to be proven. The present experiments suggest such an association.

*Conclusions.* Mice injected in a foreleg with pertussis vaccine alone or combined with precipitated diphtheria-tetanus toxoids were frequently paralyzed in the injected foreleg following infection with mouse encephalomyelitis virus. The frequency of paralysis in the inoculated extremity was 7 or more times greater than among the untreated controls.

Precipitated diphtheria-tetanus toxoid had less effect and typhoid-paratyphoid vaccine had none.

The injection of vaccines did not increase the frequency of paralysis, irrespective of location, but pertussis vaccine consistently shortened the period between the introduction of virus and the onset of paralysis.

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