AN EXPERIMENTAL STUDY OF OPSONIC IMMUNITY TO STAPHYLOCOCCUS AUREUS.[1]

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The cause of the acquired immunity to *Staphylococcus aureus* has not been definitely established. An increase of the bacteriolytic power of the serum has never been demonstrated even after prolonged immunization. Agglutinins are increased, sometimes to a very marked degree, but increase of agglutinins in many instances has little relation to increased resistance to infection. In all infections with staphylococcus there is local as well as a general leucocytosis, and it has been shown that *Staphylococcus aureus* produces a soluble thermostable substance which is strongly chemotatic for polymorphonuclear leucocytes. Besides this substance, van der Velde[2] found that *Staphylococcus aureus* produces a body called by him leucocidin, which destroys leucocytes and produces pus. All the polymorphonuclear leucocytes attracted by staphylococci to the point of infection are not destroyed, and Kirch[3] in 1889 was the first to observe that polymorphonuclear leucocytes ingest the staphylococcus. Many observers have since demonstrated that leucocytes can not only ingest but also destroy staphylococci after ingestion.

The first experiments made to explain the protective properties of staphylococcus immune serum were those of Pröscher.[4] He injected immune and normal sera into animals, and the following day introduced living staphylococci into the peritoneal cavities of these animals. Leucocytes soon accumulated, and in those animals into which the immune serum had been injected, phagocytosis was very marked, while very little phagocytosis occurred in the animals which had received normal serum. These results suggested that

[1] Received for publication September 1, 1909.

[2] *Ann. d. l'Inst Pasteur,* 1896, x, 580.

[3] Thesis, 1889, cited by Ricketts, Infection, Immunity and Serum Therapy, Chicago, 1906.

[4] Cited by Ricketts, idem.

phagocytosis played an important role in the protection afforded by such immune serum.

The cause of increased phagocytosis in the protected animal was not definitely understood until Wright and his co-workers proved that phagocytosis was promoted by the presence of opsonins. The method of Leishmann, perfected by Wright, has been used for the quantitative estimation of opsonins in the serum, but it does not demonstrate that opsonins are sufficiently increased to explain the protection found in animal experiments. Moss,[5] using this method, made an exhaustive study of opsonic immunity to *Staphylococcus aureus,* and came to the conclusion that "no high degree of opsonic immunity, such as is possible in anti-toxic and bactericidal immunity, can be produced in rabbits by ordinary inoculation procedures with the *Staphylococcus aureus.*" This has been the experience of nearly all who have worked with these methods.

In 1906, Simon, Lamar and Bispham[6] advocated a modification of the opsonic index of Wright. The opsonic power of diluted immune serum was compared with diluted normal serum of the same strength. This method undoubtedly gave a more accurate index of the opsonic power of the immune serum, but the dilutions were not carried far enough. Neufeld and Hüne,[7] and Klien[8] independently determined the opsonic power of the serum of animals immunized to *Bacillus typhosis* and allied organisms by a method of dilution which sought the point at which phagocytosis ceased altogether. By this means, a high degree of opsonic immunity was demonstrated. Marshall[9] applied a modification of this principle of dilution to the investigation of staphylococcus immunity in animals. He diluted the serum with thirty-two times its volume of fluid, and came to the conclusion that in this dilution (1 to 32) the serum causes phagocytosis with the same activity as in the undiluted state. The opsonic index of the undiluted serum, he maintained, is no guide to the manner in which a serum will behave on dilution. He did not, however, carry the dilution of the serum to the point of opsonic extinction.

[5] *Bull. of the Johns Hopkins Hosp.,* 1907, xviii, 237.
[6] *Jour. of Exper. Med.,* 1906, viii, 651.
[7] *Arb. a. d. k. Gsndhtsamte,* 1907, xxv, 164.
[8] *Bull. of the Johns Hopkins Hosp.,* 1907, xviii, 245.
[9] *Jour. of Bact. and Path.,* 1908, xii, 378.

I have demonstrated by the method of dilution that an increase of opsonic activity comparable to the increase of bactericidal, antitoxic and agglutinating powers may be produced in animals immunized with the following living bacteria: *Bacillus dysenteriæ, Bacillus tuberculosis, Streptococcus pyogenes,* and in a less degree with *Staphylococcus aureus.* In rabbits immunized to *Staphylococcus aureus,* abscess formation always occurred, but when a few doses of staphylococcus vaccine were first administered, the animals resisted small amounts of the living organism much better than those animals to which no vaccines had been given. This fact confirmed previous observations which showed that killed cultures of staphylococcus could produce a certain amount of immunity.

The following experiments have been undertaken to determine what degree of immunity can be produced by the administration of killed cultures or vaccines, and to determine whether such an immunity has a definite relation to the opsonic activity of the serum. Since it has never been possible to demonstrate any bactericidal properties in staphylococcus immune serum, the experiments were confined to the estimation of the opsonic power of the serum.

Methods.—The technique used was similar to that employed in my previous experiments, which is essentially the same as that devised by Klien. The staphylococcus emulsion, leucocytic emulsion, and blood serum were collected by the methods of Wright. The serum, however, was diluted with Ringer's solution. Specimens were prepared from each dilution in the pipettes used by Wright and after incubation at 37° C. for fifteen minutes smears were prepared from each pipette. A specimen in which Ringer's solution was substituted for the diluted serum was prepared with each set of experiments. The smears were stained with Hasting's modification of the Romanowski stain, and the number of cocci in fifty leucocytes was counted for each specimen. A large number of observations made with normal rabbit serum demonstrated that the opsonic power of normal serum had almost constantly disappeared after a dilution of one to thirty. Such extinction of phagocytosis by dilution was so constant that it was not considered necessary to make normal control dilutions with normal serum for comparison with each experiment (see Chart I). The strain of

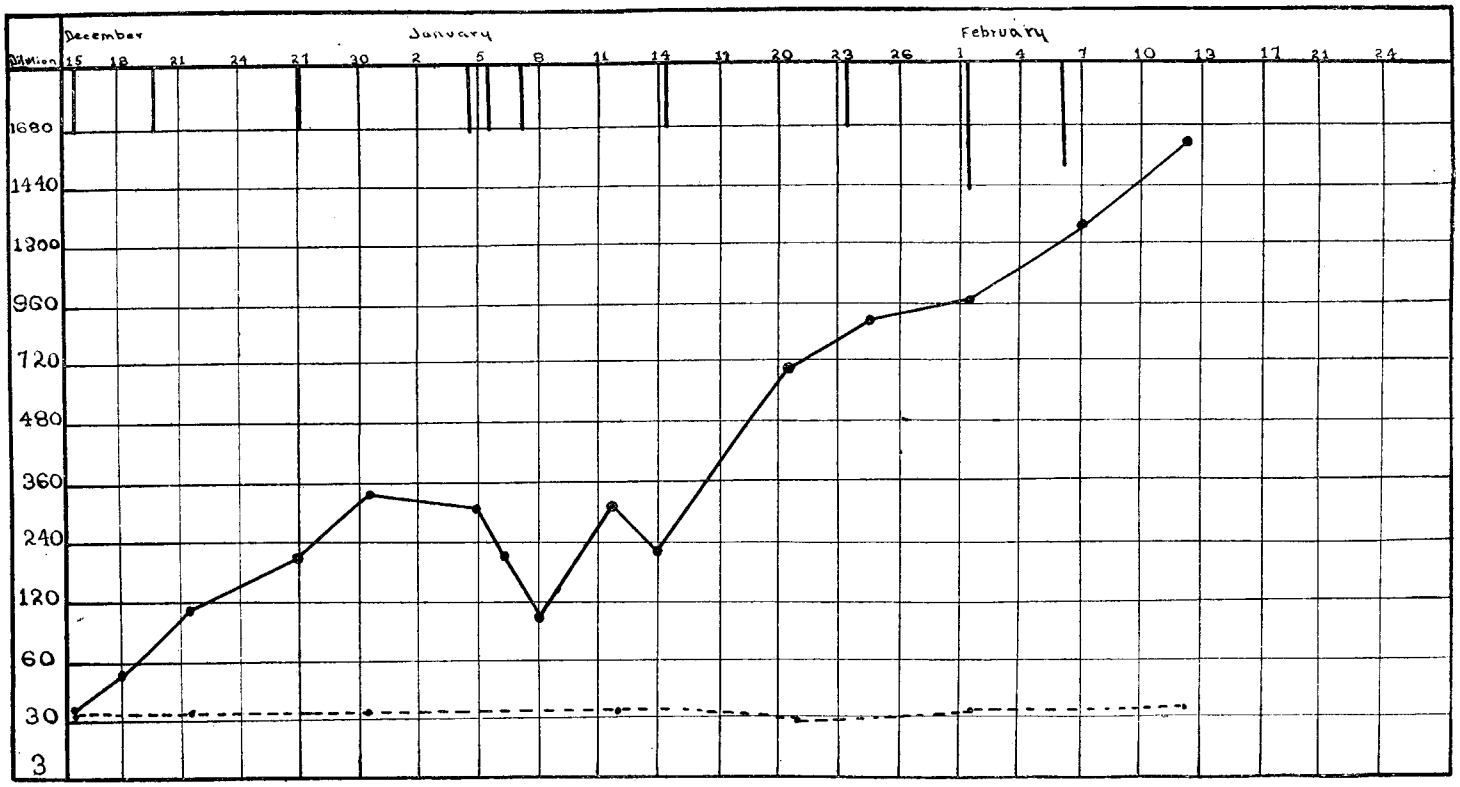

CHART. I. Heavy straight line represents opsonic curve of immunized rabbit; dotted line represents opsonins of normal rabbit.

Staphylococcus aureus used for the opsonic determinations and for preparation of the vaccines was obtained from the blood of a patient with severe pyemia. It was very virulent for rabbits and underwent phagocytosis of only moderate activity. The leucocytes were obtained from normal human blood drawn into a solution of sodium citrate, and washed three times with Ringer's solution. The vaccines were prepared from a twenty-four hour old culture, grown on plain agar. The organisms were killed by heat at 60° C. for one-half hour. One-half cubic centimeter of the vaccine was introduced into a flask of bouillon; the vaccines tested by this method were uniformly sterile. The number of organisms per cubic centimeter was not counted, but the dosage of the vaccine was determined by inoculating the growth from a certain number of agar slants.

EXPERIMENT I.—A rabbit weighing 1,950 grams was used. The opsonic power of the serum determined several times before the first vaccination was found to disappear between a dilution of 1 to 30 and 1 to 45. On December 5, 1908, a vaccine of *Staphylococcus aureus* was prepared from the growth on two agar-agar slants, and injected subcutaneously (all subsequent vaccinations were made by this method). The vaccinations were repeated at intervals of from seven to ten days (see Table I), and the opsonic power of the serum was determined at intervals of from three to five days (see Chart I).

TABLE I.

No.	Date of vaccination.	Amount of vaccination.
1	December 15, 1908.	2 agar-agar slants.
2	December 19, 1908.	2 agar-agar slants.
3	December 27, 1908.	2 agar-agar slants.
4	January 3, 1909.	2 agar-agar slants.
5	January 5, 1909.	2 agar-agar slants.
6	January 7, 1909.	2 agar-agar slants.
7	January 15, 1909.	2 agar-agar slants.
8	January 24, 1909.	2 agar-agar slants.
9	February 2, 1909.	3 agar-agar slants.
10	February 8, 1909.	2½ agar-agar slants.

The opsonic power gradually increased and after sixty days the dilution in which phagocytosis ceased was one to fifteen hundred, or practically fifty times the opsonic power of the serum before the vaccination. The opsonic power of a normal rabbit was frequently determined for control of the vaccinated animal and was found to vary little from day to day. The curve representing the opsonic activity of the serum of the vaccinated animal diverged from the curve representing the normal animal and the increase of opsonins was progressive with only one unexplainable remission, which might have been due to an error of technique.

In order to determine whether a negative phase of opsonic activity occurred after inoculation, vaccines were given at close intervals. On January 3, 5 and 7, respectively, vaccine prepared from the growth on two agar-agar slants was given and the opsonic power was estimated every second day. There was found to be a diminution in the amount of opsonins present in the serum and this diminution increased after each successive dose, but quickly disappeared when the period between the vaccinations was prolonged. This rabbit received altogether as vaccines the growth from twenty-one and a half agar-agar slants, divided into ten doses. At the site of each injection a small mass appeared after a few days. The characters of these masses will be described later. There was a slight increase of weight during the period of vaccination and the animal continued in excellent condition. On February 14, however, it was found moribund, and died during the day. An autopsy was performed one hour post-mortem. The liver was very large, of a blackish color and filled with coccidial cysts. Microscopical examination showed that the liver contained a large amount of black pigment. The other viscera were normal. Cultures from the liver, spleen, kidneys, lungs and heart's blood remained sterile. The cause of death was not determined.

Since this experiment had demonstrated that the serum develops a high opsonic power for *Staphylococcus aureus* when rabbits immunized by vaccines prepared from this microörganism were used, further experiments were undertaken to confirm this finding, and to determine, if possible, what protection such increase of opsonins might afford.

Six rabbits were vaccinated as in the first experiment, and the opsonic power estimated at irregular intervals in order to follow the progress of the immunity. The vaccines were injected subcutaneously every seven days. At the point of each inoculation a small indurated lump developed. A few of these lumps were incised and thick white pus evacuated; it was always sterile. This abscess formation was evidently due to the power of the dead staphylococcus to cause a local accumulation of leucocytes. The majority of the swellings produced by the vaccines were not opened and were gradually absorbed; in no case did spontaneous rupture occur.

The opsonic power gradually increased in a manner similar to that recorded in Experiment I, but Table II shows that the production of opsonins did not occur with equal rapidity in all of the animals. Rabbit 2, and especially Rabbit 5, acquired an increased opsonic power very quickly. Rabbit 3 produced opsonins very slowly.

TABLE II.

Number.	Weight of rabbits.	Number of vaccinations.	Total amount of vaccines.	Time under treatment.	Opsonic power before vaccination.	Opsonic power after 20 days.	Opsonic power after 45 days.	Opsonic power after 70 days.	Opsonic power after 95 days.	Opsonic power after 121 days.
1	1690 gm.	15	25 agar slants.	121 days.	1 to 45	1 to 120	1 to 480	1 to 960	1 to 1200	1 to 1500
2	1650 "	15	25 " "	121 "	1 to 45	1 to 150	1 to 720	1 to 1200	1 to 1500	1 to 1800
3	1680 "	11	19 " "	95 "	1 to 30	1 to 120	1 to 360	1 to 720	1 to 960	—
4	1950 "	11	19 " "	95 "	1 to 30	1 to 120	1 to 240	1 to 960	1 to 1200	—
5	1720 "	11	19 " "	95 "	1 to 45	1 to 210	1 to 720	1 to 1200	1 to 1800	—
6	1700 "	11	10 " "	32 "	1 to 45	1 to 150	—	—	—	—

Rabbit 6 died suddenly after thirty-two days of immunization, having attained an opsonic power of 1 to 120. An autopsy was immediately performed and the liver exhibited a condition similar to that noted in Experiment I. There was extensive coccidiosis; the organ was dark red and microscopical section revealed slight pigmentation of the cells. Cultures from the blood, liver, spleen and peritoneum were sterile.

When the serum of Rabbits 1, 2, 3, 4 and 5 had developed considerable opsonic activity, their resistance to virulent living staphylococci was tested. The staphylococcus used was that from which the vaccines had been prepared. The virulence of this strain was increased by passing it through three rabbits. In order to determine the natural resistance of rabbits to this strain of *Staphylococcus aureus,* various quantities were injected into the ear vein of nine normal rabbits. The staphylococcus was grown on plain agar-agar and the growth from each tube was gently removed in two cubic centimeters of normal salt solution. The suspension was well shaken with glass beads to break up the clumps and prevent emboli formed by masses of bacteria.

TABLE III.

Number.	Weight.	Opsonic power.	Date of injection.	Amount of injection.	Results.
1	1,490 gm.	1 to 45	June 25	1/8 agar slant.	Died after 5 days.
2	1,600 "	1 to 45	" "	1/4 " "	" " 36 hours.
3	1,500 "	1 to 45	" "	1/3 " "	" " 24 "
4	1,535 "	1 to 30	" "	1/2 " "	" " 12 "
5	1,450 "	1 to 45	July 1	1/4 " "	" " 36 "
6	1,800 "	1 to 45	" "	1/4 " "	" " 36 "
7	1,600 "	1 to 30	" "	1/4 " "	" " 24 "
8	1,650 "	1 to 30	" "	1/4 " "	" " 24 "
9	1,200 "	1 to 30	" "	1/4 " "	" " 24 "

The results of inoculation are shown in Table III.

AUTOPSY FINDINGS IN THE CONTROL RABBITS.

Rabbit 1.—There was cloudy swelling of all viscera, with numerous abscesses in the kidneys. Cultures showed staphylococci in the blood.

Rabbit 2.—The findings were similar to those of Rabbit 1, except that abscesses in the kidneys were much smaller.

Rabbits 3 and 4.—There was marked cloudy swelling, but no abscesses were found. Cultures from all the viscera and the blood showed a pure growth of *Staphylococcus aureus.*

Rabbit 5.—Findings were similar to those of Rabbit 1.

Rabbit 6.—Findings were similar to those of Rabbit 2.

Rabbits 7, 8 and 9.—Findings were similar to those of Rabbits 3 and 4.

The immunized rabbits were injected intravenously with the same strain of *Staphylococcus aureus,* five control rabbits (see Table III) being given the same amount of bacterial emulsion on the same day. The results of inoculation of the immunized rabbits are given in Table IV.

TABLE IV.

No.	Weight.	Opsonic power.	Date of inoculation.	Amount of inoculation.	Result.
1	1,690 gm.	1 to 1,500	July 1.	¼ of agar slant.	Died after 21 days.
2	1,650 "	1 to 1,800	" 1.	" " "	Etherized after 45 days.
3	1,680 "	1 to 960	" 1.	" " "	Died after 5 days.
4	1,950 "	1 to 1,200	" 1.	" " "	Died after 13 days.
5	1,720 "	1 to 1,800	" 1.	" " "	Etherized after 45 days.

PROTOCOLS OF IMMUNIZED RABBITS.

Rabbit 1.—The animal, inoculated on July 1 with one-quarter of an agar-agar slant of *Staphylococcus aureus,* was very ill until July 8, but from this date there was slight improvement until July 12. The animal gradually lost weight and died on July 22, twenty-one days after inoculation. At autopsy the animal was much emaciated, and the skeletal muscles had a pale, cloudy appearance. The liver was very dark in color. The spleen was not enlarged. The kidneys contained a few small white areas, some of which were rather indistinct and depressed below the surface, and, on section, appeared to be scar tissue. Other white areas were even with the surface of the cortex and contained a small bead of yellowish pus. Smears from this pus showed polynuclear leucocytes and a few cocci. The pelves, ureters and urinary bladder were congested.

No growth was obtained from the heart's blood, lungs, liver, spleen and scar-like areas in the kidneys. Cultures from the small abscesses of the kidneys and from the urine contained a pure culture of *Staphylococcus aureus.*

Rabbit 2.—The animal, inoculated on July 1 with one quarter of the growth from an agar slant, gradually lost weight for two weeks following the in-

oculation, being reduced to 1,400 gm., but after July 16 it began to regain its weight, and on August 14 weighed 1,580 gm. On August 14 it was killed with ether. The liver was of a blackish color and was somewhat smaller than normal. The kidneys were pale, and in the cortex there were several opaque depressions to which the capsule was adherent.

No bacterial growth was obtained from the heart's blood, lungs, liver, kidneys and urine.

Rabbit 3.—The animal, inoculated on July 1 with the growth from one-quarter of an agar slant, was very sick the next day and died five days after inoculation. The viscera were congested and showed marked cloudy swelling. The liver, as in the other experiments, was very dark. The kidneys contained a few minute white areas, which, on section, contained a drop of yellowish fluid; smears of this pus showed pus cells and cocci.

Cultures from the heart's blood, lungs, liver, spleen, kidneys and urine contained a pure growth of *Staphylococcus aureus.*

Rabbit 4.—Inoculated as in the preceding experiments, the animal exhibited no signs of acute illness, but gradually lost weight and strength and died thirteen days after inoculation. The findings at autopsy were similar to those of Rabbit 1. No growth was obtained, except from the kidney abscesses and the urine, which contained *Staphylococcus aureus.*

Rabbit 5.—Inoculated as in other experiments, the animal, like Rabbit 2, lost weight (from 1,720 to 1,300 gm.), but at the time of etherization it had regained 350 gm. (weighing 1,650 gm.). The autopsy findings were the same as those of Rabbit 2. No bacterial growth was obtained.

The inoculations of living staphylococci into normal rabbit, especially when one-quarter of the growth from one agar-agar slant culture was employed, presented several noteworthy features. Six animals were inoculatd with this quantity; all of them died within thirty-six hours after the time of injection, three living thirty-six hours, and three, twenty-four hours. The three that lived thirty-six hours had an opsonic power of 1 to 45, whereas those living twenty-four hours had an opsonic power of only 1 to 30. The regularity of this relationship between the degree of the opsonic power and the effect of the inoculation is significant in view of the results obtained with immunized animals.

The experiments with immunized animals demonstrate what a high degree of opsonic power may be produced in rabbits by vaccination with *Staphylococcus aureus.* The dosage of the vaccines was large, as it was believed that the organisms might be subject to such change during the process of sterilization that their effect in the production of immune bodies would be diminished. The vaccines were prepared in large amounts, and the dosage was graduated

as carefully as possible. In spite of this care, the amount of opsonins produced varied greatly in different animals. Variation was probably due to individual peculiarities of the animals as they had about the same weight and age and were kept under similar conditions.

Although the immunized animals received, as nearly as could be determined, the same amount of living bacterial emulsion, the results varied greatly. One rabbit lived five days; a second, thirteen days; a third, twenty-one days; and two apparently recovered completely. Such differences seemed due to the differences in the degree of immunity in different animals. The rabbit which had the lowest opsonic power died in five days, and those with the highest opsonic power recovered (see Table IV). This observation accords with the results obtained with normal rabbits, for those with the higher opsonic power lived the longer.

Since there appears to be a direct relationship between the quantity of opsonins in the blood serum and the resistance of immune and normal rabbits to virulent *Staphylococcus aureus,* the opsonic power of the serum may afford a valuable means of determining the presence and the degree of such resistance. As bactericidal bodies have not been demonstrated for *Staphylococcus aureus,* and as agglutinins play but a small part in the destruction of bacteria, it is fair to suppose that opsonins play an important part in the cure of infection with staphylococcus.

TABLE V.

No.	Source of Staphylococci.	Opsonic power of Rabbit 1.	Opsonic power of Rabbit 2.	Opsonic power of Rabbit 3.	Opsonic power of Rabbit 4.	Opsonic power of Rabbit 5.	Opsonic power of normal rabbit.
1	Pyemia (organism used for vaccination).	1 to 1,200	1 to 1,500	1 to 840	1 to 960	1 to 1,500	1 to 30
2	Abscess.	1 to 960	1 to 1,200	1 to 720	1 to 720	1 to 1,200	1 to 30
3	Arthritis.	1 to 960	1 to 1,200	1 to 720	1 to 720	1 to 1,200	1 to 30
4	Acne.	1 to 960	1 to 1,200	1 to 720	1 to 720	1 to 1,200	1 to 30
5	Cellulitis.	1 to 960	1 to 1,200	1 to 720	1 to 720	1 to 1,200	1 to 30

Do opsonins produced by vaccination with a single strain of *Staphylococcus aureus* render other strains susceptible to phagocytosis? With this question in view, the following experiment was performed. Five cultures of *Staphylococcus aureus,* obtained from different sources, were tested with the serum of each of the five immunized rabbits. The results are given in Table V.

This experiment shows that opsonins produced by vaccination with one strain of *Staphylococcus aureus* are very powerful in promoting phagocytosis of heterologous strains, but have a somewhat greater power against the homologous strain. This observation has an important bearing upon the administration of vaccines in the treatment of staphylococcus infections of the human body. It indicates the advisability of using homologous strains of staphylococcus as vaccines in order to obtain the best results, but vaccines from other strains of *Staphylococcus aureus* might be efficacious.

Since the opsonic power of the serum plays an important part in the protection of animals infected with *Staphylococcus aureus*, it is important to ascertain what degree of opsonic immunity is developed in man during the course of infection with staphylococcus. For this purpose the following cases were investigated:

Case I.—W. C., age 37, white, single, was admitted to the service of Dr. Eliot in the Presbyterian Hospital, on March 7, 1909; he has always been in excellent health, but for many years has had to urinate several times each night. He denies any venereal infection. On the morning of March 5, 1909, while at work, the patient was seized suddenly with pain in the right flank, radiating to the right hypogastrium and the right iliac region. Until entrance into the Hospital at midnight of March 6, 1909, the pain was continuous, and was only partially relieved by anodynes. There were no urinary symptoms.

Physical examination on entrance to the Hospital showed that the patient was a large, well nourished man. The abdomen was moderately distended and rigid, particularly on the right side; there was acute tenderness, moderate fulness, and increased dulness of the right flank. The white blood cells numbered 13,800, consisting of polymorphonuclear leucocytes, 76.5 per cent., transitionals 5.5 per cent., large mononuclears 1 per cent., lymphocytes 6.5 per cent., eosinophiles 0.5 per cent. The temperature was 101.5°, pulse 102, respiration 28.

An incision was made over the right kidney along the outer border of the quadratus lumborum muscle. The tissues surrounding the kidney were infiltrated with sero-purulent fluid, and the perinephritic fat appeared necrotic. In the space between the capsule of the kidney and the cortex there was a quantity of clotted blood. The kidney itself was only slightly enlarged and much congested. The cortex was studded with small whitish areas, filled with pus, the largest being the size of a pea. The kidney was removed. A culture at the time of operation showed a pure growth of *Staphylococcus aureus.*

Microscopical examination shows an increase of the interstitial tissue; the arteries are thickened, the tubules are compressed, and the epithelium is desquamated in many places. The whole organ is riddled with miliary abscesses. The wall of the pelvis is much thickened by granulation tissue, the mucosa being ulcerated and covered with a necrotic slough.

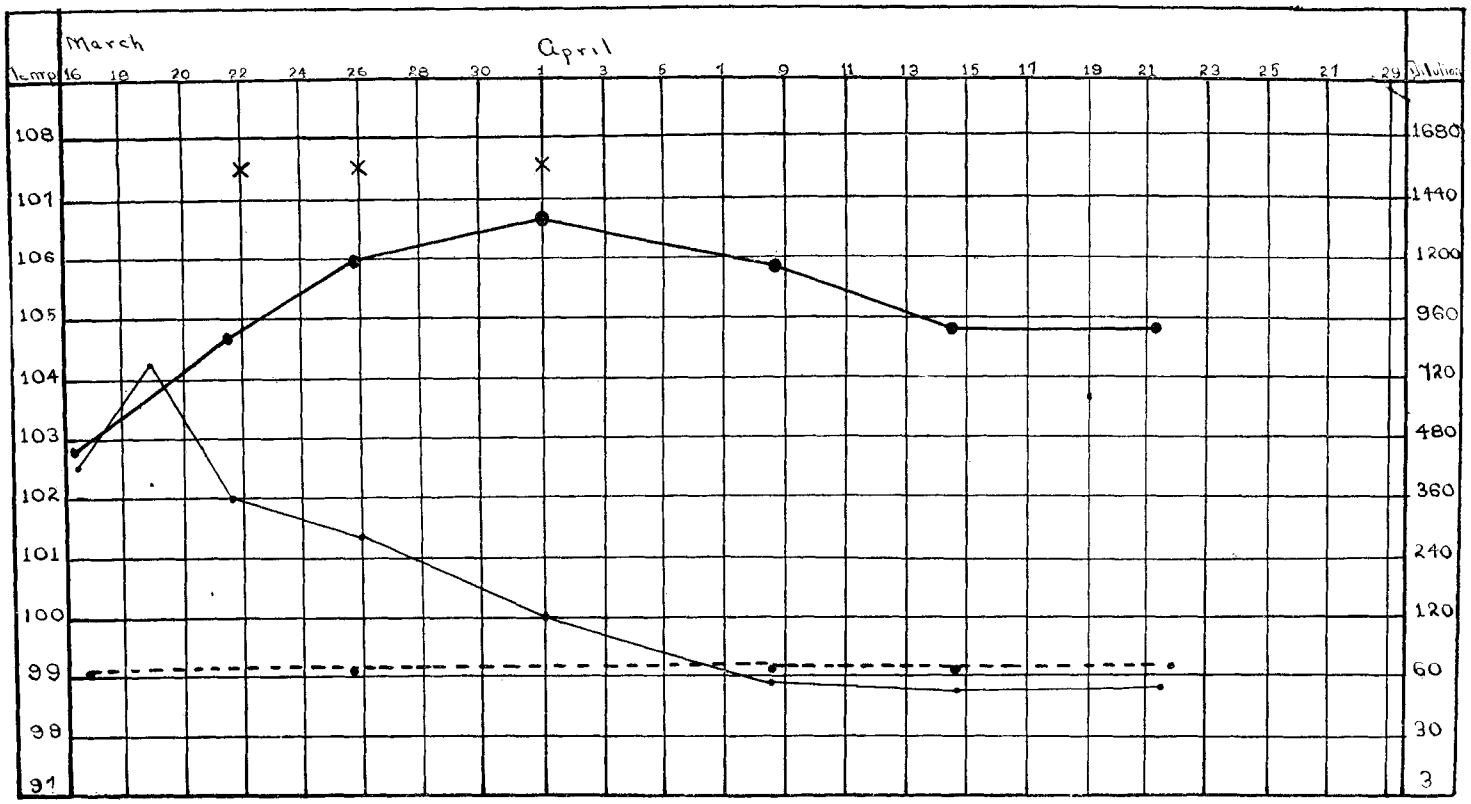

CHART II. Heavy straight line represents opsonic curve of patient; dotted line, opsonic curve of normal individual; fine straight line, temperature curve of patient; *X* represents dates of vaccination.

March 10.—The patient has been prostrated and delirious at times. He has had incontinence of urine. The temperature has gradually risen to 103.5° F., and the pulse to 120. The urine after operation was acid (sp. gr., 1,020) and contained a trace of albumin. There were hyaline and granular casts, with many pus cells and a few red blood cells.

March 18.—The wound has completely broken down, and brawny induration extends from the costal margin to the crest of the ilium. The urine has been voided in good amount, averaging 50 to 80 ounces in twenty-four hours. On this date, there is swelling of the left shoulder and axillary region, the arm, the forearm and the hand, with much tenderness along the course of the axillary vein. A blood culture made on March 14 contained a pure growth of *Staphylococcus aureus* and a culture from the urine on this date contained *Streptococcus* and *Bacillus coli communis.*

A vaccine was prepared from the *Staphylococcus aureus* found in the blood and the first dose of one hundred million cocci administered on March 22. This dose was repeated on March 26 and April 1.

April 1.—The temperature is lower and the patient's condition has improved.

April 23.—The patient continues to improve, and the wound has healed completely. The signs of phlebitis in the left arm have disappeared. The urine contains only a few casts and a trace of albumin, and is voided in good amount.

The opsonic activity of the serum was followed from March 16 until the patient left the hospital (see Chart II). There was a steady increase during the time of infection and a slight diminution during convalescence. The highest point attained was 1 to 1280, and when the patient passed from observation the opsonic power ceased at a dilution of 1 to 960. Whether the administration of the vaccine tended to increase the opsonic power is uncertain. Before the vaccines were given, the opsonic power of the serum was greater than normal; there was further increase and synchronous with this increase there was marked and continuous improvement in the patient's condition.

CASE II.—M. J., age 26, male, white, had suffered for five years with repeated attacks of furunculosis, which had resisted systemic and local treatment. On April 24, 1909, a furuncle appeared on the back of the neck. It was incised but remained as a brawny induration about 3 cm. in diameter, and discharged very little pus. On April 28, a second boil appeared about 3 cm. from the first and quickly attained a large size. Merging with the first, it formed a large indurated mass about 10 cm. in diameter. Both furuncles were again incised, but incision did not have a favorable result; the induration remained and the whole of the back of the neck, on the right side, was dark red, indurated and exquisitely tender. The cervical glands were enlarged. A culture from the first furuncle contained *Staphylococcus aureus* and from this culture a vaccine was prepared. Five hundred million cocci were injected

subcutaneously, May 1, at 9 p. m. The next afternoon there was a moderate reaction at the point of injection, which increased towards evening; at this time the induration and tenderness of the furuncles had increased and the patient felt sick. On the morning of May 3, there was a marked change; the induration of the furuncles had almost disappeared, the redness and tenderness were much less, and there was a very profuse discharge of pus. Within twenty-four hours the discharge ceased, and all that remained to indicate the points of incision were two small openings. The patient's general condition was excellent, and he has remained totally free from subsequent infections during a period which has been the longest for five years.

The opsonic power of the serum was estimated several times before and after the vaccinations (see Chart III). The opsonic

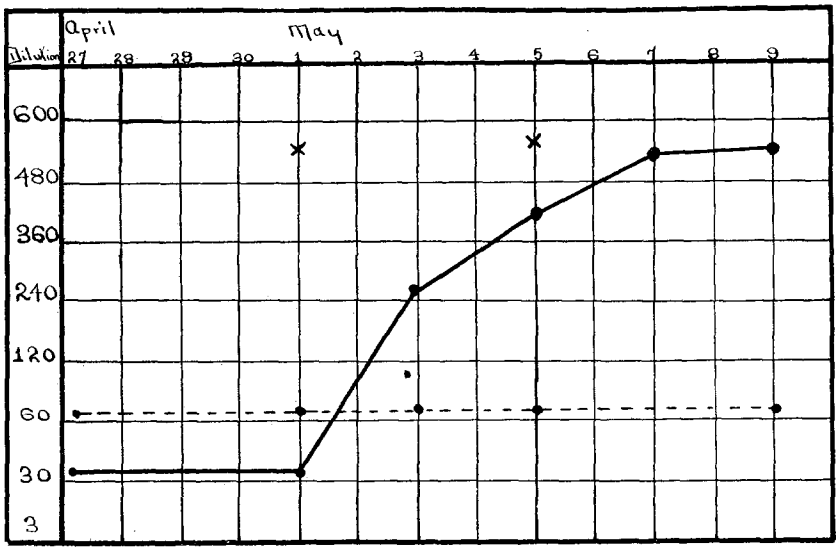

CHART III. Heavy line represents opsonic curve of patient; dotted line, opsonins of normal individual; *X*, dates of vaccination.

activity of the serum before vaccination was subnormal (1 to 30), but immediately after the first administration of vaccine the opsonic activity increased and continued to increase steadily after the second vaccination. In this case, the increase of opsonins and the synchronous improvement of the local lesion and the general condition of the patient are strong evidences in favor of the view that increase of opsonic immunity served to combat infection with staphylococcus.

CONCLUSIONS.

1. The administration of *Staphylococcus aureus,* killed by heat (vaccine), produces a high degree of opsonic immunity in rabbits.

2. Such increase of opsonin affords protection against living virulent staphylococcus in direct proportion to the amount of opsonins present in the serum and complete recovery may follow subsequent inoculation, if the opsonic power be high.

3. Frequent administration of vaccines may produce a diminution of the opsonic power of the serum.

4. Immune opsonins are most active against the homologous strain of *Staphylococcus aureus,* but are only slightly less active against heterologous strains.

5. Infections of the human body by *Staphylococcus aureus* may cause great increase of opsonins.

6. Vaccines prepared from *Staphylococcus aureus* may produce a high degree of opsonic immunity in man.