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Bacteria in Healthy Tissues

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F. W. Mott and V. Horsley

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ON THE EXISTENCE OF BACTERIA, OR THEIR ANTECEDENTS, IN HEALTHY TISSUES.

By FREDERICK W. MOTT, M.R.C.S., and V. HORSLEY, M.R.C.S. Pl. XI.

Although the determination of the existence of bacteria or their "germs" in healthy living tissues is of fundamental importance in correctly appreciating the relation that these organisms bear to certain diseases, the evidence is not unanimous in deciding the question either affirmatively or negatively. It was in considering the theory on which the brilliant practice of antiseptic surgery is founded that we were struck by this variance of opinion on a point of such elementary importance, and were led to repeat some of the published experiments and introduce variations in the method of research.

Of seven authors who have worked at this question, we find that five (viz., Tiegel, Burdon-Sanderson, Béchamp, Nencki and Giacosa) conclude that bacteria or their germs do exist in healthy living tissues, while on the other hand, two papers written by Watson Cheyne, and by Chiene and Ewart, give results in the negative direction (3, 7); and in the case where blood was investigated, the results obtained by Pasteur (11) are also negative. As regards the investigations of the observers first mentioned there is a striking concordance of results (confirmed throughout by our own experiments) not only as regards the kinds of bacteria found in the different tissues, but also in the range and distribution of bacteria in the body. The principle of the methods employed is throughout the same, viz., the removal of the tissue in a living state, placing it under conditions precluding the possibility of external contamination, and finding that the change of state has (under certain conditions) determined the appearance of bacteria. This result has been held to prove the existence of bacteria or their "spores" in living tissues, although the facts are equally explicable on the theory of a "bacterial degeneration" of the tissue itself (Bastian) (9).

Against these positive results we have the evidence of two sets of experiments, viz., those of Chiene and Ewart and of Watson Cheyne. These observers removed organs with antiseptic precautions,

and placed them either in carbolic gauze or cucumber infusion. The former of these methods has been found experimentally by Nencki and Giacosa (6) to be objectionable on account of the penetrating power of carbolic acid ; and the second of these methods is open to the same objection, since the beakers of cucumber infusion were exposed under a spray of carbolic acid. Moreover Cheyne's control experiment does not necessarily contradict this opinion, for it is a well-known fact (which his other experiments confirm) that a less degree of carbolization is required to prevent the development of organisms than that needed to exterminate them when already present.

We were not aware of the results obtained by Nencki and Giacosa (6) until some months after we had arranged our methods and commenced the present series of experiments. Thinking that possibly the difference of results obtained depended upon the methods of experimentation, we resolved to repeat the paraffin experiments of Tiegel (1) and Burdon-Sanderson (2), superadding antiseptic precautions, and to compare these with a parallel series in which the tissue was hermetically sealed in glass vessels with a minimal introduction therein of carbolic acid. The very possible fallacy due to carbolization was of course to be specially guarded against in obtaining blood, and by the method described below this objection was removed. The following method was used in removing tissues for investigation:—

Method.

In every case the animal was killed by inhalation of chloroform or ether; immediately respiratory movements had ceased it was plunged into a large bath containing a 1 in 20 (5 per cent.) solution of carbolic acid, and the whole body was then thoroughly soaked with the same solution. The necessity of working in a pure atmosphere was provided for by turning on the spray for several minutes before the foregoing manipulation. Under the cloud of 1 in 40 carbolic acid solution, the abdomen was rapidly opened and the vena cava inferior exposed. All instruments and ligatures were kept in a 1 in 20 solution of carbolic acid. After blood had been taken in the manner described below, the renal vessels on both sides were ligatured and the organ treated as follows :—

(*a.*) In repeating Tiegel and Sanderson's experiments we used pure paraffin heated to 110° C. The organ was suspended in this by the ligature on the vessels. As soon as the surface layer had cooled, the ligature was

divided and fresh paraffin poured on to the depth of a centimetre or more. In this way the organ was kept in the centre of the wax mass, and all possible channels from the exterior blocked.

(*b.*) The parallel series for comparing with the paraffin method was conducted as follows : closely-fitting stoppered wide-mouthed bottles (which if not new were thoroughly cleaned) were washed in 1 in 20 carbolic acid solution, and finally filled with the same solution. At the commencement of each experiment these bottles were emptied (in the spray) and the bottle then inverted in the bath of 1 in 20 carbolic acid solution. When the organ was removed it was rapidly placed in the bottle thus drained of carbolic acid, the stopper fitted and then sealed with boiling bottle wax, the whole operation being conducted under the spray. The vastus internus of the left side was then removed and treated in the same way.

Of each series two preparations of the same tissue were made. Of these one was placed in an incubator at 37·5° C., while the other was kept in a cupboard, the temperature of which rose from 8° C. in December to 20° in July.

Blood was obtained in the following way :—Pipettes of 3 or 4 cc. capacity were heated to redness throughout, and, being sealed while red-hot, a partial vacuum was obtained. The upper end of the vena cava being secured by a clip, the wall of the vein was raised and slightly scratched with the knife : through the wall thus weakened the pipette was easily thrust, and the point being broken off within the vein, the partial vacuum was soon filled with blood, the pipette was then drawn out and sealed in the spray. By this means blood only (Tiegel used the ligatured heart) was obtained without the least admixture with carbolic acid.

Objection has been urged by Koch (8) against the paraffin method on the ground that as the wax cools, minute fissures appear in it and thus afford a possible entry to bacteria. This objection was proved to be merely theoretical from the following facts. In control experiments small organs were chosen and immersed in a large mass of paraffin heated to 150° C. When examined after incubation, the organs presented an opaque appearance ; the tissue was evidently cooked through, and showed no trace of putrefaction or presence of bacteria. Moreover, in the paraffin preparations that were placed in the incubator, the putrefactive gases forced up the paraffin for a short distance, thus converting the cupped, cooled surface into a dome. The possibility of bacteria finding an entrance where gases could not effect an exit will scarcely be maintained.

We have made 64 experiments, using 21 cats (of which five were pregnant), four rabbits and one dog. In 12 instances, among the earlier

experiments, we used other organs besides the kidney, viz., liver, spleen, and heart, obtaining similar results but keeping the main question in view, and not the localization of bacteria in the body, We chose the kidney as a typical organ in being the furthest removed from bacteria as regards the circulation, and the vastus internus was invariably employed for muscle.

The importance of noting the influence of temperature as a factor in the development of organisms induced us to spread the experiments over a term of seven months. We have mentioned that two preparations were made in each experiment, and of these one was incubated and the other kept in a cool cupboard.

The rapidity of development of organisms in tissue which was not incubated increased with the rise in temperature of the atmosphere, while in every case, with one exception (noted below), tissues subjected to incubation rapidly developed organisms.

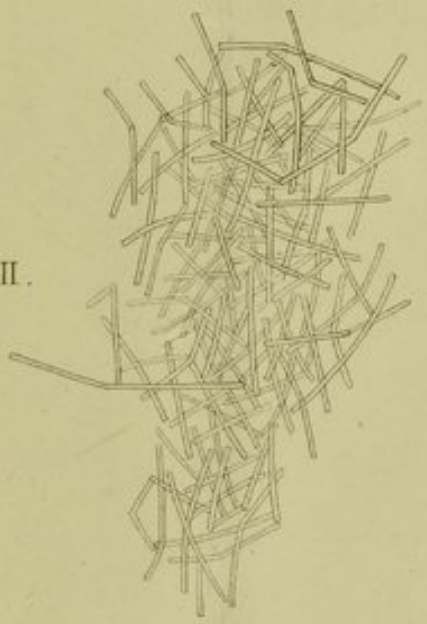
As regards the nature of the organisms formed, and their relations to the two different tissues, our results agree exactly with those of Tiegel, Burdon-Sanderson, Béchamp, and Nencki and Giacosa. Thus, coccus forms preceded rod forms, and further, while rods were found as the rule in the kidney, they were more rarely found in muscle, and never in the blood. But a new point of interest was soon elucidated, viz., that the organisms differed widely in size and shape. Among the animals which were the subject of experiment this individual difference, however, was not extended into a generic difference; the rods found in the rabbits did not differ less among themselves than the rodents' bacteria differed from those found in the cats.

We do not propose, in the present unsatisfactory condition of the literature of the subject, to give more than an outline of the various forms observed.

Two kinds of coccus (Fig. 2, Pl. XI.) were observed, and would correspond to Billroth's micrococcus and megacoccus. All degrees and sizes of rods were found, the largest measuring $\frac{1}{600}$ in. in length, and the smallest not more than $\frac{1}{8000}$ in. in length. When jointed, the segments had no constant length in the same preparation; and in only 3 per cent. of the cases were rods seen in active motion. Multiplication by fission was evidently the rule, but in a few preparations we found series of forms tending to confirm Ewart's (10) description of the development of rods from spores. Further, the relation of putridity to the development of organisms was found to be of uncertain

I.  × 400.

II.  × 700.

III.  × 400.

IV.  × 700.

V.  × 400.

VI.  × 750.

F. Huth, Lith. Edin.

nature ; thus the presence of bacteria did not necessarily determine the existence of foul gases, though the organ possessed a peculiar odour. In a few instances, especially in the case of muscle, this concurred with the existence of rod forms.

The naked-eye changes that the organs underwent were very instructive. The shining capsule or fascia soon lost its lustre, the yellow fat assuming a brownish tinge ; later on, the small quantity of blood collected at the bottom of the bottle had broken down into a grayish-brown, grumous-looking fluid. The organ itself subsequently became swollen and black, then gradually shrank, at the same time assuming a gray colour ; and finally, if kept a sufficient length of time, it was converted into a yellowish caseous mass.

The one exceptional instance in which no indubitable bacteria were found was that of a young cat. In the tissues examined were found bright particles which we did not feel justified in describing by a specific name, but which may have been a spore condition of some form or other.

The idea of using fœtuses as a means of carrying the test of the question to its furthest limit occurred to us early in the series, and we were able to make five experiments on this point. In every case bacteria appeared in the fœtal tissues, and in every case the organisms were identical with those found in the maternal tissues.

As examples of the results obtained in following the above-described methods, one or two details may not be out of place. Thus, in the paraffin series.

Exp. 11. June 16, 1880.—Animal used, kitten. The organ (kidney) of either side was treated in the same way, viz., placed in the hot paraffin, then, while one preparation was incubated at 37·5° C., the other was put into a cupboard, the temperature of which was approaching 20° C.

Result :

Incubated.	Not Incubated.
Tissue broken down, soft and pulpy. Fluid swarming with short rods (*bacterium termo* of authors) and micrococci.	Putrid, showing numbers of rods varying from $\frac{1}{4000}$in. to $\frac{1}{500}$in. in length, some of them apparently dividing transversely.

Exp. 12. June 16, 1880.—A control experiment.—Animal used, a kitten. The organ chosen was the liver, each half of which was placed in paraffin at 150° C. On examination the tissue in both incubated and

non-incubated preparations was found to be pale and dry throughout, the organ being purposely chosen small it was completely cooked, with, of course, as a result, the total absence of putrefactive changes and development of organisms.

The following experiment from the bottle series will show the changes that proceeded in the three principal tissues from the same animal.

Exp. 9. May 26, 1880.—Animal, adult cat.

	Incubated.	Not Incubated.
Tissue. Kidney.	Organ very fetid and swarming with rods varying in length from $\frac{1}{3000}$ in. to $\frac{1}{2000}$ in.	Although containing innumerable rods (narrow) this preparation had no unpleasant odour. The rods varied in length from $\frac{1}{8000}$ in. to $\frac{1}{2000}$ in., and the diameter was $\frac{1}{20000}$ in.
	Incubated.	**Not Incubated.**
Muscle.	Soft and rotten. Numerous rods similar to those in the kidney (incubated preparation).	No unpleasant smell. Numerous small rods (*bacterium termo*).
Blood.	Clear and laky, otherwise unaltered.	

This experiment shows very clearly the fact noted further on that the existence of organisms is not necessarily accompanied by the development of fetid gases, and as the above and other experiments go to show, this latter change is apparently determined by the elevation of temperature.

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EXPLANATION OF PLATE XI.

Fig. 1. Rabbit.—Narrow rods of variable length, from the kidney. × 400 diameters.

Fig. 2. Rabbit.—Two forms of coccus found in the heart, probably Billroth's meso and micrococcus. × 750 diameters.

Fig. 3. Cat.—Large group of rods from the kidney. × 400 diameters.

Fig. 4. Cat.—From a preparation of muscle (incubated) showing all the stages, apparently, of spore-development into rod forms.

Fig. 5. Fœtus (cat).—Chains of micrococci from the liver. × 400 diameters.

Fig. 6. Fœtus (cat).—Rod forms from the liver. × 750 diameters.

The objects were drawn at a distance of 9 in. from the eye, and the systems employed were, as a rule, Hartnack's Oc. No. 3, and Objs. Nos. 8 and 10 min.

In a paper published a year ago in the *Deutsche Zeitschrift f. Chirurgie* (Vol. XIII.) under the title of "Klinische Studien u. Erfahrungen aus der Chirurg. Klinik in Göttingen," the contents of which have become known to us since the above paper was in print, the author states that Prof. Meissner has recently made "a very complete and perhaps conclusive series of experiments," of which the leading results are as follows: Various organs of cats and rabbits—such as whole kidneys, spleens, pancreas, and bits of liver—taken from animals just killed, have been successfully kept, without septic change and without the use of any antiseptic, sometimes immersed in water, sometimes exposed *for two or three years* in vessels of which the air was free from dust. In the preparation of these organs nothing was done which could have the effect of killing any germs which might have previously existed in them. The only precautions used related to the purity of the water, of the glass vessels, and of the instruments used. The vessels were first plugged with cotton wool and then heated to 160° C. The water was distilled into them from a glass apparatus specially constructed with a view to the exclusion of dust. The instruments were kept in alcohol and drawn through the flame immediately before use. The operation was done in a room as free from dust as possible, without spray, and without more assistance than was absolutely indispensable. Farther details are not given. It appears that Prof. Meissner's experiments, which were communicated to the *Medicinisch-Naturwissenschaftliche Gesellschaft* at Göttingen, have not been published in full. Dr. Rosenbach speaks of his own short notice as a "Vorläufige Veröffentlichung," made by him with Prof. Meissner's permission, and so far as we have been able to ascertain nothing has appeared since. Without further information, no judgment can be formed as to the value of the results, but what has been stated is sufficient to indicate that the question is as yet undecided. It is worthy of note that all the experiments showed that the development of organisms was dependent on temperature.