

THE PRODUCTION OF ACTIVE AND PASSIVE  
IMMUNITY TO THE PNEUMOCOCCUS  
WITH A SOLUBLE VACCINE

## A PRELIMINARY REPORT\*

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The extensive employment of vaccines made from dead microorganisms has been followed by variable results; in some cases the results have been excellent, in others, absolutely negative. It seemed probable that these discrepancies might be due to the fact that in the one case an efficient quantity of the bacterial endotoxin had entered into the circulation, whereas when no beneficial results ensued it might be that a satisfactory extract of the microorganisms had not occurred. It therefore seemed logical to search for a means of dissolving the active principle of the bacteria with as little injury as possible.

time necessary to kill the control was probably due to the varying acidity that developed in the culture medium. This occurred before it was discovered that the mere presence of the calcium carbonate did not preserve the neutrality of the liquid.

In these experiments, in which I was ably assisted by Dr. L. S. Mace, only a single dose of the soluble vaccine was administered, the purpose being to establish the principle that such a soluble vaccine had really been produced. The method employed was to grow the pneumococcus for forty-eight hours in the culture medium just described to which a piece of marble had been added. To this culture equal parts of a solution of 0.2 per cent. pancreatin (Cudahy) in 2 per cent. bicarbonate of soda at 37 C. were added, allowed to stand fifteen minutes in the incubator, made faintly acid with hydrochloric acid, and filtered through a Pasteur filter. Rabbits injected with a single dose of this liquid developed relative or complete immunity to a virulent pneumo-

TABLE 1.—PNEUMOCOCCUS VACCINE NO. 40

(ALL ANIMALS VACCINATED DEC. 20, 1911)

Number of Rabbit	Quantity of Vaccine, c.c.	Date of Infection	Quantity of Pneumococci* c.c.	Date of Death	Number of Days Living	Pneumoc. in Blood †	Number of Control	Date of Death	Number of Days Living	Pneumoc. in Blood †
1029	10	12/24/12	0.005	1/15/12	22	+	1041	12/20/11	2	+
1030	10	12/25/12	0.005	12/26/11	1	+	1050	1/4/12	10	..
1028	10	12/26/12	0.005	1/2/12	7	0	1060	12/29/11	3	+
1027	10	12/27/12	0.005	1/10/12	14	0	1067	12/29/11	2	+
1026	10	12/28/12	0.005	12/29/11	1	0	1069	12/30/11	2	+
1024	5	12/29/12	0.005	1/7/12	9	..	1070	12/31/11	2	+
1021	5	12/30/12	0.005	1/5/12	6	..	1074	1/3/12	4	..
1023	5	12/31/12	0.005	1/21/12	21	0	1095	1/6/12	7	..

\* Pneumococcus 913. † In this and Table 2, where no note of the presence of germs in the blood is made, the examination was not made or the record was lost.

TABLE 2.—PNEUMOCOCCUS VACCINE NO. 41

(ALL ANIMALS VACCINATED DEC. 24, 1911)

Number of Rabbit	Quantity of Vaccine, c.c.	Date of Infection	Quantity of Pneumococci* c.c.	Date of Death	Number of Days Living	Pneumoc. in Blood	Number of Control	Date of Death	Number of Days Living	Pneumoc. in Blood
1053	10	12/29/11	0.005	1/3/12	5	+	1070	12/31/11	2	+
1060	10	12/30/11	0.005	1/4/12	5	+	1074	1/3/12	4	..
1049	10	12/31/11	0.005	1/5/12	5	+	1095	1/6/12	6	..
1040	10	1/1/12	0.005	1/14/12	13	0	1108	1/6/12	5	+
1061	10	1/2/12	0.005	1/7/12	5	0	1109	1/6/12	4	+
1039	5	1/3/12	0.005	1/12/12	9	..	1111	1/5/12	2	+
1051	5	1/4/12	0.01	1/11/12	7	0	1113	1/6/12	2	+
1047	5	1/5/12	0.05	1/16/12	11	0	1117	1/8/12	3	+
							1119†	1/7/12	2	+
1043	5	1/6/12	0.05	1/16/12	10	..	1121	1/8/12	2	+
1050	5	1/7/12	0.05	1/10/12	3	+	1112	1/9/12	2	+

\* Pneumococcus 913. † Received 0.0005 c.c.

After various methods had been unsuccessfully tried a solution was made by acting on the living microorganism with an alkaline pancreatin solution at 37 C. for a definite time, stopping the action by slight acidulation and filtering through a Pasteur filter. Studies with the pneumococcus showed that the virulence of the microorganism could be readily maintained by growing it in a 2 per cent. glucose, 4 per cent. glycerin veal bouillon, faintly alkaline to litmus, to which calcium carbonate had been added according to the method of Hiss and transplanting the culture daily. It is of interest to note that unless the culture with the calcium carbonate be shaken frequently it will become acid, as diffusion through the liquid is not sufficiently rapid to preserve the neutral reaction.

In order that there should be no doubt of the results, larger doses of the pneumococci than were necessary to kill the rabbit were employed; but the variability of the

coccus culture in about six or seven days, as is shown by the accompanying tabulated statement of two sets of experiments. (Tables 1 and 2).

On April 21, 1912, one rabbit was vaccinated with 5 c.c. and another with 40 c.c. of a solution prepared from a culture of the pneumococcus that had not grown very vigorously. On May 8, they and a control were each injected with 0.05 c.c. of a virulent pneumococcus culture. Two days later the control was dead of pneumonic septicemia with the microorganisms in the blood, whereas the vaccinated animals are still living. In later experiments in which two injections of the extract were given, a small one followed a week later by a larger dose, active immunity to the pneumococcus was more regularly produced in the rabbit.

It has been found that the vaccine will retain its qualities without any preservative at ordinary temperatures (45 to 65 F.) for at least somewhat over three months. A rabbit that had been vaccinated April 1,

\* From Cooper Medical College.

1912, with 5 c.c. of the pneumonic extract No. 40 that had been prepared Dec. 20, 1911, was inoculated, together with a control, April 10, 1912, with 0.05 c.c. virulent culture of pneumococcus. The vaccinated animal is still alive, whereas the control died in two days and pneumococci were isolated from the heart's blood. Another rabbit, injected April 11, 1912, with the same extract is likewise still living, the control having died within twenty-four hours and pneumococci having been cultivated from the heart's blood.

In order to determine whether it would be possible to produce a serum which would afford passive immunity, a dog was injected with increasing doses of the pneumonic extract from Dec. 28, 1911, to April 13, 1912, when he was bled. A rabbit injected with 4 c.c. of the dog's serum intravenously March 14, 1912, and subcutaneously with 0.05 c.c. virulent culture of pneumococcus, is still living, whereas the control rabbit was dead within three days and the pneumococci were cultivated from the heart's blood. March 24, 1912, the dog was again bled and on March 25, 1912, two rabbits received 6 c.c. of the serum intravenously and 0.05 c.c. virulent pneumococcus culture subcutaneously. Two controls died within two days with pneumococci in the blood. The one vaccinated rabbit lived until April 8, 1912, and the other until April 13, 1912, and the heart's blood of both was found to be sterile. In order to be positive that the pneumococcus with which I was operating was not sensitive to normal dog's serum 6 c.c. of the latter was injected intravenously into a rabbit and 0.05 c.c. virulent pneumococcus culture was administered subcutaneously, March 21, 1912. The rabbit died March 22, 1912, and the control March 23, 1912, and pneumococci were cultivated from the heart's blood of both rabbits.

Unfortunately the serum of the dog cannot be used on human beings on account of its toxicity, and experiments are being made for the purpose of producing antipneumonic serum from sheep and horses.

Ten patients with pneumonia were treated with an extract prepared from an emulsion of the same washed living pneumococci. All recovered. In a majority of the cases crisis set in within twenty-four hours after the first dose. A striking fact observed was a rapid fall of the number of leukocytes within twelve hours and preceding the reduction of temperature. The number of cases is far too small to warrant an estimate of the value of the treatment but certainly proves its harmlessness.

The cases are presented in Table 3:

TABLE 3.—PNEUMONIA PATIENTS TREATED WITH PNEUMOCOCCUS VACCINE		
Treatment Begun After Initial Chill.	Crisis.	
1. 32 hours.....	within 24 hours	
2. 3 1/2 days.....	within 12 hours	
3. 4 days.....	within 8 hours	
4. 4 days.....	within 12 hours	
5. 5 days.....	within 5 days	
6. 5 days.....	within 24 hours	
7. 5 days.....	within 24 hours	
8. 5 days.....	within 43 hours	
9. 5 days.....	within 50 hours	
10. 12 days.....	within 36 hours	

Since cases of pneumonia are comparatively rare in San Francisco at the present season, we have been unable to test the extract in the large number of cases necessary in order to form reliable conclusions. Because of the well-known variability of the virulence of the organism in different communities and in different seasons, it is desirable to test this extract under as diverse conditions as possible. I shall be glad to furnish the extract to hospitals and research institutions free of charge, on request.

Encouraging experiments are being conducted with various other germs treated in a similar manner. The results will be reported later.

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A GRAPHIC MENSTRUAL CHART

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It requires but a short experience in taking gynecologic case histories and in reading such histories as taken by students and resident physicians to demonstrate the difficulty

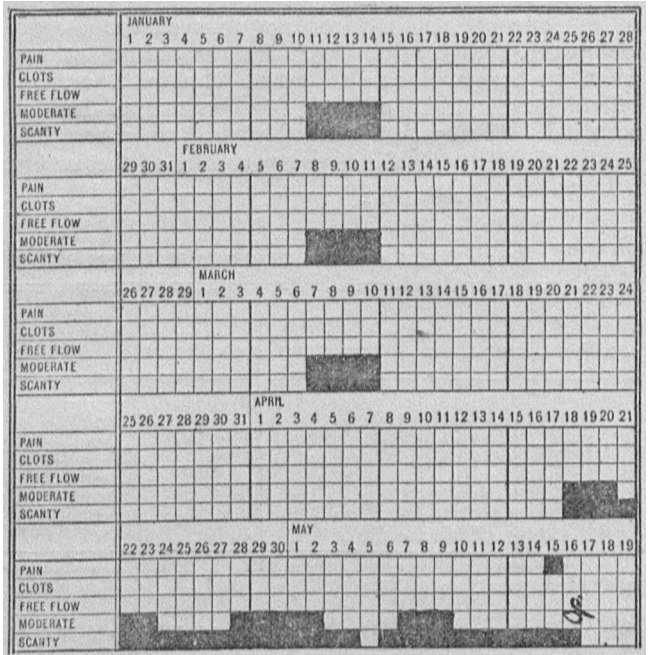


Fig. 1.—Menstrual chart of a case of ectopic pregnancy. Operation, May 10, 1912. There is space on the paper containing the chart to record the name, diagnosis, operation and such other details as are desirable.

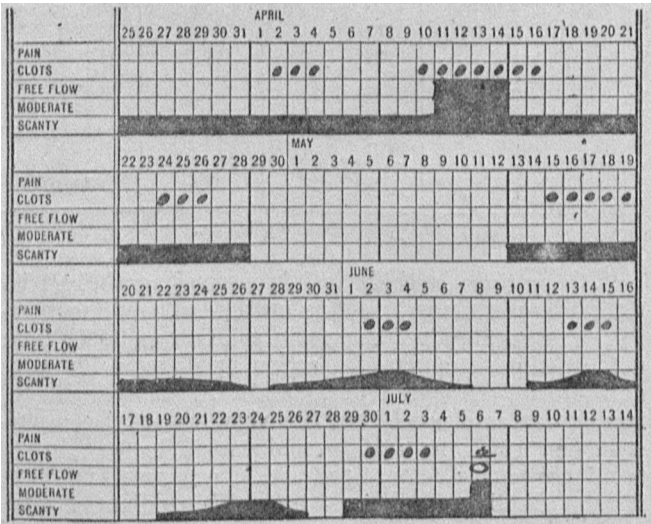


Fig. 2.—Menstrual chart of a case of hypertrophic glandular endometritis. Curettage, July 6, 1912.

of grasping quickly and remembering accurately the patient's exact menstrual history. Unless a rather wordy description is written and referred to at each visit, the same questions will be put to the patient repeatedly, and, after the history has extended over several months, only moderately accurate replies will be obtained.

After a considerable period of dissatisfaction with present conditions I endeavored to formulate a method of graphically