Effect of Pertussis, Diphtheria Toxoid and Salmonella Immunization on Experimental Poliomyelitis.* (18823)

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Recent clinical evidence in Australia and England suggests that pertussis vaccine given alone or in combination with diphtheria toxoid during the poliomyelitis season results in a significantly increased frequency and clinical severity of paralysis in the inoculated limb(1-4). No significant difference was noted between diphtheria toxoid injected alone or combined with pertussis vaccine. The great majority of the intervals between the last inoculation and onset of poliomyelitis was less than 30 days. There was no evidence that inoculations carried out 3 months or more before the onset of illness had any such effect. In all of the above reports the usual 1:2 or 1:3 ratio of arm to leg paralysis was reversed in young children who received prophylactic injections predominately in the arms within a 28-day period prior to the onset of the disease. A recent editorial(5) in the *J.A.M.A.* summarizes clinical reports on the relationship between poliomyelitis and prophylactic immunizations.

The present studies were undertaken to study the effect of pertussis vaccine alone or in combination with diphtheria toxoid on the incubation period of Swiss mice inoculated with the Lansing strain of poliomyelitis virus. Experiments showing the effect of vaccinating Lansing virus infected mice with *Salmonella typhimurium* vaccine were also done.

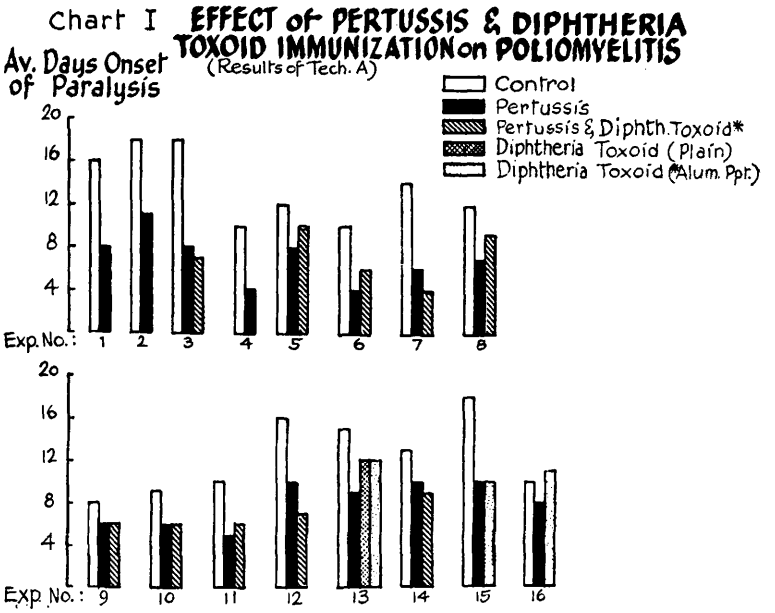
Materials and Methods. Groups of Swiss mice (Plymouth strain) weighing 13 to 15 g were inoculated intracerebrally with approximately 10 LD_{50} units of the Lansing strain of poliomyelitis virus. The identity of the Lansing strain used was verified periodically by neutralization tests with hyperimmune Lansing antiserum. Mice inoculated in each experiment varied from 20 to 80 in number and were equally divided into vaccinated and control animals. The test mice were vaccinated intraperitoneally on the first, third, and fifth days after virus inoculation with 0.5 cc of pertussis vaccine containing 7.5 billion organisms or 0.25 cc combined alum precipitated diphtheria toxoid and pertussis vaccine (3.75 billion organisms). The alum precipitated diphtheria toxoid contained ⅛ of a human immunizing dose. A few experiments were also carried out in which the mice were injected intraperitoneally with 0.25 cc of alum precipitated diphtheria toxoid alone or plain diphtheria toxoid. The alum precipitated toxoid contained ⅛ of a human immunizing dose, while the plain toxoid contained 1/12 of a human immunizing dose. The pertussis vaccines and diphtheria toxoids used were biological products prepared by the Illinois Department of Public Health for human use and were obtained through the courtesy of Dr. H. J. Shaughnessy and Mr. John Neal. The control mice in most experiments were inoculated intraperitoneally (0.5 cc) with nutrient broth after the same intervals.

The *Salmonella typhimurium* vaccine which was used was prepared from a 24 hour culture grown on trypticase agar, killed by heating at 56°C for 1 hour, and adjusted to a suspension containing approximately 1 billion organisms per cc with 0.85% saline solution. The vaccine was preserved with 1:10,000 merthiolate and stored in the refrigerator at 3 to 5°C.

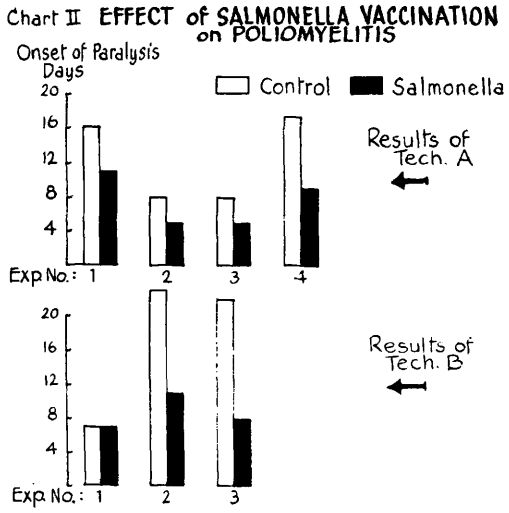
Results. Pertussis Vaccine and Diphtheria Toxoid. Results obtained in a series of 16 experiments carried out by technician A are

* Supported in part by the Michael Reese Research Foundation. Read before the Society of American Bacteriologists, May 29, 1951, Chicago, Ill.

1. Martin, J. K., *Arch. Dis. Childhood,* 1950, v25, 1.
2. McCloskey, B. P., *Lancet,* 1950, v1, 659.
3. Geffen, D. B., *M. Officer,* 1950, v83, 137.
4. Hill, A. B., and Knowelden, J., *Brit. M. J.,* 1950, v2, 1.
5. Editorial, *J.A.M.A.,* 1950, v144, 240.



given in Chart 1. The chart shows the average incubation period in days after virus inoculation for onset of paralysis in the control and various vaccinated mice. The range of incubation period for both groups varied from 3 to 30 days. Over 500 mice were studied in this series, and there was a minimum of 10 mice in each group of control or vaccinated mice in every experiment. Mice in the first 8 experiments were inoculated with the same pool of Lansing virus, while those in the second group of 8 experiments received a different batch of virus. Chart 1 also shows the results obtained in identical experiments carried out independently by technician B. Technician B, however, used a different pool of Lansing virus. The graphs in Chart 1 show that a consistent significant reduction in the average incubation period before onset of paralysis was obtained in all experiments in which mice were vaccinated with pertussis vaccine alone or combined with alum precipitated diphtheria toxoid. Statistical analysis of these data was carried out by Dr. Herbert Silverstone using the Student-Fisher t test.† According to Dr. Silverstone the differences obtained between the vaccinated mice and controls in all experiments except with the alum precipitated toxoid alone in experiment 16 are significant because the probability value (P) varied from < 0.01 to < 0.001. There was no significant difference between the groups of mice receiving pertussis vaccine alone or the pertussis-diphtheria toxoid mixture. Moreover, there was no sig-



† As the variance of each experimental group was proportional to the mean, the statistical tests of significance were conducted with the logarithms of the days of paralysis. The mean values presented are the geometric means. (Bartlett, M. S., *Biometrics*, 1947, v3, 39).

nificant difference in average survival time after onset of paralysis between the control and vaccinated mice in most experiments.

Salmonella Vaccine. We reported(6) that autolyzed brain diluent shortens the incubation period and facilitates the transfer of experimental poliomyelitis. Since then we and others have been unable to reproduce these results. We noted, however, that at the time that we carried out the original experiments with the autolyzed brain diluent the strain of mice (CFW) which we used were infected with *Salmonella typhimurium.* The signs of the infection were readily controlled by feeding the mice sulfadiazine, although the carrier state persisted. It occurred to us that perhaps it was the *Salmonella* rather than the autolyzed brain which was responsible for the enhancing effect, especially since it was found that feeding sulfadiazine to virus inoculated, *Salmonella*-free mice had no significant effect. In order to test this hypothesis, a heat-killed *S. typhimurium* vaccine containing one billion organisms per cc was prepared and injected intraperitoneally (0.5 cc) following virus inoculation in the same manner as described in the pertussis-diphtheria toxoid experiments. The results obtained were too equivocal to be significant although some experiments were highly suggestive.

Recently we have repeated these experiments by inoculating the *Salmonella* vaccine intravenously instead of intraperitoneally. Groups of mice were inoculated intracerebrally with approximately 5 LD_{50} units of Lansing virus. On the first and third days following the virus injection, they were inoculated intravenously with 0.1 cc of *Salmonella* vaccine (100 million organisms). The inoculated mice showed little or no reaction to the *Salmonella* vaccine. Similar numbers of mice were used in the control and vaccinated groups as for the pertussis-diphtheria toxoid experiments. The results obtained by technicians A and B working independently are shown in the graphs of the 7 experiments which were done in Chart II. Except for the results obtained in Exp. 1 by technician B, Dr. Silverstone states that the results are statistically significant in analysis by the t test. The probability value (P) varied from <0.01 to <0.02. In other experiments it was found that *Salmonella* vaccine inoculated intracerebrally (0.03 cc) at the same intervals following virus inoculation had a similar effect in decreasing the incubation period.

Discussion. These experiments indicate that pertussis vaccine given alone or in combination with alum precipitated diphtheria toxoid by intraperitoneal injection after previous intracerebral inoculation with the Lansing strain of poliomyelitis virus significantly decreased the incubation period before onset of paralysis. There was no significant difference between the mice vaccinated with pertussis vaccine or the pertussis-diphtheria toxoid combination. Alum precipitated diphtheria toxoid alone also significantly reduced the interval before onset of paralysis in 2 out of 3 experiments. It was impractical to inject the vaccines in the extremities in these experiments in order to determine the site of onset of paralysis and thereby duplicate the clinical observations. Obviously, a large inoculum would lead to disuse of the limb and confuse the findings. Perhaps the use of larger animals such as the rhesus monkey would successfully overcome this difficulty.

The fact that we were also able to show that heat-killed *Salmonella typhimurium* vaccine injected intravenously after previous intracerebral Lansing virus inoculation also significantly decreased the interval before onset of paralysis in 6 out of 7 experiments may indicate that the effect of vaccination on poliomyelitis is a nonspecific stress stimulus. Possibly the mechanisms involved are similar to those in the so-called "alarm reaction," *i.e.,* the higher incidence and more severe paralysis which occurs in monkeys subject to fatigue and chilling during the incubation period of poliomyelitis(7). We are unable to explain our observation that intraperitoneal inoculation of the *Salmonella* vaccine gave equivocal results, while the intravenous or intracerebral

6. Milzer, A., and Byrd, Jr., C. L., *Science,* 1947, v105, 71.

7. Levinson, S. O., Milzer, A., and Lewin, P., *Am. J. Hygiene,* 1945, v42, 204.

routes were effective. As already indicated we now believe that it was the *Salmonella* infection in our stock mice rather than the autolyzed brain diluent which was responsible for the apparent enhancing effect of the latter which we previously reported(6).

When the present studies were nearly completed, the paper by Findlay and Howard(8) appeared in which they reported similar findings in mice injected with diphtheria toxoid or pertussis vaccine plus diphtheria toxoid following intracerebral inoculation with the Lansing strain of poliomyelitis virus. Their experiments differed from ours, however, in that all of the vaccines were given intravenously rather than intraperitoneally. They also reported that T.A.B. vaccine given intravenously had a similar effect.

Summary. Swiss mice infected intracerebrally with the Lansing strain of poliomyelitis virus show a significantly decreased incubation period before onset of paralysis following intraperitoneal inoculation with pertussis vaccine, pertussis-diphtheria toxoid or diphtheria toxoid alone. The results obtained were based on a series of 20 experiments carried out by 2 technicians working independently and involving over 650 mice. Similar results were also obtained in 6 out of 7 other experiments in which infected mice were injected intravenously with *Salmonella typhimurium* vaccine. Evidence is presented that *Salmonella typhimurium* is apparently responsible for the previously reported enhancing effect of autolyzed brain tissue diluent.

Addendum. We have found that 30,000 units of procaine penicillin G in peanut oil given intraperitoneally at the same intervals as the pertussis vaccine had no significant effect on the incubation period before onset of paralysis.

8. Findlay, G. M., and Howard, E. M., *J. Path. and Bact.*, 1950, v62, 371.

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Beneficial Effect of Liver Feeding on Swimming Capacity of Rats in Cold Water.* (18824)

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Considerable data are available indicating that in addition to the known nutrients substances are present in our diet which may be required in increased amounts during conditions of stress. Such factors are apparently dispensable under normal conditions, or their requirements are so small they may readily be met by amounts present in the diet or through the synthetic activity of the intestinal flora or the animals' own tissues. Certain drugs or other "stress factors" may, however, increase requirements for these substances to such an extent that deficiencies occur, manifested by retarded growth or tissue pathology, and preventable by the administration in appropriate amounts of the missing nutrient (1,2). Whole liver is a potent source of such unknown nutrients. In the present communication data are presented which indicate that whole liver contains a factor, apparently distinct from any of the known B vitamins, which significantly increased the capacity of rats to withstand the stress of swimming in cold water.

Procedure. The basal ration employed in the present experiment consisted of sucrose,

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1. Ershoff, B. H., *Physiol. Rev.*, 1948, v28, 107.

2. Ershoff, B. H., Nutrition Fronts in Public Health, 1951. The National Vitamin Foundation, Inc., New York.