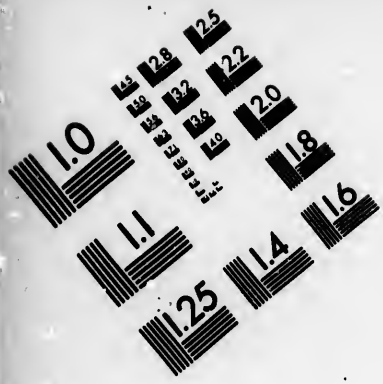

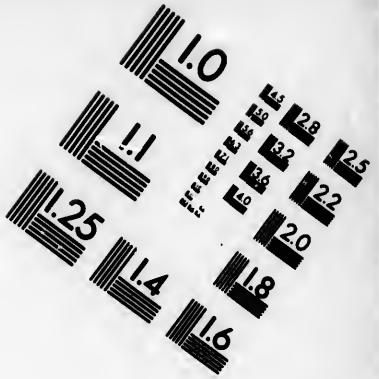

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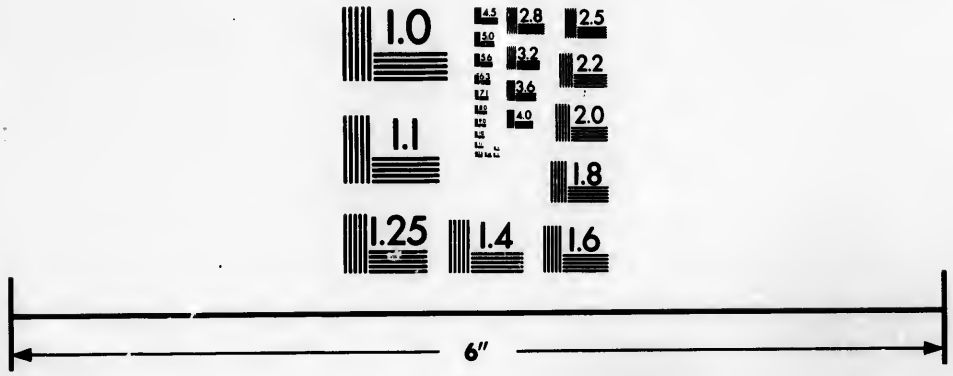

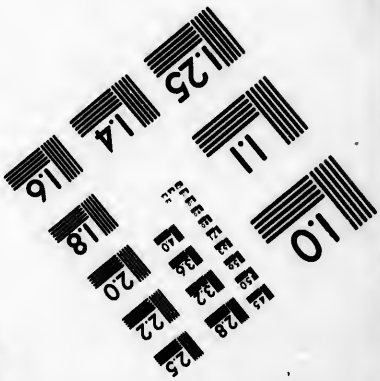

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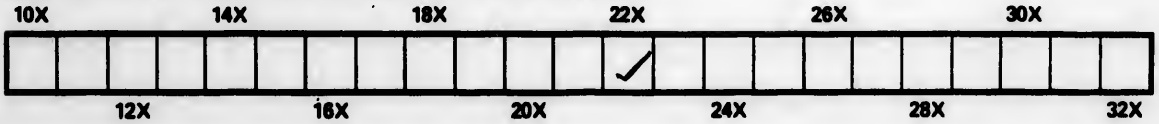

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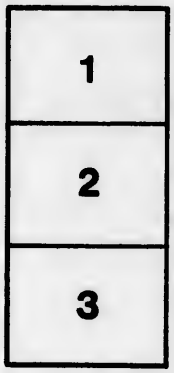

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THE BACTERIOLOGY OF HEALTHY ORGANS.

BY

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FELLOW IN PATHOLOGY, MCGILL UNIVERSITY, MONTREAL.

From the

Transactions of the Association of American Physicians

Vol. XV., page 389, 1900

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(*From the Molson Pathological Laboratory.*)

Scientific literature is full of careful observations of those observers who have studied the various species of bacteria isolated from diseased organs and tissues in man and animals, and scattered in the literature of the past two decades are a few articles which describe the micro-organisms which have been found in the living body where no evident disease was present.

The results of these later observations have been so at variance that practically two schools of bacteriologists have developed: the one studying cultures and smears from healthy organs removed immediately after death, claiming that bacteria are normally found; and the other, using slightly different methods and finding no growth on their cultures from similar organs, attributing the successful results of its opponents to errors in technique, and coming to the conclusion that these organs are normally sterile.

Meissner,[1] early in the last decade, states that "in the living tissues of the healthy animal no bacteria capable of development are present," while Zweifel,[2] quite on the contrary, affirms that in the living tissues bacteria are always present, that, however, they are anaërobic in character, their capability of development thus depending to a larger extent upon the amount of oxygen brought to the tissues by the blood.

Hauser,[3] moreover, following the work of these two men with similar observations, found, almost without exception, that the tissues of the body were sterile, whether sections of these tissues be submitted to bacteriological examinations (Gram's method) or whether cultures be made directly from the organs on the different nutritive media.

Welch,[4] in 1891, speaking with especial reference to the colon bacillus, states that he has isolated this organism from the internal organs of the body only where some distinct lesion of the intestinal mucosæ was present, and almost uniformly failed to find it outside the intestinal tract when no demonstrable lesion of the mucosa existed. He states, moreover, that the colon bacillus does not invade the blood and organs in the process of post-mortem decomposition.*

Among the more extended observations in this particular field of bacteriology are those of Neisser and Opitz, who have not only published the results of their own careful and painstaking researches, but have as well made accurate historical reviews of the bacteriological literature of the past thirty years, especially that portion which deals exclusively with the question under consideration.

Neisser[5] using rabbits and guinea-pigs removed the internal organs (liver, spleen and kidneys, heart, lung, mesentery) with as thorough asepsis as possible, cultivated them for *two days* on nutritive media, and finding that without exception these organs were free from bacteria at the end of this time, concluded that "under normal conditions no bacteria are present in the lymph or blood stream." After feeding different animals with cultures of various micro-organisms, however, and producing distinct lesions of the intestines, he found a large number of internal organs from which bacteria grew, either those with which the animal was treated or those that normally inhabit the intestine.

Opitz[6] removed the mesenteric glands of cattle killed at the abattoir at Breslau, carried them to the Hygienic Institute in the same city, and there, after carefully sterilizing the surface, cut out bits of these glands and cultivated them on agar and gelatin plates. Observing that in the majority of organs thus treated no bacteria grew at the end of *three days*, and that the forms isolated from the organs which decomposed were in all cases spore-bearers, especially the bacillus subtilis, the spores of which are exceedingly hard to kill, he concluded that the mesenteric glands of cattle are normally sterile, that is, that a passage of bacteria through the intestinal wall during digestion normally does not take place.

* Dr. Welch has, however, informed us—and he will, I feel assured, permit me to mention the fact—that in the course of an incompleted research in his laboratory, prior to the commencement of these investigations, it was found that the organs of healthy cats treated immediately after death by the perchloride method (to be described later) gave cultures of more than one form.

Neither Neisser nor Opitz record any observations on organs kept longer than three days, and the latter, in the experiments described above, naturally does not eliminate the possibility of post-mortem changes, as the transportation of the organs from one institution to another, together with the means employed to sterilize the surface, made necessary by such a transportation, consume so much time that rapidly growing species of bacteria might develop, or, on the other hand, bacteria present at the time of death might be destroyed by liberated bactericidal substances; and, to say the least, Opitz is not working with tissues absolutely normal.

For now some years studies more especially on the liver, made by Professor Adami in Montreal, have revealed the presence under the microscope of minute bodies within the cells, present both in diseased states and in organs showing no special lesion, which Dr. Adami could only conclude were of bacterial origin.[7] It thus became of interest to note whether bacteria were taken up by the healthy organism, and Dr. A. G. Nicholls, Senior Demonstrator of Pathology, undertook a study of the bacteriology of the healthy rabbit. His results seemed persistently to show that there was a passage of bacteria into the normal tissues.

Lack of adequate time, however, to carry out an extended series of observations has prevented the completion of enough experiments to determine with sufficient accuracy whether this law holds true for different species of animals under different conditions, and, consequently, the work was elaborated by the Fellow in Pathology.

It is the object of the present paper to describe the experiments made, with more especial reference to the methods employed and the general results obtained than to the particular varieties of organisms isolated.

It was decided to attempt the solution of one part only of this problem, and to determine whether bacteria are normally present in the livers and kidneys of animals killed instantaneously, and not to raise the question of the path of the bacteria from their normal habitation to the organs in which they might be found, a subject which Dr. Nicholls is more especially engaged in studying at the present time.

In addition to the experiments on animals, a number of autopsy cultures were studied, and while a considerable interval of time neces-

sarily elapsed between the moment of death and the moment of making the culture, yet the results of this set of observations seemed so much to verify the work on animals that they have been included in this paper.

It was necessary at the outset to keep two facts especially in mind.

First, it was desirable to reproduce the conditions in which the organs normally exist, and to remove them within a time after death, so short that it could be certain that post-mortem changes were not being studied; and, secondly, cultures had to be made on a variety of media both solid and fluid, so as to avoid if possible the common experience of bacteriologists who frequently see bacteria in substances examined and yet are unable to obtain any growth from them.

In this connection may be mentioned the variance in the results of blood cultures obtained by the German and English students as compared with the French, for while the former using solid media, straight or slant, on the surface of which the blood has been smeared, have obtained in many cases no growth on their media, the latter using large quantities of fluid media, especially broth, have found bacteria in a much larger proportion of cases. It may thus be possible that the blood undiluted will exhibit a strong bactericidal power which will be lost if the blood be well diluted, and thus there is frequently a decided growth in broth, for example, as compared with a sterile culture on agar. The same law may hold true for the animal juices as well as for the blood. Therefore, animals were instantaneously killed, the organs were removed with the greatest possible care to avoid contamination; they were then preserved in gelatin, agar, or broth at the room or the body temperature, and cultures were made directly from these organs on both solid and fluid media.

It was decided, moreover, to study animals in both a condition of fasting and of full digestion to determine what changes might be caused by the state of the stomach and intestines, and bearing in mind the negative results obtained by Neisser and Opitz, it was decided to preserve the organs for such a time as to be sure that a small number of bacteria developing slowly in the animal tissues might not escape detection.

The methods employed in this work will be briefly described. The first set of animals was killed early in October of last year. Rabbits were selected, starved for twenty-four hours, killed instantaneously by a

blow on the head, fastened to animal boards and saturated with water, to prevent the hair and fur from flying about the field of operation. The operator's hands were washed with soap and water, soaked in saturated solutions of potassium permanganate and oxalic acid, and then bichloride of mercury solution 1 : 1000 for several minutes.

All instruments were boiled for fifteen to thirty minutes, and with separate instruments for each step of the operation, the skin was removed, a small incision in the abdominal muscles made and the kidneys and half of the liver of each animal were excised, then passed immediately into the gas jet of a Bunsen burner, where they were held in the flame with hot-flamed instruments till the outer surface was scorched or cooked to a grayish-brown. They were then transferred immediately to specially prepared gelatin bottles. These bottles were wide mouthed, furnished with ground-glass stoppers, each perforated by a small opening plugged with cotton, each bottle containing about 50 c.c. of gelatin well sterilized, and the whole placed in the steam sterilizer at a temperature of 110°. At the operation the glass stoppers were removed, the smoking organ dropped directly from the flame into the melted gelatin and the glass stopper immediately replaced. The organs thus removed were preserved at the room temperature for eight days.

Cultures were made from day to day by drawing up a small bit of the organ in a sterile capillary pipette through the perforation in the glass stopper, and inoculating agar and broth-tubes. After eight days the organs were hardened in formal-Müller and sectioned and examined by the carbol-thionin and Gram's method.

From the first six organs thus prepared cultures were obtained on the fourth to the seventh day in four cases, and a similar set of organs was then removed from full-fed rabbits with the same technique and preserved in similar gelatin bottles. Positive results were obtained in four of the six organs of this series.

A third set of rabbits was killed, the organs removed (kidney and liver) with the same care, and I was particular, moreover, to flame the instruments in every case before touching the organs, and to cook the surface of the organ thoroughly in the flame. These organs were preserved in agar in the thermostat at the body temperature.

At the end of three days in this set of cultures the surface of the agar was covered with an abundant growth of bacteria, from which

cultures were made. The organ was then removed, its surface seared by a red-hot knife, and another set of cultures taken from its interior.

The glass-stoppered bottles were now discarded in favor of small two-inch deep Petri dishes half-filled with agar, which could be managed exactly like an ordinary plate culture. Long broth-tubes of larger calibre were also employed, furnished with broken glass in the bottom, having a cruncher of glass rod passed through the cotton-plug, and containing about 100 c.c. of neutral bouillon.

Both agar and broth were sterilized in the autoclave, and at the operation they were opened for only a fraction of a second, and the hot sizzling organ passed directly into the medium.

The organ in the broth was broken up by the glass rod, which was then pulled out through the cotton-plug and the top of the tube was covered with tin-foil. Cultures could be obtained from this as from an ordinary broth-tube.

Thus, in the latter experiments, each set of animals furnished a series of cultures on broth, on the surface of the agar and from the interior of the organ.

As a later procedure the Durham modification of the Smith fermentation-tube was adopted instead of the broth-tube, and glucose broth used instead of the plain. This modification, I may say, consists in having a small test-tube inserted in a larger one, which contains sufficient broth to fill the inner tube.

With the technique and method thus described cultures have been made from the kidneys and liver of full-fed and fasting rabbits, guinea-pigs, cats and dogs, and the various bacteria isolated from them have been identified as far as possible. Especial care has always been taken to avoid contamination. For many of the experiments the instruments had been soaked for some days in a mixture of 40 per cent. formalin* and glycerin, and then boiled immediately before operation; but the surest way of securing their absolute sterility has been to heat them in the flame for several minutes before using.

In all cases the organs have been thoroughly cooked in the flame before preservation. By means of the fermentation-tube the organ preserved in broth has often been found to evolve a considerable quantity of gas, and yet the cultures from it would show only a

* Formalin is 40 per cent. solution of formaldehyde. This antiseptic was originally recommended by Dr. F. Buller.

staphylococcus or a non-gas-producing bacillus. Smears, however, from the original tube would show various bacilli, either the colon or other forms, which were possibly overgrown by the hardier staphylococcus or some variety like mesentericus.

In many cases it has been impossible to identify the organisms isolated, so rudimentary is our knowledge of bacterial species; and while in the earlier experiments careful observations were made to determine if possible which particular variety had grown, a number of different animals being inoculated to establish this point, in the later experiments it has sufficed to describe the bacteria briefly and to separate them into rough types. I hope to describe later the character of the foregoing forms isolated in the course of these experiments.

In work of this kind the chances of contamination are many, and the greatest care must be exercised to avoid this factor. With attention to the details of asepsis, however, the organs may be removed and preserved without introducing bacteria from without into the culture media; but it is a different problem to take routine cultures from these organs from day to day over a period of several days or weeks without at times suspecting that the later growths are due to an earlier contamination. After a certain amount of experience, however, it became possible to take the cultures in the proper way, and a few organs in each series which remained sterile after being tapped for a number of times demonstrated that the bacteria which did grow in the others came from the organs themselves. To illustrate, a liver was preserved for thirty-nine days, cultures taken every third day, and yet at the expiration of this time the cultures were as absolutely free from bacteria as at first. Another organ was preserved for the same time with the same result.

The time of growth of the bacteria followed a fairly general law.

Cultures were usually obtained on the fourth, fifth, or sixth day, at which time the organ would show signs of decomposition. Rarely growths were obtained on the second and third day, but it was usually possible to foretell by the third day what particular organ would furnish a culture, both by means of the discoloration of its surface, its softness where tapped, the color it imparted to the media, and especially by gas evolved in the fermentation-tube.

As a routine measure, therefore, cultures were made every third day; the organs were preserved for fourteen days, those organs being

declared sterile which had not decomposed by this time. Contradictions to this law were frequently noted. In a number of cases there was an abundant gas-production in the broth-tubes, and yet no growth in culture media, while in other cases the organs in agar would present every sign of decomposition, yet no bacteria could be isolated. Cultures taken immediately after removal from the body were invariably sterile, and on the first and second day usually so, though 50 c.c. flasks of bouillon, so useful in blood cultures, were employed.

The results obtained in this work have been appended to this article in tabulated form for convenience of reference, and we may now analyze the various experiments, contrasting the species of bacteria found in the different animals with the different media and under the different conditions of digestion.

The Livers and Kidneys of Rabbits.

The first twelve organs cultivated, which may be comprised in the first set, were taken from fasting and full-fed rabbits and preserved in *gelatin* at the room temperature. From eight of these cultures were obtained, including staphylococci, spore-bearing pathogenic bacilli, varieties of proteus, and a paracolon. The time of growth was often late, sixteenth to seventeenth day, and many of the bacilli were spore-bearers.

Both the late period of development and the character of the bacilli grown might be expected in gelatin cultures at the room temperature. From the four organs remaining sterile, cultures were made at intervals for many days, and yet no growth resulted.

Out of a total of twelve organs eight contained bacteria, a percentage of 66⅔. The same percentage is true of the cultures made.

In the second set, kidneys and livers of rabbits preserved in *agar*, there were fifteen organs cultivated with eighteen cultures. Bacteria were obtained in fifteen of the eighteen cultures—83.3 per cent., while thirteen of the fifteen organs grew—86.6 per cent.

This difference in percentage develops from the fact that two portions of the same liver may be cultivated on different media—for example, in broth and agar, while the kidneys in every case were saved entire. In each series the different parts of the liver have been considered as one organ, while the different kidneys from the same animal necessarily are separate organs, for while the general

conditions governing the lymph and vascular supplies of the two kidneys are identical, yet the bacteriology of the two kidneys of the same animal may be quite different, as has been seen in many cases.

Cultures were obtained from the fasting animals in every case, the growth on the surface of the agar corresponding to that from the organ itself, and, as a rule, with that in the broth-tube. The predominant types are staphylococcus, mesentericus, colon, and paracolon in the fasting animals, while the mesentericus, staphylococcus, subtilis, and proteus grew from the full-fed animals. No culture of colon appeared, however, in the full-fed condition. The percentage of growth in this series is much higher that in any other set of animals, and illustrates possibly a condition of lowered resistance on the part of the rabbits used. They were taken from the laboratory supply which had been kept in confinement in unhygienic conditions for many weeks, and can hardly be said to represent the normal conditions of animals at liberty.

In these cultures the form appearing at the surface is often only one of the forms grown from the organ, as in Series III., Liver II., where a staphylococcus alone appeared on the surface of the agar, and in the organ there grew staphylococcus, mesentericus, and two varieties of paracolon. In Series IV. it may be noted that from Liver I. in both agar and broth the cultures were identical, while the third liver and kidneys were sterile in agar and grew staphylococcus in broth.

A number of cultures were taken immediately in 50 c.c. flasks of bouillon, all of which were sterile except the third kidney, from which grew a mould.

By accident one portion of one liver was preserved without singeing. Its duplicate was preserved in the regular manner; but from both of these organs, and from a part of the organ in broth, the same variety of mesentericus developed.

In general it may be seen that when an organ covered by agar decomposes, only one or two of the forms of bacilli present in the organ grow through the agar to the surface, and that while an organ in agar may not furnish a culture, yet the broth emulsion of another part of the same organ will. Either the breaking up of the organ liberates the bacteria more easily or the broth itself is a better medium for developing an organism. It is possible that the liberation and

neutralization of the bactericidal substances in the juices of the organ may be especially facilitated by a fluid medium.

Livers and Kidneys of Guinea-pigs.

The third set of animals (guinea-pigs), prepared in the usual way, grew practically the same variety of micro-organisms as did the rabbits, except that here the bacillus subtilis appeared in several of the cultures.

Its presence raised immediately the question of the proper preparation of the culture media, but its consistent appearance in cultures on both agar and broth, as in Kidney III., Series V., and its isolation from both the surface of the agar and the interior of the organ, as in Kidney II., Series VI., contraindicated the suspicion that it was an interloper.

A number of sterile organs in this series also pointed to the purity of the culture medium. In the subsequent experiments, however, the Petri dishes of agar were not only sterilized in the autoclave, but tested for a week or more in the thermostat on the suspicion that the spores of the subtilis might have been present in the agar stock.

From twenty-four cultures made here definite results were obtained in eighteen cases—a percentage of 75, while from the actual number of organs cultivated (eighteen) fourteen grew (77.7 per cent.).

Livers and Kidneys of Cats.

The cats next used were more suitable than the rabbits, as they were brought from the country, 150 miles away, kept in confinement for a few days only before operation, and thus represent a thoroughly wild condition. A different variety of organisms resulted from the cultures of this set. Staphylococcus and colon persist, subtilis and mesentericus drop out, and a number of forms like proteus, megatherium, and mycoides appear, as well as a staphylococcus and two varieties of long, narrow, spore-bearing bacilli, whose growth was so slow and weak that a careful study of their characters was impossible. Anaërobic cultures in Buchner jars likewise failed to reveal their identity.

From the twenty-four cultures here made growths were obtained or definite bacteria noted in twenty cases, a percentage of 83.3; fourteen of the eighteen organs contained bacteria (77.7 per cent.).

Livers and Kidneys of Dogs.

In the fifth set of animals the proper conditions were more nearly fulfilled than in any other experiment. The dogs were wild, had been confined only a few days; the technique of operation and the method of making cultures was better understood and handled; there was no doubt of the proper sterilization of the culture media and the Durham-Smith fermentation-tube furnished an accurate means of collecting any gas produced in a decomposing organ, thus indicating the presence of bacteria. The thorough surface cooking which the larger size of the organs permitted, and to which each organ was subjected, left no doubt of the absolute sterility of the surface.

The same flora was isolated as from the cats, especially forms like mycoides, megatherium, B. Zopfii, and many long, spore-bearing bacilli, which could be grouped together like those of the preceding set. The growth of the latter was very scanty, but the microscopical characters of the bacilli were unmistakable. Out of the twenty-four cultures here twenty-one grew (87.5 per cent.), while sixteen of the eighteen organs contained bacteria (88.8 per cent.).

Fœtal Organs.

Four organs at this time were removed from fœtal kittens and cultivated in the same way. Three remained sterile, from one grew a staphylococcus and megatherium, an apparently inexplicable result, yet the four organs were all subjected to the same procedure, especially in the sterilization of the surface.

Sterilization by Perchloride of Mercury.

At the suggestion of Dr. Welch a final set of organs was prepared with a somewhat different method, to see if possible whether any change in the procedure adopted could make any change in the actual results obtained.

Rabbits were killed, the organs were removed carefully, and then placed in dishes of bichloride of mercury 1:1000, and left for one hour. The dishes had previously been soaked for from two to eighteen hours in this solution. The organs were left in bichloride an hour, transferred to sterile water, and there left another hour, rinsed off with

boiling hot sterilized water, and placed in fermentation-tubes and sterile Petri dishes, into some of which agar was subsequently poured.

By this method the bichloride of mercury forms an insoluble precipitate of albuminate of mercury on the surface of the organ, which allows complete sterilization of the surface without destruction of the micro-organisms in the interior.

Twenty cultures prepared in this way grew in thirteen cases (65 per cent.), the forms being identical with those from the rabbits originally used, mesentericus, staphylococcus, and colon being more especially present. Of the actual number of organs employed (twelve) nine showed bacteria (75 per cent.).

Examination of Sections of the Organs.

Bacteriological examination of the hardened organs was made in all cases, and the results were somewhat difficult to interpret. In many organs from which bacteria grew the forms isolated could be easily demonstrated in the sections, especially mesentericus and staphylococcus. The bloodvessels, as a rule, were filled by the bacteria. In other cases, however, the only evidence of bacteria was the appearance, under the microscope, of many granules, both extracellular and intracellular, especially in the forms of cocci and diplococci, the numbers of these bodies being relatively great in the organs from which cultures were obtained as compared with a small number of granules seen in sterile organs, and practically none in the fœtal.

The appearance of these bodies was not determined by the variety of bacteria isolated, as they were common to all the organs, whether a staphylococcus, mesentericus, subtilis, or colon was the result of cultivation, and the entire phenomenon suggested the possibility that these granules were broken up, destroyed bacteria.

Results from Autopsies (Man).

The autopsy cultures were made by means of a simple device like a potato-cutter or cork-borer, provided with a piston, which, after sterilization by heat, was pushed through the sterilized surface of the organ, and, when withdrawn, carried with it a portion from the interior of this organ, which was placed in the fermentation-tubes. These cultivations were made some hours after death—four to six hours in the earliest case—and, of course, this element of time de-

tracts somewhat from the accuracy of the results. Twenty-six cultures were made from nine different autopsies, and in every case bacteria were grown from these cultures.

The colon, staphylococcus, and mesentericus were the common forms, together with a number of long, narrow bacilli like those seen in gangrene. No putrefactive organisms were isolated, and from the similar results of early and late cultures one is led to believe that at the time of death a few bacteria were scattered in the organs, which are capable of development and transplantation, rather than that there was a post-mortem passage of bacteria from the intestine, for example, into the organs. In several cases a pure staphylococcus was grown, yet a large quantity of gas was evolved from the original culture, smears made directly, however, showing many bacilli, which might either be the gas-producing colon or some pure anaërobe overgrown by the hardier staphylococcus. Anaërobic cultures in Buchner's jars revealed nothing further to solve this problem.

Conclusions.

There are a number of generalizations which can be made from the scrutiny of the various experiments described above, which both testify to the accuracy of these observations and explain in great part their contradiction of the conclusions of earlier investigators.

A. *Each species of animal showed its own peculiar bacteriology* quite distinct from that of other species, regardless of the conditions in which the animals were killed or in which the cultures were made.

Rabbits. From the kidneys and livers of rabbits there grew mesentericus, staphylococcus, colon, proteus, and a variety of spore-bearing pathogenic bacilli which was not isolated from any other animal. These bacteria were obtained in agar, in gelatin, and in broth, from either full-fed or fasting animals, an interval of several weeks elapsing between the cultivation of each series. The last rabbits were killed over six months after the first, the organs were preserved in a different manner, and yet the cultures were practically identical with those originally seen.

Guinea-pig. The flora from the guinea-pig was similar to that of the rabbits, and yet not identical.

There is not the same profusion of mesentericus as in the other animals, and the bacillus subtilis becomes a quite common form. A

greater number of organs remained sterile. Staphylococci and colon appeared in about the same proportion of the cultures. Both the rabbits and guinea-pigs are herbivora, and their organs show species of bacteria fairly consistent with the food on which such animals live.

Cat. In the organs of the cats there appeared quite different forms, megatherium, mycoides, Zopfii, with an occasional subtilis and mesentericus.

Staphylococci and colon were common, but the prevalent forms were of unknown species grouped about two common types, A and B—long, straight bacilli—spore-bearers, growing with great difficulty on the ordinary media, producing gas in the culture tubes, and isolated from the majority of the organs of this series of animals.

Dog. The forms seen in dogs were similar to those in the cats. There were very few staphylococci, no colon or paracolon, and the spore-bearers of the other carnivora were here replaced by long, narrow bacilli without spores, and by broad, straight bacilli, both gas producers and difficult to cultivate. Mycoides, megatherium, and B. Zopfii continue to grow in many of the cultures.

As a rule, the carnivora, dogs and cats, showed species of bacteria quite similar to each other, yet absolutely different from the cultures obtained from the rabbits and guinea-pigs—herbivora, and these results are thus quite consistent with the difference in the food used by the animals, which would determine to a large extent the intestinal flora.

B. *Each animal used, regardless of its species, showed its distinct bacteriology.* Among the rabbits the second animal of the third series, killed fasting, showed in its organs staphylococcus, mesentericus, paracolon, and proteus, while the third animal showed staphylococcus alone in all its organs.

The first animal of the fourth series showed mesentericus and staphylococcus, while from the third animal nothing grew on agar, and only staphylococcus in broth.

Among the guinea-pigs the second animal of Series VI. showed staphylococcus and colon, while the organs of the third animal were sterile throughout.

There is a corresponding similarity in the cultures obtained in the separate dogs and cats, but possibly the last set of rabbits shows examples of this law more convincing than are seen in any other series.

From the first animal there grew colon or paracolon in every organ, either alone or in combination with other bacilli. The cultures of all of the organs of the second animals were sterile; from the third animal there were cultures from the kidney only; while from the last there was a mixture of colon and staphylococcus.

From two animals, at least, there were no cultures. From five or six there was only staphylococcus in the broth, and from a similar number a single species, like mesentericus, grew from all the organs.

C. *The different organs of the various animals displayed the same bacteria on the different culture media.* Liver I., Series IV., grew mesentericus in both agar and broth, as well as the unsinged liver in agar; Liver I., Series VI., grew paracolon throughout, while Kidney I., Series VII., grew bacillus subtilis on all media.

D. *The different culture media furnished a variation in the species of micro-organisms quite consistent with the growths obtained from the organ itself.* The *surface* of the agar showed frequently one form only, which was isolated later from the organ in combination with other forms, while the broth developed bacteria from organs which were sterile in agar. For example, the surface of the agar in Liver II., Series III., grew staphylococcus alone, but in the organ there appeared paracolons, mesentericus, and staphylococcus. The broth cultures of Liver III. and Kidney III., Series IV., grew a staphylococcus, while the agar cultures were sterile.

It is thus evident that when an organ decomposes, covered by agar, some bacteria develop rapidly and appear on the surface either alone or before the others present in the organs can reach the same position, and it is a significant fact that in practically every organ cultivated in this series of experiments that particular species of bacterium developing on the surface of the agar was obtained later from the singed organ.

The only possible exception to this law is in the case of Kidney I., Series III., where on the surface of the agar a mesentericus appeared, with a staphylococcus, while from the organ a few days later the staphylococcus only grew.

E. *The condition of digestion exerts a decided but not a universal influence* on the species of bacteria isolated from the kidneys and livers cultivated. In many cases the colon group appears in the cultures

from the fasting animals, but fails to do so in the full-fed condition. This is especially well illustrated in Series III. and IV., where the fasting rabbits furnished colon in three out of six organs, while the full-fed rabbits furnished none. The prevalence of the colon group was not marked sufficiently, however, to prove this law in other species of animals, while in the carnivora it was impossible to work out a corresponding law for the unknown bacteria there isolated. The actual number of sterile organs seems to be the same, whether we are dealing with the full fed or fasting.

F. As has already been pointed out, *there was in a number of cases ample microscopic evidence to prove that bacteria had developed in an organ, and yet no cultures could be grown.*

There was a production of gas in several of the Durham-Smith tubes of Series XI., yet no growth on culture media, while the portions of liver of the third animal were discolored, soft and juicy, like those which had furnished a growth of bacteria, and yet the cultures were invariably sterile.

The microscopic examination of these hardened organs showed no bacteria, but the same granular appearance as was noted in the decomposed organs.

The entire number of animals killed was 34, furnishing 93 organs and 122 cultures. Of the 93 organs, 75 furnished definite growths on the various media, a percentage of 80.6. Of the actual number of cultures made, 122, 95 grew, a percentage of 77.8.

This apparent discrepancy is explained when we remember that one portion of an organ may grow on one medium, and a corresponding portion on another medium be sterile, the broth in all cases developing more bacteria than the agar. Each set of animals as well, when analyzed separately, showed over 65 per cent. if we consider the different cultures, and over 66 per cent. if we consider the organs. While the highest percentage of organs containing bacteria was 88.6, that of cultures growing was 87.5.

In view of what has already been said, there is some evidence that the total number of organs furnishing cultures is smaller than the number of organs which might actually contain bacteria, as several decomposed organs are marked sterile because no bacteria were grown from them.

Contamination of the cultures may be eliminated for two reasons: The utmost care was taken to remove the organs aseptically and to preserve them in sterile media in sterile dishes. The instruments were thoroughly boiled and flamed, the media were sterilized in the autoclave and then tested, the operator's hands were surgically clean, and in the actual manœuvres care was taken to prevent any possible introduction of bacteria from without. Each organ was either thoroughly singed or soaked in bichloride of mercury to sterilize the surface, and then passed immediately into the culture media prepared for it. The routine cultures were made in the most careful manner possible, and the actual preservation of several organs in the various media, gelatin, agar and broth, for from two to six weeks, and their absolute sterility at the end of this time proves that the laws of bacteriology were carefully adhered to.

Again, *the isolation of different bacteria from different species of animals, from different animals and from different organs, shows that the results of cultivation of these organs depend on actual presence of bacteria in the organs themselves, and not on the accidental introduction of germs from either the air, instruments, the hands of the operator, or the media and dishes used.*

The removal of the organ from the animal the moment after death and its transfer to the culture medium consumed less than five minutes of time, and it is thus certain that these observations represent actual living conditions.

In conclusion, why do these results contradict the results of Neisser and Opitz? The answer to this question is simple. Neisser and Opitz cultivated their organs for two and three days only, and at the end of this time declared their organs sterile. *In my cultivations at the end of three days an identical condition was determined. The cultures were sterile.* There is, then, no doubt thrown upon the facts adduced by these observers. If, however, the organs be left for several days, a week, or two weeks, bacteria will appear on the culture media with such persistence and in such profusion and specific variety as to show that they come from the actual organs and are not introduced from without.

There are two explanations for this phenomenon. On the one hand, the bacteria present in the organs may be so few in number that immediately after death they will not grow on culture media, for

it is now being admitted that to obtain a culture of bacteria in many cases it is necessary to have them present in some slight number. As Haffkine has pointed out, the transfer of bacteria from one medium to another leads to the destruction of a certain number from alteration of environment. Thus where few are present they may all succumb.

On the other hand, the bactericidal substances in the animal juices may be so powerful in the living condition that if they be transferred with bacteria to culture media they will there exert an inhibitory action on these bacteria and render a culture sterile (especially in solid media), whereas, the same juices may lose their bactericidal power if separated from the living animal, as in the case of blood serum, thus allowing the growth and multiplication of any bacteria present in the living organ. This curiously slow development of bacteria within healthy organs removed from the body affords a most interesting problem for further elucidation.

But whatever be the explanation of the facts as a result of the experiments described in this article, it must be concluded that at least 80 per cent. (80.6 per cent.) of the livers and kidneys of healthy normal animals contain bacteria which are capable of development, provided the proper culture media be adopted, and provided that these organs be cultivated for a sufficiently long time after their removal from the animals used.

My thanks are due to Dr. Adami for his great kindness and his unfailing help and advice in this series of experiments.

SET I.

SERIES I.—*Rabbits Fasting. Organs in Gelatin Bottles.*

Rabbit A 1:			
Kidney	I.	Staphylococcus.	8th day.
Liver	I.	Pathogenic spore-bearing bacillus, No. I.	4th day.
Rabbit A 2:			
Kidney	II.	I. Pathogenic spore-bearing bacillus, No. II. II. Paracolon. III. Pathogenic spore-bearing bacillus, No. III.	7th day.
Liver	II.	0	
Rabbit A 3:			
Kidney	III.	0	
Liver	III.	Staphylococcus aureus.	7th day.

SERIES II.—*Rabbits Full Fed. Organs in Gelatin Bottles.*

Rabbit B 1:			
Kidney	I.	Pathogenic spore-bearing bacillus, No. IV.	12th day.
Liver	I.	0	Sterile after 39 days.
Rabbit B 2:			
Kidney	II.	Staphylococcus.	17th day.
Liver	II.	0	Sterile after 39 days.
Rabbit B 3:			
Kidney	III.	Staphylococcus.	4th day.
Liver	III.	Proteus.	
		Paracolon.	16th day.

SET II.

SERIES III.—*Rabbits Killed Fasting. Organs in Agar Bottles in Thermostat.*

		Surface.	Organ.
Rabbit C 1:			
Kidney	I.	Staphylococcus.	Staphylococcus.
		Mesentericus vulgatus.	
Liver	I.	Long spore-bearing bacillus.	Staphylococcus.
		Paracolon A.	Paracolon A.
		Paracolon B.	
Rabbit C 2:			
Kidney	II.	Paracolon C.	Proteus vulgaris.
		Mesentericus vulgatus.	Mesentericus vulgatus.
			Paracolon B.
Liver	II.	Staphylococcus.	Paracolon.
			Paracolon B.
			Mesentericus fuscus.
			Staphylococcus.
Rabbit C 3:			
Kidney	III.	Staphylococcus.	Staphylococcus.
Liver	III.	Staphylococcus.	Staphylococcus.

Growths obtained on sixth day, previous to which the bottles were unopened.

SERIES IV.—*Rabbits Killed Full Fed. Organs in Agar Plates.*

		Surface.	Organ.	Broth tubes.
Rabbit D 1:				
Kidney	I.	Mesentericus ruber.	Mesentericus vulgatus.	Staphylococcus.
Liver	I.	Mesentericus vulgatus.	Mesentericus vulgatus.	Mesentericus vulgatus.
				Mesentericus fuscus.
Rabbit D 2:				
Kidney	II.	0	0	Mesentericus fuscus.
Liver	II.	Mesentericus vulgatus.	Mesentericus vulgatus.	Mesentericus vulgatus.
		Proteus mirabilis.	Proteus mirabilis.	Bacillus subtilis.
Rabbit D 3:				
Kidney	III.	0	0	Staphylococcus.
Liver	III.	0	0	Staphylococcus.
Liver	I.	Not singed.		
		Mesentericus fuscus.	Mesentericus vulgatus.	
		Mesentericus vulgatus.		

All cultures taken immediately in 50 c.c. bouillon sterile except kidney III., from which grew a mould. Growths obtained fourth to sixth day.

SET III.

SERIES V.—*Guinea-pigs Killed Full Fed. Organs in Agar Plates.*

		Surface.	Organs.
Guinea-pig A 1:			
Kidney	I.	Staphylococcus. Paracolon.	Staphylococcus. Paracolon.
Liver	I.	Mesentericus vulgatus. Bacillus subtilis.	Proteus vulgaris. Mesentericus fuscus.
Guinea-pig A 2:			
Kidney	II.	0	0
Liver	II.	Staphylococcus.	Staphylococcus.
Guinea-pig A 3:			
Kidney	III.	Mesentericus ruber. Bacillus subtilis.	Mesentericus vulgatus. Mesentericus fuscus.
Liver	III.	Bacillus subtilis.	Bacillus subtilis.

Growths obtained at end of seventh day.

SERIES V.—*Guinea-pigs Killed Full Fed. Organs Preserved in Broth-tubes.*

Guinea-pig A 1:			
Kidney	I.	Mould on surface.	
Liver	I.	Not singed.	Staphylococcus.
Liver	I.	Singed.	Staphylococcus.
Guinea-pig A 2:			
Kidney	II.	Mesentericus fuscus.	
Liver	II.	Bacillus subtilis and staphylococcus.	
Guinea-pig A 3:			
Kidney	III.	Bacillus subtilis.	
Liver	III.	0	

Growths obtained at end of seven days.

SERIES VI.—*Guinea-pigs Killed Fasting. Organs in Agar Plates.*

		Surface.	Organ.	Organs in broth-tubes.
Guinea-pig B 1:				
Kidney	I.	0	0	0
Liver	I.	Paracolon.	Paracolon.	Paracolon.
Guinea-pig B 2:				
Kidney	II.	Bacillus subtilis.	Bacillus subtilis.	Staphylococcus.
Liver	II.	0	Staphylococcus.	Staphylococcus.
Guinea-pig B 3:				
Kidney	III.	0	0	Mould.
Liver	III.	0	0	Mould.

Growths obtained at end of seventh day.

SET IV.

SERIES VII.—*Cats Killed Fasting. Organs in Agar Plate.*

		Surface.	Organ.	Broth-tubes.
Cat A 1:				
Kidney	I.	0	Bacillus subtilis.	Bacillus subtilis.
Liver	I.	0	Few spores; no bacilli.	Long bacilli and spores. Variety A.

		Surface.	Organ.	Broth-tubes.
Cat A 2:				
Kidney	II.	Proteus. Megatherium.	Proteus. Megatherium.	0
Liver	II.	0	Long bacilli and spores. Variety A.	Few long bacilli; no spores. Variety A.
Cat A 3:				
Kidney	I I.	0	0	0
Liver	III.	0	Mesentericus. Mycoides. Megatherium.	Long, broad, thick bacilli and spores. Variety B.

SERIES VIII.—*Cats Full Fed. Organs Preserved in Agar Plate.*

		Surface.	Organ.	Broth-tubes.
Cat B 1:				
Kidney	I.	Mould (mucor) Colon bacilli (?)	Mould. Colon bacillus.	Few bacilli and spores. Variety B.
Liver	I.	0	Staphylococcus. Colon.	Few long narrow and few broad bacilli. Variety A.
Cat B 2:				
Kidney	II.	0	Few long bacilli. Staphylococcus.	0
Liver	II.	0	Few bacilli. Spores. Staphylococcus.	Streptococcus.
Cat B 3:				
Kidney	III.	0	Fine bacilli on smear; no growth.	Few long fine bacilli. Variety A.
Liver	III.	0	Few bacilli; no growth.	Long straight bacilli; no spores. Variety B.

Gas produced in majority of broth-tubes, which were furnished with inner tubes to collect it. Both Series VII. and Series VIII. anaërobic cultures negative. Growths obtained at end of seven days.

SET V.

SERIES IX.—*Dogs Full Fed. Organs in Agar.*

		Surface.	Organ.	Broth.
Dog A 1:				
Kidney	I.	0	0	Long straight bacilli, like Zopfii, short bacilli and large coccoid bodies like staphylococci.
Liver	I.	0	Few long straight bacilli.	Few short straight bacilli.
Dog A 2:				
Kidney	II.	Mycoides. Megatherium.	Megatherium.	Long bacilli with spores like Zopfii.
Dog A 3:				
Kidney	III.	0	Long straight bacilli; no spores.	Few long straight bacilli.
Liver	III.	0	Few long straight bacilli.	Long straight bacilli like mycoides, others like megatherium.

Gas produced in all broth-tubes of Series IX.

SERIES X.—*Fasting. Organs in Agar.*

		Surface.	Organ.	Broth.
Dog B 1:				
Kidney	I.	0	Staphylococcus. Mycoides.	Long straight bacilli like mycoides.
Liver	I.	0	Sporelike bodies and long straight bacilli.	Few long and short straight bacilli.
Dog B 2:				
Kidney	II.	0	Staphylococcus.	Spores and long straight bacilli.
Liver	II.	0	Staphylococcus. Mycoides.	Long straight bacilli like mycoides and spores.
Dog B 3:				
Kidney	III.	0	0	Few sporelike bodies and long straight bacilli.
Liver	III.	0	Staphylococcus. Spores. Long straight bacilli.	0

Gas produced in all broth-tubes of Series X. except Liver III.
Growths obtained at end of seventh day.

SET VI.

SERIES XI.—*Rabbits Killed Full Fed.*

Organs removed aseptically; thrown into bichloride of mercury solution 1:1000 *for one hour; passed into sterile water for one hour, and then washed with hot sterile water.*

Organs preserved dry in Petri dishes; preserved in agar plates and in broth-tubes.

Rabbit E 1:

Kidney	I.	Dry	=	Short straight bacilli. (Colon?)
Kidney	I.	Broth	=	Short straight bacilli. (Colon?) Large bacilli. (Gas produced.)
Liver	I.	Dry	=	Colon.
Liver	I.	Agar	=	Colon.
Liver	I.	Broth	=	Colon. (Gas produced.)

Rabbit E 2:

Kidney	II.	Dry	=	0
Kidney	II.	Broth	=	0 (Gas produced.)
Liver	II.	Dry	=	0
Liver	II.	Broth	=	0 (Gas produced.)
Liver	II.	Agar	=	0

Rabbit E 3:

Kidney	III.	Dry	=	Colon and heavy thick bacilli.
Kidney	III.	Broth	=	Colon and heavy thick bacilli. (Gas produced.)
Liver	III.	Agar	=	0
Liver	III.	Dry	=	0
Liver	III.	Broth	=	Broad straight bacilli. (Gas produced.)

Rabbit E 4:

Kidney	IV.	Broth	=	Staphylococci and bacilli. (Gas produced.)
Kidney	IV.	Agar	=	Staphylococci.
Liver	IV.	Agar	=	Surface. Mesentericus. Long bacilli-like subtilis. Organ. Mesentericus. Colon-like bacilli.
Liver	IV.	Broth	=	Short and long straight bacilli. (Gas produced.)
Liver	IV.	Agar	=	Long and short thick bacilli.

TOTALS. ANIMALS KILLED.

SET I.

SERIES I. (Rabbits fasting.)	SERIES II. (Rabbits full fed.)
Kidney I. +	Kidney I. +
Liver I. +	Liver I. 0
Kidney II. +	Kidney II. +
Liver II. 0	Liver II. 0
Kidney III. 0	Kidney III. +
Liver III. +	Liver III. +

Number of cultures	12
Number of growths on cultures	8
Percentage	$66\frac{2}{3}$
Number of organs	12
Number bacteria-containing	8
Percentage	$66\frac{2}{3}$

SET II.

SERIES III. (Rabbits fasting.)	SERIES IV. (Rabbits full fed.)	
	Agar.	Broth.
Kidney I. +	Kidney I. +	Kidney I. +
Liver I. +	Liver I. +	Liver I. +
Kidney II. +	Kidney II. 0	Kidney II. +
Liver II. +	Liver II. +	Liver II. +
Kidney III. +	Kidney III. 0	Kidney III. +
Liver III. +	Liver III. 0	Liver III. +

Number of cultures	18
Number of growths on cultures	15
Percentage	83.3
Number of organs cultivated	15
Number of bacteria-containing	13
Percentage	86.2

SET III.

SERIES V. Guinea-pig (full fed).		SERIES VI. Guinea-pig (fasting).	
Agar.	Broth.	Agar.	Broth.
Kidney I. +	Kidney I. +	Kidney I. 0	Kidney I. 0
Liver I. +	Liver I. +	Liver I. +	Liver I. +
Kidney II. 0	Kidney II. +	Kidney II. +	Kidney II. +
Liver II. +	Liver II. +	Liver II. +	Liver II. +
Kidney III. +	Kidney III. +	Kidney III. 0	Kidney III. +
Liver III. +	Liver III. 0	Liver III. 0	Liver III. +

Number of cultures	24
Number of growths	18
Percentage	75.0
Number of organs	18
Number of bacteria-containing	14
Percentage	77.7

Set IV.

Series VII. (Cats fasting.)

Agar.	Broth.
Kidney I. +	Kidney I. +
Liver I. +	Liver I. +
Kidney II. +	Kidney II. 0
Liver II. +	Liver II. +
Kidney III. 0	Kidney III. 0
Liver III. +	Liver III. +

Number of cultures 24
Number of growths 20
Percentage 83.3

Number of organs 18
Number of bacteria-containing 14
Percentage 77.7

Series VIII. (Cats full fed.)

Agar.	Broth.
Kidney I. +	Kidney I. +
Liver I. +	Liver I. +
Kidney II. +	Kidney II. 0
Liver II. +	Liver II. +
Kidney III. +	Kidney III. +
Liver III. +	Liver III. +

Set V.

Series IX. (Dogs full fed.)

Agar.	Broth.
Kidney I. 0	Kidney I. +
Liver I. +	Liver I. +
Kidney II. +	Kidney II. +
Liver II. +	Liver II. +
Kidney III. +	Kidney III. +
Liver III. +	Liver III. +

Number of cultures 24
Number of growths 21
Percentage 87.5

Number of organs cultivated 18
Number containing bacteria 16
Percentage 88.8

Series X. (Dogs fasting.)

Agar.	Broth.
Kidney I. +	Kidney I. +
Liver I. +	Liver I. +
Kidney II. +	Kidney II. +
Liver II. +	Liver II. +
Kidney III. 0	Kidney III. +
Liver III. +	Liver III. 0

Set VI.

Series XI. (Rabbits full fed.)

Kidney I. Dry +	Kidney II. Dry 0	Kidney III. Dry +	Kidney IV. Broth +
Kidney I. Broth +	Kidney II. Broth 0	Kidney III. Broth +	Kidney IV. Agar +
Liver I. Broth +	Liver II. Dry 0	Liver III. Agar 0	Liver IV. Agar +
Liver I. Dry +	Liver II. Broth 0	Liver III. Dry 0	Liver IV. Broth +
Liver I. Agar +	Liver II. Agar 0	Liver III. Broth +	Liver IV. Agar +

Number of cultures 20
Number of growths 13
Percentage 65.0

Number of organs cultivated 12
Number of bacteria-containing 9
Percentage 75.0

Summary.

Total number of animals	34
" number of organs cultivated	93
" number of cultures	122
" number of growths on cultures	95
Percentage	77.8
Number of organs containing bacteria	75
Percentage	80.6

Autopsy Cultures. Royal Victoria Hospital.

II., 1900. Male, 45. Plastic peritonitis with pneumothorax.

Eight hours after death.

Kidney,	Colon.	
Liver,	Colon.	
Spleen,	Staphylococcus.	

III., 1900. Female, 42. Chronic interstitial nephritis and uræmia.

Twenty-five hours after death.

Liver,	Colon.	
Spleen,	Colon and paracolon.	

VI., 1900. Male, 64. Chronic phthisis.

Sixteen hours after death.

Kidney,	Colon and staphylococcus.	Gas produced (Cultures).
Liver,	Colon.	Gas produced (Cultures).
Spleen,	Staphylococcus.	
	Paracolon.	No gas produced.
	Long putrefaction (2) forms.	(Cultures.)

VIII., 1900. Female, 10. Tuberculous meningitis and miliary tuberculosis.

Sixteen hours after death.

Kidney,	Staphylococcus.	
Liver,	Staphylococcus.	
Spleen,	Staphylococcus.	

IX., 1900. Male, 58. Mitral stenosis and incompetency.

Twenty-four hours after death.

Kidney,	Staphylococcus.	Gas produced.
	Colon.	
	Long thick bacilli.	
Liver,	Colon.	
	Long forms.	Gas produced.
	Broad forms.	
Spleen,	Colon.	Gas produced.

X., 1900. Female 30. Cholecystenterostomy. Death from exhaustion; no peritonitis.

Eight hours after death.

Kidney,	Staphylococci.	
	Mixture of bacilli which did not grow.	Gas produced.
	Colon.	

Liver,	Staphylococci. Mixture of bacilli. Colon.	Gas produced.
Spleen,	Staphylococci. Mixture of bacilli.	Gas produced.

XI., 1900. Female, 18. Cerebral abscess following otitis media.

Sixteen hours after death.

Kidney,	Staphylococcus. Mesentericus fuscus.	Gas produced.
Liver,	Staphylococcus. Mesentericus fuscus (vulgatus).	Gas produced.
Spleen,	Staphylococcus. Long narrow bacilli.	Gas produced

XII., 1900. Female, 43. Alcoholic neuritis.

Six hours after death.

Kidney,	Staphylococcus Mesentericus fuscus.	No gas produced.
Liver,	Staphylococcus. Colon. Mesentericus fuscus.	Much gas produced.
Spleen,	Staphylococcus. Colon. Mesentericus vulgatus.	Much gas.

XIII., 1900 Female, 65. General peritonitis following stricture and ulceration of rectum.

Eight hours post-mortem.

Kidney,	Staphylococcus. Mixture of bacilli.	Gas produced.
Liver,	Mesentericus fuscus. Mixture of bacilli.	Gas produced.
Spleen,	Mesentericus fuscus.	Gas produced.

ANAËROBIC CULTURES. ROYAL VICTORIA HOSPITAL.

X., 1900. Eight hours after death.

Kidney,	Staphylococcus.
Liver,	Staphylococcus.
Spleen,	Paracolon.

XI., 1900. Sixteen hours after death.

Liver,	Colon.
Liver,	Staphylococcus. Long bacilli.
Spleen,	Staphylococcus. Long bacilli.

XII., 1900. Six hours after death.

Kidney,	Staphylococcus.
Liver,	Staphylococcus.
Spleen,	Colon.

XIII., 1900. Eight hours after death.

Kidney,	Paracolon. Mixture of bacilli. Long narrow bacilli without visible growth.
Spleen,	Staphylococcus.

AUTOPSY. ROYAL VICTORIA HOSPITAL.

II.—1900	Kidney	+
	Liver	+
	Spleen	+
III.—1900	Kidney	?
	Liver	+
	Spleen	+
VI.—1900	Kidney	+
	Liver	+
	Spleen	+
VIII.—1900	Kidney	+
	Liver	+
	Spleen	+
IX.—1900	Kidney	+
	Liver	+
	Spleen	+
X.—1900	Kidney	+
	Liver	+
	Spleen	+
XI.—1900	Kidney	+
	Liver	+
	Spleen	+
XII.—1900	Kidney	+
	Liver	+
	Spleen	+
XIII.—1900	Kidney	+
	Liver	+
	Spleen	+

ANAEROBES.

X.—1900	Kidney	+
	Liver	+
	Spleen	+
XI.—1900	Kidney	+
	Liver	+
	Spleen	+
XII.—1900	Kidney	+
	Liver	+
	Spleen	+
XIII.—1900	Kidney	+
	Liver	+
	Spleen	+

Total: 26 organs cultivated, all contain bacteria.

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