AN EXPERIMENTAL STUDY OF THE DIRECT INOCULATION OF BACTERIA INTO THE SPLEEN OF LIVING ANIMALS; AND A CONTRIBUTION TO THE KNOWLEDGE OF THE IMPORTANCE OF A LESION IN ANIMAL TISSUE FOR THE LODGMENT AND MULTIPLICATION OF BACTERIA WITHIN IT.

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The methods of experimental infection which are at the present time mostly used are subcutaneous or intraperitoneal inoculation or intravenous injection of the micro-organisms employed. The final object to be attained is the introduction of the organisms into the circulation, and the different results obtained by these various methods of inoculation are largely due to a mechanical factor—to the rapidity with which the organisms and their products are absorbed from the locality in which the injection was made.

It occurred to the writers that new characteristics in the forms of infection might be obtained from a systematic study of inoculations made primarily into one of the internal organs.

It seems fair to suppose that specific metabolic processes take place in all organs having special functions, and it is not illogical to assume that the specific catabolic processes of an organ might have a characteristic effect upon bacteria, more particularly when they are unchanged by previous contact with the blood or lymph.

The spleen, above all the internal organs, seems to harbor the specific micro-organisms in certain of the infectious diseases, and for this reason, notwithstanding the fact that the exact function of the spleen seems to be little understood, this organ was selected for our experiments as the seat of direct infection. From its peculiar anatomical structure, some hold the opinion that the spleen acts as a filter

and holds back bacteria from the blood passing through it. It might also be claimed that the bactericidal power of the blood is increased in the spleen, for on entering the organ, the blood leaving the capillaries comes in close contact with the lymphoid tissue where leucocytes abound, and it is quite generally believed that a close connection exists between leucocytes and the bactericidal action of body fluids.

Now, on the other hand, H. Buchner* claims that wherever disintegration of the red blood corpuscles occurs, the alexines fail to display their full power; and according to the prevailing opinion the erythrocytes are broken down in large numbers in the spleen. Hence it might be assumed, as a fair hypothesis, that while the non-corpuscular alexines are easily carried from the spleen by the blood, that the corpuscular elements, including the micro-organisms, remain in the spongy tissue of the spleen, and the bacteria, well nourished by the broken down erythrocytes, resist the attacks of the remaining alexines.

Our first problem then was to determine the fate of bacteria when inoculated directly into the spleen.

The animals experimented on were chiefly rabbits; the inoculations were mostly of broth cultures of B. coli communis, and the results obtained were controlled by inoculations of broth cultures of B. typhosus and Staph. pyogenes aureus. The inoculations, made with a hypodermic syringe, were directly into the substance of the spleen, the needle being introduced into the ventral end, *i. e.* that end of the spleen lying nearest the abdominal wall. The operation for exposing the spleen was made under ether anesthesia, the incision in the median line of the abdomen causing little or no loss of blood. In none of our numerous experiments did a secondary infection at the site of operation occur, the wound in the abdominal wall healing *per primam.*

The slight bleeding from the spleen, caused by the puncture of the hypodermic needle, was arrested either by a ligature thrown around the needle or by the actual cautery.

In due time each animal was killed with chloroform and the autopsy

* *Centralbl. f. Bakt. u. Parasit.*, vi, No. 1.

performed immediately under strict aseptic precautions. After opening the thorax and abdomen the spleen was at once removed and placed in a sterilized Petri dish, and cultures were then made in 10 per cent nutrient gelatin, reacting 1.5 per cent acid to phenolphthalein, from the blood in the left ventricle, hepatic vein, liver and left kidney, as a routine procedure; other cultures being made from time to time as hereafter noted. Gelatin plates were then made from the spleen, which for convenience was divided into three parts; the ventral end, or that through which the inoculation was made, being designated I, the middle third, II, and the dorsal third, III. Three large "loops" of the juice of the spleen were used for making the plates and other plates were made with bits of tissue torn out from the substance of the organ. All plates were kept under observation for not less than seven days. Cover-glass smears were also made from the tissues plated.

*Experiment 1.** Medium-sized rabbit; 5 minims of 24 hours broth culture of B. coli communis injected into spleen. Autopsy after 40 hours. Plates from parts I, II and III of the spleen remained sterile.

Experiment 2. Small rabbit; 6 min. of 48 hours broth culture of B. coli comm. injected into spleen. Autopsy after 40 hours. Spleen plates; I showed 4 colonies; II, 2 colonies; III, 5 colonies. Plates from liver, 6 colonies; from hepatic vein, 2 colonies; and from left ventricle, 4 colonies.†

Experiment 3. Small rabbit; 6 min. of 24 hours broth culture of B. coli comm. injected into spleen. Autopsy after 14 hours. Spleen plates; I, 3 colonies; II, 7 colonies; III, 0. Plates from liver, 0; from kidney, 1 colony.

Experiment 4. Large rabbit; 10 min. of 24 hours broth culture of B. coli comm. injected into spleen. Autopsy after 14 hours. Spleen plates; I, 11 colonies; II, 43 colonies; III, 3 colonies. Plates from liver, 32 colonies; from hepatic vein, 15 colonies; from left ventricle, 133 colonies; and from left kidney, 0.

The experiments are numbered in the order in which they are reported, not in the order in which they were made.

†**The number of colonies reported are the average of two or more plates made from the same locality, the number occurring on each of the several plates not being far from the average obtained.**

Experiment 5. Small rabbit; 5 min. of 20 hours broth culture of B. coli comm. injected into spleen. Autopsy after 3 hours and 20 minutes. Spleen plates; I, 0 colonies; II, 13 colonies; III, 3 colonies. Plates from liver, 2 colonies; from hepatic vein, 0; from left ventricle, 5 colonies; and from left kidney, 0.

Experiment 6. Small rabbit; 25 min. of 4 days broth culture of B. coli comm. injected into spleen. Rabbit died in 3½ days; autopsy six hours after death. Spleen plates; I, 200; II, 350; III, 150 colonies of B. coli communis; no other colonies present.

Experiment 7. Large rabbit; 25 min. of 48 hours broth culture of B. coli comm. injected into spleen. Died after 24 days. Plates from the three parts of the spleen, from the liver, hepatic vein, left ventricle and left kidney all remained sterile.

It seems unnecessary to mention further experiments of this kind; the few here quoted from our protocols show that large numbers of bacteria injected into the spleen soon disappear.

The organisms injected into the spleen were neither kept back mechanically, as in a filter, nor did they find within the spleen a soil favorable to their multiplication. Although the 5-minim dose of broth culture, which was injected, corresponds to about one-third of the entire volume of the spleen and contained many millions of bacteria, the plates made from the juices and tissue of the spleen were for the most part sterile, and even in the plates made only 3 hours and 20 minutes after the inoculation, but few colonies developed. Controls of the cultures employed for the inoculations were made in several instances by adding a small loopful of the culture to 5 cc. of sterile broth and immediately plating three loopfuls of this mixture in nutrient gelatin. The number of colonies developing on these plates varied from 3000 to 10,000.

These control experiments assured us that many millions of bacteria disappeared from the spleen within a short time.

The question now arose: Is this large number of bacteria rapidly destroyed in the spleen, or are these bacteria carried out from the spleen in immense numbers by the blood current?

The best method to exclude the latter proposition would have been to tie all the splenic veins, but this we could not do in the small animals

experimented with. In a single case an attempt was made to prevent the flow of blood from the spleen by tying the portal vein.

Experiment 8. Medium-sized rabbit; ligated portal vein and then injected 5 min. of broth culture of B. coli comm. into the spleen. Animal died in 30 minutes. The spleen was removed, placed in a sterilized Petri dish and plated 14 hours later. Juices and tissue were plated from five different incisions made into the spleen. All plates showed an innumerable growth of B. coli and no contaminations were found.

In the other experiments it seemed preferable to tie the splenic vessels themselves, but we were obliged to ligate the arteries together with the veins.

Experiment 9. Large rabbit; tied all splenic vessels and injected into the spleen 5 minims of broth culture of B. coli communis. Autopsy after 40 hours. Spleen plates I, II, III all show an innumerable growth of B. coli communis.

Here the bacilli could not migrate into the circulation, and indeed there was every evidence of an active growth of them within the spleen. We have to remember, however, that the supply of fresh blood and with it the supply of fresh alexines were cut off, while the amount of alexines present in the spleen at the moment of ligating the vessels was probably out of proportion to the number of bacteria introduced into the spleen. It is now generally accepted that the effect of the bactericidal elements in the blood is but little apparent in the presence of large numbers of bacteria, and that after the bactericidal powers are exhausted the surviving bacteria multiply with great rapidity.

Anatomists teach that the capillaries in the spleen resolve and the blood enters the tissue; and the assumption seems justified, and is indeed tacitly made, that quite a fair communication exists between the interstitial spaces of the spleen. We therefore resorted to the plan of ligating only a part of the vessels pertaining to the spleen, anticipating that the migration of bacteria from the ligatured portion would be considerably restricted, while there might be some, perhaps an abundant supply, of the bactericidal factors to this part provided by

the neighboring parts of the spleen through which the blood was allowed to flow.

Experiment 10. Small rabbit; tied about ⅓ of the vessels on the ventral end of spleen, and through the ventral (ligated) end injected 5 min. of a 24 hours broth culture of B. coli comm. into the middle portion of spleen. Tip of spleen tied. Autopsy after 40 hours. Tip of spleen was found detached from the rest of the organ and imbedded in the mesentery; the ventral third of the spleen, the vessels of which had been ligated, was considerably thickened and paler than the remaining part of the organ, and distinctly separated from it by a ridge or line of demarcation. The plates made from the ligatured part of the spleen (I) were overcrowded with colonies and the plates from parts II and III showed growth of colonies which were too abundant to count. Colonies were most abundant in plates from part I, distinctly less abundant in plates from part II, and still less numerous in the plates from part III.

Experiment 11. Medium-sized rabbit; blood-vessels of the ventral third of spleen ligatured and 6 min. of a broth culture of B. typhosus injected through the ventral end into the middle third of spleen. Autopsy after 40 hours. The ligated end of the spleen was hard, dark red and dull on the surfaces, and the hard portion was separated from the normal part by a sharp ridge. Spleen plates; I, innumerable colonies; II, 1900 colonies; III, 1150 colonies. Plates from liver developed 91 colonies; from hepatic vein, 90 colonies; from left ventricle, 20 colonies; and from left kidney, 4 colonies, of B. typhosus.

In some experiments, in which the vessels of the middle portion of the spleen were ligated and the animal was allowed to live for seven or eight days, the entire middle portion of the spleen had become very much thickened, of the greyish yellow color and remarkably dry, while both ends of the organ, the circulation in which we endeavored not to interfere with, appeared normal in every respect.

Thus we see that our expectation of a collateral interstitial nutrition was not realized, an interesting fact which, however, we cannot discuss further here.

On the other hand, our experiments show that plates from the non-ligated portions of these partly ligatured spleens developed a considerable number of colon or typhoid colonies, quite in contrast to our experience with spleens no part of which were ligated.

In studying over our entire material it seemed to us that the as-

sumptions which give the fittest interpretation of all the facts in our possession—at least for the present—are: (1) That the blood within the spleen is less bactericidal than the blood within the balance of the circulation, on account, perhaps, of the nourishment afforded the bacteria by the large numbers of disintegrated erythrocytes within the spleen; and (2) That the structure of the spleen offers but little hindrance to the rapid migration into the circulation of large numbers of bacteria injected into this organ.

Our experiments, which showed that but few bacteria could be found in the spleen only a few hours after large numbers had been injected into it, must be explained by assuming that great numbers of them were soon carried away from this organ through the splenic veins by the blood stream; the fate they met was probably that which awaits those large numbers of bacteria which are injected directly into the circulation.

In our second set of experiments, in which the bacteria were injected into a spleen, part of the vessels of which had been ligated, the presence of bacteria in the non-ligated (normal?) portion of the spleen may be explained by assuming that many of those which got into the ligated part remained there and, from the abundance of nutriment, multiplied rapidly. From this source a fresh supply of bacteria was constantly given out to the adjacent, more normal part of the spleen, whence the blood, flowing through the organ, carries the bacteria out almost as soon as they are supplied to it.

Although it seems to be a fact that but little interchange of nutritive fluids takes place between the several regions of the spleen, the bacteria are apparently able to wander over by their own motility (colon and typhoid), or to grow through, from one region into another.

The fact that, only a few hours after injection into the spleen, so few bacteria could be found in the blood, does not argue against the assumption that all the injected bacteria were carried out into the blood stream, because they disappear in the same manner when injected directly into the veins.

Wyssokowitsch * found five hours after injecting 1 cc. of typhoid

* *Ztschr. f. Hygiene*, i (1886), 1.

culture into the jugular vein of a rabbit that 0.2 cc. of the blood contained only 5 bacilli.

The presence of 1900 colonies in plates made from a point in the spleen adjacent to a part the vessels of which had been tied, while not more than 90 colonies were found in plates made from the liver and the hepatic vein, may well be interpreted in favor of the assumption that blood within the spleen is less bactericidal than blood in the vessels nearest to this organ.

Another point in favor of this assumption seems to be the fact that the bacteria thrive in a ligated spleen, while, according to de Giaxa and Guarnieri,* bacteria succumb to the bactericidal effects of blood in the vessels between two ligatures as well as in the extra-vascular blood.

Finally, we have to report that in a number of cover-glass smears made from non-ligated spleens, which were most carefully examined microscopically, no bacilli were discovered; and it would seem to us improbable, even if only a considerable portion of the bacteria were killed by the alexines, that no bodies of the bacteria, which would take a stain, should be found in the smears.

The views here put forward would seem not to be in harmony with the statements of Wyssokowitsch.† This author found, after injecting numerous micro-organisms into the jugular vein, that they soon disappeared from the circulation, but that a number of them settled in the spleen, liver and bone-marrow, a fate which, according to Ponfick ‡ and others, they share with non-organized corpuscular bodies, such as cinnabar, ultramarine, etc., injected into the circulation.

Wyssokowitsch, however, states further that certain bacteria, for example the typhoid bacillus and others, soon become destroyed within the spleen. He states that twenty-four hours after injection of the typhoid bacillus into the blood, cultures made from the spleen show no living bacilli; in other words, he claims that the spleen acts as a filter upon the organisms in the blood passing through it, and the bacilli succumb to the bactericidal influences within the spleen.

With regard to the first point we freely admit that corpuscular

* *Annales de Micrographie*, iii (1890-91), 481.

† Loc. cit.

‡ Virchow's *Archiv*, 1869.

elements may find within the spleen a greater obstacle to their free movement than in any other part of the blood current, and that a larger accumulation of organisms may occur in the spleen than in any other part of the circulation; yet the results of our experiments have prompted us to assume that the retained bacteria are easily carried out again from the spleen, and that the " filtration " is comparatively of but little account and not permanent. As to the disappearance of the bacteria from the spleen, we maintain that Wyssokowitsch has not offered the slightest proof of his assumption that the destruction of the bacteria occurs within the spleen. This disappearance of the bacteria can be as well explained by supposing that they have been gradually carried out from the spleen by the blood into the vessels, where they have succumbed to the bactericidal power of the circulating blood. We wish it understood that we by no means claim that the spleen possesses no degree of bactericidal influence and that no destruction of bacteria takes place in this organ. On the contrary, at the beginning of our experiments, the total sterility of the inoculated spleens led us to believe that the bacteria were destroyed by the bactericidal elements of this organ, and when during our investigations the highly important publications of R. Pfeiffer * and of A. Wassermann † appeared, showing that the elements of active immunization in cholera and in typhoid fever appear in the greatest amounts in the spleen, lymphatic glands and bone-marrow; and inasmuch as immunization in typhoid fever and in cholera does not mean antitoxic but bactericidal effects, we were further strengthened in our belief that we, too, had found bactericidal effects of remarkable power within the spleen. Yet a careful analysis of the results of all our experiments led us to the present conclusion that the bactericidal power within the spleen acts rather less intensely than in the rest of the circulation.

As a matter of fact, this view seems to us to harmonize far better with the principles and findings of Ehrlich, Pfeiffer and Wassermann. The normal bactericidal elements are not identical with the bacteri-

* Pfeiffer and Marx, *Deutsch. med. Wochenschr.*, 1898, p. 47.
† *Berliner klin. Wochenschr.*, 1898, p. 209.

cidal bodies of active immunization; they are derived from different sources. The bactericidal elements of the normal blood are seemingly generated in one way or another by the leucocytes, while Pfeiffer has established the fact that the bactericidal bodies generated by immunization stand in no relation to the leucocytes. Following the theory of the so-called "side-chain-immunity" of Ehrlich,* it is assumed by Behring † and by Wassermann that these bactericidal bodies are generated by the organ-cells, which, reacting to certain stimuli, produce an excess of these bodies. The stimulating agents are the bacteria and their products.

This, in a few words, is the essence of the views of these writers so far as concerns us here. We believe now that we are not going too far when we add the hypothesis that the greatest overproduction of the bactericidal bodies occurs in these organs, in which the bacteria are permitted to multiply most actively. In other words, when the bactericidal elements of the normal blood are less active, less influential, then the most active generation of bactericidal bodies of immunization takes place. It is in this sense, then, we can claim that our assumption of the diminished effect of the normal bactericidal action within the spleen harmonizes with the statements of Pfeiffer and of Wassermann, that the bactericidal bodies of immunization are abundantly produced within the spleen.

In one of our experiments with injections into the non-ligated spleen, numerous bacilli were found to be present in the liver.

In this case an attempt had been made to reach the vagus nerve below the diaphragm, requiring some pressure upon the liver, and it occurred to us that the mechanical injury the liver had received might have been the cause of the retention and perhaps multiplication of the bacteria within it. We therefore attempted to produce by cauterization circumscribed lesions of the surface of the liver before injecting bacteria into the spleen, and our experiments in this line show conclusively that by mechanical injury to the tissue we have it

* Die Wertbemessung des Diphtherieheilserums, *Klinisches Jahrbuch*, vi (1897).

† *Deutsch. med. Wochenschr.*, 1898, p. 68.

in our power to cause bacteria which are circulating in the blood to settle in any part of the surface of the liver we may select. In all the cases experimented upon the cauterized part of the liver contained innumerable organisms; in the normal part of the organ immediately adjoining the lesion they were still numerous, while in remote parts but few, if any, were to be found. The organisms found in the lesions were pure cultures of the species which had been injected into the spleen. In cases in which the animal was allowed to live for 30 to 40 hours, it was easy to establish the fact that an innumerable quantity of bacteria were present in the lesion, while none were found at the site of inoculation. The perfect sterility of the spleen and the immense number of bacteria found at the site of the lesion showed that at least some of the bacteria migrated from the spleen in a living condition.

The liver, however, is the next station at which bacteria escaping from the spleen would arrive, and the question suggested itself whether a sufficient number of bacteria escaped from the spleen to allow of their reaching and settling in a more remote organ, and whether or not lesions of other organs would react in a similar manner to those of the liver.

To this end lesions were produced by cauterization in several organs: in the kidney, the uterus, the testicle, and in a circumscribed area in the peritoneum, and in the subcutaneous tissue, etc. In all places where suitable lesions were produced, the specific organisms were present in large numbers, as illustrated by the following experiments.

Experiment 12. Large rabbit; cauterized pregnant uterus through incision in abdomen and injected 2.5 min. of a 3 days broth culture of B. coli comm. into spleen. Autopsy after 3½ days. Spleen plates; I, 1 colony; II and III, 0. Plates from liver, 0; from hepatic vein, 1 colony; from left ventricle, 1 colony; from left kidney, 0; from the site of lesion in the uterus and from the fluid in the cavity of the uterus at site of lesion, innumerable colonies.

In this experiment the quantity of culture injected was small, the rabbit and the spleen were both large, and the plates made from the blood and uninjured organs were sterile—conditions which would

favor the interpretation that the bacteria were all destroyed in the spleen, but the abundant growth in the lesion of the uterus demonstrates that a considerable number of bacilli must have escaped into the general circulation to found this thriving colony in a remote region.

Experiment 13. Medium-sized rabbit; cauterized right kidney and uterus, and injected 5 min. of a three days broth culture of Staph. pyogenes aureus into the spleen. The tip of the organ through which the needle was inserted was tied. Autopsy after 40 hours. Spleen plates from tied tip showed 460 colonies; from part I, 192 colonies; part II, 7 colonies; part III, 4 colonies; plates from the liver, hepatic vein, left ventricle and left kidney were sterile; from the right kidney, necrotic portion and adjoining portion, innumerable colonies; and from the necrotic portion of the uterus over 10,000 colonies developed. The tip of the spleen contained a considerable number of bacilli, as did also the adjoining part, a fact which was observed in nearly all experiments in which the tip of the organ was tied, and which probably is explained by the cutting off of the blood supply by the ligature.

Our observations on the action of bacteria in lesions made in the internal organs seemed to be of such importance, that we conducted a series of experiments by injecting bacteria into the general circulation, after producing lesions in one or more of the internal organs.

Experiment 14. Large rabbit; ventral end of spleen and border of liver squeezed with hot forceps; 25 min. of a 20-hour broth culture of B. coli comm. injected into ear vein. Autopsy after 40 hours. The cauterized portions of the spleen and of the liver were necrotic. Plates from the blood in left ventricle showed 1 colony; from left kidney, 0; from hepatic vein, 48 colonies; from normal part of liver, 2 colonies; from the necrotic part of the liver the colonies were innumerable; from part I, of spleen, the necrotic portion, innumerable colonies; from part II the colonies were also innumerable, but distinctly fewer than were found in part I, and from part III only 39 colonies developed.

Experiment 15. Large rabbit; cauterized right kidney, border of liver, two areas in spleen, one near each end, a circumscribed area of the peritoneum, and the subcutaneous tissue in the right inguinal region. Twenty-five minims of a broth culture of B. coli communis was then introduced into the ear vein and the animal killed after 64 hours.

Plates made from blood in the left ventricle showed no growth; from hepatic vein, 0; from a normal portion of the liver, 0; from necrotic part of the liver, innumerable colonies; spleen plates showed an innumerable number of colonies from the necrotic portions and a very abundant growth, though distinctly less, from the intermediate parts; plates from the left kidney showed no growth, while those from the normal part of the right kidney showed 410 colonies; the development from the necrotic portion of the right kidney and the apparently healthy tissue adjoining it was innumerable, though distinctly less in the latter case than in the former. Plates made from the cauterized portion of the peritoneum and from the necrotic subcutaneous tissue, all showed an innumerable growth.

Experiment 16. Medium-sized rabbit; squeezed with hot forceps the ventral end of the spleen, the lower border of the liver and the lower end of the right kidney. Twelve minims of broth culture of B. coli comm. were then injected into the ear vein. The autopsy was made after 41 hours and plates from blood in the left ventricle developed no colonies; from hepatic vein, 72 colonies; from normal part of liver, 570 colonies; from the necrotic part of this organ, innumerable colonies; from an adjoining portion, 2671 colonies; from the left or normal kidney, 0; from the squeezed portion of the right kidney, innumerable colonies. Plates made from various parts of the spleen developed very few colonies.

The few colonies found in plates from the spleen may perhaps be explained by the fact that the squeezed portion of the spleen was dry and leathery in appearance and very tough when cut into, and it may be that the circulation was so completely cut off from this part that the bacteria had but little chance to reach the lesion in the organ, and were mostly carried out again into the circulation. It seems probable that only when some more or less normal tissue remains within the necrotic part, *i. e.* tissue through which the blood can flow, that the bacteria can gain access to the injured tissue and be deposited there. It is of course a very difficult matter always to regulate the amount of pressure exerted, so as to produce the same kind of a lesion in every experiment.

In this connection it seems important to mention that in experiments in which half of the vessels of the spleen were tied and the inoculation made into the circulation through the ear vein, plates made from the spleen, whether from the ligated or the non-ligated

part, remained sterile. The sterility of the ligated portion probably finds its explanation in the reasons set forth above. Injections made directly into or through a ligated portion of the spleen offer entirely different conditions, for here the bacteria are brought directly into the ischæmic part and could not escape except through their own motility or by growing out through enormous multiplication. It seems likely that the abundant growth within the ligatured portion of the spleen was due largely to the lesion of the tissue caused by ligation of the vessels which favored the retention and development of the organisms.

The principle underlying the fact that a lesion in an organ favors the retention and development of bacteria within it is not new. J. Rosenbach,* Becker,† and others, have shown that the intravenous injection of Staph. pyogenes aureus can cause osteomyelitis in a fractured bone. The experiments of Wyssokowitsch,‡ and of Prudden,§ have established that a mechanical lesion, and according to Prudden that also a chemical lesion, of the cardiac valves causes the staphylococcus and the streptococcus, when intravenously injected, to settle there and develop ulcerative endocarditis. According to Wyssokowitsch, however, the M. tetragenus and other organisms did not settle in the injured valve. In our experiments with the three species of bacteria mentioned, we made no attempt to determine how they would act in relation to injured heart valves, but were enabled to cause them to settle in any tissue experimented with, by producing a suitable lesion. This seems to us a point of great practical importance, inasmuch as any physiological or artificial injury, such as parturition or operation, carried out under the most scrupulous asepsis, can become infected, if in any place in the body there exists a focus containing micro-organisms; and in this connection it must not be forgotten that attenuated micro-organisms may regain their virulence in a tissue lesion.‖ How many cases of puerperal infection or of post-operative

* *Zeitschr. f. Chirurgie*, x.
† *Deutsche med. Wochenschr.*, 1884.
‡ Virchow's *Archiv*, ciii.
§ *Amer. Journ. Med. Sciences*, 1887, p. 70.
‖ Nocard and Roux, *Annales de l'Institut Pasteur*, 1887.

sepsis might here find their explanation! Without, however, entering into a discussion of the numerous instances in pathology which can be interpreted profitably by our facts, we would add that, judging from a few experiments we have made, it would appear that lesions of the internal organs can become infected also from a subcutaneous focus.

In one experiment in which subcutaneous injection of the B. coli comm. was employed, plates made from the injured portion of the liver contained over 2000 colonies, while the necrotic spleen showed a very meagre growth. In another similar experiment the plates from the necrotic portion of the spleen contained over 6000 colonies, from a cauterized portion of the peritoneum about 1000 colonies, and in plates from the cauterized uterus there were innumerable colonies.

The failure of Wyssokowitsch to produce ulcerative endocarditis from subcutaneous injections of pyogenic bacteria may perhaps be explained by the fact that certain bacteria are but slowly absorbed from the subcutaneous tissue; if the injury to the valves had been caused a few days after the injection instead of simultaneously, more time would have been allowed for the bacteria to get into the circulation, and the result might have been different.

The frequent mention of the presence of B. coli comm. in the cauterized portions of the liver might lead to the suspicion that this ubiquitous organism was present in the liver before the intrasplenic or intravenous injections were made. Aside from the numerous instances in which the uninjured organs were found free from this bacillus, other studies bearing directly on this point were made. A number of rabbits were opened, the liver and spleen were cauterized and the abdomen closed, just as in the other operations alluded to, but without any injection of micro-organisms whatever. After a few days the animals were killed, and plates made immediately from the injured and uninjured organs and from the blood, and in all the cases the plates were found to be sterile. It therefore seems to us probable that the cases in which numerous colon bacilli were found in the normal liver were from platings made some time after death had occurred.

In conclusion we desire to say that, although in our many experiments we have produced numerous foci containing very many bacteria, and thus have offered a number of different sources for the abundant supply of bacteria to the blood, the blood, nevertheless, has remained perfectly sterile or contained but very few bacteria. The blood is a liquid tissue, and may we not, perhaps, assume that the blood, like other tissues, permits the growth of bacteria within it only when it has been in some way injured?

SUMMARY.

I. Cultures of B. coli communis, B. typhosus and Staph. pyogenes aureus, when injected into the tissue of the normal spleen, soon disappear from that organ, and indeed from the normal body generally.

II. Bacteria injected into a spleen after the whole or a part of the vessels have been tied, multiply in the spleen with great rapidity and continue to supply bacteria to the blood, whence in the healthy body they soon disappear.

III. Bacteria injected into the spleen, or subcutaneous tissue, or into the blood current through the ear vein, in cases in which moderate lesions have been made by cauterization or compression in the spleen, liver, kidney, uterus, testicle, peritoneum, or subcutaneous tissue, usually find lodgment in these lesions and multiply there.

IV. Even in cases in which numerous foci existed, from which the blood was constantly provided with a fresh supply of bacteria, only few bacteria were found at any time in the blood.