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DEPARTMENT OF THE ARMY
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Title: New model experiments for the prevention of intestinal infections
(Neue Modellversuche zur Infektabwehr des Darmes)

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April 1969

Oral immunization with inactivated microorganisms has been attempted thus far only in the case of infections of bacterial origin. Results were obtained long ago in experiments with typhoid, paratyphoid, dysentery, and cholera (1). The mechanism of action involved in this type of vaccination was still unknown up until a few years ago and even then the information obtained from animal experimentation and epidemiological studies was of an empirical nature only. Only recently has one attempt to elucidate the nature of the localized immunity of the intestines using scientific methods. (2-4)

Oral immunization using inactivated enteropathogenic virus has thus far not been attempted. In animals experiments, it was quite convincingly demonstrated that bacteriophages, when employed as a pathogenic model, will pass through the mucous membranes of the intestinal tract quite rapidly and regularly and appear in the blood stream after only a few minutes (5 - 9). On the basis of the results obtained in the aforementioned experiments, it was concluded that inactivated virus should also exert a Herbst effect (Volkheimer, 10) and be absorbed into the intestines and thereby become effective antigens as a result of their contact with lymph vessels and the blood in spite of the absence of specific processes of infection and reproduction.

If this hypothetical considerations are correct, then oral immunization with inactivated viruses should be possible. For my first experiments in this direction, I chose the parapoliomyelitis infection of the mouse which is caused by the Columbia-SK virus of Jungeblut. This model has the advantage that the experimental animal, the mouse, becomes ill and dies regularly in a characteristic manner after an oral infection. I examined the reabsorption viremia of this model of infection (11). The origin of the virus, the techniques for infection, the dosage and the method of evaluation can be found in the reference mentioned.

The four vaccines examined thus far and the results obtained in animal experiments are summarized in the Table. In the specific oral immunization with heat-inactivated Col. SK virus, brains from mice that had become ill and showed typical paralysis were homogenized and the supernatant was inactivated by heating at 60 C for one hour. The loss in infectivity was determined by intracerebral injections of the material into young mice. In this manner, one could assure that there were no reproducible Col. SK virus remaining which could lead to a light immunizing infection.

The virus titer was determined before inactivation by intracerebral injection into young mice. More than half of the animals consistently died when they were injected with the supernatant from a homogenized brain at a dilution of 10^{-7}. On the other hand, they survived when injected with a dilution of 10^{-8}. The inactivated virus vaccine was given in quantities of 0.25 ml at daily intervals for six days and was applied orally with a throat swab. Three days after vaccination, oral infection was introduced. The results of the experiment were determined by measuring the mortality of the experimental animals at the end of the experiment which was six days after oral infection. A comparison of columns 4 and 5 in the table shows that mice which have been vaccinated orally with the inactivated vaccine have significantly more protection than the control animals. The effect of vaccination is very high - 71.2 (column 9). Columns 6 and 8 show that the differences in mortality is statistically significant in spite of the relatively small number of animals employed.

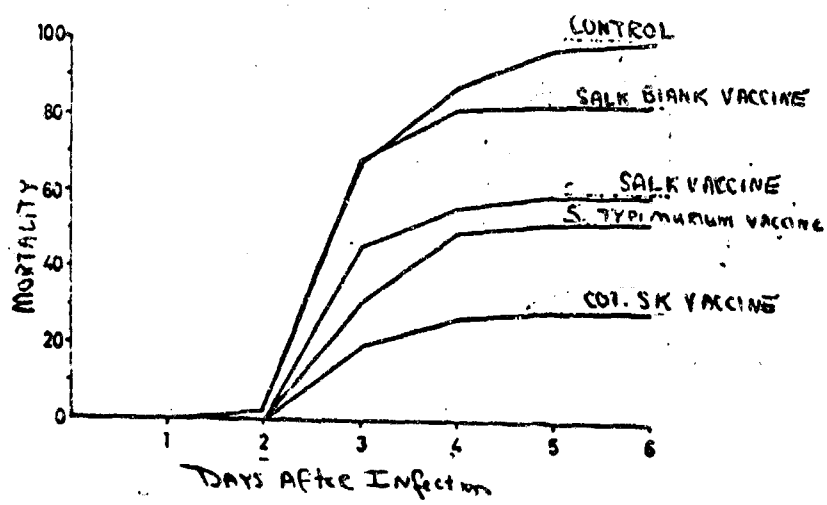
Using the model of the S. typhimurium infection of the mice, I was able toldemonstrate that not only specific oral immunization is effective by also nonspecific immunization with other salmonellae or with Coli bacteria(12)

The question of specificity therefore was examined also in the virus model. First of all, it was obvious that one should use inactivated polyiomyelitis virus for unspecific oral immunization. For this purpose, a commertial trivalent Salk vaccines was used. The vaccine was used in equal volumes and at the same time intervals and the animals were treated at the same point in time. This holds true also for the two following experimental groups. The third horizontal column of the Table shows that im this experimental group, significantly more experimental animals were employed. The difference in mortality between the vaccinated and non-vaccinated animals is not as great as in the first group and corresponds to a value of 39.7. Column 8 of the Table shows that the decrease in mortality by oral immunization is statistically significant at a high degree. Because we are dealing here with nonspecific protection, it can also be assumed that vaccine additive such as tissue culture constituents, antibiotics, or preservatives may have modified the reaction of the intestinal epithelium to Col. SK infection. As a result, a control series of tests in which a blank vaccine which had exactly the same composition as the Salk vaccine except that it contained no inactivated Polio virus was used, was carried out. The difference in the final mortality was small and the value of 19.9 lies well within the statistical deviation. From a comparison of these two sets of results, it can be concluded that the effective substance in the Salk vaccine is the poliomyelitis virus.

In following up the question concerning the nonspecific effect of oral immunization in the case of this virus model, a vaccine was chosen which was completely unrelated to the Col. SK virus either with regards to its antigenic composition or its pathogenic effect. My choice in this regards was the inactivated Breslau vaccine. The reasons for selecting this vaccine cannot be discussed in this paper. The nonspecific oral immunization with inactivated S. typhimurium bacteria was unexpectedly successful with regards to oral infections with virulent Co. SK virus as can be seen in the second horisontal

column of the summarizing table. The decrease in mortality as a result of this vaccination was almost half and the statistical value of 47.4 was quite significant and markedly better than that obtained after oral vaccination with the trivalent poliomyelitis vaccine.

The results that have been obtained thus with various antigens are shown in Figure 1 where the final mortality of the control animals has been set at 100. The visual effect of this figure confirms the results shown in the Table.



The specific oral immunization with heat inactivated Col. SK virus caused the largest decrease in mortality. Salk and Breslau vaccines follow and are quite close to each other. The results obtained with a blank vaccine are identical to the controls within statistical deviations. This graphic representation, furthermore, shows an additional effect of oral vaccination. The three curves of the specific and nonspecific immunization rise distinctly later than those of the controls and the blank vaccine. This indicates that the oral vaccines prolong the median survival time or , in other words, that the vaccinated animals, although they died, received protection in as much as they died later than did the control animals.

TABLE I

SUMMATION OF THE VARIOUS STUDIES WITH ORAL IMMUNIZATIONS AGAINST AN ORAL INFECTION WITH COL. SK VIRUS

Antigen	Number of mice	Number of single studies	Mortality in % Vaccinated	Mortality in % Unvaccinated	Statistical Security: Observed difference D_o	Statistical Security: Largest allowable probable difference D_a	$D_o:D_a$	Value
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)
heat-inactivated Col. SK virus	185	4	9.0	31.3	22.3%	17.5%	1.28	71.2
heat-inactivated S. typhimurium bacteria	802	7	24.3	46.1	21.8%	9.8%	2.23	47.3
Commercial trivalent Salk vaccine[1]	1402	14	29.0	48.0	19.0%	7.6%	2.50	39.7
Salk blank vaccine[2]	275	2	23.8	29.7	5.9%	17.5%	0.34	19.9

[1] The Salk vaccine for the first five studies was from the S.A. R.I.T. N.V., Genval, Belgium while that used in the remaining studies was from the Behring Co., Marburg.

[2] I would like to thank Dr. Hennessen, Behring Co., Marburg, for the donation of this control vaccine.

The mean mortality values have been evaluated in the summary table as well as in Figure 1 (compare column 3 of the table). In this manner, a general view of the results was obtained. However, this procedure can lead to erroneous conclusions when extreme results from a single experiment distort the mean values. As a result, in Figure 2 the stability of the effect of oral immunization has been presented graphically. This method of observation could also lead to the wrong conclusion. Nonspecific vaccination with S. typhimurium whole antigen clearly showed the most stable action. The values obtained from seven single experiments are very close to the mean value as can be seen from Fig. 2 and in column 7 of the table. In comparison, the values obtained with the Salk vaccine fluctuate much more with relation to the mean value. Also, of the three experiments with very high values, two experiments gave values which could be considered to be "outliers" in that the mortality in these experiments was higher than that observed with the controls (unvaccinated animals). The four single experiments with oral vaccination with Col. SK virus do not permit a definite statement with regards to specific immunization; the results obtained in the third experiment which deviate strongly do not have a great deal of significance since the number of animals in that experiment was very small. This can also be seen in Fig. 2. The values obtained from this experiment hardly influenced the mean value. In the following discussion, it is important to keep in mind the fact that it is more important to consider which nonspecific vaccination protects most reliably against a virulent infection rather than which one protects best.

No confirmation of our results on the basis of experimental animal studies reported in the literature is possible since oral immunization with inactivated virus has not been reported on. Here we must satisfy ourselves with some hypothetical thoughts concerning the still unknown mechanism of action with these antigens. On the basis of the experiments conducted thus far on bacterial

intestinal infections, coproantibodies may be responsible for the effects of oral immunization (2-4). Accordingly, one should first examine the virus model. The specific effect of antibodies becomes important only in the case of specific vaccination with Col. SK virus. When examined from a statistical point of view, coproantibodies could explain only the differences in the mortality values between specifically and nonspecifically immunized animals. In the case of the reduction of mortality following nonspecific oral vaccination, one has to examine other possibilities to explain this effect. In the case of vaccination with the Salk vaccine, one could assume that, after contact of the virus with the intestinal epithelium, an interferon or an interferon-like substance is excreted into the intestinal lumen where it has some type of a protective action. Since the

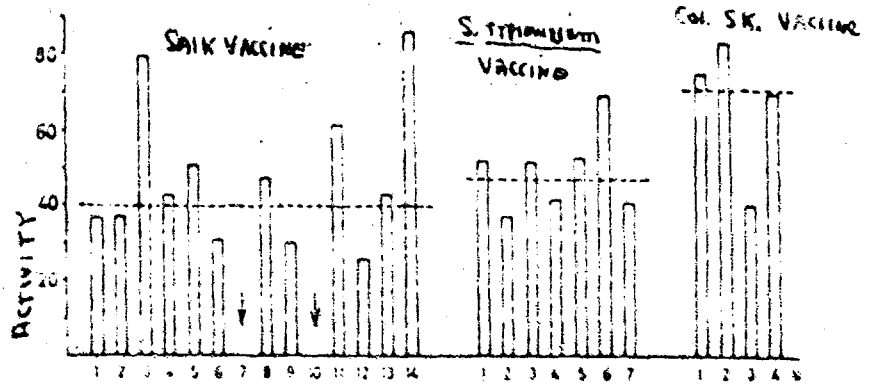


Figure 2.

formation of interferon can be stimulated also by inactive viruses and since its action is not specific, this assumption can be considered as a possibility. At first glance, one would consider this possible explanation only in the case of nonspecific immunization with inactivated poliomyelitis virus but not in the case of immunization with S. typhimurium. However, since Stinebring and Youngner reported (12) that interferon could be detected for a short time in the plasma of mice which had been administered S. typhimurium antigen intravenously, it is reasonable to look for interferon or an interferon-like

substance in animals after oral vaccination with inactivated S. typhimurium bacteria. We have started experiments with this in mind.

In summary, I would like to propose that it is possible to increase eith specifically or nonspecifically the defense of the intestine against enterotr ic viral infections by means of oral vaccination with inactivated microorganisms

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REMARKS DURING DISCUSSION

H. Raettig (Berlin): During the entire course, it can be only a question of interference. The time just allows for that. Only through this phenomenon could one explain the protective action by heterologous viruses since interfe nce is host-specific but not agent-specific. Interferon can even be stimulated

cellular DNA and RNA as has been shown in 1963 by Rotsne, Kohlhage and others. It can even be stimulated by polysaccharides as has been shown recently by Kleinschmidt.

H. Stickl (Munich): With reference to the question of nonspecific mechanisms in the prevention of infection, there are two models for the competetive occupation of cell receptor sites that have been studied: the influence of the immunocytolysis reaction by reaction with a noncorresponding antigen and the protective action of bacterial lipopolysaccharides in experimental influenza infections of the mouse.