Active Immunization against Pertussis. Immunization with Live Antigen.* (19293)

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(Introduced by L. E. Holt, Jr.)
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Active immunization against pertussis has made great strides in recent years, due largely to the use of freshly isolated Phase I cultures. There is still some discrepancy in the reports as to its efficacy(1-4), but it is quite clear that neither the degree of protection nor the duration of immunity is comparable to that conferred by an attack of the disease. The inferior immunization power of killed vaccine may be attributed to some alteration of the antigen resulting from the procedure used to kill the organisms. It occurred to us that the use of a live vaccine might be feasible in the case of *H. pertussis*. Since the organism is known not to be invasive, it should not be capable of producing the actual disease when injected at a site other than the respiratory tract. An examination of the literature disclosed two attempts to use a live pertussis vaccine. Nicolle and Connor(5) reported the use of a live vaccine, administered subcutaneously, in the treatment of this disease. They presented no convincing evidence of the efficacy of this procedure; marked local reactions were not encountered. More recently, Lapin(6) carried out intradermal inoculations with live *H. pertussis* in human subjects. He obtained no evidence of spreading infection, but encountered local reactions definitely more severe than those resulting from killed vaccine.

In our studies we undertook (1) to compare the effectiveness of live and killed vaccines in protecting mice, and (2) to explore the feasibility of vaccination with living organisms in man.

Animal experiments. The general plan of these experiments was as follows: White mice, 10-12 g in weight, obtained from Rockland Farms were inoculated intramuscularly with graded doses of living or killed pertussis organisms in equal numbers. After a varying period of time these mice were challenged by intracerebral inoculation(7) of living organisms, and the survival rates studied. It was decided to employ the intramuscular route of immunization, although the intraperitoneal route is most commonly employed when killed vaccine alone is tested. Large doses of live pertussis organism were found to cause toxic deaths when given to mice intraperitoneally. When administered intramuscularly doses up to 1.5 billion organisms in 0.1 ml volume caused local necrotic lesions and death only infrequently. Two sets of experiments were carried out in which the strain of organism used and the killing procedures were varied slightly. In Exp. I a total of 270 mice were used; 180 mice were immunized, 90 mice served as controls. The organism used for immunization was *H. pertussis* strain No. 18323 obtained from Dr. Pearl Kendrick, Mich. Dept. of Health(7). The live vaccine was made as follows: Lyophilized strain No. 18323 was grown on modified Bordet-Gengou Media(8). The 24-hour growth (6th subculture) on this media was removed and suspended in 1% Casamino Acids, P.H. 7.0-7.2. The suspension was centrifuged at low speed to remove clumps of bacteria, and the turbidity adjusted by means of a Klett photoelectric colorimeter to an equivalence of 15 billion org. per cc. Suitable dilutions for inoculation were made from this stock suspension. The dilutions were immediately injected to insure maximum viability. The stock killed vaccine was prepared several weeks earlier in a similar fashion. Merthiolate to a concentration of 0.001% was added, and the suspension stored in an ice chest. It was found to be non-viable after approximately 3 weeks storage at 5°C.

Groups of 30 mice were inoculated with 3 different doses of live and killed organisms and after a period of 21 days were challenged intracerebrally with approximately 100 LD/50 of the homologous strain No. 18323. The

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TABLE I. Experiment I. Protective Effect of Live and Killed Vaccine in Mice.

Immunizing dose, billions	Killed vaccine, survival/total	Live vaccine, survival/total
.06	5/21	5/23
.30	1/21	7/24
1.50	2/25	15/26

All animals were challenged intracerebrally with 50000 organisms strain #18323. Virulence control —LD_{50} (in control mice at age of immunization) = 598 organisms. Age control—LD_{50} (in control mice at age of challenge) = 523 organisms. Non-immunized mice challenged intracerebrally with 50000 organisms, Strain #18323 = 0% survival.

result is shown in Table I.

Among the animals given killed vaccine there was a larger proportion of survivals with the smallest dose than with the larger doses. However, the survival rate in all 3 groups of this vaccine was of such low magnitude that there was no clear dosage-survival rate relationship. The results might therefore be attributed to chance (random) fluctuation. The chi square test was applied to a comparison of the live and killed vaccine and gave the following results:

Dose

0.06—Obviously not significant

0.3 —Not significant at 5% level.

1.5 —Highly significant; $P < 0.001$.

A second series was carried out to confirm this observation and in addition, to determine whether there were any differences in duration of the acquired immune levels. 424 mice were used, 360 being immunized and 64 serving as controls. In this series a freshly isolated strain of *H. pertussis* was employed. The live vaccine was prepared as described above. The preparation of the killed vaccine was varied slightly from the technic previously employed. To a 72-hour Bordet-Gengou culture suspended in saline, Merthiolate was added, and the stock suspension was rendered non-viable at 5°C.

Groups of 15 mice each were injected with specified doses of live and killed organisms and, after periods of 10, 17, 24 and 120 days they were challenged intracerebrally with 50,000 organisms of *H. pertussis* strain No. 18323. The results of this series are shown in Table II.

The data of Table II were subjected to analysis of variance to differentiate between the following sources of variation:

(1) Killed versus live vaccine
(2) Times of challenge
(3) Immunizing dosage levels
(4) Interaction of (1) and (2)
(5) Interaction of (1) and (3)
(6) Interaction of (2) and (3)
(7) Triple interaction, (1), (2) and (3)

The only items that reached the 5% point of significance were No. (1) and (3). For these the variance ratios (Snedecor's F) were: Between killed and live vaccine—F = 9.97; probability between 5% and 1% points. Dosage levels—F = 20.99; probability between 1% and 0.1% points.

If the differences in time of challenge, No. 2, had any effect, it was not great enough to reach the ordinary standard of significance. Both with the live and killed vaccine whatever immunity was attained at 10 days persisted without consistent change at 120 days.

Since all the inter-actions were non-significant, the significant differences between the effects of doses are equally applicable to both types of vaccine and all times of challenge. The significant differences between killed and live vaccine can be taken as equally applicable at all dosage levels and all times of challenge.

The magnitude of the differences which are proved significant in the above analysis can be indicated by pooling the data and estimating percentages of survivals. When the results of the 4 challenges are combined with respect to size of immunizing dose, it is seen that with the smallest dose (0.06B) the substitution of live for dead vaccine increases the survival rate from 11.3% to 21.0%; in the intermediary group (0.3B) the increase is from 29.3% to 59%; and with the largest dose (1.5B) from 53.8% to 65.9%. When all animals of each type of vaccine are pooled, irrespective of dose, the survival rates are: dead vaccine—31.3% and live vaccine—46.7%. The difference is 15.4%.

It was of interest to determine whether the better results with live vaccine were attributable to multiplication of the organism at the site, after inoculation. The following experiment was therefore performed to test this hypothesis. A group of 24 white mice, 15-16 g, obtained from Rockland Farms were inoculated intramuscularly with 200,000 live

TABLE II. Experiment II. Protective Effect of Live and Killed Vaccine in Mice.

Time of challenge, days	Immunizing dose, billions	Killed vaccine, survival/total	Live vaccine, survival/total
10	.06	2/15	4/14
	.30	7/15	9/14
	1.50	6/15	5/14
17	.06	2/15	5/15
	.30	3/15	9/15
	1.50	9/14	7/9
24	.06	0/14	1/14
	.30	1/14	6/13
	1.50	8/14	5/8
120	.06	2/9	2/14
	.30	6/14	9/14
	1.50	5/9	10/10

Time of challenge	Immunizing dose, billions	Killed vaccine		Live vaccine	
		Survival/total	% survival	Survival/total	% survival
All challenges combined	.06	6/53	11.3	12/57	21
	.30	17/58	29.3	33/56	59
	1.50	28/52	53.8	27/41	65.9
	All doses	51/163	31.3	72/154	46.7

All animals were challenged intracerebrally with 50000 organisms strain #18323.

TABLE III. Total Inoculum—200000 Organisms.

Time killed	Avg No. viable organisms present
30 min	28500
4 hr	5625
7	6975
24	1050
48	82.5
72	0
120	0
144	—

organisms of strain No. 18323 (24-hour culture—suspended in 1% Casamino Acids). At time intervals of 30 minutes to 144 hours, groups of 3 mice were killed, the injected muscle excised, ground, and suspended in 3 cc of 1% Casamino Acids. Aliquot portions were plated on Bordet-Gengou media. After 5 days incubation, the total number of viable organisms was determined. The results are shown in Table III. Viable organisms remained at the site of inoculation over a period of 48 hours but it did not appear that multiplication had taken place.

Human studies. Encouraged by the animal experiments described above we have carried out some preliminary exploratory observations with live vaccine on human subjects, both adults and children. We are now engaged in immunizing a group of children with live vaccine. Results of these studies will be available at a later date. There are obvious practical difficulties in the preparation of a live vaccine for general use. Efforts to overcome these difficulties will depend on the demonstration of efficacy of immunization with live vaccine in the human disease.

Summary. (1) Live pertussis vaccine proved to be more effective than killed vaccine in mouse protection tests. (2) The use of live vaccine in human subjects is under investigation.

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