ON LOCAL AND GENERAL IMMUNITY:

AN INVESTIGATION BASED UPON EXPERIMENTAL ERYSIPELAS IN ANIMALS; TOGETHER WITH SOME OBSERVATIONS ON THE MODE OF GROWTH AND LIFE-HISTORY OF THE *STREPTOCOCCUS ERYSIPELATIS*.

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- I. Introduction.
- II. Methods.
- III. Mode of growth of *Streptococcus erysipelatis*.
- IV. Cause of cessation of growth of the streptococcus in beef-broth.
- V. On the changes in virulence produced by varying the artificial media.
- VI. Method of increasing the virulence of the streptococcus.
- VII. On the effect of intra-abdominal injections of *Streptococcus erysipelatis*.
 - (a) Disappearance of the micro-organisms from the peritoneal cavity.
 - (b) On metastatic erysipelas.
- VIII. On the immunity conferred upon the abdominal cavity against virulent cultures of *Streptococcus erysipelatis* by previous injections of attenuated virus.
- IX. On the effect of inoculating the ears of rabbits with streptococci—
 - (a) The occasional appearance of relapses and their nature.
- X. Comparing the results of inoculations into parts which had been directly affected by erysipelas with those of similar inoculations into other parts of the same animals.
 - (a) On general immunity, conferred by an attack of cutaneous erysipelas.
 - (b) On local immunity, conferred by cutaneous erysipelas, as shown by the effect of second inoculations of ears which had recently been the seat of erysipelas.
- XI. On the cause of the inflammatory reaction which resulted when ears, locally protected by recent erysipelas, were reinoculated with living streptococci.
- XII. On the production of general immunity by intra-peritoneal injections of attenuated cultures of *Streptococcus erysipelatis*.
- XIII. On the relation of inflammatory reaction to immunity.
- XIV. On the relation of inflammation to recovery from erysipelas.
- XV. On immunity produced by the chemical products of the *Streptococcus erysipelatis*.
- XVI. Conclusions.

I. INTRODUCTION.

WHEN pathogenic micro-organisms have once succeeded in establishing themselves in the body of a living animal, and by growing and multiplying have caused the symptoms of disease, we might naturally expect that they would continue to flourish there until they had destroyed their host; yet many microbic diseases, under favourable circumstances, terminate in recovery. At some period during the course of these affections a change takes place in the body of the patient, which makes it a less favourable place for the microbes to live in than before; they consequently disappear, and recovery ensues. Moreover, for some time afterwards, the patient is found to be less susceptible to the attack of the same microbe. The supposition, that immunity is due to the same change which brought about recovery from the first attack of the disease, has commended itself to many observers. Now, it is well known that, in erysipelas, recovery in the part first affected may commence while the disease is actively spreading elsewhere. Here a local change must be the cause of the local recovery, and it seemed worth while to inquire whether this is followed by any local immunity. It was in order to investigate this point that the present research was undertaken.

But erysipelas in favourable cases terminates before the whole surface of the body has been affected, and consequently this ultimate recovery must be due to some change which affects the whole of the body, and this, on the supposition alluded to above, should, for as long a time as it persists, cause general immunity. That erysipelas confers an immunity of short duration, though overlooked by clinical observers, has been fully demonstrated by Fehleisen, Roger, and others, but none of these observers distinguish between local and general immunity.

Fehleisen, who inoculated patients suffering from cancer with erysipelas, found it impossible to communicate the disease to those who had recently recovered from an attack. The longest period of immunity which he observed was a few months only. More recently Roger has shown that it is possible to immunise rabbits by means of intravenous injections of living broth cultures, or of filtered cultures heated to 110° C. And he points out that, after an attack of cutaneous erysipelas, a second inoculation produces either a mild form of the disease, or merely a small abscess.

Since it is true that an attack of erysipelas is followed by general immunity, it will be impossible to obtain evidence of local immunity, unless the parts which have been directly affected have acquired a protection which is more efficient or of longer duration than that acquired by the rest of the body. We might indeed be able to obtain evidence of local immunity in a part which had recovered before the disease had ceased to spread elsewhere, but, in the animals at our disposal, erysipelas did not extend widely enough to make this possible. We hope to be able to show that this difficulty did not prove a serious

obstacle, but, before describing this part of our work, it will be necessary to say something of the methods employed, and desirable to make some observations upon the life history and mode of growth of the streptococcus.

II. METHODS.

In our experiments we used rabbits, which not only possess the advantage of being susceptible, but also of having large ears, which are very convenient for observing the effects of an inoculation.

Our original cultures were obtained from cases of erysipelas in man; one from a patient suffering from the facial form of the disease, and two others from children in whom the inflammation had spread widely over the surface of the body.

The method of obtaining these cultures was the following:—A part was selected where the disease was actively spreading by a definite margin. This was sterilised by washing successively with soap and water, ether, and perchloride of mercury, 1 in 1000. Absolute alcohol was then applied to remove the mercury, and when the skin was dry a small puncture was made with a sterilised needle. A drop of fluid was then squeezed out, and this was transferred by means of a platinum wire to one or more agar tubes and spread over their surfaces. Some difficulty was experienced in obtaining any fluid, and it was found advisable to make the puncture at a distance of 1 in. from the actual margin where the swelling was greater.

The tubes were incubated at a temperature of 37° C., and minute colonies of streptococci became visible in 1 or 2 days. In the many tubes sown on these occasions, only one foreign colony appeared. We also, on one occasion, made use of Fehleisen's method of snipping off a piece of the affected skin with sterilised scissors, but we found that this was attended with more risk of contamination; due, no doubt, to the fact that it is impossible completely to sterilise the epidermis. And we preferred the puncture method, on account of its simplicity and certainty. Moreover, it causes very little discomfort to the patient, a matter of some importance if it is to be used as a diagnostic test of erysipelas in man. Many new cultures were obtained in the course of our work from the ears of rabbits affected by erysipelas, and in these cases a slight modification of the method just described was employed. A small area of skin was sterilised by the momentary application of a hot glass rod, which caused no appreciable discomfort. A fine capillary glass tube was then pushed into the subcutaneous tissue, and the fluid which entered it was subsequently blown out on to the surface of nutrient agar.* With due care this method was found to be quite free from risk of contamination. From these cultures others were obtained on beef-broth containing peptone, solidified blood serum, and nutrient gelatine.

In order to produce erysipelas, liquid or solid, cultures were rubbed

into scratches, made with a sterilised needle, or liquid cultures were injected by means of a hypodermic syringe, but the following method was found to give the most satisfactory result. A small portion of the surface of the skin having been sterilised in the way just described, a puncture $\frac{1}{4}$ in. long was made under the skin with a needle. A straight platinum wire, rubbed over the surface of a culture on a solid medium, was then introduced into the puncture, and as it was withdrawn the ear was held between the finger and thumb in such a way as to cause the streptococci which had adhered to the wire to be left behind. The puncture was then sealed with collodion. Intra-abdominal injections were made by means of a capillary glass pipette, pushed through the abdominal wall, after the skin had been sterilised with a hot rod. This procedure was found to be unattended by risk of wounding the intestine.

The inflammatory changes produced by inoculating the rabbits' ears were recorded in notes and drawings, and comparative measurements of the intensity of the inflammation obtained by observing the changes in the volume of the affected parts. These measurements were made in the following way. A cylindrical vessel, of a size just sufficient to admit the swollen ear, was filled with water, and into it the ear was immersed until the brim was in accurate contact with the animal's head. The ear having been withdrawn, its volume was then estimated by measuring the quantity of water which was required to make up for that which had been displaced. Inasmuch as rabbits' ears, while affected by erysipelas, are very liable to become temporarily congested when interfered with in any way, it was necessary, in order to avoid errors which might arise from this cause, to handle them as little as possible, and to employ cold water for their immersion. For the purpose of comparing the changes in volume of ears which, at the time of inoculation, were of different sizes, it was thought desirable to record, not the volumes actually measured, but the ratios of these to the original volume of the ear. Curves were accordingly drawn, in which the ordinates represent these ratios, and the abscissæ equal intervals of time. When these were compared with the notes taken at the same periods as the measurements, they were found to give an accurate representation of the intensity and rate of progress of the acute stages of the inflammation, but no indication of its subsidence, because considerable thickening in all but mild cases remained for many days after redness and heat had disappeared.

III. ON THE MODE OF GROWTH OF THE STREPTOCOCCUS ERYSIPELATIS.

In beef-broth containing peptone the *Streptococcus erysipelatis* grows rapidly at a temperature of 37° C. At times the resulting growth is scanty, at other times copious. In the former case the liquid remains clear throughout. In the latter, it is turbid at first, but becomes clear after a day or two when the micro-organisms have settled at the bottom of the tube.

The duration of the life of the individual coccus is probably very short, for if a tube be sown in the ordinary manner from a broth culture, which has for 3 or 4 days been free from turbidity, growth but seldom takes place. For this reason it was necessary to transfer our cultures to new tubes every few days. Later, however, we found that it is possible to obtain a growth from an old culture, by taking a considerable quantity of the sediment of microbes and putting it into new broth. When this was done, we had no more difficulty in obtaining a culture from one 90 days, than from others a week or 10 days old. These experiments were repeated on many occasions, always with the same results; and we came to the conclusion that the majority of the cocci die about the end of the first week, but that a few remain alive for a considerable period.

The sediment, which collected at the bottom of a flask when active growth ceased, usually diffused itself readily throughout the liquid on shaking, but it was sometimes collected into flocculent masses, which did not readily break up.

The microscope showed that individual cocci varied much in size. Large and small beads were seen in the same chain; and chains—some composed of large and some of small beads—were often present in the same flask. Moreover, in some cultures the cocci were generally of a larger size than in others, and it was in these cultures that the sediment collected into flocculent masses.

Von Lingelsheim,¹ who has recently studied the mode of growth of streptococci, mentions this formation of flocculi. He found that there was a greater tendency to their formation in cultures sown directly from the animal body than in those sown from cultures which had been kept for a long time on artificial media; and he looked upon their presence as a favourable indication of vitality and virulence. On the other hand, in our experience the formation of these flocculi occurred only in cultures which had been grown for many generations in beef-broth, and which possessed little or no pathogenic power.

In broth, without the addition of peptone, there was very little growth.

On agar the colonies varied much in size. As is the case with many other microbes, they remained small when thickly clustered together, and grew larger when situated at a greater distance from their neighbours; they seldom, however, reached the size of a small pin's head. Occasionally all the colonies of one tube were of a larger size, and this was the case when a film of blood containing cocci was spread over the surface of the nutrient medium. They then absorbed the pigment from the blood immediately around them, and appeared surrounded by a colourless halo. In tubes which were fairly dry the colonies were sharply defined and discrete, but on moist agar their outlines were irregular, and they tended to run together and form a uniform film on

¹ *Ztschr. f. Hyg.*, Leipzig, 1891, bd. x. s. 329.

the surface. No difference was found in the virulence of cultures composed of large as compared with those composed of small colonies, but the growths on dry tubes appeared to be more powerful than those on moist.

IV. ON THE CAUSE OF THE CESSATION OF THE GROWTH OF STREPTOCOCCUS ERYSIPELATIS IN BEEF-BROTH.

In order to find out why the streptococcus ceases to grow in broth at the end of the first few days, we undertook some experiments similar to those made by Pasteur¹ on chicken cholera many years ago. The clear fluid of an old culture was drawn up into a pipette and put into sterile tubes. These, together with control tubes of fresh broth, were then sown with various micro-organisms, including the streptococcus. In none of the latter did any growth appear, but *Bacillus anthracis* and other peptonising microbes flourished in the fluid. This led us to add 1 per cent. of peptone in concentrated solution to the old broth, with the result that it became capable of supporting as copious a growth of the streptococci as appeared in the control tubes.

It appears from this that the culture ceases to grow because its supply of nutriment is exhausted. In support of this view we are able to bring forward some evidence that there is no bactericidal body in the broth of an old culture which is capable of preventing further growth.

Buchner found that the bactericidal property of serum is destroyed by exposure to a temperature of 50° C. for one hour.² And it seemed probable that if any similar substance existed in the fluid of our old cultures it would be destroyed by heat. We therefore heated this fluid in the autoclave to 110° C. for 15 minutes, and again sowed it with streptococci. No growth appeared. In other tubes of the same material, which had been exposed to a temperature of 130° C. for 1 hour, a trace of growth did indeed develop after they had been sown. This we attributed, not to the destruction of any bactericidal body, which would probably have taken place at the lower temperature, but to the formation of peptone out of some albuminous body (possibly alkali albumen) in the broth. Further evidence of the absence of any bactericidal substance from the fluid of an old culture was obtained by evaporating it, *in vacuo*, to small bulk, and adding enough fresh broth to restore its original volume. In this mixture the streptococcus grew as freely as in the control tubes sown at the same time.

From these results we conclude that the cessation of growth of a culture of streptococci in peptone broth is due solely to the exhaustion of medium; an opinion identical with that arrived at by Pasteur as the result of his experiments on chicken cholera.

¹ *Compt. rend. Acad. d. sc.*, Paris, 1880.

² *Centralbl. f. Bakteriöl. u. Parasitenk.*, Jena, bd. v.

V. ON THE CHANGES IN VIRULENCE PRODUCED BY VARYING THE ARTIFICIAL CULTURE MEDIA.

In our experiments on rabbits we first made use of broth cultures, because they were more convenient for injection.¹ When liquid cultures were injected under the skin of the ears, they were found to be incapable of causing erysipelas; even 10 drops produced only a slight transient inflammation or local suppuration.²

Considerable variation was observed in the virulence of different broth cultures when introduced into the peritoneal cavity. For instance, one animal died 24 hours after receiving 1 c.c., another in the same time after 2 c.c., and a third 18 hours after 5 c.c., but this was quite exceptional. In the majority of cases it required 10 c.c. to kill an animal, and recovery sometimes occurred after the injection of 30 c.c. of broth culture.

When broth had been sown with streptococci, taken directly from the body of an animal, it was more virulent than that which had been sown from another broth culture. Observations on the different degrees of virulence of cultures of various ages gave conflicting results. Old growths were in some instances harmless, in others powerful. When the micro-organisms were transferred directly from the body of an animal to agar, or solidified serum, their virulence was not appreciably diminished, and we did not observe any attenuation in cultures grown for several generations on solid media. It was noticed also that cultures increased in virulence when transferred from beef-broth to agar, so that they would then produce erysipelas. We had not, however, discovered these facts at the time of our earlier experiments, and were obliged to increase the virulence of broth cultures in other ways.

VI. METHOD OF INCREASING THE VIRULENCE OF THE STREPTOCOCCUS.

We attempted to increase the virulence of a broth culture of streptococci, first by cultivating them in rabbit's serum. In this medium they grew well, but their pathogenic power was not increased. We next grew them in tubes of beef-broth, from which all oxygen had been expelled, but without success. We succeeded better when we grew them in the abdominal cavities of several rabbits in succession. A large injection of a beef-broth culture having resulted in the death of the first animal, the peritoneal exudation containing cocci was taken up in a pipette and injected into the abdomen of a second rabbit. When the second

¹ Unless otherwise stated, the broth cultures used for injection were 2 or 3 days old, and their vitality was proved by re-sowing.

² Since this was written we have obtained from a man suffering from erysipelas, a culture of streptococci, which, when cultivated in beef-broth, even for two or three generations, was capable of causing erysipelas in rabbits.

animal died its peritoneal fluid was injected into a third, and so on, until the culture had passed through 3 or 4 rabbits. It was then found to have reached such a degree of virulence that 2 or 3 drops of the peritoneal exudation, put into the abdomen of another animal, produced death in about 12 hours or even less; and when the same material was rubbed into little scratches in a rabbit's ear, in the manner in which vaccine lymph is usually applied, it produced severe erysipelas.

Nevertheless, if sown on broth, its virulence immediately diminished, and it was necessary to inject into the abdomen 10 c.c. of such a culture to produce death.

VII. THE EFFECT OF INTRA-ABDOMINAL INJECTIONS OF STREPTOCOCCUS ERYSIPELÆTIS.

Shortly after intra-abdominal injections of 10 c.c. or more of living broth cultures, the animals began to show symptoms of illness. In about an hour they had become less active than their fellows and refused food; a little later they might be seen crouching in the corner of the cage, rocking to and fro with each respiratory movement. The temperature fell immediately after the injection, sometimes as much as 2° or 3° F. In the course of a few hours it rose above the normal, and in more than one instance it reached 106° F. on the second day.¹ In fatal cases it fell considerably, before death, which occurred in from 4 to 45 hours from the time of injection. Diarrhœa was commonly observed in those rabbits which survived for more than 12 hours.

Post-mortem examination revealed the presence of more or less inflammatory œdema in the subcutaneous tissues about the puncture. Within the abdomen the signs of peritonitis were comparatively slight, a few c.c. of turbid fluid, sometimes blood stained, were found. A little lymph was often seen gluing the large intestine to the parietal peritoneum, near the track of the pipette, and the same material was sometimes found about the edges of the spleen. The omentum which, as a rule, appeared to have suffered more severely, was commonly found drawn up, œdematous, and reddened by hæmorrhages. The intestines were distended with watery material. The lungs were often congested, and the pleural and pericardial cavities contained an excess of fluid.

(a) *On the disappearance of the streptococci from the peritoneal cavity.*—In many cases the peritoneal exudation contained multitudes of free streptococci; in others, they were found only in the interior of the cells; and in a few none could be seen. Growth commonly, but not invariably, occurred when the fluid was sown on nutrient media.

¹ The normal temperature of a rabbit is 102°–103° F.

We observed that in those animals which succumbed quickest, free cocci were very numerous in the peritoneal exudation; and in those which survived longest they were either absent, or contained within phagocytes. In very young rabbits they disappeared earlier than in older ones. To this rule there were frequent exceptions, and we must rely on the accompanying table to show upon what evidence it rests.

TABLE I.

CLASS I.—IN WHICH BEEF-BROTH CULTURES WERE USED FOR INJECTION.			
Rabbits 8-12 weeks old. Weight 800-1100 grms.	Dose.	Time of Death.	Streptococci in the Peritoneal Cavity.
A	18 c.c.	4½ hours	Very numerous.
B	45 c.c.	5 "	Do.
C	18 c.c.	8 "	Do.
D	3 c.c.	12 "	Numerous.
E	5 c.c.	15½ "	Do.
F	38 c.c.	18 "	Do.
G	5 c.c.	18 "	None observed, not sown.
H	15 c.c.	19 "	Very numerous.
I	13 c.c.	20 "	Do.
J	15 c.c.	20 "	Not examined.
K	11½ c.c.	22 "	Numerous.
L	14 c.c.	23 "	Do.
M	1 c.c.	24 "	None observed, not sown.
N	(20 days old.) 2 c.c.	24 "	Very numerous.
O	10 c.c.	30 "	Few, many in cells.
P	5 c.c.	36 "	Numerous.
Rabbits 4-5 weeks old.			
Q	20 c.c. (43 days old.)	4 "	None observed, but growth on sowing.
R	3 c.c.	9 "	None observed, not sown.
S	9½ c.c.	42 "	Do. but growth on sowing.
CLASS II.—IN WHICH PERITONEAL EXUDATION CONTAINING COCCI WAS MIXED WITH BEEF-BROTH, INCUBATED FOR A FEW HOURS AND USED FOR INJECTION.			
Rabbits 8-12 weeks old.			
A	6 c.c.	7 hours	Very numerous.
B	6 c.c.	8 "	Do.
C	2½ c.c.	8 "	Not examined.
D	12 c.c.	11 "	Do.
E	4½ c.c.	16 "	Present.
F	5 c.c.	16 "	Do.
G	1 c.c.	36 "	Not examined.
H	8 c.c.	44 "	Present in cells only.
Rabbit 4-5 weeks old.			
I	½ c.c.	28 "	Do. do. growth on sowing.

TABLE I.—*continued.*

CLASS III.—IN WHICH PERITONEAL EXUDATION, DIRECT FROM ANIMALS WHICH HAD RECENTLY DIED OF AN INTRA-PERITONEAL INJECTION, WAS USED.			
Rabbits 8-12 weeks old. Weight 800-1100 grms.	Dose.	Time of Death.	Streptococci in the Peritoneal Cavity.
A	1 c.c.	5½ hours	Very numerous.
B	(?)	7 "	Do.
C	4 drops	12 "	Do.
D	3 c.c.	12 "	Not examined.
E	1 c.c.	13 "	Do.
F	2 c.c.	15 "	Very numerous.
G	3 c.c.	15 "	Do.
H	½ c.c.	18 "	Present in cells only.
I	5 c.c.	54 "	Not examined.
J	4 drops	4 days	Do.
Rabbits 4-5 weeks old.			
K	3 drops	10½ hours	Present.
L	3 drops	17 "	Do.
M	Grown from a serum tube	7 days	Few in cells only, growth on sowing.

Streptococci were often found in the pericardial and pleural fluid.

In the blood, after death, they were commonly present. In 3 only, out of 13 cases in which we sowed the blood on nutrient media, did we observe no growth. The fact that 20 to 30 colonies appeared on the surface of agar tubes sown with a single drop of blood gave a rough indication of their relative numbers.

In those cases, in which no streptococci could be demonstrated by culture experiments to be present in the peritoneal exudation, they were found in the blood, and it was evident that even in many of those animals which died of septicæmia after an abdominal injection, the streptococci had more or less completely disappeared from the peritoneal cavity.

In order to find out how quickly they disappear from this situation in non-fatal cases, 2 rabbits which appeared about to recover were killed. In the first, which had received 5 c.c. of a broth culture (2 days old) 30 hours before, only one chain of free streptococci was found after prolonged search, but many cocci were contained in cells, and broth sown with this fluid grew a good culture. The second rabbit having shown no signs of illness after an injection of 6 c.c. of an anærobic broth culture (2 days old), received, next day, 10 c.c. of a similar material, 1 day old, swarming with streptococci. When killed 5½ hours later, not only could no streptococci be seen either free or in cells, but no growth developed in broth sown with the abdominal fluid.

(b) *On metastatic erysipelas.*—Out of 41 animals which had been inoculated in the peritoneal cavity for the first time, with cultures of streptococci, 3 developed erysipelas in the ear a few days later.

In the first case 28 c.c. of a beef-broth culture were injected into the peritoneal cavity of a rabbit weighing 1160 grms. A considerable quantity of the fluid was forced out of the abdomen into the subcutaneous tissue, and a fluctuating swelling was thus produced. This was rapidly absorbed, but a ring of inflammation appeared around the place where the swelling had been, and spread until it was $1\frac{1}{2}$ in. wide; after this no further extension occurred. On the seventh day erysipelas appeared in the right ear, and increased in severity till the death of the animal on the ninth day.

In the second case, a rabbit weighing 840 grms. received 16 c.c. of a beef-broth culture. On the following day it appeared quite well. Twenty-four hours later it was again injected with 15 c.c. of a more virulent broth culture. On the following day there was an erysipelatous patch about $1\frac{1}{2}$ in. in diameter about the seat of inoculation, and 12 hours later erysipelas appeared in the right ear. It ran a mild course, with slight disturbance of general health, and the animal recovered.

The third instance of metastasis occurred in a rabbit weighing 720 grms., which had had injected into the abdominal cavity 6 c.c. of a broth culture, sown direct from the peritoneal fluid of an animal, and therefore more virulent than that used in the two cases just described. For the next 4 days it remained quiet and ate little. On the eighth day erysipelas appeared in the right ear and spread over the head; on the ninth day it had reached the opposite ear, and the animal died the same evening. Streptococci were grown from fluid taken from the spreading margin on the left ear during life, and from the blood after death.

VIII. ON THE IMMUNITY CONFERRED UPON THE ABDOMINAL CAVITY AGAINST VIRULENT CULTURES OF *STREPTOCOCCUS Erysipelatis* BY PREVIOUS INJECTIONS OF ATTENUATED VIRUS.

In order to find out how far a previous injection of a weak culture of streptococci increases the resisting power of the peritoneum, we injected into the abdominal cavity of a number of rabbits, which had recovered from their first injection, a second dose of more virulent cultures; some of the animals received 3 or even 4 injections; control animals were used in all cases.

The results of these experiments show that the previous injections had considerably increased the resistance of the peritoneum. In 15 out of 18 experiments, the animals, which had received a previous injection, recovered from a second, third, or fourth injection of most virulent material, in quantities as large, and in some instances considerably larger, than those which killed the controls. On one occasion the dose was too weak to kill either animal. In the three instances in which the animals died of the second or third injection, we must suppose

TABLE II.—*Showing the Immunity conferred upon the Abdominal Cavity by Intra-Peritoneal Injections of Cultures of Streptococcus erysipaelis.*

FIRST INJECTION.			SECOND INJECTION.			THIRD INJECTION.			FOURTH INJECTION.		
Animal.	Weight in Grammes.	Dose.	Result.	Interval.	Weight.	Dose.	Result.	Interval.	Weight.	Dose.	Result.
I.	800	20 c.c. B (? age)	Recovery	1 day No control	?	45 c.c. B	Recovery	1 day Control A	?	30 c.c. B 10 c.c. B 45 c.c. B	Recovery Death 30 hours Death 5 hours
II.	840	15 c.c. B (3 days old)	Recovery	5 days Control	800 1100	6 c.c. P+B 6 c.c.	Recovery Death, 7 hours	10 days Control	830 875	5½ c.c. P+B 2½ c.c.	Both found dead next morning
III.	1175	20 c.c. B (3 days old)	Recovery	5 days	1025	8 c.c.	Recovery				
IV.	1040	19 c.c. (43 days old)	Recovery	5 days Control	1040 1035	8 c.c. P+B 8 c.c.	Recovery Death, 40 hours				
V.	840	16 c.c. B (5 days old)	Recovery	5 days Control	835 875	15 c.c. P+B 9½ c.c.	Recovery Death, 41 hours	7 days Control	920 940	12½ c.c. B 12½ c.c.	Recovery 2nd day Recovery 3rd day Dose not large enough
VI.	515	One agar culture	Recovery	5 days Control	510 480	5 c.c. B 5 c.c.	Recovery Death, 24 hours				
VII.	?	20 c.c. B (? age)	Recovery	6 days Control	425 925	½ c.c. P ½ c.c.	Recovery Death, 17 hours				
VIII.	880	10 c.c. B (12 days old)	Recovery	6 days Control	850 930	1 c.c. P+B 1 c.c.	Death, 18 hours Death, 28 hours	This rabbit the peritoneal cavity was full of cysticerci			
IX.	940	12 c.c. B (1 day old)	Recovery	9 days Control	700 730	3 c.c. P+B 3 c.c.	Recovery Death, 9 hours				
X.	925	9 c.c. B (2 days old)	Recovery	14 days Control	1080 1230	6 c.c. P+B 5½ c.c.	Recovery Death, 40 hours				
XI.	890	30 c.c. B (2 days old)	Recovery	15 days Control	835 895	5½ c.c. P+B 4½ c.c.	Both found dead next morning	Dose ably too			
XII.	1320	9½ c.c. B (? age)	Recovery	15 days Control	1300 1060	Similar dose of same serum culture	Recovery Death, 7 days				
XIII.	1010	Soluble products	Recovery	8 days Control	510 480	5 c.c. B 5 c.c.	Recovery Death, 24 hours				

N.B.—B=Beef-broth culture. P=Peritoneal exudation fluid containing cocci, from an animal which had just died of an abdominal infection of a living culture. P+B=Mixture of peritoneal exudation fluid with a little beef-broth inoculated for a few hours at 37°C.

that the dose was too large, and that, although the immunity conferred on the peritoneum by previous injections is considerable, yet it is not complete: the animals may die if the virus be very strong.

That this increased resistance of the peritoneum to the streptococcus is a specific immunity to that microbe and no other we do not assert. Bearing in mind that Klein¹ was able to produce immunity against injections of the cholera spirillum into the abdominal cavities of guinea-pigs by previous injections in the same situation of various other microbes, we are inclined to think that the inflammation rendered the peritoneum more resistant to bacterial invasion in general; but on this point we have no evidence. It is interesting to notice that clinical observations on man accord with these views. Treves² points out that abdominal operations on persons who have previously suffered from peritonitis are attended with less danger of septic complications than like operations on the healthy peritoneum.

The question, whether an injection of the streptococcus into the peritoneal cavity confers immunity on other parts of the body than those directly affected by it, will be more conveniently discussed when we have described the effect of inoculating the ears of fresh rabbits.

IX. ON THE EFFECT OF INOCULATING THE EARS OF RABBITS WITH STREPTOCOCCUS ERYSIPELÆTIS.

We have already mentioned that when broth cultures were injected under the skin of the ear, transient redness, and sometimes the formation of a minute quantity of pus, were the only consequences which followed. The more virulent material which we found necessary to use, in order to produce erysipelas, was either the peritoneal exudation of a rabbit which had died of an abdominal injection, or the growth from the surface of an agar tube. Between these two materials there is an important difference. The former doubtless contains, in addition to the microbes, the extra-cellular products of their vital activity; while the latter contains presumably nothing but the microbes themselves. A corresponding difference, in the mode and rate of progress of the inflammation which resulted from the injection of these two materials, was also observed.

The peritoneal exudation was either injected, in quantities of one to three drops, under the skin of the ear, or rubbed into scratches. The agar growths were, in our earlier experiments, suspended in a minute quantity of fresh broth, and used in the same manner; in our later experiments a platinum wire, rubbed over the surface of the culture, was inserted into a puncture made with a sterilised needle.

When the peritoneal exudation was used, the following appearances were observed. After 3 or 4 hours the skin about the seat of inocula-

¹ *Brit. Med. Journ.* 1893, p. 632.

² *Ibid.* February 8rd, 1894.

tion was found to be warmer than elsewhere and slightly red and swollen. These appearances were not sharply limited, but gradually faded towards the healthy parts. In about 12 hours there was slight œdema, and the vessels of the ear appeared blurred when seen by transmitted light. The swelling gradually increased, and the ear became hot, tense, and somewhat greasy. The inflammation reached its maximum, in severe cases, on the eighth or ninth day; in mild cases it began to diminish a few days earlier. The subsidence of the active signs of inflammation was rapid; heat and tension quickly disappeared, and the surface, no longer moist and greasy, became dry and scaly, but the œdema lasted for a considerable time. After this had gone the ears remained, in all but very mild cases, much thickened for several weeks, and the hair fell out, so that sometimes, after severe erysipelas, the ear became quite bare.

We were disappointed at the absence of the definite spreading margin, so characteristic of erysipelas in man. This we supposed to be due to the action of poisonous bacterial products present in the peritoneal exudation injected, which, by rapidly diffusing and causing inflammation, had obscured the effects of the more gradual advance of the microbe.

This supposition received support on three occasions, when the erysipelas spread to the opposite ear. In this situation, to which it was unlikely that the poisonous products had travelled in a direction contrary to the natural lymph stream, the inflammation spread by a definite margin, in the œdema fluid of which we found streptococci.

When erysipelas was produced by the inoculation of cultures on solid media, the inflammation spread by a similar definite margin from the commencement. The first sign was a small pale and slightly raised œdematous area extending a few mm. on each side of the puncture. In most cases this was first seen about 6 hours after the inoculation, and in 18 hours this patch had reached the size of a shilling, its yellowish-white colour contrasting with the more translucent bluish appearance of the surrounding parts, and the raised edge clearly defining its limits. Moreover, the orifices of the glands became very obvious, and between their depressions the skin was thrown into delicate wrinkles, giving, on a small scale, the appearance of leather made of pigskin. By transmitted light the central part of the patch appeared red, but the redness always stopped short of the margin. The vessels of the affected part were obscured; in the rest of the ear they were clearly seen; as a rule they were but little injected, unless the ear had been recently handled. The main central vessels, however, were slightly blurred, some lymphangitis having occurred along their course, and the auricular and submaxillary glands were enlarged. The margin of the inflammation gradually advanced over the ear, the whole of which became affected on the third day. About this time the ear drooped. The subsequent course was the same as in those inoculated with peritoneal exudation. A little pus was often

seen about the seat of the puncture, and in one instance small abscesses appeared in other parts of an ear which was unusually severely affected. In three cases the onset was delayed until the fourth or fifth day, and on more than one occasion erysipelas began at the root and spread towards the seat of inoculation.

On the Occasional Appearance of Relapses and their Nature.—In four instances inflammation reappeared in parts which were returning to their normal condition. This phenomenon is in apparent contradiction to the theory that erysipelas confers local immunity, and it is therefore desirable to describe these cases in detail, and to inquire whether this inflammation be true erysipelas.

The first instance of relapse occurred in a rabbit which had been inoculated in the ear with peritoneal exudation. On the following day a little inflammation appeared about the seat of inoculation, but this faded in a few hours, and the ear remained normal until the fourth day. The ordinary appearances of erysipelas were then observed to be present over the posterior third of the ear from root to tip. The inflammation then advanced towards the anterior border, and gradually faded in the part first affected. On the eighth day the anterior third was red and swollen, and the posterior two-thirds were almost normal in appearance. On the ninth, however, the whole ear was found to be hot and swollen, and on the tenth the animal died.

The remaining instances of relapse occurred when the signs of active inflammation had disappeared from the whole ear. On the tenth day in two cases, and in the other on the seventeenth, after the first inoculation, the parts which had just recovered from erysipelas again became hot, red and swollen; this recurrent inflammation appeared simultaneously in the whole ear, and rapidly subsided in about 48 hours. Two of the animals recovered, the other died about 5 days later, probably from some other cause, for no streptococci could be grown from the blood. Similar relapses have been observed in cases of erysipelas in man. In these the inflammation reappears simultaneously over a considerable area, no advancing margin is observed, and it lasts but a short time.

We shall have to point out later that when the products of the streptococcus are injected into an ear, which has recently recovered from erysipelas, a somewhat severe though transient inflammation appears almost simultaneously over the whole ear, differing markedly from the milder inflammation produced by the introduction of these poisons into other parts; and we would suggest that the relapses which we observed in our rabbits, and which occur occasionally in man, are not true erysipelas, that no second invasion of the part by micro-organisms takes place, but that the appearances are caused by diffusion of the products of the microbes growing in a part recently invaded; some cause, such as a general disturbance of health, due to some intercurrent disorder, having led either to a weakening of the resistance of the patient,

and to the consequent increase in the quantity of poisons produced, or to a failure to neutralise these poisons *in situ*.

It is to be regretted that we did not examine the œdema fluid of the ears by culture experiments during these recurrent inflammations, but at that time we did not recognise the importance of doing so.

Primary erysipelas in the ear was fatal in 19 out of 35 cases. In two of these death was probably due to other causes. Omitting the latter the time of its occurrence was between the third and tenth day after the inoculation.

Streptococci were found in the blood of 7 out of 11 cases examined after death by culture experiments. Our experience in these respects differs from that of Fehleisen,¹ who produced in rabbits erysipelas which never resulted in death; but it agrees with that of Roger,² who found that erysipelas in these animals was often fatal, and that it was possible to grow streptococci from their blood after death.

X. COMPARING THE RESULTS OF INOCULATION INTO PARTS WHICH HAD BEEN DIRECTLY AFFECTED BY ERYSIPELAS WITH THOSE OF SIMILAR INOCULATIONS INTO OTHER PARTS OF THE SAME ANIMAL.

Having succeeded in causing erysipelas in rabbits' ears, we proceeded to determine whether any local or general immunity had been conferred. We accordingly inoculated, at the same time, both ears of animals which had recovered from an attack in one ear, and observed the differences in the inflammations which resulted.

Most of the animals used in these experiments had recently recovered from their first attack, and the thickening which it had caused in their ears had not yet disappeared. This thickening varied considerably in different cases, and depended as much on the severity of the first attack of erysipelas as on the shortness of the interval between the inoculations.

Control animals were inoculated in nearly all cases, and great care was taken that in each experiment both ears of the animal whose immunity was to be tested, and also that of the control, should receive exactly the same quantity of the same culture.

The table on pp. 55, 56, 57 gives the results of these experiments.

We must first inquire what amount of general immunity had been conferred by the previous attack of erysipelas, before we can draw any inference about local immunity. We will, therefore, proceed to describe the results of inoculations into ears which had not been directly affected by the previous inflammation. We may here state, for convenience of description, that in all cases it was the right ear which had been the seat of the primary erysipelas.

(a.) *On general immunity conferred by an attack of cutaneous erysipelas.*

¹ "Ætiologie des Erysipelas," 1883.

² Roger, *Rev. de méd.*, Paris, 1892.

—The effects of inoculating the left ears of rabbits which had recently recovered from erysipelas in the right, varied considerably in different instances (Table III.). We have, accordingly, arranged these experiments in three classes, according to the intensity of the inflammation which resulted. In 6 animals, placed in Class I., erysipelas developed and ended fatally. Streptococci were found in the œdema fluid taken from the ears at some distance from the seat of inoculation, and also in the blood after death. In 4 animals, placed in Class II., the left ears became inflamed, and the appearances at first were like those of commencing erysipelas, but in 2 or 3 days the inflammation completely disappeared. In 4 animals, placed in Class III., nothing but a little redness, and in 2 of them a drop or two of pus resulted from the second inoculation.

In 13 of the 15 control animals, erysipelas developed, ran the usual course, and ended fatally in 6.

TABLE III.—*Showing the Effect of Inoculating with Streptococcus erysipelatis both Ears of Rabbits, which had recovered from Erysipelas in the Right Ear.*

CLASS I.—IN WHICH THE SECOND INOCULATION RESULTED IN AN INFLAMMATORY REACTION IN THE RIGHT, AND ERYSIPELAS IN THE LEFT EAR ; SHOWING LOCAL BUT NO GENERAL IMMUNITY.					
FIRST INOCULATION.			SECOND INOCULATION.		
	Right Ear.	Interval.	Right Ear.	Left Ear.	Control.
A.	These two rabbits were inoculated with equal quantities of the same culture. Both developed erysipelas of a mild type, which ran a similar course in each, and subsided on the fifth day.	After 6 days they were again inoculated with equal quantities of same culture. One in the right, and the other in the left ear.	Not inoculated.	Erysipelas, death eighth day, cocci in blood.	Did not take.
			Inflammatory reaction (3 days).	Not inoculated.	
B.	Same as the above A', but subsiding on the sixth day.	Same as the above, but the interval was 12 days.	Not inoculated.	Erysipelas, death sixth day, cocci in blood.	Erysipelas, recovery sixth day.
			Inflammatory reaction (mild). Death, however, occurred on the fourth day from another disorder. No cocci were found in blood.	Not inoculated.	

TABLE III.—*continued.*

CLASS I.— <i>continued.</i>					
FIRST INOCULATION.			SECOND INOCULATION.		
	Right Ear.	Interval.	Right Ear.	Left Ear.	Control.
C.	Erysipelas (4-day course).	9 days.	Inflammatory reaction.	Erysipelas, death fifth day.	Erysipelas, death fifth day.
D.	Erysipelas (6-day course).	10 days.	Inflammatory reaction.	Erysipelas, death fourth day, cocci in blood.	Erysipelas, death eighth day, cocci in blood.
E.	Erysipelas (6-day course).	12 days.	Inflammatory reaction (3 days).	Erysipelas, death seventh day.	Erysipelas in 2 controls, one died.
F.	Erysipelas (8-day course) with relapse on the fifteenth day.	17 days.	Inflammatory reaction (mild).	Erysipelas, death third day.	Erysipelas, 10 days, recovery.
CLASS II.—IN WHICH THE SECOND INOCULATION RESULTED IN AN INFLAMMATORY REACTION IN THE RIGHT, AND COMMENCING BUT ABORTIVE ERYSIPELAS IN THE LEFT EAR; SHOWING COMPLETE LOCAL AND LESS GENERAL IMMUNITY.					
A.	Erysipelas (7-day course).	9 days.	Inflammatory reaction (3 days).	Inflammatory reaction commencing more slowly than in the right, ending fifth day. Suppuration.	Erysipelas 6 days (recovery).
B.	Erysipelas (9-day course).	15 days.	Inflammatory reaction (2 days).	Began like erysipelas, aborted third day and suppured.	Erysipelas (severe) in 2 controls, 1 died.
C.	Erysipelas (12-day course).	17 days.	Inflammatory reaction (2 days).	Inflammation like erysipelas, ending fourth day.	Erysipelas (12-day course).
D.	Erysipelas.	30 days.	Inflammatory reaction (3 days).	Inflammation more severe than in the right, ending third day.	Inflammatory reaction (3 days).

TABLE III.—*continued.*

CLASS III.—IN WHICH THE SECOND INOCULATION RESULTED IN AN INFLAMMATORY REACTION IN THE RIGHT, AND LESS REACTION IN THE LEFT EAR; SHOWING COMPLETE LOCAL AND GENERAL IMMUNITY.					
FIRST INOCULATION.			SECOND INOCULATION.		
	Right Ear.	Interval.	Right Ear.	Left Ear.	Control.
A.	Erysipelas (10-day course).	12 days.	Inflammatory reaction (severe, ending fifth day.	Local redness and suppuration.	Erysipelas, death fifth day.
B.	Erysipelas (8-day course).	13 days.	Inflammatory reaction (2 days).	Local redness and suppuration.	No control.
C.	Erysipelas (6-day course).	22 days.	Inflammatory reaction.	Local redness.	No control.
D.	Erysipelas (very severe and prolonged).	37 days.	Inflammatory reaction (3 days).	Local redness.	No control.
CLASS IV.—IN WHICH THE SECOND INOCULATION RESULTED IN ERYSIPELAS IN BOTH EARS, SHOWING ABSENCE OF LOCAL AND GENERAL IMMUNITY, THE INTERVAL BETWEEN THE TWO INOCULATIONS BEING TOO LONG.					
A.	Erysipelas (mild) ending fifth day. A second inoculation produced an inflammatory reaction ending third day.	22 days from the first inoculation.	Erysipelas commencing sixth day, well developed on the seventh day.	Erysipelas began on the second day, no progress till sixth day, well developed on the seventh day.	Erysipelas, death eighth day.
B.	Erysipelas (severe).	118 days.	Erysipelas (severe) subsiding slowly from seventh day.	No inoculation.	No control.
C.	Erysipelas (severe).	135 days.	Erysipelas subsiding seventh day.	No inoculation.	Erysipelas 5 days (recovery).

(b.) *On local immunity conferred by cutaneous erysipelas, as shown by the effect of second inoculations of ears which had recently been the seat of erysipelas.*—The results of second inoculations of the ears *directly affected by the previous erysipelas* were constant. We cannot record a single instance of erysipelas appearing in those which had *recently* recovered from the first attack. In two instances, where the interval between the first and second inoculations was over 100 days, erysipelas did develop, and ran

the usual course; and in one other case in which the primary erysipelas had been very mild, and had subsided on the fifth day, erysipelas was produced after an interval of 22 days. In these 3 animals (Table III. Class IV.) we may suppose that the period of immunity was over, and it is important to note that at the time of the second inoculation no thickening remained.

In all the other cases the only result was a transient inflammatory reaction, lasting 2 or 3 days, and we could get no evidence of any invasion of these ears by streptococci, from an examination of agar

tubes sown with the œdema fluid of their ears, as we were able to do in cases of true erysipelas.

We may sum up the results of these experiments by saying that the first attack of cutaneous erysipelas had conferred a distinct general immunity upon more than one-half of the animals experimented on, but that the rest proved as susceptible as the control animals. On the other hand, an absolute local immunity had been conferred upon the parts directly affected by the first attack, unless the interval had been long enough to permit of the entire disappearance of all inflammatory thickening.

FIG. 1.

The reason for the variation in the amount of general immunity conferred did not very clearly appear. But it seems reasonable to suppose that it depended, partly at least, upon differences in the severity of the first attack, and it will be noticed in the tables that in those animals which proved to have no general immunity (Class I.), the primary erysipelas had run a shorter and less severe course than in those which proved to be better protected (Classes II. and III.) We must also mention that 2 out of the 6 rabbits which showed no general

immunity, were, at the time of the second inoculation, suffering from a disorder, the chief symptom of which was a profuse discharge of saliva and nasal secretion; and it is possible that the disturbance of general health diminished their resistance to the streptococcus.

The accompanying figures give some curves, in which are compared the relative intensity and duration of the inflammation which resulted from a second inoculation, in the ear locally protected by a previous erysipelas, and in the other.

The abscissæ represent days, the ordinates the ratios of the volumes actually measured, in the manner already described (p. 42), to the volume of the ear immedi-

FIG. 2.

FIG. 4.

FIG. 3.

FIG. 5.

ately before the second inoculation. For example, if at the time of the second inoculation the volume of an ear was 10 c.c., and on the fourth day it had increased to 20 c.c., the ratio $20:10=2$ will indicate that the ear has become doubled in size, as the result of the inoculation. In this way the proportional increase in volume of the two ears is compared.

It must be remembered that, at the time of the second inoculation, the ears previously affected were still swollen and steadily diminishing in volume. For such ears, then, a horizontal curve of volume indicates, not that the second inoculation was without effect, but that its effect was to check the diminution which was taking place. Moreover, the method of measuring volumes was not sufficiently delicate to give any evidence of slight degrees of inflammation, which were made quite obvious to the hand and eye by increased heat and redness.

Figs. 1, 2, 3, 4, and 5 are from experiments A, B, D, E, F (Table III. Class I. pp. 55 and 56). They illustrate the fact that these rabbits, which had recently recovered from erysipelas in the right ear, were completely immune to inoculations of the streptococci into these ears, but that they succumbed to erysipelas when similarly inoculated in the opposite ears.

Figs. 3 and 4 show in addition the transient inflammatory reaction which occurred in the protected ears. In the other experiments the reaction in the protected ears was too slight to cause any appreciable increase in volume.

XI. ON THE CAUSE OF THE INFLAMMATORY REACTION WHICH RESULTED WHEN EARS LOCALLY PROTECTED BY RECENT ERYSIPELAS WERE REINOCULATED WITH LIVING STREPTOCOCCI.

It has already been stated that when ears, which had been locally protected by recent erysipelas, were again inoculated with living streptococci, a more or less severe inflammatory reaction followed, and that this appeared before any inflammation was observed in the opposite ears of the same animals, treated in the same way, or in those of the controls. And we have given as a reason for thinking that this inflammation was not mild erysipelas, the fact that no multiplication and distribution of the organisms in the lymphatic spaces of the ear could be observed. It remained to be seen whether the poisonous products which had been introduced with the living cocci were capable of causing this reaction. We accordingly inoculated, with concentrated filtered cultures, both ears of rabbits which had recently recovered from erysipelas in one ear (right). The details of the four experiments which we made are given in Table V.; and the changes in volume which resulted in two of them are illustrated by curves (Figs. 6 and 7).

TABLE V.—Comparing the Effects produced by inoculating Filtered Cultures into both Ears of Rabbits which had recovered from Erysipelas in the Right Ear.

Animal.	Result of First Inoculation with Streptococci.	Interval between Inoculation.	Dose in Minims.	Inoculation of both Ears with Broth from filtered Cultures, concentrated <i>in vacuo</i> to $\frac{1}{15}$ of its original volume.	
	Right Ear only.			Right Ear.	Left Ear.
A.	Erysipelas (severe).	18 days.	15	Severe inflammatory reaction, subsiding third day.	Less severe inflammatory reaction than in the right, slower in its development, but subsiding same day.
B.	Erysipelas (severe).	13 days.	4	Severe inflammatory reaction, subsiding second day.	Inflammatory reaction, never quite so severe as in the right, but not subsiding till fourth day.
C.	Erysipelas (severe).	16 days.	15	Severe inflammatory reaction, subsiding second day.	Very slight reaction.
D.	Erysipelas (severe).	26 days.	3	Severe inflammatory reaction, subsiding third day.	Inflammatory reaction much less severe than in the right, subsiding third day.

The following curves contrast the changes in volume which occurred in the two ears of a rabbit, which had recently recovered from erysipelas in one of them (right), as the result of injecting filtered cultures of streptococci, concentrated to $\frac{1}{15}$ of their original volume:—

FIG. 6.—Dose=15 drops.

FIG. 7.—Dose=4 drops.

In these experiments a marked but transient inflammatory reaction occurred in the ears previously affected by erysipelas, while a less severe, but in some cases a more prolonged, reaction occurred in the others; and they clearly showed that the ears locally protected against erysipelas react more strongly than their fellows, which are less

effectually immunised, to the irritation caused by the chemical products of the streptococcus.

In the majority of our experiments, however, inoculations had been made with the cocci from agar cultures, and presumably therefore little or no extra-cellular products of microbic activity had been introduced. Klein¹ has demonstrated the existence of intracellular poisons in certain microbes, and this has been confirmed by Kanthack and Wesbrook.² And it occurred to us to inquire whether an injection of dead streptococci, in quantities as small as those used in the experiments alluded to, was capable of causing inflammation, and if so whether we should observe the same differences in the early reaction of the two ears which we observed when living microbes were used. We accordingly inoculated the two ears of a rabbit which had recently recovered from erysipelas in one ear (right) with minute quantities of streptococci from an agar culture sterilised in the following way. The cocci were suspended in a minute quantity of sterilised water, and drawn up into a capillary pipette. This having been sealed, was put into a water bath at a temperature of 55° C., and allowed to remain there 10 minutes. That the cocci had been destroyed by this procedure was proved by sowing them on agar tubes. No growth appeared in these, while a control tube, sown with the material before heating, developed a good growth. A single drop of the fluid containing the dead cocci was then injected into each ear.

TABLE VI.—*Result of Inoculating with Streptococci killed by Heat both Ears of a Rabbit which had recently recovered from Erysipelas in the Right Ear.*

Interval since Erysipelas in Right ear.	Dose.	Result in Right Ear.	Result in Left Ear.
18 days.	1 drop.	Severe inflammatory reaction, ending third day.	Milder inflammatory reaction, commencing slowly but lasting till fifth day.

FIG. 8.—Curves of volume from the same case (in which dead streptococci were used).

FIG. 9.—Curves of volume obtained when living streptococci were used. (From one of the cases in Table III. Class 2.)

In this ear the result produced was exactly the same as if a similar quantity of living cocci had been injected. The usual inflammatory

¹ *Brit. Med. Journ.* March 25th, 1893.

² Kanthack and Wesbrook, *Brit. Med. Journ.* Sept. 9th, 1893.

reaction appeared after a few hours, reached its maximum on the second day, was fading on the third, and had disappeared on the fourth. In the left, a little patch of redness about the seat of inoculation was seen on the second day, had slightly increased on the third, and did not disappear till the fifth. This experiment showed that the inflammatory reaction which we observe, when immunised ears are injected with living streptococci, may be caused by the poisons contained in the microbes actually introduced. This, together with the fact that we could obtain no growth of micro-organisms when we sowed the oedema fluid of such ears on nutrient media, finally convinced us that no multiplication of the microbes takes place when they are introduced into immunised ears.

XII. ON THE PRODUCTION OF GENERAL IMMUNITY BY INTRA-PERITONEAL INJECTIONS OF ATTENUATED CULTURES OF STREPTOCOCCUS ERYSIPELATIS.

We may now proceed to inquire whether rabbits which had recovered from intra-peritoneal injections had acquired any general immunity. In order to investigate this point we inoculated their ears with virulent cultures, control animals being in all cases similarly treated.

In the experiments given in Table VII. the results were similar to those obtained when a rabbit which had recovered from erysipelas in the right ear was inoculated in the left, before the period of general immunity had elapsed; that is to say, a more or less severe inflammatory reaction appeared before any inflammation was observed in the controls, and subsided on the second or third day. In two only of the nine cases did erysipelas result, and in one of these, the long interval of 80 days having elapsed between the inoculations, the period of immunity was probably at an end. On the other hand, erysipelas appeared in all the control animals, and ended fatally in six.

In these cases the general immunity was similar to but more complete than that conferred by an attack of cutaneous erysipelas. It was more complete, possibly because the area of peritoneum affected was very much larger than that of a single ear; but we must also point out that the majority of the animals had had several intra-peritoneal injections before their general immunity was tested, while in the other series of cases the ears shared only in whatever general immunity had been caused by one attack of erysipelas in the opposite ears.

[TABLE.]

TABLE VII.—*Showing the General Immunity conferred upon Rabbits by Intra-Peritoneal Injections of Cultures of Streptococcus erysipelatis.*

Animal.	Number of previous Intra-Peritoneal Injections.	Interval between Inoculation of Ear and the last Intra-Peritoneal Injection.	Result of Inoculation of Right Ear.	Control Animal.
I.	2	7 days.	Inflammatory reaction (lasting 3 days).	Erysipelas, severe (subsiding ninth day).
II.	2	6 days.	Inflammatory reaction (3 days).	Erysipelas, severe (subsiding ninth day).
III.	2	2 days.	Inflammatory reaction (3 days).	Erysipelas (death eighth day).
IV.	2	6 days.	Inflammatory reaction (4 days).	Erysipelas, mild (subsiding eighth day).
V.	2	10 days.	Inflammatory reaction (2 days).	Erysipelas (death eighth day).
VI.	4	3 days.	Inflammatory reaction (4 days).	Erysipelas (death, eleventh day).
VII.	4	3 days.	Inflammatory reaction (2 days).	Erysipelas (death, fifth day).
VIII.	1	4 days.	Erysipelas (subsiding seventh day).	2 Controls { 1. Erysipelas (death, fourth day). 2. Erysipelas (subsiding eighth day).
IX.	1	80 days.	Erysipelas which spread to opposite ear (death eighth day, cocci in blood).	Erysipelas (death, eighth day).

XIII. ON THE RELATION OF THE EARLY INFLAMMATORY REACTION TO IMMUNITY.

The theory that inflammation is a protective process has commended itself to many observers, and it seems worth while to inquire whether our experiments give any support to this view. We have already pointed out that when the ears of immunised and of normal rabbits were inoculated, the appearances of inflammation were first seen in the former. This difference was very striking when ears locally protected were compared with those of the control animals. The inflammatory reaction varied considerably in intensity in different cases, but was constantly present, and in most instances was subsiding at a time when inflammation had made but little progress in the controls. It will be remembered that these cases proved to be absolutely immune. Again,

when ears which shared in the general immunity which followed one or more intra-abdominal injections were inoculated and compared with those of control animals, a similar early inflammatory reaction appeared in the former before inflammation had made much progress in the latter. The difference in these cases was not quite so striking, and was altogether absent in the case of the animals (Table IV. Nos. 8 and 9) which developed erysipelas, and also in the case of No. 4, which proved immune, and whose inflammatory reaction was delayed until the third day.

On the other hand, this early inflammatory reaction was not nearly so striking in the case of ears which shared in whatever general immunity followed cutaneous erysipelas in the opposite ear, and we may repeat that these ears proved less immune than those alluded to above.

We may state then, briefly, that the rapidity of the onset of inflammation which followed the inoculation with streptococci was in proportion to the immunity observed, and we think it probable that the early inflammation, by drawing to the spot a large number of leucocytes, and of possibly bactericidal serum,¹ was the means by which the part resisted the micro-organisms before they had sufficient time to multiply and generate their poisons; in other words, that a rapid inflammatory reaction is the chief factor in the production of the immunity, both local and general, which we observed in our experiments.

XIV. ON THE RELATION OF INFLAMMATION TO RECOVERY FROM ERYSIPELAS.

It has long been known that in erysipelas the streptococci may be found near the margin of the inflamed area with greater certainty than in parts which have been longer affected. We have made some experiments in order to determine how long they remain alive at any one spot, and have found that œdema fluid, taken from a part which, on the previous day, corresponded to the visible margin, almost invariably contained living cocci, while fluid taken from the same spot 24 hours later—that is to say, when redness has replaced the paleness for about 12 hours—usually contained none. In one instance, however, they were found in a part where redness had existed for 24 hours, but they had disappeared by the following day. These observations furnish additional evidence that recovery of the parts first attacked commences while the disease is spreading elsewhere.

We would ask, What is the cause of this local recovery, and also of the ultimate cessation of the spread of erysipelas? In the earlier part of this paper we suggested that the cause of recovery and subsequent immunity are probably identical, and we have given reasons for thinking that immunity is brought about by an inflammatory reaction which destroys the micro-organisms and arrests the disease at its outset.

¹ Roger has shown that *Streptococcus erysipelatis* grows well on the serum of immune rabbits, but becomes attenuated in virulence.

It now remains to be seen whether there is any evidence that recovery is also due to inflammation. Let us first consider the case of recovery which occurs in the parts first affected by erysipelas while the disease is still spreading elsewhere. This we shall speak of for the sake of brevity as "local recovery." If we watch the changes which occur in a small portion of a rabbit's ear, from the very commencement of erysipelas in it, we notice that the part first becomes œdematous, but remains pale, and that it contains living streptococci in large numbers. After about 12 hours, the pallor is replaced by redness, and when this redness has persisted for about 24 hours, living micro-organisms can no longer be found there. It seems reasonable to suppose, therefore, that the inflammation has brought about their destruction; that it is, in fact, the cause of local recovery.

The cause of the ultimate cessation of the disease appears to us to be similar.

We have seen that when erysipelas is actively spreading, the affected area is bounded by a pale œdematous zone, in which the micro-organisms can readily be found; in other words, that the advance of the virus precedes the advance of the redness, and we have also seen that when a part efficiently protected by general immunity, produced by erysipelas elsewhere, is again inoculated, an inflammatory reaction occurs which is characterised by the absence of the pale zone containing cocci, and that the inflammation occurs sooner than in the control animals inoculated for the first time. Is it not, therefore, reasonable to suppose that this power of reacting quickly to the irritation of the streptococci is acquired during the course of the disease, and is the means of arresting it. We suppose that, as the cocci spread in the lymphatic spaces of the tissues, they are followed by inflammation more and more closely, until reaction at last becomes coincident with invasion, and the disease is brought to an end, for on more than one occasion we have observed that, towards the end of an attack, the inflamed area was no longer bordered by a pale zone of œdema, but redness extended to the very margin of the affected part. We did not observe this more frequently, because erysipelas starting in the ear of a rabbit does not cease to advance until it has spread to the head and neck, where the character of the skin renders the margin less distinct, and where it can only be seen at all after the hair has been removed.

It was not without considerable hesitation that we arrived at the conclusion that inflammation was the cause both of recovery and of immunity. We were aware that an actively inflamed part has long been regarded as a place of lessened resistance and particularly susceptible to invasion by micro-organisms, and we ourselves have seen a rabbit's ear, while inflamed by previous immersion in warm water, become attacked by erysipelas. We have pointed out that in our experiments inflammation produced by introducing streptococci into a locally immunised ear is followed by rapid destruction of the microbes, while, on the other

hand, we find that streptococci can attack an ear already actively inflamed and produce erysipelas, and it was necessary to inquire into the cause of this apparent contradiction before we could maintain with any confidence the theory that inflammation is protective.

Modern pathology teaches that a transient injury, however severe, is unable of itself to produce inflammation; that to cause inflammation the irritant must act for a considerable time. The view that inflammation is a protective reaction on the part of the tissues, the purpose of which is to get rid of the source of irritation, is becoming more and more widely accepted.¹ A wound caused by a transient injury does not inflame if it remains aseptic; and whatever inflammation follows a bruise is to be attributed, not directly to the injury, but to the presence of extravasated blood and other dead matter in the injured tissue.

Let us examine, in the light of this theory of inflammation, what takes place in a rabbit's ear which has been injured by immersion in warm water. It at first becomes intensely hyperæmic, but this passes off in a few minutes. It is then found to be studded with petechial hæmorrhages, and very soon becomes swollen with œdema. This hæmorrhage and œdema we may regard as the direct result of damage done to the tissues, and not as a part of the inflammatory process which follows later, and whose purpose it is to remove these effete matters. If micro-organisms be introduced during the early part of the inflammatory stage, they find in the œdema fluid a favourable medium for their growth, and the inflammation which already exists, probably only sufficient to effect the purpose for which it was called forth, has now to cope, not only with the extravasated matters, but also with the cocci. Further inflammation is accordingly excited, but not more rapidly than in uninjured tissues to which streptococci have found their way; and so, as in this latter case, the micro-organisms gain a start and erysipelas is the result. It is quite otherwise when an ear immunised by previous erysipelas is again inoculated with streptococci: then, as we have shown, the inflammation starts far more quickly, and is able to destroy the microbes before they have had time to multiply and generate their poisons.

Bearing these considerations in mind, we came to the conclusion that the fact that streptococci may attack a part already inflamed is not really opposed to the theory that inflammation is protective.

XV. ON THE IMMUNITY PRODUCED BY CHEMICAL PRODUCTS OF THE STREPTOCOCCUS Erysipelatis.

The injection of a filtered beef-broth culture into the peritoneal cavity, was found to render an animal immune to a subsequent injection

¹ Thus Metchnikoff defines inflammation as a "phagocytic reaction on the part of the organism against irritants," "Comparative Pathology of Inflammation," 1891, Eng. Trans. p. 189.

of a living culture in the same situation, a similar dose of which proved fatal to a control animal (Table II. p. 50).

Roger¹ has pointed out that an injection of a filtered culture of streptococci into the veins, renders a rabbit more susceptible than before to the action of the living virus, and that this increased susceptibility persists for at least two months. The experiment which we have mentioned above will not appear incompatible with his results, if we bear in mind that the probable effect of the intra-abdominal injection of the filtered culture was an inflammation of the peritoneum, which gave rise to a local immunity, and we must point out that we made no attempt to determine whether the injection had conferred any protection upon other parts.

The same material, when injected into the ear, caused inflammation of greater or less intensity, according to the dose employed, and this was confined to the neighbourhood of the seat of injection, and rapidly subsided. We hoped, by this means, to be able to cause a local immunity in the tissues in advance of the spreading erysipelas, and so check its further course. Five attempts have already been made, but up to the present time we have not met with much success. In all instances we inoculated the ear with sterilised products near the root, and subsequently inoculated the tip with living virus. The erysipelas which developed appeared to spread over the whole ear, but we did not determine whether the part directly affected by the first inoculation had been invaded by micro-organisms, nor could we tell whether this inoculation with sterilised products had checked the advance of erysipelas, because in rabbits the disease seldom spreads beyond the ear. It is worthy of note, however, that none of these animals died, although locally the disease was severe.

CONCLUSIONS.

1. That cultures of *Streptococcus erysipelatis* cease to grow in beef-broth after 3 or 4 days, and that the majority of the cocci die a few days later; but a few remain alive and capable of producing a new growth when sown on new culture media, for 90 days, and probably longer.
2. That the cause of cessation of growth in beef-broth is an exhaustion of the nutrient material, and not the production of a bactericidal substance.
3. That cultures of *Streptococcus erysipelatis* maintain their virulence when grown on solid media, but become greatly attenuated when grown in beef-broth.
4. That the virus which has become attenuated by cultivation in beef-broth may be intensified by growing in the bodies of animals.
5. That the injection of streptococci or their products into the

¹ Roger, *loc. cit.*

abdominal cavity confers immunity to a second injection, in the same situation, of more virulent cultures, in quantities fatal to control animals.

6. That cutaneous erysipelas completely protects the parts directly affected against subsequent inoculations of the virus. In other words, it confers an absolute local immunity, while on the rest of the body it confers a general immunity which is less constant, sometimes protecting completely, at other times modifying, the course of the disease, while in some instances it is entirely absent.

7. That intra-abdominal injections of attenuated cultures of streptococci confer a somewhat more perfect immunity upon the rest of the body.

8. Both local and general immunity are of short duration, and do not last more than a few weeks.

9. That when streptococci are introduced into rabbit's ears, protected by recent erysipelas, an inflammatory reaction quickly appears, and has already subsided before inflammation has made any considerable progress in the control.

10. That the rapidity of onset, and the intensity of this inflammatory reaction, is most marked in ears locally protected, less marked in those which share in the general immunity produced by intra-peritoneal injections, and least marked in those which share in the general immunity produced by erysipelas in the opposite ear; that is to say, it is in proportion to the immunity observed in these three classes of cases.

11. A similar difference in the inflammatory reaction of immunised and normal parts is observed when filtered cultures or dead streptococci are introduced.

12. That this power of quickly reacting is an important factor of immunity both general and local.

13. That this same power of reacting is acquired during the course of the disease, and is the cause of recovery.