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ON THE GROWTH OF BACTERIA IN THE
INTESTINE.*

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It has for some time been well known that if cultivations be made of the bacterial contents present in the intestine in different parts of its course, the colonies will be found to differ in number and character according to the part investigated. Generally speaking, bacteria are much more abundant in the large than in the small intestine. Giæxa was one of the first to point out this difference as it is to be observed in guinea-pigs.

The power of regulating bacterial growth possessed by the intestine is further revealed by observations on the kind of bacteria present. The universal presence of bacilli belonging to the coli communis and proteus groups points to the fact that the conditions maintained in the normal intestine permit the survival of these bacteria, while numbers of other species ingested with the food are more or less destroyed. This regulation of bacterial growth is disturbed in a number of diseases; but especially in those originating primarily in the intestine. In investigating the etiology of such diseases, the question will naturally arise how far the morbid processes are due to the growth of the ordinary bacteria after the normal power of control has been impaired, and how far there is involved the growth of a microbe introduced from a special source and possessing the character of a specific irritant. The investigation must further include a study of the nature of the controlling mechanism and the conditions under which its activity becomes impaired.

These problems are presented in the pathology of typhoid fever, and on this account we decided to repeat the observations on the normal intestine in order to define more clearly in some respects our knowledge of the regulating mechanism.

The method we employed was to make direct observations on the number of bacteria in the contents of the intestine at short intervals in its course. The intestine was removed immediately after death and stretched out on a table. At the points selected for examination the peritoneal surface was sterilized by a hot iron, and an incision was made with a sterilized knife. From the contents which flowed from the incision a platinum wire loopful was taken and transferred to melted agar at 40° C, and this was poured out in a Petri's box. After incubation for twenty-four hours at 37° C., the colonies were counted, and in a number of cases planted out for further study. In each case a liberal loopful was taken, and when the plate was made fragments of intestinal contents might be seen scattered through the agar. The animals used were chiefly dogs and rabbits, but a few observations on other animals are included. Anaërobic plates also were made in a number of cases, and incubated in a Bulloch's anaërobic chamber. We have not given separate curves for these observations as the results conformed closely to those obtained by aerobic plates.

The numbers of colonies in the plates are not to be regarded as an exact measure of the number of bacteria present. As regards the faeces it has been shown that the number of colonies which develop in an ordinary plate cultivation is far short of the number of bacteria present. The same principle applies to the contents of the small intestine. The method is, however, applicable to the species of the B. coli and B. proteus which occur universally in the intestine. The members of

these groups flourish in the anaërobic conditions presented by the contents of the intestine, and they are very easily cultivated in artificial media also. The variations which are found in the growth of bacteria which can be cultivated may therefore be taken as a reliable index of bacterial growth as a whole in the intestinal contents. The average of the results obtained by enumeration we have expressed by means of curves.

Observations on Dogs.

The dogs had been kept on biscuit, but had had no food for twenty-four hours before our observations were made. It is obvious that to a certain extent the number of bacteria in the intestine must be dependent on the number ingested with the food.¹ In the condition of hunger, therefore, we may expect the evidence of inhibitory effect to be more clearly seen than immediately after food has been taken.

The number of bacteria which grew on an agar plate inoculated with a loopful of intestinal contents taken at regular intervals.

- (1) Continuous line = average results of ten dogs with healthy intestine.
- (2) Dotted line = average result of six dogs infected with intestinal worms.
- (3) Interrupted line = case of dog with normal intestine, showing very few bacteria throughout.
- (4) Wavy line = case of dog in which the intestinal wall was congested.

The first curve is constructed from observations on a series of dogs in which the intestinal canal was normal, as far as could be determined by the naked eye. The material used for making plates was the fluid or semi-solid contents of the intestine. When this was deficient the surface of the mucous membrane was scraped, and a portion of the scraping was planted.

The first point which follows from the examination of this curve is that the growth of bacteria in the small intestine is very limited in comparison with the amount of nutrient material present. In general the growth of bacteria is small till the region of the lower ileum is reached. Here the number of bacteria is greatly increased, and below the ileo-caecal junction they are found in the numbers which are characteristic of the faeces. A clear distinction is therefore to be drawn between the small and the large intestine. Inhibition of bacterial growth occurs in the stomach, and there the bactericidal effects are generally supposed to be associated chiefly with the presence of free hydrochloric acid. In the intestine, however, in spite of the loss of acidity from the addition of alkaline secretions, there still remains the power of inhibiting bacterial growth.

It is hardly conceivable, therefore, that the acidity of the intestinal contents has much to do with the regulation of

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bacterial growth. The contents of the large intestine are acid in reaction more frequently than those of any other part of the alimentary canal, except the stomach, and yet here we find invariably the most abundant bacterial growth.

The fact that the restraining power is diminished in the region of the lower ileum in normal animals has a direct bearing on the incidence of disease in the neighbourhood of the ileo-caecal junction. It is at this point that a disturbance of the regulation of bacterial growth would naturally show its first effects.

We had the opportunity of comparing with these results, observations on a series of intestines which were infested by worms. The irritation caused by the presence of parasites gives rise to an increase in the absolute number of bacteria throughout the intestine; but the inhibitory effect is not yet wholly lost. The relatively sterile area is reduced. The increase in the number of bacteria takes place in these cases some distance higher up than in the normal intestine.

In another case (Curve 3) there was, for some undiscoverable reason, an inflammatory condition of the mucous membrane of the intestine. The number of bacteria in this case was much increased throughout, and there was distinctly less difference in the different areas. The sterilizing or inhibitory power of the secretion had been reduced by the inflammation.

For comparison with this case we have given also the curve of bacterial growth from a dog in which the bacteria were extremely few. Here the numbers were almost nil until the large intestine was reached, when a plentiful growth was obtained.

These results show that non-specific irritation may set up abundant bacterial growth in the intestine in areas in which it is normally present to only a limited extent.

A point of interest in relation to the effects of parasites may also be mentioned here. The fact that their presence is accompanied by an increase in bacterial growth throws light on the constitutional effects to which they often give rise in patients, and in particular may possibly afford an explanation of the haemolytic effects associated with bothriocephalus latus and ankylostoma duodenale.

The bacterial growth in the large intestine in the normal animal is as a rule plentiful. Sucksdorf has pointed out² that in men fed on sterile food the number of bacteria in the faeces averages 10.395 per m.g. The absorption of fluid which converts the contents of the intestine into solid masses at the same time probably restrains the activity of bacterial growth. In certain animals where desiccation of the contents is very complete—as in guinea-pigs—the relatively sterile condition of the small intestine still prevails in the large.

Observations on Rabbits.

The rabbit's intestine represents that of the herbivorous animal. Observations on the secretion of bile have shown that the amount secreted is relatively enormously greater in the case of the rabbit than in the dog. The germicidal power of the bile, as we will see, is an important factor in the regulation of bacterial growth in the intestine, and the figures in these tables, when compared with those for dogs, show that the contents of the rabbit's intestine are in general more nearly sterile than those of the dog.

The effect of hunger in these rabbits was not observed, as the animals were allowed to eat their food as usual. Generally speaking, we have the same results as we find in the case of dogs, and to express the difference in growth at the different areas the curve is similar.

The number of sterile plates is remarkable. The effect of desiccation of the contents in the large intestine is seen in the case of some of the rabbits, and sterile plates were occasionally obtained even with the contents of the large intestine. One or two cases require special mention.

In one case (Curve 2) there were two parasites, small tapeworms, in the intestine, one being in the small intestine near the middle, the other at the lower end. Round the site of the tapeworm in the small intestine there was increased growth of bacteria, but above and below this area there was the ordinary condition of relative sterility. This seems to show that the inhibitory effect is due to the secretion of the intestinal glands, and not to the action of the acid juices of the stomach or the bile, since the effect is reduced by the presence of the worms, but is re-established at some distance

lower down. The tapeworms in this case were only about 3 in. long.

Another case (Curve 3) of a similar kind showed in two of the segments a number of small white worms. The plates from the contents of these segments exhibited a copious growth of colonies, while above and below this area the contents presented the ordinary degree of sterility.

- (1) Continuous line = the average result from the examination of rabbits.
- (2) Dotted line = rabbit with tapeworms in the intestine. Site of worms marked T. W.
- (3) Interrupted line = rabbit with a number of small white worms in the lower half of small intestine segments 7 and 8.

- The intestine having been divided into segments numbered from above downwards, this diagram expresses the percentage of cases in which a loopful of contents was sterile.
- (1) Continuous line = percentage of sterile plates from eighteen healthy rabbits.
 - (2) Dotted line = percentage of plates which contained less than five bacteria from eighteen healthy rabbits.

The small intestine having been divided into ten segments numbered from above downwards, this diagram shows the percentage of cases in which a loopful of contents was sterile.

- (1) Continuous line = percentage of sterile plates from ten healthy dogs.
- (2) Dotted line = percentage of sterile plates from six dogs infected with worms.

A third case of interest was one in which the rabbit had been inoculated by a fairly virulent species of *B. coli* communis obtained from the spleen of a typhoid patient. The bacillus was found in the bile and in the gall bladder. All the plates inoculated with intestinal contents contained innumerable colonies. This observation throws light on the significance of finding *B. coli* communis and *B. typhi* in the gall

bladder in disease. In these morbid conditions the bile, as well as other secretions of the intestinal glands, loses its normal inhibitory power.

In connexion with this case we made a number of observations on the effect of bile on the growth of *B. coli communis* and *B. typhi*. We found that when gelatine is simply smeared with bile, and subsequently inoculated with *B. coli communis* and *B. typhi*, there is no distinct inhibition of the growth; but when bile is mixed with gelatine so as to form a considerable proportion of the medium, say 50 per cent., no growth occurs. This subject has, however, been worked out in reference to the bile salts in a recent investigation by McConkey. Clearly, however, the action of the bile is only one of the factors in the process. The examination of the rabbits with parasites indicates that the secretion of the intestine itself in any given segment controls the growth in that area.

A number of similar observations on the mucous contents of a relatively sterile part of the intestine gave grounds for believing that they are possessed of an inhibitory power even stronger than that of bile alone. We have not, however, made observations where we have had to deal with the action of intestinal secretions free from admixture with bile.

The experiments on guinea-pigs give the same results as those on the rabbits and do not call for remark.

We had the opportunity of studying an example of the human intestine in a condition favourable to the purpose of the research. We have examined a number of cases in the *post-mortem* rooms where the patient has been dead for twenty-four hours or more; but these are of little or no value for our immediate purpose for two reasons: (1) The patient is abnormal; and (2) the time which elapses after death allows the growth of bacteria to occur to such an extent as to make observations useless. One suitable case which came under examination was that of a cyclist who had been killed in a street accident. The plates made a few hours after death showed that in this healthy human intestine the conditions of bacterial growth were the same as those in the intestine of the dog or rabbit.

REMARKS.

The results of the investigation just recorded are of interest because of their bearing on the pathology of a number of diseases. It is clear, as we have shown, that one of the first places in which a disturbance of the regulation of bacterial growth takes place is the region of the ileo-caecal junction. The power of inhibiting bacterial growth having become impaired, the bacteria which occur normally in the large intestine will become still more abundant, and approximately similar changes will take place in the small intestine. In the region of the ileo-caecal valve the change will occur to a greater extent than in the higher portions of the small intestine. This conclusion follows from a study of the contraction of the relatively sterile area in the small intestine seen in cases of dogs infected with parasites.

In the human subject the region of the ileo-caecal valve is very liable to attacks of inflammation in the shape of appendicitis and typhlitis in their various forms, and we are probably near the truth in finding in these diseases an illustration of the mode of bacterial increase which we have just described. Further illustrations of the same principle present themselves in the incidence of the lesions of typhoid fever and tuberculosis of the intestine. It is in the lower end of the small intestine that there occur the earliest and gravest of the structural changes associated with these diseases. We may therefore surmise that bacterial invasion of a specific kind follows in these cases the same law as the simple disturbance of regulation due to such non-specific causes as we have investigated. Bacteria possessing specific pathogenic power if taken by the mouth will, like other bacteria so ingested, be restrained from multiplying until the lower ileum has been reached. In other words, the various forms of bacterial irritation of the intestine conform more or less to a type the determining factor of which is the normal power of inhibition of bacterial growth in the small intestine, and in a study of this and the manner in which it is impaired, we obtain important light on the occurrence of these diseases, and the tendency which they show to localize themselves chiefly in the lower ileum.

A large number of observations have been made on the

bacteria which, though harmless inhabitants of the normal intestine, increase, especially in the small intestine, in various morbid conditions. The principal members of the group are *B. coli*, *B. proteus*, various micrococci, and the bacillus enteritidis sporogenes of Klein. When the regulation of bacterial growth is disturbed, large numbers of these microbes are found in the small intestine, and they become endowed with increased virulence. In many forms of catarrh of the intestine this increased vigour of bacterial growth is the sole change that can be discovered. The attempt to find a microbe characteristic of such conditions has failed.³ Again, in typhoid fever, along with the growth of the *B. typhi* there occurs an increase in the small intestine of *B. coli* and other bacteria normally present there. The bacterial change, therefore, which occurs in simple catarrh of the intestine, has its place in this disease along with the specific bacterial effects due to the growth of the typhoid bacillus.

It was, as we have already remarked, to enable us to appreciate this part of the pathology of typhoid fever that the present research was undertaken. In this JOURNAL, in 1899, we published a paper giving the result of an examination of the serum reactions on a series of typhoid patients. In this we showed that a very considerable percentage of the patients gave, not only Widal's reaction, but also more or less pronounced reactions with varieties of *B. coli communis*. We thus confirmed the observations of Stern⁴ published in 1898, and subsequent investigators have added largely to that confirmation. The interest of this research is that in the *B. coli* reaction we have a means of obtaining direct proof of the part which *B. coli* plays in certain cases of typhoid fever. Long before the evidence of the serum reaction was brought forward it was held by several pathologists that *B. coli* had a share in the causation of typhoid fever. Various observations on the presence of *B. coli* in the spleen and other organs of typhoid patients, and Sanarelli's experiments on the influence of coli toxins in raising the virulence of *B. typhi* had given ground for this conclusion. The study of the regulation of bacterial growth in the intestine which we have just detailed shows, it seems to us, the mode in which normal bacterial processes in the intestine may increase and thereby contribute to a disease without invading the tissues or the blood.

To study this question from another point, we carried out a number of inoculations with races of *B. coli*, in order to compare the reactions developing in consequence in the animal's (rabbit's) blood with those of the blood of a patient in typhoid fever. This method failed, however, to throw much light on the problem we had before us, but this was probably due to the fact that various races of *B. coli* differ very much in their reaction to agglutinating coli serums. It often happens that even when a race which does not easily agglutinate is used for inoculation, the resulting serum will give a reaction with other races of *B. coli* at a dilution much higher than the maximum dilution which will agglutinate the inoculating race. It is consequently impossible to use the serum reaction of the coli group of bacilli in the same definite way as may be permitted with the typhoid group, in which the tendency to agglutinate is much more uniform. There is accordingly an element of uncertainty in interpreting the meaning of coli reaction in a given instance.

Further, in carrying out these inoculations, we tested the rabbit's blood with races of *B. typhi*, and in confirmation of Sternberg's results,⁵ we found that the serum occasionally had acquired the power of agglutinating *B. typhi* at a dilution at which it had no effect on the *B. coli* used to inoculate. We do not consider we have yet the data to enable us to interpret this result.

REFERENCES.

- ¹ Sucksdorf, *Arch. f. Hyg.*, 1886. ² *Ibid.* ³ Gordon, *Epidemic Diarrhoea, Practitioner*, August, 1902. ⁴ *Centralbl. f. Bakt.*, Band xxiii, S. 673. ⁵ *Zeit. f. Hyg.*, Band xxxiv.

At the meeting of the Royal Meteorological Society held on December 17th, Mr. H. W. Dines, President, in the chair, M. C. A. Angot of Paris, and Professor W. L. Moore of Washington, U.S.A., were elected honorary members. A paper on the Climate of Cyprus, by Mr. W. C. O. Bellamy, M.I.C.E., was read, and one on the Eclipse Cyclone of 1900, by Mr. H. H. Clayton, of the Blue Hill Observatory, U.S.A.