SOME OBSERVATIONS ON THE STUDY OF THE INTESTINAL BACTERIA.

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The alimentary canal may be regarded from the point of view of bacterial processes within it, as a singularly perfect incubator; an incubator in which there is provided at different levels such a range of reaction and diversity of food that not only are the conditions suitable for the growth of the normal habituated intestinal bacteria but often also for those organisms, capable of developing at body temperature, which are ingested with the food of the host.

An idea of the truly enormous daily bacterial proliferation which takes place in the intestinal tract may be obtained if one remembers that a considerable portion of the fecal mass is made up of the bodies of bacteria, dead and living. At the same time the multiplicity of types and variety of physiological requirements of this intestinal flora are indications of the excellence of the incubator and a strong reminder of the influence which the unrestrained activity of these organisms might conceivably exercise upon the general condition of the host.

The possibilities of bacterial invasion through the intestinal portal of entry have not been overlooked by investigators, and, indeed, among the most brilliant chapters of medicine are those concerning the etiological relationships which have been demonstrated between certain pathogenic bacteria of exogenous origin and specific diseases of the intestinal tract, for example, typhoid, cholera and dysentery.

The very importance of these discoveries has been a potent factor in diverting attention from the studies of the normal intestinal flora with its wealth of problems relating to the principles which govern the activity of these bacteria. Even at the present time the sequence of events which permits the establishment of
these exogenous invaders in the alimentary canal and the exact conditions through which they are able not only to extend and maintain themselves but even to replace wholly or in part the normal flora, are unknown.

It is possible to trace the influence of these epoch-marking studies in the subsequent history and development of Intestinal Bacteriology.

It appears to be a fact that the majority of bacteria of exogenous origin, pathogenic for man (excluding the anaerobes) are relatively inert from the standpoint of chemical activity. On the other hand, these organisms grow in more or less distinctive ways in artificial media, and, usually, they may be recognized by their cultural aspect, their inability to bring about deep-seated changes in their nutrient environment, through specific serum reactions or by their power to initiate characteristic lesions in susceptible animals. In these respects these exogenous organisms contrast in a noteworthy manner with many prominent types of the normal intestinal bacteria.

The more prominent of the latter are distinguished by their chemical or physiological activity and their identification depends far more upon their ability to bring about well-marked chemical changes in their nutrient environment than upon their cultural properties or serum reactions.

The lack of appreciation of this fundamental difference which exists between the relatively inert pathogens and the chemical activity of the more important types of the normal intestinal flora, together with the notoriety that attaches to the former, explains the unprogressive attitude which has characterized many researches on intestinal bacteriology.

While it must be admitted that the purely academic methods of research have resulted in scores of more or less complete morphological and cultural descriptions of bacteria of intestinal origin this knowledge is fragmentary and unclassified. It is devoid of data which would permit one to correlate the presence of these organisms with the diet or condition of the host, or even to form a judgment concerning their numerical relations with other intestinal organisms.

This "bacteriocentric" conception is not illogical when one is dealing with the exogenous pathogens mentioned above, but
it is unproductive of definite results when it is applied in its unmodified form to the study of the normal intestinal flora. It is becoming more and more evident that the problem of intestinal bacteriology must be approached from the dynamical rather than from the cultural standpoint.

Dr. Theobald Smith has stated the case admirably in the following terms: "It is what bacteria do rather than what they are that commands attention, since our interest centers in the host rather than in the parasite."

It is the purpose of this paper (having called attention to the inadequacy of purely academic methods) to indicate in a general way the procedures and use of media through which one may obtain a more comprehensive idea of the significance of bacterial activity in the intestinal tract. For the sake of simplicity, it will be assumed that the host is an experimental animal (preferably a monkey, since its physiology more nearly approaches that of man) under absolute control. Its diet can be regulated at will and its excretions, particularly the urine and feces, can be collected in an uncontaminated state. The diet of this animal may be either purely protein in nature (e.g., hard boiled eggs) or may be carbohydrate. For the latter it has been found that milk with some added dextrose is excellent. This combination contains considerable protein, but, as has been shown in a previous communication, the flora developed is acidophilic and not proteolytic in nature. It should be stated parenthetically that a diet consisting wholly of carbohydrate would be less suited for bacterial development since bacteria need some nitrogen in their food.

The host (monkey) is fed daily with protein or carbohydrate, as outlined above. As this food passes through the alimentary canal from mouth to anus, it is subjected to the action of ferments elaborated by the host. Also, at different levels of the tract it is decomposed in part by various types of bacteria. The predominating types of bacteria which take part in this decomposition are determined largely by the nature of the diet.

When a change is made in the animal's diet from protein to

2 Kendall: This Journal, vi, pp. 257-269, 1909.
carbohydrate, or the reverse, it would seem at first sight that two possibilities exist with respect to the behavior of the bacterial flora towards these alterations in pabulum. First it would appear that the types represented might undergo relatively little change, owing to the fact that they accommodate their metabolism to either form of diet; or, secondly it is conceivable that there might be a shifting of the dominant organisms so that upon the protein diet the proteolytic bacteria will be prominent, while acidophilic bacteria\(^1\) will become dominant as the carbohydrate is increased.

Previous experiments\(^2\) have shown that the latter possibility is the one most commonly realized, namely that there is a parallelism between the nature of the diet and the character of the bacterial types represented in the intestinal and fecal flora. Definite evidences of this activity of the intestinal flora are not wanting. In the excretions (particularly in the urine) there occur substances which are the products of bacterial metabolism. These end products of bacterial digestion may be burned in the body, excreted direct, or combined with some substance or substances elaborated by the host to render them less toxic and excreted in this combined form. The presence of these metabolic products in the urine is influenced by two principal factors—bacterial activity and intestinal absorption. Other considerations enter into the problem, and for this reason the qualitative, rather than the quantitative estimation is all that is to be considered in this connection. It follows that the recognition of these end products, which are in reality indicators of certain definite types of bacterial activity, is of the greatest importance. Hitherto this correlation between diet, intestinal flora and end products has been largely overlooked, and the natural result has been that the corroborative evidence which these indicators furnish has not been utilized.\(^3\)

\(^1\)For a discussion of the bacterial changes associated with a change in diet see Kendall: This Journal, vi, pp. 266–268, 1909.

\(^2\)See Kendall: loc. cit.

\(^3\)It is undoubtedly true that some connection between diet and bacteria on the one hand, bacteria and end products on the other, has been surmised, but the lack of definite information available at the present time is strong evidence of the truth of the assertion that the three phases—diet, bacteria and end products—have not been considered in their interdependent relations.
Having determined by experiment that a given diet (for example, simple protein) is associated with a definite type of bacterial activity, and that coincidentally certain of these indicators are present in the urine of the host, it becomes a relatively simple matter to isolate individual strains of this fecal flora which will reproduce, either alone or symbiotically with other strains, these same end products. This is accomplished by growing the mixed fecal bacteria in media of the same fundamental composition as that of the diet which originally nourished them. An enrichment of the dominating types usually takes place, the abrupt change from intestine to media, with its resulting lack of development is partially overcome, while every possible opportunity is given for the selective development of the desired types. Thus the plating, which must be relied upon for the final separation of the cultures in a state of purity, is far more successful than when plating direct, without the preliminary enrichment, is resorted to. These end products, then, become the criteria through which it is possible to decide with definiteness the participation, indifference or antagonism of each of these types of bacteria in the process under consideration.

In the present undeveloped state of the subject it will be impossible to formulate a definite procedure applicable to all cases. It is very probable, indeed, that from the nature of the phenomena involved, such an undertaking would be disappointing in its results. The best that can be done will be to outline the course of a definite experiment, indicating the procedures through which it is possible to arrive at the desired conclusions.

Before this is done, however, I wish to mention briefly those developments and extensions of present methods which have made it possible to bring the work to its present state. They are: the association of certain products of bacterial metabolism (which are present under specific conditions in the urine of the host) with the activity of certain definite types of organisms upon definite foodstuffs; the corroborative use of artificial media for the demonstration of the completeness and direction with which the bacterial complex in the intestinal tract follows the changes in the character of the diet, and the employment of these media for the selective enrichment and isolation of those varieties of organisms which are most intimately concerned in these changes and the elaboration of these end products.
Study of Intestinal Bacteria

The following experiment, which was repeated several times, always with the same results, may be quoted to demonstrate the general procedure followed in this work.

A monkey was placed upon a diet consisting of milk plus dextrose. Bacteriologically considered, this diet was essentially carbohydrate in character—there were very few proteolytic bacteria present in the fecal flora, which was of the acidophilic type; the fecal bacteria which developed on this regimen grew less readily in artificial media than was the case with either a mixed or a protein diet.

The acidophilic nature of the fecal flora was brought out in a striking manner by inoculating with the mixed fecal flora milk fermentation tubes, broth fermentation tubes containing dextrose, lactose and saccharose, gelatin and a series of dextrose broth containing varying amounts of acetic acid.

The milk tubes showed coagulation, but no further action was apparent. In the broth fermentation tubes there were slight turbidities with very little or no gas. The gelatin tubes contained only a very slight growth after many days, while the acid dextrose tubes showed moderate development, even in the highest acidities. The milk and gelatin are particularly noteworthy. Milk, and to a lesser extent, gelatin, are excellent media for the development of proteolytic bacteria, while the acidophilic flora grow much less readily on artificial media than do the protein bacteria. Hence the lack of bacterial development in these media is the strongest evidence of the inhibition, or even replacement of proteolytic organisms by the acidophilic flora.

The urine was found to be free from indican, phenolic bodies and other products of intestinal putrefaction.

The animal was then placed upon a purely protein regimen with an ample allowance of water.

The conditions changed rapidly. The milk fermentation tubes became the seats of great bacterial activity when they were inoculated with the mixed fecal flora. The milk was greatly peptonized and much gas was formed; liquefaction was marked in the gelatin tubes (stab inoculations); the fermenta-

tion tubes showed large amounts of gas (even 90 and 100 per cent being not infrequently produced in eighteen hours); while there was a gradual diminution in the acidophilic flora grown in acid broth tubes. This diminution was manifested chiefly by the inability of the organisms to grow in the highest acidities. These growths took place very rapidly, eighteen to twenty hours being ample time for the described phenomena to develop in their completeness.

Coincidentally products of the decomposition of protein began to appear in the urine. Indican and phenolic bodies were particularly sought for and found in increasing amount as the proteolytic flora became established. Urorosein was not found.

The replacement of the acidophilic flora, then, was demonstrable in the following manner:

a. There was a microscopic change in the fecal flora. The strongly Gram-positive fields, consisting largely of the medium sized rod-shaped, acidophilic organisms were replaced by large, Gram-positive rods; smaller, Gram-positive and Gram-negative rods [subtiloid bacilli]; coccal forms in small numbers, and oval, Gram-negative bacilli, referable morphologically to B. coli and related aerogenic bacilli.

b. Culturally, whereas the acidophilic (carbohydrate) flora grew very poorly or not at all in gelatin; slowly, with at most coagulation, in milk; moderately, with little or no gas in fermentation media; and considerably in even the highest acidities (\(\frac{8}{10}\)) in dextrose broth, the exact reverse was the distinguishing feature of the protein diet. Gelatin was promptly liquefied; peptonization and considerable gas-production were features of the milk tubes; heavy turbidities and large volumes of gas were produced in dextrose, lactose and saccharose, while the higher acidities of the acid dextrose bouillon cultures were devoid of growth.

c. Chemically, on a carbohydrate diet, with the resulting acidophilic flora, the urine was free from products of intestinal putrefaction. As the protein regimen was established and the proteolytic bacteria became habituated to the changed conditions in the intestinal tract, indican and phenolic bodies gradually became prominent in the urine.

It will therefore be seen that through the use of this general procedure it is possible to demonstrate perfectly definite, con-
sistent correlations between the nature of the diet, the morphology, cultural and physiological relations of the intestinal flora, and the type of and products of bacterial metabolism on each of these diets. These relations are distinctive and sharply defined.¹

**SUMMARY.**

The procedures in this paper are outlines of general principles applicable to the determination of the more important types of bacterial activity in the intestinal tract and for the isolation of the principal agents concerned in these processes, rather than specific methods to meet special cases. An extension of these principles, along appropriate lines, however, will furnish a definite line of approach to the study of the majority of problems relating to the intestinal flora.

These procedures are based upon the correlation which exists between diet, bacterial flora and end products of bacterial activity which appear in the urine. The nature of the diet practically determines the dominant types of intestinal bacteria, and these organisms in turn, acting upon the digestive products of the diet elaborate the end products of their activity which appear in the urine.

With the exception of a few anaerobes (which derive their oxygen from the combustion of carbohydrates) the majority of the prominent types of the normal flora which develop on a protein diet grow luxuriantly in media free from carbohydrate, while those developing on a carbohydrate regimen grow poorly, or even not at all, unless carbohydrate is present. Hence by inoculating portions of the mixed fecal flora with gelatin and milk and observing the degree and rapidity of peptonization, it is possible to form a judgment of the character of the proteolytic flora. At the same time these media furnish conditions so favorable for the growth of these organisms that they can be regarded as selective for the isolation of the proteolytic flora.

On the other hand, through the use of media containing carbohydrate and particularly the acid dextrose broth, one obtains a

¹ A detailed account of these experiments carried out on monkeys, using these procedures, will be published later.
fairly specific enrichment of the acidophilic flora, characteristic of a carbohydrate regimen.

Furthermore, through the use of these selective media it is possible to form a judgment of the completeness of the bacterial response to the nature of the diet. For example, if the experimental animal is on a carbohydrate regimen, the presence or absence of growth in protein media will indicate the presence or absence of proteolytic bacteria, since the acidophilic organisms do not grow well in these media and cannot, therefore, inhibit the growth of these organisms. Conversely, with a protein diet, the presence or absence of acidophiles may be determined by inoculating the mixed fecal flora into acid dextrose broth, which is unfavorable for the development of the proteolytic types. These determinations may be made roughly quantitative for the different types by inoculating definite amounts of the mixed fecal flora into appropriate media.

The end products of bacterial activity which appear in the urine are important for two reasons: they indicate the types of bacterial activity in the intestinal tract, and their reproduction in artificial media by pure cultures derived from the intestinal flora furnish strong presumptive evidence of the participation of these organisms in the process.
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