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Vaccination of Rabbits Against Pneumococci with Special Reference to Oral Immunization.*

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The use of pneumococcus vaccines by *oral* administration is largely based upon the investigations of Ross[1] and in an article on prophylactic immunization of rabbits against pneumococcus pneumonia and septicemia Kolmer and Rule[2] found that 7 daily doses of vaccines by oral administration engendered a high degree of immunity against Type I pneumococcus, somewhat less against Type II and still less against Type III, as determined by the intratracheal injection of virulent cultures of these types. Kolmer and Amano[3] also observed that rabbits may be effectually immunized against meningitis and septicemia following intratympanal and intracisternal injections of virulent Types I, II and III pneumococci, by the oral administration of vaccines given once a day for a week. Particularly encouraging results were observed with HCl acid-killed, sodium taurocholate-dissolved and heat-killed milk cultures of virulent pneumococci and it was recommended that when time permits, humans with pneumococcus paranasal sinusitis and otitis be prepared for operative procedures by the oral immunization of autogenous vaccines as a possible protection against extension of the infection to the meninges.

These investigations were continued with rabbits to determine: (1) dosage of vaccine by oral administration in relation to acquired immunity; (2) duration of immunity after oral immunization; (3) to compare the immunity response from subcutaneous injections with oral administration of the vaccines.

Since "acid-killed" pneumococcus vaccines appeared to engender the highest degree of immunity in rabbits[2] they were mostly employed. The organisms were cultivated in a broth medium for 24 hours and sufficient N/1 hydrochloric acid added to give an N/15 concentration. After standing for 2 hours at room temperatures the

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[1] Ross, V., *J. Exp. Med.*, 1930, **51**, 585; *Immunol.*, 1926, **12**, 219, 237; *J. Lab. and Clin. Med.*, 1927, **12**, 566; PROC. SOC. EXP. BIOL. AND MED., 1926, **24**, 273.

[2] Kolmer, J. A., and Rule, A. M., PROC. SOC. EXP. BIOL. AND MED., 1932, **30**, 107.

[3] Kolmer, J. A., and Amano, K. W., *The Laryngoscope*, August, 1932.

vaccines were subcultured for sterility. Numerically they averaged about 500 million per cc. The dosage was 0.2, 0.5, 1.0, 2.0, and 5.0 cc. per kilo administered daily for 7 doses by stomach tube. Numerically the dosage varied therefore from approximately 100 to 2500 million per kilo of weight. One week after the last dose each animal, including normal controls, was given intratracheal injections of living broth cultures. Immediately or within a few hours after death the heart blood was cultured for pneumococci and complete necropsies performed with special reference to the lungs.

Dosage in Relation to Acquired Immunity of Rabbits.—Table I summarizes the results of an experiment with Type I pneumococcus. Rather large doses were required for engendering immunity amounting approximately to 7 doses of 2 cc. of 24 hour broth cultures, or approximately 1000 million acid-killed cocci per kilo of weight.

TABLE I.
Dosage in relation to oral immunization of rabbits against Type I Pneumococcus Pneumonia and Septicemia.

Dosage* per Kilo. cc.	No. Immunized	Survived**	Died***	% Survivals
0.2	8	4	4	50
0.5	8	4	4	50
1.0	8	6	2	75
2.0	8	7	1	88
5.0	8	8	0	100
Controls not Immunized	8	0	8	0

* Seven at daily intervals by stomach tube.
** After intratracheal inoculation with 0.5 cc. of 1:30 dilution of 6 hour broth culture given one week after last dose of vaccine.
*** All with positive heart blood cultures.

All of the rabbits succumbing died in from 3 to 6 days after intratracheal inoculation with living highly virulent culture and all showed positive heart blood cultures. However, even doses as small as 0.2 cc. of vaccine per kilo produced a rather high degree of immunity in 50% of the animals while a dose of 1.0 cc. per kilo immunized 75%, 2.0 cc. about 88%, and 5.0 cc. 100% of rabbits. (Table I.)

With vaccines of Type II and Type III pneumococci the results were less definite. (Table II.)

Both strains were less virulent for rabbits than Type I since about half of the inoculated normal controls survived. But the results have shown that acid-killed vaccines by oral administration engendered immunity in doses varying from 0.5 to 5.0 cc. per kilo, the

TABLE II.

Dosage in relation to oral immunization of rabbits against Type I and II Pneumococcus Pneumonia and Septicemia.

Dosage* per Kilo. cc.	Type II No. Immunized	Survived **	Died	% Survivals	Type III No. Immunized	Survived ***	Died	% Survivals
0.2	4	2	2	50	5	2	4	40
0.5	4	3	1	75	5	3	2	60
1.0	4	3	1	75	5	4	1	80
2.0	4	4	0	100	5	5	0	100
5.0	4	4	0	100	5	5	0	100
Controls not Immunized	4	2	2	50	5	3	2	60

* Seven at daily intervals by stomach tube.
** After intratracheal inoculation with 2 cc. of a 6 hour broth culture given one week after the last dose of vaccine.
*** After intratracheal inoculation with 2.5 cc. of a 6 hour broth culture given one week after the last dose of vaccine.

2.0 cc. dose per kilo being approximately the minimal dosage when given daily for 7 consecutive days. Group IV pneumococci were not included.

Number of Doses in Relation to Acquired Immunity in Rabbits. Rabbits were given 1, 2, 3, 5, and 7 daily consecutive doses of 2 cc. of the Type I vaccine by stomach tube. Seven days after the last dose, each, along with normal controls, was inoculated intratracheally. As shown in table, 3, 1, 2 and 3 doses were without appreciable effect while 5 daily doses were approximately the minimum required for completely effective immunization.

TABLE III.

Number of doses of Type I Pneumococcus Vaccine by oral administration in relation to acquired immunity of rabbits to Pneumococcus Septicemia and Pneumonia.

No. Doses*	No. Immunized	Survived**	Died	% Survivals
1	8	1	7	12
2	8	0	8	0
3	8	0	8	0
5	8	7	1	88
7	8	8	0	100
Controls not immunized	8	0	8	0

* 2.0 cc. each by stomach tube.
** After intratracheal inoculation with 0.5 cc. of 1:30 dilution of 6 hour broth culture given one week later.

Duration of Immunity After Oral Immunization of Rabbits. We expressed the opinion[2] that immunity of rabbits after 7 daily consecutive doses of Type I vaccine administered by stomach tube, may last 4 months or longer. This would appear to be about the longest

duration in experiments of this character, remembering that some immunity may be present which is masked by the rather severe test following intratracheal inoculation of sufficient amounts of living culture to produce the death of all normal controls within 72 hours. Rabbits given 5 daily consecutive doses of acid-killed vaccine of 2.0 cc. by stomach tube, developed a rapid immunity as shown by the survival of all when inoculated intratracheally one week later. Four weeks later this immunity was appreciably reduced, and it was apparently gone, in the terms of the severe experiment, within 6 months as shown by the results with Type I vaccine summarized in Table IV.

Subcutaneous vs. Oral Immunization of Rabbits. Two vaccines were employed. The first for subcutaneous injection was an unheated broth suspension containing approximately 20 million of

TABLE IV.
Duration of Immunity of rabbits to Type I Pneumococcus following 5 daily doses of 2.0 cc. of Vaccine by oral administration.

Interval after Immunization*	No. Immunized	Survived**	Died	% Survivals
1 week	6	6	0	100
4 "	6	5	1	83
8 "	6	4	1	66
4 mo.	6	2	4	33
6 "	6	0	6	0
Controls not immunized	6	0	6	0

* With 5 daily doses of 2.0 c.c. by stomach tube.
** After intratracheal inoculation with 0.5 cc. of 1:30 dilution of 6 hour broth culture.

Type I and an equal number of Type II and Type III pneumococcus per cc. and sterilized with 0.5% tricresol. The second for oral administration contained the same numbers of the 3 strains killed in an N/15 concentration of hydrochloric acid. Both were administered in a dose of 1 cc. per kilo but the results have indicated that the doses were too small, since our own work reported herein has shown that larger doses are required and the same has been apparently observed by Cecil and Steffen[4] in their work on the vaccination of monkeys against pneumococcus pneumonia.

Twelve rabbits were given a subcutaneous injection of the first vaccine in dose of 1 cc. per kilo every 5 days for 6 doses. One week later 4 were inoculated intratracheally with living Type II and 2 survived (50%) and the remaining 4 were inoculated with a virulent Type III with 1 survival (25%).

[4] Cecil, R. L., and Steffen, G. I., *J. Exp. Med.*, 1921, **34**, 245; 1923, **38**, 149.

These results are summarized in Table V. Rabbits vaccinated with chemically killed mixed pneumococcus vaccine by subcutaneous injection yielded a better immunity response than rabbits vaccinated with equal numbers of an acid-killed pneumococcus vaccine by stomach tube administration in the same dose per kilo of weight. Better results would probably have been observed had larger doses been given but these experiments were finished before we realized the importance of this dosage factor. However, the results are not without value as indicating the superior immunizing capacity of subcutaneous injections of vaccine.

Summary. 1. Vaccination of rabbits against Types I, II and III pneumococcus by oral administration of acid-killed vaccines requires rather large doses amounting approximately to 1000 million per kilo of weight, although smaller doses (100 million per kilo) were sometimes effective, especially in the case of Type I pneumococcus. 2. One dose of 1000 million Type I acid-killed vaccine by oral administration sometimes produced effective immunization but best results were observed with a minimum of 5 doses at daily intervals. 3. Pneumococcus immunity after oral administration of Type I acid-killed vaccine is of short duration as determined by survival after intratracheal injections of virulent culture. One month after vaccination the degree of immunity is decreased, and practically extinct at the end of 6 months. 4. The subcutaneous injection of rabbits with a chemically killed vaccine containing a total of 60 million of Types I, II and III per cc. gave a better immunity response than an acid-killed vaccine of the same strength by oral administration in dose of 1 cc. per kilo of weight.

TABLE V.

Subcutaneous versus oral immunization of rabbits with mixed Types I, II and III Pneumococcus Vaccines.

Route of Immunization	No. Immunized	% Survivals after Intratracheal Inoculation With Type I	With Type II	With Type III
Subcutaneous	12	75	50	25
Oral	12	50	0	0
Controls (12) not immunized	—	0	0	0