

Therefore the fetish-stone, as a sort of spiritual sponge, was introduced.

And I would here enter a plea for the primitive medicine man. He is not usually the arrant knave or juggler so frequently pictured by travellers. His so-called 'tricks' are not attempts at deception. They are solemn operations by which he is himself as much deceived as are any of his witnesses. We are told that these earliest practitioners suck, knead or cut their patients, and end by pretending to find and extract, and by triumphantly holding aloft, some grub, insect or other small object—frequently a minute fetish-stone like the one I have described, that 'they claim' to have actually extracted from the diseased part. We aliens are the only ones of their witnesses who are deceived by them in the way we accuse them of deceiving, for what they really attempt to do is either to expose, or otherwise make as uncomfortable as possible, the animate seat of the disease, and then to furnish it with a decoy, as it were, a vehicle or body of escape, as a killed and squeezed-out body of one of its own kind, or else in the form of its kind as seen in some ancient and more potent and nearly natural object resembling it. Sometimes, again, living insects or worms, or fetishes that are supposed to be living, ravenous and inimical to the worms of disease, are introduced, that they may prey upon and destroy these worms and the seed-substance of their kind. This is especially apt to be the case when thick pus is abundant and parasites are forming; for the squeezed-out pus itself resembles worms more or less, portions of it even in mass, being streaked, seeming to contain their forms in embryo. It, also, is therefore held to be the seed-plasm or substance of worms, and the proof of this is alleged to lie in the fact that, if exposed, like dead flesh, it speedily turns to worms.

The subsequent treatment received by

the man whose case I have described, at the hands of his primitive doctors, was quite as much in keeping with this sort of philosophy as had been their operation. His wound was, of course, dressed, cleaned, copiously sprayed, and, I may add, 'Spiritually disinfected,' every day. But, in addition to this, he was put on diet—the freshest or 'newest' possible corn food—and was, for the first four days, deprived of salt (this, too, being abundant in pus-like excreta) and all flesh-food, and was thereafter until perfectly cured—for he recovered with amazing rapidity—denied all meat containing fat and other non-muscular tissue, since these, as well as old and so-to-say 'decrepit' seeds, are supposed to be, of themselves, peculiarly liable to 'worm-turning.'

FRANK HAMILTON CUSHING.

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*THE INFLUENCE OF ENVIRONMENT UPON  
THE BIOLOGICAL PROCESSES OF THE  
VARIOUS MEMBERS OF THE COLON  
GROUP OF BACILLI: AN EXPERI-  
MENTAL STUDY.*

THE results found in the following pages have been made possible by a grant from the Bache fund of the Smithsonian Institution. The disposal of the grant mentioned is left to the discretion of Dr. John S. Billings and Dr. S. Weir Mitchell. The topic of this research was submitted to these gentlemen at the beginning of the year, and from time to time they have been kept informed of the progress of the work. It is with their approval that this paper is presented for publication. The research of the past year has been a continuation of the studies begun in the fall of 1895, upon the variability of bacteria.

The colon group of bacteria have been chosen for this study, and particular attention has been paid to those forms which appear to be modifications of the typical colon

bacillus which have undergone functional derangement as a consequence of their environment. A short résumé of our experimental work is given in this paper, but we fear that it is insufficient in regard to the details of experiments and the results obtained to maintain our conclusions.

From the observations and deductions of several competent investigators, the pathogenesis of the colon bacillus is due to cultivation in the living fluids of the animal body, which are supplied with an unusual amount of proteid substance by the process of inflammation. We have made experiments with the members of the colon group to ascertain to what extent the proteolytic activity can be increased by growth upon an artificial medium containing large amounts of proteid material. The medium used for our experiment was a bouillon free from sugar and containing a large per cent. of fresh peptones derived from beef muscle by the action of trypsin. These peptones, according to Péré, are capable of forming more indol than those produced by the action of pepsin or any other enzyme.

Series of cultures comprising, severally, the typical colon bacillus, transitional forms of the colon group, and the typical typhoid bacillus, have been carried through a series of cultures in this medium, each culture being changed to fresh media every seventy-two hours, and the old culture tested for indol for the purpose of obtaining an approximate estimation of the proteid digestion.

The result of these experiments was that under these conditions the proteolytic function is increased in activity, so that cultures of the typical colon bacillus gave, in the second generation, after growth for three days in 25 c. cm. of the medium, an indol reaction of a blood-red color in some instances, and requiring in every culture from 10 to 15 c. cm. of a 0.1 per cent. indol solution to give the same depth of color to 25 c. cm. of the sterile medium. Transitional forms of the

colon group which have hitherto been supposed to be devoid of the indol-producing power, after cultivation for three or four generations in this medium gave a marked indol reaction. Seventeen different specimens of the typhoid bacillus, obtained by us from some of the best known laboratories in this country, gave also a marked indol reaction after passing through a series of changes in this medium.

*Result of excess of one function.* The result of the excessive discharge of one function, viz., proteolysis, which has been produced by growth in the fresh peptone bouillon free from sugar, is, first, an increase of the proteolytic activity as shown by the increased amount of indol produced in typical colon cultures, and the production of indol by organisms supposed to be devoid of this property: viz., atypical forms of the colon bacillus, and the typical typhoid bacillus; secondly, after the maximum of indol production is reached, a decrease in proteolytic activity occurs, shown by the lessened amount of indol, and, finally, after several generations, the attenuation and premature death of the organism takes place.

*Indol production as an index of pathogenesis.* An experiment relating to this question has been carried on for over a year with a virulent culture of the colon bacillus. The culture was tested for indol and virulence as soon as it was isolated. The indol reaction was very marked, and 0.5 c. cm. of a bouillon culture proved invariably fatal to guinea pigs of 500 grammes weight. It was then kept for five months on agar-agar with but few changes, when it was again tested. A faint indol reaction was obtained, and 1 to 2 c. cm. of a bouillon culture was required to kill an animal of 300 to 500 grammes weight. This culture was then placed in the alkali-peptone solution and changed to fresh medium twice in seven days, when tests were again made. The color of the indol reaction was very intense, and 0.3 c. cm. of an

alkali-peptone bouillon culture about twenty-four hours old killed animals weighing from 200 to 300 grammes. This experiment was repeated with the same result, viz., the indol production and pathogenesis were both increased by growth in a medium containing an unusual amount of proteid material so prepared as to be especially suitable for bacterial assimilation. The amount of indol produced by this culture was not greater, however, than is found in other cultures of the colon bacillus which are non-pathogenic. The estimation of the virulence of this organism by the amount of indol formed seems for this reason impracticable.

*Cultures from typhoid spleens.* We have found by our experiments that the colon bacillus grown in an excess of proteid material in an artificial medium undergoes a disturbance of equilibrium in function, with first an increase of proteolytic activity and later an apparent suppression. Cultures obtained from the pathological tissues of man and animals also present characteristics differing from those of the typical colon bacillus obtained from the healthy colon. For the purpose of demonstrating this modifying effect upon cultures of bacteria belonging to the group under discussion, we have obtained cultures from organs that have been undergoing inflammation for a considerable period of time, viz., typhoid spleens, and have noted their reactions both upon carbohydrate and proteid foods. We have tried to classify these cultures according to these reactions, and with the following results: Cultures corresponding to the typical colon bacillus in reaction have not been found among the cultures studied. A group of cultures were obtained which gave one or more of the prominent reactions of the colon bacillus, and appeared to belong to the transitional series of the colon group. A second group gave the characteristic modifications in function that are found in attenuated cultures of the colon group. A

third and larger group gave the reactions of the typhoid bacillus. May not the cultures of the first and second group be colon cultures which have been modified in function by growth in an excess of proteid food?

Our final test for the differentiation of our cultures obtained from typhoid spleens is the serum test, to which the investigations of Widal have recently drawn so much attention that it has become a well-known and popular diagnostic procedure in typhoid fever. We have reversed this test, and have used a specimen of blood which was obtained from a genuine case of typhoid fever, and which had given a characteristic reaction with the typhoid culture used by us for the diagnosis of typhoid fever by the so-called method of Widal for testing our cultures.

The result of this test showed that only about one-third of our typhoid cultures gave what is regarded by us as a positive reaction, although every culture except one showed a distinct agglutination of the bacilli and some degree of cessation of motility. A comparison of the reactions given by the typhoid cultures with those of our cultures from typhoid spleens shows that of 28 cultures derived from typhoid spleens, and giving all the cultural reactions of typhoid bacilli, 18 gave a positive reaction, while 10 did not fulfill the requirements of this reaction in regard to cessation of motion. Two cultures classified as transitional colon cultures gave as typical a reaction as any of the cultures tested.

Our tests upon the series of typical and atypical colon bacilli show that in one culture of each series a reaction was obtained which seemed as striking as any reaction obtained by us with typhoid cultures. Several of the other cultures differed from the control drop by presenting indications of a reaction.

#### CONCLUSIONS.

Assuming the typical colon bacillus and

the typical typhoid bacillus to represent the types of this group that present the greatest divergences in biological peculiarities, we conclude, as others also have done, that there is a series of closely related forms that may be regarded as intermediate or transitional, and which serve to establish a biological relationship, either near or remote, between these two typical members.

From our own studies we are inclined to regard the typical colon bacillus as the type of this group, for the reason that its functional equilibrium, as observed in the intestine, is so permanent a quality that it may readily be perpetuated under what is ordinarily regarded as favorable artificial circumstances, and that with the continuance of such conditions there is no conspicuous tendency on the part of this organism to deviate from what we regard as its norm; whereas, on the other hand, with all the other members of the group with which we have worked there is not only a lack of uniformity in the adjustment of the functions, but such as exists is readily disturbed under artificial environment, though it must be borne in mind that even with the typical colon bacillus we have also shown functional modifications to be possible under particular conditions.

That when the members of the colon group are cultivated under circumstances favorable to the development of both the function of fermentation and that of proteolysis, fermentation invariably takes precedence and no evidence of proteolysis is manifested until after fermentation has ceased.

That the cultivation of all the members of the colon group under circumstances that favor the development of one function, viz., that of proteolysis, at the expense of another, viz., that of fermentation, results first in apparent increase of vigor; but this is of temporary duration and is quickly followed by the decline and death of the cell.

The result of this increased activity of

the proteolytic function is the formation of much larger amounts of indol by typical colon cultures than has ever been obtained by us by any other method.

By the method of experimentation through which we were enabled to accentuate the proteolytic activity of the typical colon bacillus, as caused by an increase of indol formation, we have also induced the function of indol formation not only in atypical colon bacilli that have been devoid of it but in every specimen of typical typhoid bacilli to which we had access as well.

We feel justified in regarding one of the differential tests between the typhoid and colon bacillus, notably that of indol formation on the part of the latter and the absence of this function from the former, as of questionable value, for the reason, as shown above, that by particular methods of cultivation indol production has been shown to accompany the development of a number of specimens that we have every reason to regard as genuine typhoid bacilli.

As a result of our own experiments, together with the observations of others, there can be no doubt that the bacillus *coli communis* at times possesses pathogenic properties, and that by artificial methods on treatment it may often be brought from a condition of benignity to one of virulence,

The spleen of a typhoid patient has always been regarded as the only trustworthy source from which to obtain the typical typhoid bacillus. While we believe this to be true, still our investigations show that other members of the colon group may also be present in this viscus; in fact, from such spleens we have isolated practically all of the varieties of this group with which we are acquainted.

From our experience the value of the serum test for the differentiation of typhoid and colon bacilli would seem to be questionable. We are inclined, however, to attribute the irregularities recorded above as

due more to the method of application than to defects of the principles involved; for, as stated, by the use of dried blood, as in our experiments, it is not possible to make the test with constant and accurate, or even approximately accurate, dilutions of the serum. Our irregularities may be in part due to this defect. We therefore lay less stress upon this than upon the other features of our work.

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#### THE VIRGINIA COLONY OF *HELIX NEMORALIS*.

THE now well-known colony of the European *Helix nemoralis* at Lexington, Va., has twice before been the subject of articles by the present writer. (*Nautilus*, November, 1889, and December, 1894.) It was shown in these articles that many new variations, different from those observed in Europe, had occurred; and the colony consequently became of great interest to students of evolution. Unfortunately, Professor Morrison, who was studying these snails, removed from Lexington in 1890, and, having the misfortune to lose his collection by fire, paid no further attention to the matter. It is only now that I am, through the kindness of Mrs. John M. Brooke, of Lexington, in a position to give some account of the colony subsequent to the date mentioned.

In order that every reader may understand what follows, I will explain the system of band-formulæ used for recording the variations. The typical shell has five bands and the formula is 12345. The absence of a band is indicated by 0, thus 10345. Two or more bands united are bracketed together as (12)345. A rudimentary band is expressed by a small figure below the line, as 1,345. A split band is expressed by doubling the number, as 123345. An extra

band, not assignable to any of the normal five, is expressed by a  $\times$ , as 123 $\times$ 45.

Mrs. Brooke sends me a series of shells gathered by herself at Lexington in 1896 and 1897. They are as follows:

#### A. With a yellow ground-color = *libellula*.

<i>libellula</i> 12345.....	70.
" 123(45).....	21.
" 10345.....	10.
" 1,345.....	9.
" 12045.....	6.
" 00000.....	5 sent, but Mrs. Brooke says they are plentiful.
" 12,45.....	4.
" 1,0345.....	3.
*" 12,3(45) nov. formula..	2, the form- ula shows only near the mouth of the shell.
" 12,345.....	2.
" (123)(45).....	2, juv.
" (12)3(45).....	2, one is juv.
* <i>libellula</i> (12) <sub>2</sub> 3 <sub>34</sub> 45 nov. formula,..	1, the formula shows only near the mouth.
" 12,3(45).....	1.
" 00345.....	1, juv.
" 1,045.....	1.
" 02345.....	1.
" 123 <sub>xx</sub> (45).....	1.
" 00300.....	1.
" 1,0300.....	1.
" 123 <sub>x</sub> (45).....	1, juv.
*" 1(22)045 nov. formula..	1.

#### B. With fawn-colored ground = *petiveria*.

<i>petiveria</i> 12345.....	1. = <i>brissonia</i> , Mo- quin-Tandon.
" 123(45).....	1. = <i>arcelinia</i> Locard.
" (12)3(45).....	1. = <i>brookea</i> , n. n.

#### C. With pink ground = *rubella*.

<i>rubella</i> 00000.....	2. Quite pale. Mrs. Brooke says they are extremely scarce.
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The three forms above marked with an asterisk are new to the Lexington list, and are all new formulæ of the split-band type, like the previously found new variations in the colony. All of the rest were formerly obtained by Professor Morrison.

The examination of the above list brings out the apparent fact that the new split-band variations are now comparatively rare in the colony, though still much more fre-