The common bacterial infections of the digestive tract and the intoxications arising from them / by C.A. Herter.

Histology Store

THE COMMON BACTERIAL INFECTIONS OF THE DIGESTIVE TRACT


The MC Co.

THE COMMON BACTERIAL INFECTIONS OF THE DIGESTIVE TRACT

AND THE INTOXICATIONS ARISING FROM THEM

BY

C. A. HERTER, M.D.

PROFESSOR OF PHARMACOLOGY AND THERAPEUTICS IN COLUMBIA UNIVERSITY, CONSULTING PHYSICIAN TO THE CITY HOSPITAL, NEW YORK

New York
THE MACMILLAN COMPANY
LONDON: MACMILLAN & CO., LTD.
1907

Set up and electrotyped. Published April, 1907.

Norwood Press
J. S. Cushing & Co. — Berwick & Smith Co.
Norwood, Mass., U.S.A.

PREFACE

This little volume embodies views recently presented at the New York Academy of Medicine, in a lecture before the Harvey Society for the Diffusion of Medical Knowledge. Although the data on which this lecture was based have been summarized in another publication, I wish to present them more fully in the present volume in order that some important details, not suited for publication in a medical journal, may be brought to the notice of practitioners and investigators. The book does not aim at a systematic discussion of the extended and somewhat confused field of gastro-enteric infection, either from a clinical or a bacteriological standpoint. Neither does it make any claim to present fully the literature of this subject.

I have laid considerable stress on methods developed in my laboratory with a view to obtaining a better insight into the bacterial conditions of the digestive tract than has been hitherto possible. This I have done in the belief that when these methods are utilized in practice they will prove of real service in gaining a truer conception of the nature of the bacterial processes that are operative in disease. I am confident that the painstaking application of these methods will furnish practitioners with new and

reliable indications as to the progress of many cases of infection of the digestive tract.

Without the coöperation of my associates I could not have obtained the data given in this volume. I am under especial obligations to Dr. Helen Baldwin, Miss M. L. Foster, Dr. A. J. Wakeman, Mr. H. C. Ward, and Dr. William R. Williams for the efficient help which they have in various directions given me.

December 1, 1906.

CONTENTS

THE COMMON BACTERIAL INFECTIONS OF THE DIGESTIVE TRACT

THE COMMON BACTERIAL INFECTIONS OF THE DIGESTIVE TRACT

GENERAL CONSIDERATIONS RELATIVE TO THE BACTERIAL FLORA OF THE HUMAN DIGESTIVE TRACT IN HEALTH

If one examines with the microscope the contents of any portion of the large intestine of a human being or of any mammal, the richness of the material in microorganisms is strikingly apparent, especially in stained preparations. Their number has been estimated at one hundred and twenty-six billions for the daily human excreta. It is true that if the material is selected from the lowest portion of the gut, many of the microörganisms of a cultivable nature can be shown by suitable cultural methods to be no longer living, but rather to be undergoing a process of disintegration, partly owing to a solution in their own juices — a process of autolysis. But even the dead and dying bacterial inhabitants of the lower intestinal tract point to the multiplicity of bacterial life at higher levels.[1] And not only are these

[1] Strasburger, J., "Untersuchungen über die Bakterienmenge in menschlichen Fäces," *Zeitschr. f. klin. Med.*, xlvi, p. 413, 1902. It has been estimated that the proportion of dead bacteria in normal human fæces is often as high as ninety-nine per cent. It

bacterial inhabitants numerous but they represent many species and varieties.

The knowledge that the digestive tract is so rich in bacterial forms of life has led many physiologists to inquire into the biological meaning of this remarkable fact. Pasteur expressed a belief that these bacterial inhabitants are essential in some way to the life of the individual which harbors them. Nuttall and Thierfelder,[1] in their well-known experiments, attempted to rear guinea-pigs delivered by Cæsarian section and fed on quite sterile food. As the animals lived and increased in weight, the experimenters concluded that the intestinal bacteria are not essential to normal nutrition. This view, as will be presently seen, gets support from the observations of Levin[2] that some animals of the Arctic region, as polar bears, have no bacteria in the digestive

appears, however, that this is an under-estimate of the living bacteria. It may possibly hold true of the aerobic bacteria which will grow on ordinary media. But we know that there are often many aerobes and anaerobes in the intestine which do not grow on ordinary media. In some instances there are very many strict anaerobes (*e.g. B. aerogenes capsulatus*) which appear only on specially prepared media on anaerobic plates. These, of course, do not come into any count made in ordinary ways. Even the numbers of living colon bacilli are subject to great variations. In the same individual there may be at one time a considerable proportion of cultivable (living) bacilli. In a state of constipation the number of cultivable colon bacilli may become very small. This death of bacteria in the lower bowel during constipation doubtless depends mainly on a failure of food supply and absence of moisture.

[1] "Thierisches Leben ohne Bakterien im Verdauungskanal," *Zeitschr. f. physiol. Chem.*, xxi, p. 109, 1895; xxii, p. 62, 1896; xxiii, p. 231, 1897.

[2] "Bakteriologische Darmuntersuchungen," *Skandinavisches Archiv f. Physiol.*, xvi, p. 249, 1904.

tract. Even in temperate regions there are animals whose alimentary tracts are comparatively free from bacterial life. This is said to be the case with the parrot. Other observers have, however, reached a different conclusion from that of Nuttall and Thierfelder. Schottelius [1] found that chickens fed on sterile food were retarded in development and showed normal growth only when given food containing bacteria. Similar results were obtained by Madam Metchnikoff [2] in experiments on tadpoles. Very carefully conducted experiments by Moro [3] on the larvæ of the turtle (*Pelobates fureus*, Wagler) lead to the same conclusion; namely, that intestinal bacteria are necessary to normal nutrition.

It must be admitted, I think, that none of these experimental studies are really conclusive as to the necessity of bacterial action in the digestive tract for the maintenance of health in adult mammals of the highest type — man and various domestic animals. Experiments on tadpoles and chickens cannot with confidence be applied to the case of man. The experiments on guinea-pigs can more justly perhaps be taken as typical for mammals, but as the experiments of Nuttall and Thierfelder were extended over only a short period of time, they can hardly be held to prove that bacteria are

[1] "Bedeutung der Darmbakterien für die Ernährung," *Archiv f. Hyg.*, xlii, p. 48, 1902.

[2] "Note sur l'influence des microbes dans le dévelopment des tétards," *Ann. de l'Inst. Past.*, xv, p. 631, 1901.

[3] "Morphologische und biologische Untersuchungen über die Darmbakterien des Säuglings," IV. "Der Schottelius Versuch am Kaltblüter," *Jahrb. f. Kinderheilk.*, xii, p. 467, 1905.

either essential or non-essential to the maintenance of prolonged health during the period of adult existence. The evidence given by the sterile intestinal contents of certain Arctic animals is apparently conclusive for the conditions in which this experiment of nature has been carried out. Here we have animals born and living in surroundings where bacteria are very few in number, and it is probably on account of the great rarity of micro-organisms in the air and the small number in the water [1] that the intestinal contents contain so few bacteria. These animals are able to live indefinitely in a state of robust health. Levin examined the intestinal contents of Arctic animals in Spitzenberg. The digestive tract was found to be in most instances entirely sterile in white bears, seals, reindeer, eider ducks, penguins, etc., although very small numbers of organisms resembling the colon bacillus were found in one white bear and in two seals which were examined.

Clearly then, in this case, the intestinal bacteria are not required to carry on the ordinary digestive processes and normal nutrition. It has been supposed that the intestinal bacteria aid in the digestion of cellulose, which they are undoubtedly able to decompose fermentatively. The argument in favor of the importance of this function of the intestinal bacteria loses much of its force if it be true, as lately maintained by Bergman,[2] that most of the

[1] It was estimated that there was one organism in 11 c.c. of water, whereas in the river Seine it was estimated that there are about 2,000,000 in the same volume of water.

[2] "Studien über die Digestion der Pflanzenfresser," *Skandinavisches Archiv f. Physiol.*, xviii, p. 119, 1906.

cellulose eaten by herbivora (in which the digestive function of the bacteria chiefly comes into question) is provided with intracellular enzymes capable of decomposing cellulose.

The real significance of the normal intestinal flora probably lies not in any immediate relation to processes of digestion, but in a wholly different direction.

It is impossible to avoid the entrance of bacteria into the digestive tract. As will be seen when we come to consider the normal flora of the alimentary tract, the obligate bacteria (*e.g. B. lactis aerogenes, B. coli, B. bifidus*) have adapted themselves to the secretions of this part of the body and ordinarily hold their own against new-comers. By virtue of their adaptation they are not ordinarily harmful to their host, but on the contrary, they are under some circumstances capable of doing a service by giving rise to conditions that discourage the growth of many harmless and harmful species which the human animal cannot readily exclude from his digestive tract. I believe the chief significance of the obligate intestinal bacteria lies in their potential capacity for thus checking the development of other types of organisms capable of doing injury.

Under ordinary conditions of life, in temperate climates, and still more so in hot ones, a human being is liable to take into the alimentary tract, with food and drink, microörganisms which are capable of doing injury if they find opportunity to multiply in the digestive tract. Water, milk, cheese, oysters, game, preserved and fresh meats, etc., are liable to contain injurious

bacteria. Among such bacteria are pyogenic streptococci and staphylococci, the paratyphoid bacilli, typhoid bacilli, the dysentery bacilli, *proteus vulgaris*, and the spore-bearing anaerobes — *B. putrificus* (Bienstock), *B. aerogenes capsulatus*, *B. botulinus*, etc.

Defensive Action of the Digestive Juices. — The normal human organism is provided with more or less efficient (though by no means fully understood) methods of defense against these bacterial invaders. The secretion of the gastric juice in normal abundance, after a meal, provides a degree of acidity which acts as an effective check upon the growth of many non-sporulating bacteria, and is actually destructive to most varieties at least in a measure. Probably the proteolytic action of the peptic ferment and the tryptic enzymes leads to a very quick destruction of any bacteria whose vitality has been lowered by contact with the acid of the gastric juice. If, however, bacteria are administered in very large numbers, there is a chance that a certain proportion of them will run the gauntlet of these defenses and find their way into the lower part of the small intestine and into the colon. This seems especially liable to happen in those cases where the microbes are taken into the empty, non-secreting stomach, or into a stomach with defective motility which secretes little gastric juice with a low content of hydrochloric acid — and there are many such stomachs among persons over forty years of age and in fair health. Thus the bacteria (with any spores that may have developed from them or have been ingested as such) find their way to the region of the colon

and here are confronted with immense numbers of the chief obligate race of bacteria of the digestive tract — the representatives of the *B. coli* type. Another group of obligate organisms closely allied to *B. coli, B. lactis aerogenes,* is present in the upper part of the small intestine and becomes gradually less abundant with its descent into the colon and finally appears in relatively small numbers in the fæces, if at all. As the bacteria of the *B. lactis aerogenes* type grow less numerous, the representatives of the *B. coli* group grow more abundant and beyond the ileocæcal valve largely dominate the intestinal flora.

Exactly what happens when the accidental, saprophytic forms of bacterial life — the "wild races," as the French call them — come to close quarters with the "obligate," well-adapted parasitic forms of the intestine, we do not at present know. There are, however, numerous facts which point to well-defined biological antagonisms between the "wild" forms and the representatives of the *B. coli* group.

ON THE ANTAGONISM BETWEEN *B. coli communis* AND OTHER MICROÖRGANISMS

The members of the *B. coli* group are organisms of varying morphology, characterized by a certain hardiness in growth on ordinary media, by the free production of gas and acid on various sugars, by the coagulation of milk, and usually by the formation of indol, and by a sluggish motility in some fully grown forms and active

motility in members of young surface colonies.[1] They do not retain the Gram stain. The members of the *B. lactis aerogenes* group are distinguished from those of the *B. coli* group by unimportant morphological differences. More significant differences exist in respect to biochemical characters. Among these differences are a somewhat greater ability to form gas on sugar media, a more rapid coagulative action on milk (often with capsule formation), the ability to make gas from potato starch, the more frequent failure to make indol, and the greater luxuriance of growth on gelatin. In general, then, the fermentative activities of *B. lactis aerogenes* are somewhat greater than those of the *B. coli* group, while the putrefactive powers are distinctly less. Harden [2] has lately shown that there is a constant difference in the behavior of *B. lactis aerogenes* and *B. coli* when grown anaerobically on sugar bouillon, in regard to the ratio of alcohol and acetic acid produced. This difference appears to strengthen the right of *B. lactis aerogenes* to be regarded as a distinct organism from *B. coli*, although the relationship between the two is close. The behavior of these two groups toward other organisms is probably very similar, but as the antago-

[1] For a detailed discussion of *B. coli communis* see Escherich (Th. Escherich u. M. Pfaundler, "Bacterium coli commune," Kolle & Wassermann's "Handbuch der pathogenen Mikroorganismen," p. 334, 1902); also Theobald Smith ("Note on *Bacillus Coli Communis* and Related Forms"), *Amer. Journ. of the Med. Sci.*, September, 1895, who gives important data as to fermentative characters.

[2] "The Chemical Action on Glucose of the Lactose-fermenting Organisms of Fæces," *Journ. of Hyg.*, v, p. 488, 1905.

nistic action of the *B. coli* group has been more carefully observed I shall speak of this only, especially as the physiological behavior of its members is the more important on account of their wider distribution in the digestive tract.

The most far-reaching contention relating to the defensive action of the *B. coli* group is based on the recent observation of Conradi and Kurpjuweit[1] that the members of this class make thermostabile and thermolabile substances which have a powerful antibacterial action, being still active in a dilution of 1 to 10,000 parts, and hence comparable to the antibacterial action of carbolic acid. The inhibitory action of these substances is stated to be not confined to alien bacteria, but relates also to the *B. coli* group. The inhibition in growth observed in old cultures was attributed to this substance. Moreover, it was claimed that it is owing to such bactericidal substances that the members of the *B. coli* group tend to die out as they pass toward the lower end of the bowel. It is easy to satisfy oneself that the fæces of a healthy person contain more living colon bacilli if the intestinal contents have somewhat rapidly passed through the colon than if the usual sojourn of material in the lower bowel has occurred. It is also noteworthy that relatively few living representatives of the *B. coli* class are present in the movements of healthy persons with obstinate constipation. The cause

[1] "Ueber die Bedeutung der bakteriellen Hemmungsstoffe für die Physiologie und Pathologie des Darms," *Münch. med. Wochenschr.*, lii, pp. 2164, 2228, 1905.

of this high mortality among the colon bacilli is ascribed by Conradi and Kurpjuweit to increased opportunities to make bactericidal substances.

In their experiments these writers found that the growth of the typhoid bacillus and of the paratyphoid bacillus is definitely inhibited by the activities of the colon bacillus, and this restraint is referred by them to the specific inhibitory products of the latter microbe. It will be shown in subsequent pages that the claims of Conradi and Kurpjuweit have not been successfully sustained. The observations related by them are of great interest, but the interpretation placed on them appears to be erroneous.

The inhibitory action of *B. coli* upon the putrefactive anaerobes is shared by a closely related group of bacteria first described as *B. bifidus* by Tissier.[1] This group of bacteria was originally included by Escherich with the colon bacilli, but for reasons which will be mentioned later, has now been separated from them. Tissier found that *B. bifidus*, under certain conditions, inhibits

[1] "La Flore intestinale normale et pathologique du nourrisson," *Thèse de Paris*, 1900.

There are certain bacteria that have the ability to check the growth of *B. coli*. Streptococci derived from human fæces I have repeatedly observed to repress the growth of *B. coli* from the same individual, in the anaerobic limb of the fermentation tube. *B. lactis aerogenes* may be repressed in the same way. Heinemann ("The Significance of Streptococci in Milk," *Journ. Infect. Dis.*, iii, p. 173, 1906) has found that streptococci from milk interfere with the development of *B. lactis aerogenes*. Gabricewski and Maljatai ("Ueber die bakterienfeindlichen Eigenschaften des Cholerabacillus," *Centralbl. f. Bakt.*, xiii, p. 780, 1893) observed that *B. coli* is inhibited by the growth of cholera vibrios.

the growth of the anaerobic microbe which he describes as *B. perfringens* and which appears to be no other than the organism known in the United States as *B. aerogenes capsulatus* (Welch) and in Germany as the gas-phlegmon bacillus (Fraenkel).

The *B. bifidus* of Tissier is closely related to the organism described by Moro as *B. acidophilus* and shares with the latter the ability to grow in a more strongly acid medium than can be withstood by meat bacteria. Recent studies of these bacteria by Bjeloussow[1] have led him to identify these acidophile microörganisms with certain acidophile bacteria studied by him, and to which he, as well as Mereschkowsky,[2] attaches considerable importance as protective inhabitants of the digestive tract. The capacity to grow in a medium containing one-half or even one per cent. of acetic acid is an indication that the acidophile bacteria might assume a dominant position in the digestive tract if provided with suitable food materials for the production of acid. It is stated that if large numbers of these bacteria be administered to a dog by mouth, other flora may to a large extent be temporarily suppressed. But as in the case of feeding other kinds of microörganisms the leading part can only be maintained by continuously feeding large numbers of the acidophiles. Whether

[1] "Zur Biologie und Methodik der Ausscheidung der sogenannten acidophilen Bakterien," Diss. No. 76, St. Petersburg (Russian).

[2] "Zur Frage über die Rolle der Mikroorganismen im Darmkanal," *Centralbl. f. Bakt. Orig.*, xxxix, pp. 380, 581, 696, 1905, and xl, p. 118, 1906.

such feeding would be practicable in man on a scale sufficiently great to secure definite results in the suppression of harmful types, such as the putrefactive anaerobes, can only be determined by experiment. At the present time it is known that the acidophiles are represented in all parts of the tract, especially in the large intestine, but we cannot yet say with confidence what is their physiological rôle or what would be their influence on the organism if their numbers should be greatly increased through feeding.

The various facts that have now been advanced render it certain that microörganisms of the *B. coli* type are able under some conditions to check the growth of pathogenic microörganisms which are often found among the intestinal flora. The conditions that obtain in the colon differ, however, with respect especially to the nutrient pabulum and the digestive secretions, from any attainable experimental conditions, however cleverly these may be designed to imitate what occurs in nature. We have therefore to exercise some caution in transferring these results, without due consideration, to the human digestive tract. Nevertheless the evidence now available suggests that in health the colon bacillus, both in man and the higher mammals, exerts an important function in combating the development of the injurious saprophytes with which even in ordinary health the human intestinal tract almost necessarily abounds. A long, largely anaerobic intestinal tract, permitting gradual resorption of the contents, is a physiological necessity in order that a loss of water and its detrimental consequences may

be spared the organism. The presence in the colon of immense numbers of obligate microörganisms of the *B. coli* type may be an important defense of the organism in the sense that they hinder the development of that putrefactive decomposition which, if prolonged, is so injurious to the organism as a whole.

We have in this adaptation the most rational explanation of the meaning of the myriads of colon bacilli that inhabit the large intestine. These bacilli are essential to the life of the individual mammal as a defense against bacterial foes which it is impracticable to wholly exclude from the digestive tract, and not as agents in directly facilitating the processes of digestion in the narrow sense. This view is not inconsistent with the conception that under some conditions the colon bacilli multiply to such an extent as to prove harmful, through the part they take in promoting fermentation and putrefaction. It seems to me not unlikely that the reaction of the fluids of the digestive tract may influence the character of the activities of the colon bacilli, an alkaline reaction favoring their putrefactive functions if peptones be present.

The following facts, first noted by Moro and Murath, point to the existence of bacterial inhibitory powers on the part of the fæcal flora of nurslings and bottle-fed children, and deserve mention. If one allows fæcal matter from a normal nursling to stand in a thermostat in a closed test-tube, the original not disagreeable odor lasts for days or even weeks, although the material as a rule contains small numbers of true putrefactive bacteria. Indeed, it is possible to inoculate such normal material

with Bienstock's *B. putrificus* quite richly without any evidence of putrefaction, although the organisms are kept under anaerobic conditions. Although this observation points to the absence of putrefactive processes, it must be remembered that the growth of anaerobes is relatively difficult where the quantity of moisture is limited, as in the case of the foregoing experiments, and also that the quantity of nutrient material available for the growth of putrefactive anaerobes is small in the movements of normal milk-fed children. A similar result is obtainable after inoculating concentrated fæces-agar with various bacteria, such as *pyocyaneus*, *prodigiosus*, the bacillus of Friedländer, the vibrio of Metchnikoff, and various strains of the typhoid bacillus. That is to say, such fæces-agar richly inoculated with the foregoing organisms fails to show any growth, whether the acid reaction of the medium be retained or whether neutralization has taken place. On the other hand, it has been noted that *B. coli*, *B. fluorescens*, *Staphylococcus intestinalis*, *streptococcus*, *proteus*, and the paracolon organisms, as well as *putrificus* are apt to grow somewhat better. From this observation one may perhaps reach the conclusion that the bacteria of the intestinal tract possess an elective antagonistic action against foreign types of microörganisms.

An effort has been made to obtain an adequate explanation for this behavior, and especially to determine whether the inhibition for bacteria here noted depends on the production of definite bactericidal substances. The methods employed by Moro and Murath were

similar to those of Conradi and Kurpjuweit.[1] In the series of observations made by them the growth of the following organisms was observed on the specially prepared plates: *B. coli communis*, *B. lactis aerogenes*, *B. typhi*, *B. Shiga-Kruse*, *B. Flexneri*, *Staphylococcus pyogenes aureus*, *Diplococcus intestinalis*, *Soor*, *B. prodigiosus*, and *B. pyocyaneus*. The results were by no means uniform, but showed that the normal nursling's stool exerts an intense inhibitory action against various bacteria. For example, *B. typhi* and *B. Kruse* failed to grow even in dilutions of 1 : 400. On the other hand, the representatives of the obligate bacteria of the intestine,

[1] One volume of the fresh fæces is diluted with nine volumes of bouillon. In order to measure the fæces one may use a glass capsule of 1 c.c. capacity, which is then emptied by means of a glass rod of suitable size, into the bouillon. Various quantities of the yellowish emulsion are now permitted to flow into test tubes containing agar cooled to 42° C., so that the fluids so obtained represent dilutions of $\frac{1}{10}$, $\frac{1}{25}$, $\frac{1}{50}$, $\frac{1}{100}$, $\frac{1}{200}$, and $\frac{1}{400}$ of the original fæcal material. After thorough mixing, the agar is plated in Petri dishes. The surface of these plates is then promptly inoculated with approximately the same numbers of different kinds of bacteria.

The material to be inoculated is prepared by inoculating one normal platinum loop of a twenty-four-hour agar culture into 10 c.c. of bouillon, and emulsifying it. From this emulsion of bacteria a smaller loopful is smeared upon the surface of the agar plates. It is convenient to divide up the agar into half a dozen regions by means of a platinum needle. The surface of each one of these islands may be smeared with a different culture of bacteria so that the growth of several different types of organisms may be observed on one plate. The bacteria inoculated on one of these islands does not extend to others. The growth is observed at the end of twenty to twenty-four hours, and controls are made with material of the same origin, diluted to one-tenth, boiled, and then incubated for twenty-four hours.

B. coli communis and *B. lactis aerogenes*, usually grew fairly well and suffered little inhibition. This was a regular occurrence throughout the experiments. Indeed, the phenomenon occurred so regularly that it was employed as the basis for distinguishing between typhoid bacilli and colon bacilli. In the case of *Staphylococcus pyogenes*, which is found in every nursling's stool, there is observed very little inhibitory action. In the case of *B. pyocyaneus* it was found that even in such strong concentrations as 1 : 10 there was good growth with the production of pigment. It appears, therefore, that in this case the medium favored the growth of *pyocyaneus* to a certain extent. The dysentery bacillus of Flexner in some cases behaved like the typhoid bacillus, but in other cases showed growth in a dilution of 1 : 100. The action of this medium was therefore more marked upon the whole in its action against the typhoid bacillus and the Kruse bacillus than against the Flexner bacillus.

The *Diplococcus intestinalis*, a constant inhabitant of the nursling's tract, behaved like the *Staphylococcus pyogenes* in developing only moderately. The growth of the thrush organism was more strongly inhibited than *B. prodigiosus*. The development of color was checked on all the plates in which the fæcal concentration was fairly strong.

In general, the inhibitory substances to which the restraining effect is attributed appeared to be less active in the fæces of the nursling than in those of grown persons.

A difference between the inhibitory action of the fæces

of nurslings and of bottle-fed children could not be observed. In the case of a child suffering from a chronic catarrhal condition of the gastro-enteric tract and marasmus, a great diminution in the inhibitory action was observed. It seems possible that this might be interpreted on the basis of a diminished growth of colon bacilli rather than by the production of inhibitory substances.

Experiments made with meconium showed that this material held no bactericidal substances. On the contrary, when mixed with agar it became an excellent nutrient medium for bacteria, although by itself it is only a poor medium for most varieties.

It is interesting to observe that *B. bifidus*, which forms the chief representative of the nursling's flora, appeared to be unable to make these inhibitory substances. The question arises in the mind of the critic in connection with this experiment, whether in the observations reported there was in reality a good growth of *B. bifidus*, which is an organism not easily cultivated on ordinary media.

Moro and Murath attribute the inhibitory action of the nursling's fæces entirely to the presence of organisms of the *B. coli* type. A comparison of typhoid bacillus with the colon bacillus with respect to the ability to inhibit the growth of various microörganisms showed that the typhoid bacillus possesses little or no capacity to effect this inhibition.

Criticism of Experiments of Conradi and Kurpjuweit. — The interpretation to be placed on the phenomenon of

inhibition described by Conradi and Kurpjuweit and by Moro and Murath is not yet entirely clear. That the observed inhibition is due to complex cellular products — the "autotoxines" of Conradi — is questionable in view of the studies of Manteufel,[1] of Passini,[2] and of Oebius.[3] Manteufel showed that in many cases where the surface of a plate indicated no growth on inspection, and where consequently it would be assumed that an inhibitory effect had been exerted, in reality many small microscopical colonies were present. In other words, the character of the growth may be of such a nature as to mislead one into the belief that inhibition has occurred where in reality such inhibition is absent or slighter than has been supposed. In these cases the size of the individual surface colonies was smaller but their number was increased and the surface could certainly not be regarded as sterile. Further observations made it very probable that the exhaustion of the culture medium incidental to the growth of the fæcal flora is in part the cause of the failure of the surface colonies to grow. The assumption that there are produced in the bouillon culture thermolabile, unfilterable, and dialyzable substances which check the growth, appears unwarranted in the light of certain experiments with reference to this

[1] "Untersuchungen über die 'Autotoxine' (Conradi) und ihre Bedeutung als Ursache der Wachstumshemmung in Bakterienkulturen," *Berl. klin. Wochenschr.*, xliii, p. 313, 1906.

[2] "Die bakteriellen Hemmungsstoffe Conradis und ihr Einfluss auf das Wachstum der Anaerobier des Darms," *Wien. klin. Wochenschr.*, xix, p. 627, 1906.

[3] "Ueber spontane Wachstumshemmung der Bakterien auf künstlichen Nährböden," *Med. Klinik*, ii, p. 598, 1906.

point. It also appears doubtful whether there are heat-resisting substances formed by *B. coli* which are of value in checking surface growths made under the conditions already mentioned. It was found that there was no difference in the surface growth of *B. coli* if the agar medium containing twenty-four-hour cultures of *B. coli* was heated for thirty minutes at 90° C. or 100° C. Of course in this experiment it was necessary to exclude the factor of an exhausted medium previous to making the surface cultures, since in the presence of such an exhausted medium organisms would naturally not grow upon the surface. These various experiments speak against any specific inhibitory substances. It would, indeed, be a remarkable and apparently contradictory phenomenon if organisms should form substances in the course of their growth which are more injurious to themselves and their own species than to foreign ones. Yet this view is apparently assumed by Conradi to be correct. In order to explain the dominance of the colon bacilli in the intestine he has had to make the assumption that these organisms have given rise to substances against which they have become immunized — a complex assumption. It is also singular that the autotoxines of Conradi should act in an inhibitory manner without causing actual death, if it be true, as claimed, that these autotoxines are inhibitory in an even higher degree than carbonic acid. Conradi and Kurpjuweit, in spite of this fact, attribute the bactericidal action of fresh fæces to these inhibitory substances. It is difficult to understand how, if these supposed restraining substances are

really only inhibitory and not bactericidal, leaving the bacteria still capable of growth when brought into better nutrient media, so many of the bacteria in the fæces have been killed. The proof remains to be furnished that the death of these bacteria in the lower part of the intestinal tract and the inhibition at higher levels are due to the same cause.

It is only fair to say that Conradi and Kurpjuweit have not succeeded in establishing their contention that the inhibitory action of colon bacilli is due to specific toxines which they form. The inhibitory action described by them is certainly due in part to the exhaustion of the media by the freely growing colon bacilli and in part to the production of acid, indol, phenol, or other putrefactive or fermentative substances. It would be going too far, however, to deny that substances at present unknown to us may possibly have a part in bringing about the phenomena of inhibition.

It is clear that the colon bacilli can act protectively to the digestive tract only when they are able to grow freely. If the conditions are such in the tract that they cannot multiply abundantly, they can neither exhaust the nutritive materials on which other races grow nor make products endowed with inhibitory powers. There are many examples of disease in which the colon bacilli disappear not merely from the fæces, but from the diarrhœal stools. Other races take their place. This condition is quite unintelligible unless we assume that the colon bacilli no longer find a suitable nutrient medium in the mixture of digestive juices and food. Without

radical changes in the character of the food the colon bacilli have in many instances been observed to resume their prominent position in the intestinal contents. It is reasonable to believe that the return of more normal secretory conditions in the digestive tract is a very influential factor in determining the reinstatement of the legitimate flora. If this be true, the therapeutic value of introducing colon bacilli into the intestine (with food or by high colon injection) with a view to utilizing their restraining action upon injurious races must be less than would at first sight appear to be the case. There is need for much careful research in regard to this question.

Influence of Reaction on the Growth and Products of Intestinal Anaerobes. — If mixed fæcal flora be grown in sugar bouillon and in sugar bouillon containing calcium carbonate for one week at 37° C., it will be found that a considerable amount of volatile fatty acids has been formed in each case. The quantity will usually be found to be greater, often considerably greater, in the flask containing calcium carbonate than in the flask without it. Similar results are obtained if one substitutes magnesium carbonate for the calcium salt. The observed difference in the quantity of acids is therefore probably not due to a specific effect of the calcium salt, but to its action in maintaining a neutral reaction.

In a series of observations of the sort just mentioned, which were carried out by Dr. A. J. Wakeman, two things are worthy of note. First, it was found that in nearly all instances the molecular weights of the volatile fatty

acids were at least somewhat higher in the case of the cultures in the sugar-bouillon-carbonate media than in the corresponding growths in sugar bouillon. This fact suggests a relatively greater activity of putrefactive bacteria in the neutral media, for it is known that such bacteria tend to form the higher rather than the lower volatile fatty acids. This view was confirmed by the study of the bacteria dominant in the two series of flasks. Secondly, it was found by Dr. Wakeman that in almost every instance the proportion of non-volatile acids (mainly lactic) was greater in the sugar-bouillon cultures than in the neutralized cultures. The most reasonable interpretation of these results is that a neutral medium favors the development of putrefactive bacteria (especially of the anaerobes), whereas an acid medium (such as is maintained in the sugar-bouillon flasks) restrains the growth of these bacteria and favors the growth of the lactic acid makers. It is probable that in the case of the neutral flasks some of the lactic acid formed is converted into butyric acid.

These observations and similar ones with pure cultures indicate that in the digestive tract the growth of putrefactive anaerobes must be favored by a neutral reaction and restrained by the presence of acid. The favorable influence of milk food, containing lactic-acid formers, in controlling putrefactive decompositions in the digestive tract finds its explanation partly in the inhibitory action exerted by such bacteria and in part in the presence of preformed lactic acid in the food at the time it is ingested.

AEROBIC AND ANAEROBIC CONDITIONS IN THE DIGESTIVE TRACT

There are many conditions which influence the character and extent of bacterial decompositions in the alimentary tract. The chemical character of the food (quite aside from the bacteria it may contain), the solubility of the food in the digestive juices, the volume and composition of these digestive juices, all affect in important ways the ultimate fate of the food in its relation to microörganic decomposition. Some phases of these influences will be discussed in these pages in connection with special problems, although it must be owned that our knowledge of them is far from being full, owing partly to the great difficulties of experimental investigation. Intimately intermingled with these factors of food and secretory activity is the influence of aerobic and anaerobic conditions in the digestive tract upon the nature of the bacterial activities that occur there.

Pasteur, with his sharp insight into biological phenomena, was first in recognizing the ability of certain microörganisms (including a butyric acid producer, yeast plants, aspergillus, and some mucors) to live in the absence of oxygen and thereby first to distinguish between aerobic and anaerobic life. He embodied his views in a famous epigram, the validity of which has been much discussed — "Fermentation is life without oxygen." This statement, although too sweeping and exclusive, has in it a large element of truth and has a special

significance in its application to the bacterial processes in the digestive tract. This significance comes from two fundamentally important facts: first, that the initiation of putrefactive decompositions in the digestive tract (as elsewhere) depends very largely, though probably not exclusively, on the activities of obligate anaerobes; secondly, that an important portion of the digestive tract is most of the time under anaerobic conditions.

The importance of the rôle taken by obligate anaerobes in bringing about putrefactive decomposition in native proteids is only beginning to be appreciated. Bienstock [1] made an important contribution to biology when on definite experimental evidence he insisted on the inability of the ordinary facultative anaerobes to attack native proteids, and showed that obligate and strict anaerobes like *B. putrificus* are able, quite unaided, to break down proteids. Dr. Rettger,[2] in his numerous experiments on putrefactive decomposition, confirmed and extended the work of Bienstock. He showed that an egg-meat mixture is not attacked by *B. lactis aerogenes*, *B. coli*, *Streptococcus pyogenes*, *B. alcaligenes fœtidus*, *proteus vulgaris*, etc., whereas it is regularly decomposed by strict anaerobes such as *B. putrificus*, *B. maligni œdematis*, and *B. anthracis symptomatici* (bacillus of quarter-evil). This is a severe test, and there are putrefactive anaerobes which cannot attack the egg-meat

[1] "Untersuchungen über die Aetiologie der Eiweissfäulnis," *Archiv f. Hyg.*, xxxvi, p. 335, 1899; *ibid.*, xxix, p. 390, 1900.

[2] "Studies on Putrefaction," *Journ. of Biol. Chem.*, ii, p. 71, 1906.

mixture but which are nevertheless intensely putrefactive when grown in a medium containing a low percentage of sugars as well as native proteids. This is true of *B. aerogenes capsulatus*. I can confirm the observation that *B. coli* cannot decompose media consisting of native proteids even on long standing. On the other hand, there is no more striking type of putrefaction than that induced in proteids by *B. putrificus* upon egg-meat mixture.

At the present time I know of no evidence to show that native proteids can be actively decomposed by any of the ordinary facultative anaerobes, and in respect to the digestive tract the facts all point to the correctness of the view that we largely owe the initiation of proteid cleavage there to the agency of the strict anaerobes. But it must be remarked in passing that it does not follow from what has just been said that intestinal putrefaction is carried on through the sole activity of strict anaerobes. The fact that these organisms are the great destroyers of native proteids does not tell the story of the entire putrefactive process. The intestine abounds with microörganisms which are able to attack albumoses and peptones and to effect a further degradation of the proteid molecule, thus entering into a symbiotic action with the strict anaerobes.

It appears desirable to state at this point just what is meant by the expression "anaerobic life" as applied to the strict anaerobes to which reference has been made. When we speak of anaerobic life, we are liable to vaguely picture a state of existence in which the microörganisms

concerned are subsisting without access to any oxygen whatsoever, and the term "strict anaerobe" tends to give fixity to such a conception. Such an idea, however, would be far from expressing the actual truth, since there is no known exception to the rule that living microörganisms cannot maintain themselves without producing carbon dioxide, a process which involves the oxidation of organic materials within the cell. But the quantity of oxygen required to maintain life may be extremely small, and it is in this sense only—which is expressed in the term "micro-aerophile," suggested by Beijerinck[1]—that we are justified in speaking of anaerobic microorganisms. It has been calculated by Matzushita[2] that anaerobes may live although the surrounding air contains not more than 0.0031 per cent. of oxygen. This fact is liable to mislead us if we accept it as meaning more than that the presence of very little oxygen in the air is consistent with the maintenance of some forms of microörganic life. For, on the one hand, it seems clear that microörganisms may obtain their small portion of essential oxygen not from the air, but from a medium containing a decomposable substance which, like glucose, contains oxygen which becomes available for the support of life. In other conditions the body of the microörganism may contain material which is capable of yielding oxygen for a time, in minute amounts.

[1] *Arch. Néerland*, ii, 1899.

[2] "Zur Physiologie der Sporenbildung der Bacillen, nebst Bemerkungen zum Wachstum einiger Anaeroben," *Archiv f. Hyg.*, xliii, p. 327, 1902.

It is conceivable that under such conditions as those just mentioned, it becomes a matter of indifference to the anaerobes how little oxygen is contained in the surrounding air. On the other hand, it is well known that, paradoxical as it seems, the strictest anaerobes known are able to grow freely in apparently close contact with a fairly abundant supply of oxygen, provided aerobic bacteria are also present. Pasteur, to whom this noteworthy symbiotic phenomenon was known, ascribed it to the circumstance that the aerobes remove the oxygen present in so thorough a way that the anaerobes are unhindered in their growth. This doctrine, which found wide acceptance, has lately been questioned by Kedrowski [1] (and others), who attribute the ability of anaerobes to grow symbiotically with aerobes to the formation of a "ferment" by the latter, which renders the anaerobes immune to the action of oxygen. It cannot be said that this hypothesis has received substantial support, and we are compelled to return to the teaching of Pasteur or to some modification of it.[2] There is a limit to the amount of oxygen which the aerobes

[1] "Ueber die Bedingungen unter welchen anaërobe Bakterien auch bei Gegenwort von Sauerstoff existiren können," *Zeitschr. f. Hyg.*, xx, p. 358, 1896.

[2] See Walter von Oettingen, "Anaerobie und Symbiose," *Zeitschr. f. Hyg.*, xliii, p. 475, 1903. This writer contends that the primary action in aerobic and anaerobic symbiosis is the splitting action of the anaerobes in sugar with their associated inability to appropriate the oxygen contained in carbohydrates, that oxygen being eagerly seized by the aerobes for their use. It is hard to reconcile this view with the retarded growth of the anaerobes (as compared with the aerobes) in some symbiotic experiments. In this paper will be found the literature on the subject.

can divert from the anaerobes, and it is easy to check the growth of anaerobes which are not facultative by passing oxygen with some freedom through the fluid medium, no matter how many aerobes are present.

A study lately made by Willimsky [1] on the adaptation of aerobic bacteria to anaerobic conditions shows that aerobic bacteria like the spirillum of cholera, *B. alkaligenes*, *B. fluorescens non liquefaciens*, are able to adapt themselves to an atmosphere in which there remains only a minimal trace of oxygen and that this adaptation is the more efficient the more slowly the oxygen is withdrawn, sudden withdrawal of oxygen greatly slowing the multiplication of the organisms. The multiplication of organisms was found, however, to be much less rapid where oxygen had been thus withdrawn than where it is present. When an absolutely anaerobic condition was brought about, the organisms died, and their destruction was the more rapid, the more abrupt the withdrawal of oxygen.

The symbiosis of aerobes and anaerobes is a biological phenomenon of much consequence in determining the distribution of anaerobic bacterial processes in the digestive tract. Without such symbiotic action the development of strict anaerobes would be confined to those parts of the digestive tract into which oxygen rarely passes, and then only in small amounts. The large intestine is seldom visited by free oxygen, but it is probably usual in man for the small intestine to contain a little

[1] "Ueber des Verhalten der aëroben Keime gegenüber der absoluten Sauerstoffentziehung," *Archiv f. Hyg.*, liv, p. 375, 1905.

air. Boycott [1] in his observations on the gases of the intestine found that the small intestine of the cat generally contains a little oxygen, while the large intestine holds either no oxygen or a smaller percentage than is present in the small intestine. Sometimes the proportion of oxygen in the gas from the large intestine was a little above one part per hundred. Boycott noticed that oxygen passes very readily out of the intestine, partly by diffusion, but mainly by being appropriated by the mucous membrane. There is apparently very little direct exchange with the blood. In the stomach and cavity of the mouth there is always a considerable quantity of oxygen — unless, indeed, the oxygen in the stomach be displaced by the development of an abundance of carbon dioxide, as sometimes happens. It is probably safe to assume that in the mouth the free presence of oxygen constantly acts as a deterrent to anaerobic growth. In spite of this, however, anaerobic life is possible, and the indications are accumulating which point to the important part played by anaerobes in setting up pathological states in the mouth. Caries of the teeth, which was formerly referred to aerobic bacterial action, seems clearly the result of the invasive action of anaerobes on the tooth pulp. In certain derangements of digestion, I have noticed that butyric acid fermentation or putrefaction takes place with great rapidity in the food particles that have lodged between the teeth, especially if carbohydrate food has been taken. It must be

[1] "Observations on the Gaseous Metabolism of the Small Intestine of the Rabbit," *Journ. of Physiol.*, xxxii, p. 343, 1904–05.

admitted that proof is wanting that butyric acid formation in the mouth is always due to anaerobes. The growth of anaerobes in the mouth is favored by want of cleanliness there, since the accumulation of food-masses encourages anaerobic conditions beneath the surface. Probably the intelligent and free use of the tooth-brush is a most potent means of discouraging anaerobic growth in the mouth. In removing decomposing food-masses one not merely admits air to the anaerobes, but also removes many aerobes which, through the symbiotic action already mentioned, facilitate the multiplication of the former.

In the normal human stomach the conditions for the development of anaerobic bacteria are usually poor, partly on account of the presence of air, but mainly owing to the action of the gastric juice. Nevertheless the interior of a food bolus must often afford good opportunities for the growth of anaerobes that happen to be taken with the food. In a stomach which secretes little or no hydrochloric acid, and which is sluggish in emptying its contents, the chances for anaerobic development are good, and hence we frequently find, under these circumstances, that there are evidences of putrefactive decomposition of food that has been unduly retained in the stomach (*e.g.* production of sulphuretted hydrogen, mercaptan, butyric acid, etc.). Sometimes, too, we have definite infections of the stomach wall with bacteria which are facultative anaerobes (*e.g.* streptococci), and which are capable of inducing acute lesions of the stomach. On the whole, however, I think one may say

that in the course of chronic gastric affections, the number of anaerobic microörganisms in the stomach is seldom great.

We are able to form an opinion as to the bacterial conditions that prevail in the stomach because this organ is readily accessible through the aid of the stomach-tube. Of the conditions of bacterial life in the small intestine we know really very little, because of the inaccessibility of the contents of this portion of the digestive tract during life. It is, of course, well established that the duodenum and jejunum contain few bacteria and that these are usually not of the kind that produce pathogenic effects or induce putrefaction. For example, Cushing and Livingston [1] found that in gunshot wounds of the upper part of the small intestine, the bacteria which escaped into the peritoneal cavity did little or no harm — a result in sharp contrast with what happens after injuries at lower levels of the digestive tract. Again, if we take the intestinal contents from the jejunal or duodenal level of the gut of a person recently dead of an acute disease not especially implicating the digestive tract (*e.g.* pneumonia), it will be found that the bacteria, while acting readily on sugars, owing to the presence of *B. lactis aerogenes*, do not induce putrefactive decomposition on proteid media. There are exceptions to this

[1] "Experimental and Surgical Notes upon the Bacteriology of the Upper Portion of the Alimentary Canal, with Observations on the Establishment there of an Amicrobic State as a Preliminary to the Operative Procedures on the Stomach and Small Intestine," "Contributions to the Science of Medicine by the pupils of William Welch," p. 543, 1900.

rule, but they do not alter the general applicability of the statement that putrefactive microörganisms are commonly few in the upper two thirds of the small intestine. The chief factors in bringing about this state of relative freedom from anaerobic organisms are apparently the defensive action of the stomach juices and the rapid passage of the chyme through the upper intestine, which offers little chance for the multiplication of anaerobes, or indeed of bacteria of any kind. To what extent the moderate amount of oxygen present inhibits bacterial growth is uncertain.

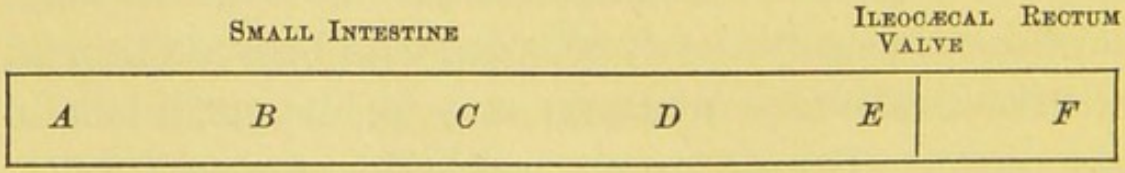
In the human ileum, the character of the bacterial decompositions in healthy persons appears to differ rather widely according to a variety of conditions, some of which are still obscure. Perhaps on account of the approach to the ileocæcal valve, which offers some degree of mechanical difficulty to the passage of the contents of the small intestine, there is an accumulation of bacteria in the ileum. This mechanical obstacle, in the human intestine and in that of many lower animals (*e.g.* dog), consists partly in the narrowing of the lumen of the gut, but partly also in the presence of inspissated intestinal contents on the further side of the valve, in the colon itself. In man there is within a foot or two of the colon a marked increase in the number of bacteria in the ileum, and this increase relates not merely to the total number of bacteria present, but also to the varieties. The mere accumulation of material affords opportunity for anaerobic conditions of life, and we find in fact that the numbers of strict anaerobes in the lower part of the

human intestine and in that of dogs is much increased in the ileum as compared with higher levels. Hence we find that the mixed fæcal bacteria taken from this level of the lower ileum are capable of inducing putrefactive changes in native proteids and in more simple nitrogen-holding media. This is true of the human ileum in health and at all periods of life, but the accumulation of putrefactive bacteria is even more pronounced in certain cases of disease — in other words, anaerobic conditions of bacterial life are exaggerated in pathological states. We may indeed look on the ileum as the debatable land of the digestive territory. In ideal health this region contains relatively few anaerobes and pathogenic bacteria; in acute and chronic intestinal pathological processes the ileum is probably inhabited by flora capable of producing substances which may damage the organism either locally or generally, and among these the anaerobes are apt to be prominent.

In the large intestine we find the most dense accumulation of bacteria and the best conditions for anaerobic growth. The transition from small to large intestine is in this respect very striking, more regularly so, perhaps, in dogs than in human beings. In dogs the large intestine is usually closely crowded with bacteria beyond the ileocæcal valve, whatever may be the conditions in the ileum. The anaerobic conditions are well maintained throughout the large intestine, and it is here that we find the greatest numbers of anaerobes and the most pronounced evidence of putrefaction. There is, however, a gradual fall in the number of living bacteria beyond the

ileocæcal valve, so that in the rectum the numbers of cultivable bacteria are very much less than in the ascending colon. But although in the ascending colon we have usually the greatest numbers of bacteria, it should be noted that their variety is often not so great as in the ileum.

With a view to learning something about the anaerobic conditions in the intestine, and their relation to the reducing power of bacteria, a number of experiments were made in which dogs were fed on meat containing methylene blue.[1] The animals were killed and the contents of the intestine examined under boiled water in order to determine at what point in the intestine reduction of methylene blue had occurred. The following typical result was obtained in one instance. A dog weighing fourteen and one-half pounds had received methylene blue in water, about two hundred milligrams in all. On the third day he received one hundred milligrams of methylene blue, placed inside a piece of cooked meat. He ate heartily of this meat and four hours after the meal the animal was killed and the intestine examined. The following diagram of the large and small intestines indicates the points at which the contents were examined.



The stomach was found to be about one-half full, and intensely blue. In the duodenum, at *A*, the intestinal

[1] I am indebted to Dr. A. J. Wakeman for the series of protocols on which these statements are based.

contents were intensely blue, the color diminishing in intensity toward *B* until at *C* the color had almost disappeared and had entirely disappeared between *C* and *D*. The contents of the intestine obtained from *D* blued only when exposed to air. When boiled with hydrochloric acid, water, and hydrogen peroxide, they became intensely blue. The contents of the colon (between *E* and *F*) were not blue under water, but became blue very quickly when exposed to air. The mucus adhering to the mucous membrane of the intestinal tract behaved in general like the intestinal contents as regards the color. This experiment indicates that beyond the middle of the small intestine the conditions become rapidly anaerobic or very nearly so, since the reduction of methylene blue in this concentration could not occur in the presence of the air. The main factors in the reduction process are, of course, the bacteria, and it is noteworthy that the reduction of the methylene blue begins at just that level of the intestine in which the bacteria begin to be numerous. There is probably in these cases not merely a reduction of the methylene blue, but decomposition of this salt, with the liberation of hydrochloric acid and the production of the methylene blue leucobase. This is indicated by the behavior of the leuco-body with hydrochloric acid.

THE BACTERIA OF THE HUMAN DIGESTIVE TRACT AT DIFFERENT AGES IN APPARENTLY HEALTHY INDIVIDUALS

While it is true that at all periods of life the human digestive tract is the seat of the life activities of myriads

of microörganisms, it is also true that the biological characters of these microörganisms are not the same at all times of life. If we compare the bacterial flora of apparently healthy persons of about the same age, we may find considerable differences in those parts of the digestive tract that are easily accessible to study, and yet, upon the whole, the resemblances in the biological characters of the bacteria so compared will ordinarily be more striking than the differences, provided the comparison be made between the flora of individuals living in a temperate climate and on the kind of food that is usual in countries commonly regarded as civilized. But if it were practicable to compare the bacterial flora of the same individual at different periods of life, it would be found that there are wide and perhaps characteristic variations — variations dependent in part on differences in food, but in part on other influences. Such comparisons have probably seldom been made in the same individual over a long period of time, but comparisons between different persons of unequal ages, but in general similarly conditioned, have been repeatedly made and support the statement that the normal bacterial flora characteristic of different ages, present different biological characters and are responsible for different types of decomposition in the digestive tract.

THE BACTERIAL PROCESSES IN THE DIGESTIVE TRACT OF NORMAL NURSLINGS AND BOTTLE-FED INFANTS

Nursling Infants. — In babies fed on mother's milk the alimentary tract is the seat of conditions of bacterial activity that possess a high degree of interest for one who wishes to obtain an insight into the physiology of digestion. For in nurslings one finds a relatively simple bacterial flora which gives a clew to the more complex and puzzling bacterial conditions that characterize normal adult life and many states of disease. The healthy nursling's bacterial flora is not merely relatively simple as regards the varieties of microörganisms; it is also remarkably definite in the individual child during the nursling period as well as in different children who are nourished on mother's milk.

By means of the Gram stain, carefully employed, it is possible to divide all the bacteria present at a given level into two groups, those which retain the stain and thus appear dark blue in the microscopical field (Gram-positive bacteria) and those which do not retain the stain (Gram-negative bacteria), and are hence susceptible to coloration by a counter-stain (a red dye such as fuchsin or saffranin being usually chosen). As these different ways of behaving to the stain correspond to biochemical peculiarities of the bacteria, we have at our

disposal a rough means of differentiation which is often of great service in the microscopical identification (in so far as this is possible) of bacterial types. If this method of study be supplemented by making aerobic and anaerobic cultures on gelatin plates, sugar-agar plates, and blood-agar plates (with or without sugar), one obtains a fair idea of the really representative bacteria present in the alimentary tract. Some of the bacteria present may, however, fail to grow on any of these media.

Gram-stained microscopical fields prepared from the normal slightly acid, orange-yellow, rather soft fæces of a nursling child from any portion of the large intestine, present essentially the same characteristic appearances. In the first place, the bacteria are seen to be extremely abundant — more so than in the fæces of normal adults and very much more so than in the meconium. A very large proportion of them are Gram-positive. The great majority of these Gram-positive bacteria present the same appearances and may give to a field the appearance of consisting of a nearly pure culture of one bacterial type. These bacteria are slender, often slightly curved bacilli of moderate size. They constitute the characteristic microörganisms of the human nursling's digestive tract and have been named (for reasons which will later appear) *B. bifidus* by Tissier, who first described them and took them out of the group of colon bacilli, with which they had been grouped by Escherich. Mixed in with *B. bifidus* is another Gram-positive bacillus, which is closely related, but grows readily on a strongly acid medium and has hence been designated *B. acidophi-*

lus (Moro). These bacilli are not surely distinguishable from *B. bifidus* in the Gram-stained field. Although *B. bifidus* thus dominates the typical field, other Gram-positive bacteria can always be found. Of these the most important (though very few in number) are large, plump bacilli, occurring usually singly, but sometimes in pairs. This is the *B. aerogenes capsulatus* (*B. Welchii* or gas-phlegmon bacillus of Fraenkel). As will be seen later, it may play an important part in the pathology of the digestive tract. Another Gram-positive organism which is regularly present in small numbers in the field is a diplococcus which frequently grows in chains. This coccus is commonly associated with a similar organism which is Gram-negative. The chief remaining Gram-negative bacteria are small coccal or coccoid forms and shorter and longer bacilli which by cultural methods can be shown to have the biological characters of the *B. lactis aerogenes* group and the *B. coli communis* group.

Thus by means of the Gram method of staining one finds that the typical field from the nursling's stool is predominantly Gram-positive and consists very largely of slender bacilli which but for their retention of the dye might be regarded as colon bacilli, and were indeed so regarded by Escherich, who in his original studies employed gentian violet as the sole staining agent. The fact that the bacterial fields from the nursling are Gram-positive is now familiar, but it came to those who first observed it as a distinct surprise. It was known that the *B. coli* group of bacteria are Gram-negative, and as

it was naturally assumed that the main bacteria of the large intestine of the nursling must be organisms of this group (as in the case of bottle-fed children), the failure of the nursling's fields to conform to this assumption at once threw doubt on the view that the nursling's dominant fæcal bacteria are really colon bacilli. The view was put forward that the Gram-positive character of the fields is due to the influence of the large fat content of the mother's milk,[1] but this had soon to be abandoned. It was only through the use of suitable cultural methods that the mystery was solved.

If one inoculates agar, gelatin, or bouillon media with the mixed flora got from a healthy nursling and cultivates the microörganism in the presence of air, bacteria of the *B. coli* type show themselves regularly to be the dominant forms, although they may be associated with diplococci, and sufficiently refined methods give evidence of the presence of the closely allied *B. lactis aerogenes*. If, however, one grows the mixed flora on a suitably prepared acid medium,[2] it becomes easy to restrict or even quite check the growth of the members of the *B. coli* group and to demonstrate the presence of a type of microörganisms which grows readily in this acid medium, has a morphology like that of the slender bacillary colon forms, coagulates cow's milk but does not act on human milk, and is definitely Gram-positive. This microorganism, or rather group of microörganisms, accounts

[1] Alex. Schmidt, "Zur Kenntniss der Bakterien der Säuglingsfaeces," *Wien. klin. Wochenschr.*, v, p. 643, 1892.

[2] Acid beerwort or acetic-acid bouillon, as suggested by Moro.

for a small number of the Gram-positive bacilli seen in the microscopical field. Moro, who first described these bacteria under the title of *B. acidophilus*, supposed that their presence explained fully the normal Gram-positive bacillary field, but this proved to be an error.

On subjecting the mixed bacterial flora to strict anaerobic conditions, Tissier found he could regularly cultivate from the nursling's fæces certain slender, Gram-positive bacilli of moderate length and thickness which not infrequently presented a bifurcated extremity and to which he accordingly gave the name *B. bifidus*. There can be no doubt that a large majority of the bacteria seen in the microscopical fields may be classed as *B. bifidus*. The bacteria belonging to the *B. bifidus* group possess a varied morphology which it seems desirable to notice here in view of the physiological importance of these bacteria during infancy. The appearance of the organisms is somewhat different according as we meet with them in the fæces or in culture media. In the microscopical fields they are recognizable under three chief forms: (1) a plain bacillary form,[1]

[1] The individuals of this class are rods of varying dimensions (length 3–5 μ; breadth, 0.2–0.4 μ). They are usually straight or slightly bent with somewhat pointed or rounded ends. Occasionally, owing to attenuation of one end and excessive development of the other, they assume a comma form. There is nothing characteristic about the arrangement of the bacilli in smears. Although distinctly Gram-positive, they vary much in the degree in which they retain the dye, both in material from the same infant at different times, and in different individuals. The entire bacterium is frequently not stained with even intensity, some parts of the organism retaining the color well, while other portions give it up. The result is the appearance which Escherich expressed

(2) a bifid form, and (3) a cephalated (knobbed) or headlet form. The simple form is by far the commonest,

in the course of his studies in the name "punctate" bacilli. These unevenly colored bacteria are commonly stained in their middle portion, while their ends are colorless, and this condition arises presumably on account of a central concentration of the protoplasm, which leaves relatively empty adjoining spaces. There are, however, other variations in the distribution of the colored protoplasm within the bacillus, which it is unnecessary to describe here. Frequently it will be noted that several bacilli are joined end to end, forming one long, unevenly stained structure. As the decolorized portion of the simple form of *B. bifidus* is still able to take up the counter-stain (*e.g.* carbol fuchsin) with avidity, we get apparently Gram-negative bacilli in those cases where the protoplasm has been rarefied throughout.

In addition to the simple forms of *bifidus*, a careful examination of the smear will sometimes reveal the presence of the bifid form from which the group takes its name. Sometimes the bifid form is very scanty or absent; at times it is moderately abundant; but it is never in health the dominant form. As pointed out by Moro, the bifurcation is peculiar in these bacteria in being a true division of the end or ends of the organism rather than a lateral branching, such as one sees in certain actinomyces. It is not necessary to describe here the numerous variations in morphology exhibited by these bifid bacteria. (For fuller details see Moro, "Morphologische und biologische Untersunchungen über die Darmbakterien des Säuglings," *Jahrb. f. Kinderheilk.*, lxi, p. 687, and p. 870, 1905; also Tissier, *loc. cit.* and *Ann. de l'Inst. Pasteur*, xix, p. 109, 1905; and Rodella, "Répartition des Microbes dans l'Intestin du Nourrison," *Ann. de l'Inst. Pasteur*, xix, p. 404, 1905.)

A third form of bacterium, which apparently belongs in the same group with the simple and bifid forms just described, is characterized by a small enlargement at one or both ends — the headlet or "Köpfchen" form. These headlet bacteria are seldom numerous in the fæces of normal nurslings, and it is usually necessary to look carefully over the field to find them, although they are seldom quite absent. The bodies of these bacteria are so like the bodies of the simple form in their morphology that one is tempted to regard them as simply slightly modified examples of this simple form — a view which is borne out by the results of

and in many preparations is the exclusive representative of the *bifidus* type.

cultural studies. Indeed, the microscopical picture itself shows transitional forms — bacilli with slight indications of the accumulation of protoplasm at one end and headlet forms which are bifid — which look like the simple and the headlet forms. Dumbbell forms are at times seen in which a headlet has developed at either end of a bacillus. In preparations which have been fixed by heat and treated with Lugol's solution it is possible to demonstrate the presence of granules in the bodies of the bacteria of the *bifidus* group, located at the peripheral ends of the organism and generally limited to this position. In hanging drops made from fresh material one finds that many of the rod-shaped bacteria possess a sluggish motility which is soon lost.

Certain relationships between the polymorphous Gram-positive bacteria which dominate the microscopical fields (prepared from the nursling's fæcal bacteria) can be made out by means of anaerobic cultures upon suitable media, such as agar or sugar-agar, containing acid. Pure cultures of the *bifidus* bacteria are not, however, always quite easy to obtain, as the *B. coli* group, and some diplococci, which are facultative anaerobes and grow well on the media just mentioned, are apt to restrict the growth of the less hardy *bifidus*. The development of colonies on sugar-agar is slow, for the tiny white lens-shaped smooth-edged growths first appear in the deeper parts of the sugar-agar after about three days. (Colonies like these in all respects except in having a toothed border are sometimes seen, and consist of mixtures of *B. bifidus* with *B. acidophilus*. On further inoculation from such colonies there grows, according to Kahn, only *B. acidophilus*.)

On microscopical examination of these colonies, it is apparent that we have to deal with differently shaped bacteria in different colonies and that even in the same colonies there is a tendency to polymorphism. Sometimes the bacilli (always Gram-positive) correspond closely to the simple form already described, both in their form and in the variations in their staining peculiarities. Some colonies are made up of bacteria very much larger than those of the simple form, and frequently knobbed or bifurcated or both. There seems to be little doubt that we are dealing here with various forms of *B. bifidus*, and the frequent development of bifid and headlet bacteria of varied morphology on the agar medium

The *B. putrificus* of Bienstock can sometimes be obtained from the nursling's intestine by employing a

which has been inoculated from the simple form of *bifidus* constitutes the best evidence of close relationship of these varieties. In lactose-bouillon fermentation tubes which have been inoculated from the fæces of nurslings one finds in the sediment large numbers of the bifid form of *B. bifidus*, after incubation for twenty-four hours.

In anaerobic sugar-agar plates made from the fæces of a human nursling, one finds colonies which develop before those made up of the *bifidus* type, especially colonies of *Staphylococcus pyogenes albus* (which sometimes grow luxuriantly on the surface), and at times one finds varieties of streptococci. An opaque, white, rumpled layer may be seen on aerobic plates made from the first dilutions of the fæcal material, which shows itself, under the microscope, to be made up of bacilli possessing motility which on further study prove to be the common potato bacilli (*B. mesentericus vulgatus*, Flügge).

In the study of the bacterial flora of the intestinal tract (especially in its lowest portions, colon, sigmoid, and rectum) the routine employment of pasteurization at 80° C. for fifteen or twenty minutes previous to making aerobic and anaerobic plates, is of much value. Most vegetative forms of bacteria present in the human intestine are killed by this exposure to heat, and only those races survive which can form spores and have done so. The application of this procedure is important both in the study of physiological conditions and for the detection of pathological peculiarities of the intestinal flora. On employing this method in the study of the intestinal flora of nurslings, it is found that by the use of suitable culture media, several forms of spore-bearing bacteria are present as regular or almost regular inhabitants of the lower intestine.

Among the microörganisms which are brought to notice by these methods is a variety which makes on agar small, cottony colonies, of somewhat indefinite outline, giving rise to small gas-production which may cause slight rents in the medium. These colonies consist of Gram-positive bacilli, often colored only in places by the dyestuff, which sometimes carry a knob at one end, which can, in certain instances, be shown to be spores. These spore-bearing bacilli are to be regarded as identical with the headlet bacteria already mentioned, and are thus the representatives of the resistant

medium of sterilized egg-white, but its presence in more than small numbers is exceptional and it may be entirely absent.

A more important anaerobic spore-bearing micro-organism than *B. putrificus* is *B. aerogenes capsulatus*,[1] which can be demonstrated by its growth on sterilized milk to which an abundance of fæcal bacteria has been added or by its ready growth on blood-agar with or without the addition of sugar. So long ago as 1894 Flügge found that the fæces of children nourished wholly on mother's milk contained Botkin's *Bacillus butyricus*, and we now know from the critique of Grassberger and Schottenfroh that Botkin's bacillus in reality represents two distinct microörganisms, each of which is capable

or spore-forming variety of *B. bifidus*. The headlet bacteria have a morphological resemblance to another spore-forming anaerobe, *B. putrificus*, which by appropriate methods of culture can sometimes be isolated from the nursling's stool. The resemblance is so close that in fæcal smears submitted to microscopical examination it is hardly possible to make a distinction with confidence.

[1] This organism is known by different names in different countries. The name *B. aerogenes capsulatus* was given to it by Professor Welch and is the name by which it is best known in the United States. The organism is identical with the gas-phlegmon bacillus of Fraenkel and probably with the granulo-bacillus *immobilis liquefaciens* of Grassberger and Schottenfroh. In France the organism is known as *B. perfringens* (Veillon and Zuber). In England Klein described an organism which he called *B. enteritidis sporogenes*, but it is doubtful if he was dealing with pure cultures, although it appears probable that he had *B. aerogenes capsulatus* in his cultures. A trinomial name is considered objectionable by botanists, and hence Migula proposed the name *B. Welchii* for *B. aerogenes capsulatus*, but the latter name is now so firmly established in the United States that it is doubtful if it will be displaced. Welch's bacillus is often spoken of as "the gas-bacillus."

of inducing decomposition with the production of butyric acid. Of these the *granulo-bacillus immobilis liquefaciens* is probably identical with *B. aerogenes capsulatus* (but not pathogenic), and was described by Passini in 1902 as occurring in the fæces of normal nurslings. This is undoubtedly true, but I wish to emphasize the fact that the organism normally occurs under these conditions in only very small numbers as compared with bacteria of the *bifidus* type. One may look through many Gram-stained fæcal fields without finding any evidence of its presence. The physiological significance of this fact will later be pointed out. The other organism which appears to have been included in Botkin's description of his *B. butyricus*, is one closely related to *B. aerogenes capsulatus* in morphology. It is, however, motile, does not liquefy gelatin, and readily forms spores — features which distinguish it sufficiently for the present enumeration [1] of the anaerobic flora. This organism has been found in the fæces of nurslings, but is of much less importance than *B. aerogenes capsulatus*. Occasionally, too, the nursling's fæces have been found to contain the bacillus of malignant œdema (Passini) and a very closely related microörganism which differs from the bacillus of malignant œdema in being Gram-positive and in making skatol.

One may summarize the results of cultural studies of the bacterial flora of normal human nurslings as follows. The stools and the contents of the large intestine from

[1] It is known as the *granulo-bacillus mobilis non-liquefaciens* of Grassberger and Schottenfroh.

these infants number among their inhabitants the following: *B. bifidus communis* and the headlet bacteria (which are probably varieties of *B. bifidus*), *B. coli communis*, and *B. lactis aerogenes*, *B. acidophilus*, *B. aerogenes capsulatus*, and certain intestinal streptococci.[1] It should be noted that of these bacteria the dominant form, *B. bifidus* (and in the upper part of the large intestine, *B. coli communis* and *B. lactis aerogenes*), is one which forms lactic acid on sugar media, is antiputrefactive, and does not attack proteids. It should also be observed that *B. aerogenes capsulatus*, the most important representative of the putrefactive type of intestinal bacteria, is an extremely scarce organism, and finally, that spore-holding bacteria and free spores are of rare occurrence (relatively) in the fæces and contents of the large intestine.

What has been already said regarding the results of microscopical and cultural investigation of the nursling's fæces makes it clear that the picture which we get from the former method is quite different from that furnished by the latter. Whereas one would conclude from the microscopical field that he was dealing chiefly with one race of bacteria (*B. bifidus*) supplemented by very few other forms, cultural methods which include anaerobic

[1] Less constant inhabitants are the following, *Staphylococcus pyogenes albus*, intestinal diplococci, *B. putrificus*, the motile butyric acid bacillus (*Amylobacteria* of Brüber or *granulo-bacillus mobilis non-liquefaciens* of Grassberger and Schottenfroh); *B. mesentericus vulgatus* (Flügge); certain actinomyces, especially *Actinomyces chromogenes;* sarcinæ; *Oidium albicans*, and various yeasts.

studies on suitable media show that the flora is in reality more varied and that forms physiologically important are present of which one obtains no suggestion from the fields alone. Such a picture, taken by itself, is misleading in certain respects, but representative in others. The microscopic fields of the fæces showing mainly one form represent with a fair degree of accuracy the bacterial conditions in the fæces, for here the other types are in reality very feebly represented. The cultural methods which it is almost impossible to employ in such a way as to make them give an accurate insight into the actual quantitative relations of the bacteria present, might easily mislead one into supposing that the subsidiary varieties thus brought to light are more abundant in the fæces than is actually the case. But if we look at these cultural results as indications, not of the quantitative relations of the fæcal flora, but rather as indices of the conditions prevailing at higher levels of the large intestine, where one type is no longer so dominant, these results become instructive instead of confusing.

Distribution of the Bacterial Flora in the Digestive Tract of the Nursling. — Although the distribution of the bacteria of the digestive tract in normal or approximately normal nurslings has not yet received much attention, it is a subject which cannot be ignored, for it is capable of giving us information in regard to the nature of the decompositions that are possible at different levels of the tract in health, these decompositions being often of a different nature in disease from those which occur in

health. Escherich, one of the few students of this subject, found that the number of intestinal bacteria increases gradually from above downward in the small intestine, undergoing a sudden and extreme increase at the level of the cæcum. Even as high as the duodenal level moderate numbers of microörganisms could be found corresponding to the types of the *B. coli* and *B. lactis aerogenes* groups. Schmidt, making use of Weigert's method for staining fibrin, noticed that a preponderance of Gram-positive over Gram-negative forms does not begin until the level of the colon is reached. From the middle of the colon downward, the characteristic Gram-positive fæcal flora begins to dominate the picture and appears exclusively present in the rectum — a striking evidence of the fundamental difference between the flora of the upper and lower portions of the intestine.

I have had an opportunity to make microscopical examinations (using Gram's method of staining) of the contents of the digestive tract of nursling infants dying within the first six months from conditions not closely connected with the digestive tract (*e.g.* acute bronchopneumonia). On comparing the results of these examinations with those obtained by Moro by the use of the Escherich-Weigert fibrin stain, they were found to be so nearly alike in their leading features as to make it probable that the following description, based on these two sets of observations, is generally applicable to the case of healthy nursling children.

In normal nurslings the mouth contains few bacteria, and such as it contains are for the most part organisms

derived from the skin and nipple — *Staphylococcus pyogenes aureus*, bacilli of the *B. coli* group and *B. lactis aerogenes*. In the stomach the number of bacteria is also small, and the bacterioscopic picture shows usually a few positive or negative diplococci or streptococci or negative cocco-bacilli or positive or negative bacilli, suggesting the *B. coli* and *B. lactis aerogenes* group. The duodenum and jejunum generally contain remarkably few microörganisms, owing mainly to the rapidity with which the food passes through this region. When food in the jejunum is present in larger amount than is usual, the numbers of bacteria are correspondingly increased, but it is the rule that the greater part of the small intestine is almost free from bacteria and that they do not become really numerous until the level of the lower ileum is reached. Here there is almost regularly an abrupt increase in the bacterial flora. In the cæcum (and likewise in the vermiform appendix) the bacterial increase is enormous. The normal bacteria of the greater portion of the small intestine are Gram-negative, short bacilli of the *coli* and *lactis aerogenes* variety, mixed sometimes with a few positive and negative coccal forms. In the lower ileum the Gram-negative forms become mixed with those of the *bifidus* type, especially the punctate bacilli. The transition from lower ileum to cæcum marks, as a rule, a striking change in the proportions of *coli* and *bifidus* types, since the former lose their dominant numerical position. This ascendency of the *bifidus* type is so great in the transverse and descending colon that all other bacteria in these regions are numerically incon-

spicuous and, as in the rectum, this type has the appearance of being present in pure culture.

The bacterial flora of the intestinal tract of the nursling is thus only moderately numerous as regards variety. The bacteria are concentrated in the regions that lie between the lower ileum and the anus, the ileocæcal region presenting more kinds of bacteria than the lower colon and more organisms capable of being cultivated. The comparatively small numbers of bacteria found in the small intestine has its explanation partly in the small amount of food that lodges there, partly perhaps in the bacteriolytic action of the succus entericus. Wherever particles of transformed casein are found, there also will be found bacteria in abundance, but with the exception of the lower ileum the small intestine does not harbor food masses to any considerable extent. The bacteriolytic action of the succus entericus is moderate but appreciable. The epithelial cells are said to contain an antitryptic ferment, and this passes to some extent into the succus entericus, where it is perhaps capable of exerting a restraining influence on that peptonization of proteids which is the first essential step toward putrefactive decomposition.

Various attempts have been made to form a numerical estimate of the numbers of living and dead bacteria present at different levels of the colon and to determine what proportion of bacteria have been rendered lifeless during their descent through the colon. Eberle, who compared the numbers of bacteria seen in microscopical preparations with the results of plate cultures,

concluded that from four and five-tenths to ten per cent. of the fæcal bacteria are capable of growing on the common culture media. Klein got only about one per cent. to grow; Schmidt and Strasberger, basing their estimates on a different method of procedure, which involved weighing masses of bacteria in order to avoid the errors incidental to counting, came to the conclusion that only about 0.07 per cent. of the bacteria are viable. It is, however, easy to see that none of these methods can claim to give even approximately correct results, because we have in the nursling's fæces many anaerobic bacteria which grow only under special conditions, and acidophile bacteria which require special media for their cultivation, and these special conditions have not yet been furnished by those who have made numerical estimates. Moreover, it is extremely probable that on their way through the large intestine many bacteria undergo autolysis and wholly disappear. Hence the problem of determining the fate of the bacteria in their descent through the nursling's colon is one beset with difficulties so considerable as to make numerical comparisons an unpromising field for study. There can, however, be no doubt that, as already stated, the living bacteria of the intestine usually undergo a marked diminution in numbers on their passage through the colon and that a large proportion of them seen under the microscope in preparations from the fæces are not viable, even on anaerobic plates. The histological signs of disintegration are often visible. Among the causes of the death of intestinal bacteria, under normal conditions,

the diminution of food and the lack of moisture probably are among the most effective.

A satisfactory study of the products of the mixed fæcal flora from normal nurslings has not yet been made. One fact nevertheless stands out, namely, that on sugar bouillon containing blood the volatile acid or acids produced give a molecular weight corresponding closely to that for acetic acid. The presence of only insignificant amounts of higher volatile fatty acids points to the absence of considerable numbers of anaerobic putrefactive bacteria, since these form the higher acids, provided anaerobic conditions be secured.

In harmony with this is our observation that ten or twenty cubic centimeters of a ten per cent. suspension of fæcal bacteria from a normal nursling may be injected into a rabbit, which is then incubated, without inducing a gas-liver from putrefactive anaerobes (*B. aerogenes capsulatus*).

The mixed fæcal flora when grown on plain bouillon makes indol, doubtless owing to the multiplication of colon bacilli.

The Infection of the Nursling's Digestive Tract and the Relation of the Microörganisms to the Permanent Bacterial Flora. — One naturally asks the question: What is the origin of the normal intestinal flora of the nursling child? How does it happen that the meconium, which is a sterile medium derived from the intestinal juices and epithelial cells, becomes the seat of bacterial life? As the mother's milk is normally free from bacteria when it issues from the breast, one cannot attribute the

first infection of the digestive tract to necessary bacterial inhabitants of the milk. But since a portion of the milk may come in contact with the nipple, and the child's lips come into contact with the skin of the breast as well as the surface of the nipple, it is clear that any bacteria living on or near the nipple may find their way into the digestive tract of the child. It might be supposed that the gastric juice or the enterokinase of the small intestine would form an obstacle to the passage of living bacteria into the intestine, but it has been well demonstrated that these defenses even in the fully developed form in which they exist in adults, are only partial, and that many living bacteria readily pass from the stomach into the small intestine in newly born children. In the case of very young children, the secretion of a bactericidal gastric juice is probably but a feeble protection against the passage of living bacteria into the intestine, and even were it in part effective, there might frequently be opportunity for bacteria, located in the interior of curds, to pass from the stomach into the intestine. Moreover, if the bacteria were present in large numbers in the stomach, some of them would almost certainly pass into the intestinal tract, although, as has already been seen in discussing the distribution of the bacteria of the intestinal tract, it is usual to observe a considerable falling off in the numbers of microörganisms as one passes from the stomach to the duodenum and jejunum. It seems probable, then, on the grounds already stated, that an infection of the intestine by bacteria occurs through the mouth. This probability was rendered a certainty by

the observations of Escherich on a case of atresia ani in which he was able to find in the intestine the obligate milk stool bacteria, *B. coli* and *B. lactis*. Moro, furthermore, found *B. acidophilus* in human milk and on the areola in nursing women and drew the inference that this organism reaches the intestine by way of the mouth. The passage of this particular organism which is so resistant in acid media is, of course, not surprising. Other observations were made by Moro upon human milk in the hope of finding there *B. bifidus communis* and of thus explaining the presence of this microörganism or group of bacteria in the intestine of nursling children; but although he made numerous anaerobic sugar-agar cultures from various samples of human milk (where no precautions had been taken to cleanse the nipple), he was unable to obtain anything except negative results, although relatively large quantities of milk were regularly used in making the cultures. Moro was indeed unable to cultivate any strictly anaerobic bacteria from human milk. On the other hand, he regularly found *Staphlococcus albus*, which is well known to be present on or near the epidermis; sometimes colon bacilli and pseudodiphtheria bacilli; and in exceptional cases streptococci, sarcinæ, and *Micrococcus candicans;* and once a fluorescent bacillus, but never anaerobes.

A further observation bearing on this question was made by Moro. A newly born child received only sterilized milk from the second until the sixth day. The excretion of meconium took place in the usual way and the characteristic bacterial types were found in it.

The first milk stool appeared on the fifth day and contained the physiological flora. It is evident, therefore, that the organisms present in this case must have had another source. The possibility of the presence of *B. bifidus* in the mouth of the child was then excluded by the observation that anaerobic organisms do not grow from the mouth secretion of newly born children. As it thus appeared clear that the infection of the intestinal tract by *bifidus* does not occur by way of the mouth, there remained only one possibility; namely, that the infection occurred through the anus. A study of the organisms of the meconium has made it appear very probable that this is the mode of infection with *B. bifidus*.

The bacteria of the meconium were first carefully studied by Escherich, who pointed out that the originally sterile meconium very easily acquires bacteria characterized by their relative sparcity and considerable variety and the presence of certain spore-bearing organisms.

Among the constant flora are coccal forms in relatively large numbers; some short bacilli, including *B. coli communis*, and some varieties of spore-bearers. One form of spore-bearer is the so-called headlet bacterium already mentioned, and a thicker form which was identified by Kohn as *B. subtilis*. On gelatin plates the most abundant organisms are usually *B. coli communis*, which is sometimes associated with liquefying streptococci. In fact, the presence of liquefying bacteria in the meconium is rather characteristic of the meconial period, and this peculiarity, under normal conditions, ceases abruptly

with the onset of the milk-stool period. *B. lactis aerogenes* was not found in the meconial stools. It appears from Moro's observations, which I can corroborate, that bacteria of the *B. coli* type may appear in agar plates as early as ten hours after birth. Somewhat later, various coccal forms may appear; in the course of forty-eight hours the bacterioscopic fields may show bacilli which are Gram-positive and which in some instances show typical bifurcations and through anaerobic culture on sugar-agar may prove to be *B. bifidus communis*. Still a little later, *B. aerogenes capsulatus* may make its appearance, and about this time, too, spore-bearing cylindrical bacilli may appear. The very early occurrence of *B. bifidus* in the meconium is a striking fact, since it appears hardly possible to explain its presence there so soon after birth on the supposition that *bifidus* has entered by the portal of the mouth. It appears, therefore, upon the whole, extremely probable that the infection of the gut in newly born children with *B. bifidus communis* is by way of the anus and that the air is the source of the infection. It is interesting to note that even before the appearance of *B. bifidus* in the meconial stools the colon bacillus is represented there. The presence of the colon bacillus, like the presence of *B. bifidus*, cannot be explained at this early period by an invasion through the mouth, although, as already stated, the colon bacillus may enter the intestine by way of the mouth at a later period. Both these organisms, *B. bifidus* and *B. coli*, possess motility, and it is probably by virtue of this motility that they find their way into the lower intestinal

tract, and into the meconium on which they are able to live, although it is only a fair culture medium. The meconium thus contains forms which are the forerunners of the permanent intestinal bacteria. In the case of *B. aerogenes capsulatus*, it appears to me that its presence in the meconium can hardly be explained on the ground of its possessing motility, since it is questionable whether this organism is ever at all motile, and certain that as a rule it is non-motile. It is possible that it may be found in the meconium in extremely small numbers as a result of contamination at the time of the passage of the meconium, since *B. aerogenes capsulatus* is so widely distributed and so common an inhabitant of the air. Possibly, also, the same explanation would apply to the presence of *B. putrificus*, a spore-bearing organism which is common in dust.

We see thus that while non-motile forms of organisms like *B. lactis aerogenes*, *B. acidophilus*, staphylococci, and streptococci can find their way into the intestinal tract only by way of the mouth, through the milk (being unendowed with motility), the members of the colon bacillus group may invade the digestive tract either through the mouth or the anus, and, finally, that *B. bifidus*, the chief obligate bacterium of the milk-stools of nurslings and a regular and permanent inhabitant of the meconium, reaches the intestinal tract only by way of the anus. It is noticeable that the meconium contains a considerable proportion of spore-forming bacteria; that is, of bacteria capable of resisting unfavorable influences. Whether they find their way into the intes-

tine as spores or in their vegetative form is not clear and is probably not important. They do, however, sporulate in the meconium, which may perhaps be interpreted as indicating that the meconium is a poor rather than a good nutrient medium for these organisms, although I do not feel sure that this interpretation is absolutely safe. It appears that the meconium is in general a poor medium for bacteria, and this fact explains satisfactorily its relative poverty in bacteria. The poor nutrient character of the meconium may be due in part to its inspissation and in part to its relatively high content of bile acids, which would tend to prevent the rapid multiplication of invading organisms. It is doubtless true that the meconium, in consequence of these properties, exercises a selective action on the invaders of the lower intestine, discouraging all those vegetative forms that are non-resistant either through the inability to adapt themselves to the peculiar conditions of the medium or through the inability to form spores. The meconium is therefore to be regarded as possessing physiological functions instead of being an indifferent substance. The main physiological function of this meconial plug is apparently the exclusion of numerous foreign types of bacteria and the encouragement of those organisms which are destined to become the permanent inhabitants of the nursling's digestive tract.

Bacterial Flora of Bottle-fed Children. — If one makes a comparison of the bacteria of the digestive tract of children fed on cow's milk with the flora which has

already been described as characteristic of the digestive tract in breast-fed children, one finds many points of resemblance but also some typical and important differences. In general it may be said that the number of bacterial forms present in the digestive tract of children nourished with cow's milk is considerably greater than in the case of breast-fed children. This is true even where children are fed on cow's milk that has been sterilized by boiling or by pasteurization. And the difference in number is even greater in those cases where sterilization has not been practiced, since this procedure destroys a large proportion of the bacteria present in the milk and especially the lactic acid producers. Where sterilized milk is employed the increase in the number of bacteria in the digestive tract as compared with the number of varieties present in the intestines of breast-fed children is dependent at least in part upon the presence of anaerobic bacteria or facultative varieties capable of forming spores.

That the number of bacteria should ordinarily be greater in children fed on cow's milk than in children fed on mother's milk can cause no surprise, when one remembers how abundant are the microörganisms present in most specimens of cow's milk. The number ordinarily ranges from one or two thousand bacteria per cubic centimeter [1] in cow's milk that has been collected with the utmost precautions in regard to cleanliness of preservation, up to several million per cubic centimeter.

[1] Under very special conditions milk can be obtained which has a far smaller number of bacteria.

Although the character of the bacteria is much more important than their number, those specimens of milk which contain the largest numbers are also most likely to contain the forms which, aside from the inciters of typhoid fever and dysentery, are most injurious; that is, especially, the streptococci and the putrefactive anaerobes. Ordinarily good milk frequently contains from thirty to one hundred thousand bacteria per cubic centimeter. Thus we see that there is good reason why the mouth and stomach are liable to contain much larger numbers of bacteria in the case of children fed on cow's milk than in the case of breast-fed children, and it is further clear that the actual number will vary somewhat with the character of the milk that is employed. As in the case of breast-fed children, there is a marked diminution in the number of bacteria found in the upper part of the small intestine as compared with the bacteria in the stomach. This diminution, which probably depends in part on the bactericidal action of the gastric juice and intestinal juices, tends to bring about in the small intestine a greater equality in the number of bacteria in breast-fed and bottle-fed children than is observed at higher levels. Nevertheless, even in the lower part of the small intestine and throughout the large intestine, the number of bacterial species appears to be as a rule greater in the case of children fed on cow's milk than in the case of nurslings.

Many of the bacterial forms which have already been described as regular inhabitants of the nursling's intestinal tract are also inhabitants of the digestive tract of

bottle-fed children. We find especially that this resemblance holds true with regard to the *B. lactis aerogenes* group, the *B. coli* group, the *B. acidophilus* group, and the group of organisms which we have come to know as *B. bifidus*. As regards *B. bifidus*, however, we find that there is a striking difference between the nursling and the bottle-fed child. In nurslings the *B. bifidus* is the dominant type throughout the greater part of the large intestine; in the bottle-fed child the group of *B. bifidus* is fairly well represented, as a rule, throughout the large intestine and also in the lower part of the small intestine, but it is by no means the dominant organism. The place of preponderance is, in this case, occupied by organisms of the *B. coli* type, and we thus find that the microscopical picture in the case of the bottle-fed child shows us a field in which a majority of the microörganisms present are Gram-negative instead of Gram-positive, as is the case in nurslings. In addition to these leading types of organisms in the case of bottle-fed children we have to include certain positive and negative diplococci, some of which are seen in chains while others appear as diplococci only. An organism described by Kruse,[1] and known as the *Streptococcus lacticus*, is almost always present and appears to be identical with the streptococcus of Hirsh-Libbmann. It possesses the power of coagulating milk. Among the less constant aerobic bacteria are the white staphylococci. Sometimes a

[1] "Das Verhältnis der Milchsäurebakterien zum Streptococcus lanceolatus (Pneumoniecoccus, Enterococcus, u.s.w.)," *Centralbl. f. Bakt.*, 1st Abt., Orig., xxxiv, p. 737, 1903.

prominent inhabitant is *B. cloacæ*, which is a more active gas former than *B. coli*. In fermentation tubes containing milk it may induce an active gas production and disruption of the coagulum which suggest the action of *B. aerogenes capsulatus*. Sarcinæ are often found, both in the small and large intestine, and white yeasts are occasionally present. As was first pointed out by Rodella,[1] there are several forms of bacteria present in the large intestine which are capable of dissolving and peptonizing casein. These forms, which are sometimes facultative anaerobes, sometimes obligate anaerobes, are usually distinctly more abundant in bottle-fed children than in nurslings. *Staphylococcus pyogenes albus* is one of the peptonizing forms bacteria often present and possesses the ability to liquefy both casein and gelatin. Probably in the case of bottle-fed children this organism is less important as a liquefier than some of the facultative and strict anaerobic bacilli.[2]

[1] "Ueber die Bedeutung der im Säuglingsstuhle vorkommenden Mikroorganismen mit besonderer Berücksichtigung der anäeroben Bakterien," *Zeitschr. f. Hyg.*, xli, p. 470, 1902.

[2] The facultative and strict anaerobes are much more abundant in the large intestines of bottle-fed children than in the intestines of nurslings. I attach especial importance to the presence of *B. aerogenes capsulatus* which, as already mentioned, is present in only small numbers in the case of breast-fed children. The number of these organisms is not necessarily larger in the intestines of children fed on cow's milk, but as a rule even in children considered quite normal, their number, though still small, is greater than in the case of nurslings. As compared with the number present in the fæces of adults these bacteria are few. Another anaerobic organism which is sometimes found is the motile butyric acid bacillus of Schottenfroh and Grassberger. Furthermore, *B. putrificus* can be found in some instances in the stools of bottle-

Products of Decomposition in the Intestinal Tract of Bottle-fed Children. — It has already been pointed out that the products of intestinal decomposition in normal nurslings are remarkably small in amount when we consider the large numbers of bacteria that inhabit the lower part of the intestinal tract. Almost the same thing holds true of the intestinal tract of bottle-fed children. If we make extracts of the contents of any portion of the large intestine from a normal, bottle-fed child, we find by the most delicate methods merely a trace of indol, or even no trace at all. Traces of skatol are much less frequent than traces of indol. Corresponding to the fact that indol is in health always slight in amount and frequently absent, is the observation that aqueous extracts from any part of the contents of the large intestine give no reaction with an acid solution of dimethylamidobenzaldehyde. It will be shown when dealing

fed children, but their number is usually small and their presence inconstant. The bacillus of malignant œdema has also been found in the stools of children fed on cow's milk, but it is likewise inconstant, and I believe ordinarily of little physiological importance. The anaerobes just mentioned — the motile butyric acid bacillus, *B. aerogenes capsulatus*, *B. putrificus*, and the bacillus of malignant œdema, are all spore-producers, although they do not produce their spores with equal readiness. *B. aerogenes capsulatus* indeed sporulates only under special conditions and probably seldom in the intestinal tract of a normal bottle-fed child. The number of spores present in the fæces of bottle-fed children who may be regarded as normal is ordinarily very small as compared with the numbers found in some conditions of disease. The character of the spores formed is at present uncertain; that is to say, it is difficult to decide in the case of free spores to what organisms they belong. Where sporulating bacilli are present, they are probably not members of the group of *B. aerogenes capsulatus*.

with the contents of the digestive tract in adults that a strong red coloration with this aldehyde is commonly obtained in persons in fair health and that a moderate reaction is usual in persons who are well. The reaction depends in part on the presence of indol and perhaps in part also on the presence of urobilinogen. In the case of a bottle-fed child, there is usually only a faint pink tinge when the conditions of the reaction are such as will give a distinct red in the case of material from adults. On standing, however, for several days, a blue-gray coloration may set in, the nature of which is not at present clear.

If we acidify a watery suspension made from any portion of the contents of the intestinal tract, we find that a moderate amount of volatile acid is obtained in the distillate. The molecular weight of such acids lies usually between sixty and sixty-five; that is to say, it corresponds closely to the molecular weight for acetic acid. There is no doubt that acetic acid forms by far the larger amount of the acids normally present in the distillate prepared as just mentioned. The presence of acetic acid rather than of higher fatty acids indicates that such bacterial processes of decomposition as occur within the intestinal tract are of a fermentative rather than of a putrefactive nature. It is a fact of interest that if we take the intestinal contents of a normal horse and make a distillation for the determination of the volatile acids present, this volatile acid also corresponds in molecular weight to the figures corresponding to acetic acid. The same thing is true of young pigs. These facts, and others

which might be brought forward, plainly point to the presence of acetic acid fermentation as a normal process in many healthy animals. If the material employed be distilled after the addition of alkali instead of acid, one obtains the volatile alkali; namely, ammonia. The quantity of ammonia found is moderate and about suffices to neutralize the acetic acid which is present.

Action of the Mixed Fæcal Flora upon Various Media. — As already mentioned in describing the action of the mixed fæcal flora from the nursling, the products of bacterial action are determined in important ways by the nature of the medium, and both their quality and quantity are influenced in important ways by the readiness with which bacteria grow upon the various media. If we grow the mixed flora from a normal bottle-fed child upon sugar bouillon, there is always a production of gas in the fermentation tube, and this production of gas is usually greatest in lactose bouillon. A less production of gas is seen in dextrose and levulose and the slightest is observed in saccharose. By making strongly alkaline with sodium hydroxide one may measure the amount of carbon dioxide that has been formed, and such observations have shown that from one-third to two-thirds of the gas produced under these circumstances is carbon dioxide. The composition of the remaining gas is not yet definitely known, but it consists mainly of hydrogen and probably in part of methane. It will be found on using litmus paper that there has been a production of acid at the same time that gas was produced. Acetone is not usually detectable in sugar-bouillon

cultures. If the sediments in fermentation tubes prepared in the manner just mentioned be examined microscopically, it will be found in every case that there has been a fairly abundant growth of microörganisms of the *B. coli* type. The staphylococci and Gram-positive diplococci will also be found to have multiplied in the anaerobic limb of the tube. As a rule there is little or no evidence of the development of strict anaerobes.

The gas production and the acid formation which have been noted are probably to be ascribed almost exclusively to the action of organisms of the *B. coli* group. The presence of streptococcal forms in abundance appears to inhibit the formation of gas, probably by checking the development of the colon bacilli.

If suspensions of the mixed flora be inoculated into the fermentation tubes containing bouillon without sugar, the results of the bacterial decomposition are different. Here we find either very small gas production or none whatever, and such acids as may be produced are, moreover, bound by alkali, so that the medium, originally neutral, either remains so or becomes faintly alkaline. There is a moderate production of ammonia. This ammonia is united mainly to acetic acid. There is also a production of hydrogen sulphide under these circumstances. Not rarely there is a small quantity of mercaptan, but as a rule the amount of mercaptan produced is little more than a trace, and very frequently — perhaps usually — it is wholly absent. The formation of indol is a constant phenomenon on the bouillon medium, and

in the course of twenty-four or forty-eight hours a moderate quantity is usually formed. Skatol has not been observed, but traces of phenol were regularly found.

Although the putrefactive decompositions in the intestinal tract are normally very limited in both nurslings and bottle-fed children, they are often somewhat higher, as the foregoing pages indicate, in the case of breast-fed children. The reasons for this are not entirely clear.

The urine of healthy bottle-fed children does not differ from that of nurslings in any essential particulars. The ethereal sulphates perhaps tend to be excreted in rather larger amounts in proportion to the total nitrogen and preformed sulphates. Indican is usually absent or present only in traces.

THE BACTERIAL CONDITIONS IN THE DIGESTIVE TRACT DURING CHILDHOOD, ADOLESCENCE, ADULT LIFE, AND SENESCENCE

THE bacterial conditions in the digestive tract between the period of childhood and that of old age differ in health so considerably from the conditions that exist during the period of milk feeding that they call for separate consideration. The difference depends mainly on the character of the diet, which grows more varied at the end of the milk period and stays so throughout the remainder of life. With the more varied diet comes increased opportunities for the entry of many sorts of bacteria into the digestive tract. It is impossible to briefly picture the bacterial conditions in the digestive tract in such a way as to take account of the many individual peculiarities which are met, but fairly typical descriptions are possible. It must be distinctly understood that these descriptions are based on well-cared-for individuals and not on the study of neglected persons or persons following peculiar occupations which subject them constantly to irregular conditions of life.

Period of Childhood and Adolescence. — The bacterial conditions in childhood and during adolescence differ somewhat from those of later life, and it is essential to recognize these differences in any discussion of such conditions. During normal childhood and adolescence

all the bacteria described as present in the tract during the period of bottle-feeding are likely to be still present, for the use of cow's milk is generally continued into the epoch of a mixed diet. Microörganisms of the *B. bifidus* type are usually much less numerous in the intestine in childhood and adolescence than in the cow's-milk period, but other types of bacteria are commonly more numerous. This is true of the putrefactive anaerobes, certainly of *B. aerogenes capsulatus* and probably of *B. putrificus*.[1] Still the number of these anaerobes is small[2] and putrefactive processes in the intestine

[1] An organism corresponding to Nencki's *B. liquefaciens ilei* is usually abundant in the lower ileum, ascending colon, and perhaps the cæcum. I have not yet had an opportunity to study the biochemical characters of this organism, but suspect that it may be a vegetative form of *B. putrificus*.

[2] The following notes relate to typical microscopical fields prepared from the digestive tract of a boy aged fourteen years:—

CASE OF SUDDEN DEATH, MALE, ÆT. 14

Autopsy showed heart dilated, aorta 1.5 cm. in diameter; stomach intensely congested; intestines normal. The skull was not opened. There was no history of infectious disease to explain the cardiac dilatation, and no endocarditis. No cause for this sudden death was revealed by the autopsy findings. The slides were made two hours after death.

Microscopical Findings

I. Stomach. Very few bacteria are seen. There are small numbers of positive diplococci, sometimes in chains, all medium-sized. A few short, negative bacilli and a few small, negative diplococci are also seen.

II. Duodenum. There are a few positive diplococci; otherwise no bacteria. Considerable masses of epithelial cells and very few small negative diplococci are found.

III. Jejunum. A few medium-sized, positive diplococci and

are not active. This is shown by the character of the urine and by the nature of the decomposition products

negative cocci, ill-defined as regards staining, are seen. Epithelial cells are numerous. The number of bacteria here is extremely small.

IV. Ileum. Bacteria are more numerous here, but the number of varieties is small. There are a few forms resembling *B. coli*, short, stout, negative bacilli; also bacilli of about the same width and three to four or five times the length of typical *coli;* also a medium-sized or rather large, positive diplococcus and a few small, positive bacilli about the size of colon bacilli. The long, slender, Gram-negative bacillus is rather prominent. Sometimes it takes the form of a fairly long thread. Considerable epithelium is present in this section.

V. Cæcum. This field presents an entirely different picture from the preceding. The bacteria are numerous. The field is mixed about evenly as regards the staining. Negative bacteria consist of three forms — small diplococci which are moderately numerous; short bacilli, suggesting colon bacilli in the typical form; slender, negative bacilli, more slender than colon and two or three times as long, sometimes in threads. The positive forms consist of oval diplococci medium-sized, occasionally large, single, oval cocco-bacilli, a few forms suggesting colon bacilli (but these are not numerous) and a very few organisms the size and form of *B. aerogenes capsulatus*, though tending to be a little small for this organism; and a few positive bacilli of this type containing spores located between the middle and end. No free spores are observed. The rather long, stout negative bacilli observed at higher levels are also seen occasionally. There are a few slender, negative bacilli showing irregularities in staining; that is, "punctate" bacilli.

VI. Sigmoid flexure. Bacteria are numerous. The negative forms are more prominent than in the last level. Organisms of the colon type are numerous, as are also slender, negative bacteria. Positive diplococci are considerably less numerous than in higher levels. There are moderate numbers of organisms suggesting *capsulatus*, but their number is, however, very small in comparison with the total number of bacteria. Spore-holders are not seen, nor are free spores.

VII. Rectum. The fields are mixed. Colon bacilli are numerous,

in the fæces. Indol and phenol are found in the fæces in only very small quantities — a few milligrams in one hundred grams of the moist fæces. Frequently, indeed, only a trace of indol is detectable in the intestinal contents. The character of the urine as regards putrefactive products corresponds to the fæces. The ethereal sulphates are low in proportion to the preformed, and there is little or no indican in the urine. There is also little phenol. The reaction with dimethylamidobenzaldehyde is slight or moderate. Frequently it is so slight that its existence is questionable. During a period of temporary digestive derangement the ethereal sulphates may show a considerable increase, and indican may be present, but as the derangement subsides the urinary indications of putrefactive decomposition in the intestine likewise grow less and gradually fall back to the previously existing normal level.

Period of Adult Life. — During adult life factors usually enter into the lives of human beings which are not without influence upon the bacterial conditions that prevail in the stomach and intestine. Great differences exist in the habits of different persons at this time of life, and these differences are in a degree reflected in the nature of the bacterial processes that

as are also slender, negative bacilli. There are moderate numbers of positive diplococci and diplobacilli. Organisms of the *capsulatus* type are a little more numerous than at the last level. There are a very few free, oval spores, and a few spore-holding bacilli, very much enlarged, suggesting clostridia. There are also some few slender Gram-positive bacilli about the length of *B. aerogenes capsulatas* or longer, but about one-half the diameter of *capsulatus*.

hold sway in the digestive tract. In adult life the individual experiences new responsibilities, new ambitions, new dangers, an enhanced emotional life, and in very many instances a marked change in the direction of more sedentary habits incidental to a larger proportion of indoor life. The dietary is apt to undergo an alteration in the direction of increased and frequently injudicious liberty and the use of tea and coffee, also the use of tobacco and alcoholic drinks is either increased or begun. For a time these influences may not make themselves definitely felt, but sooner or later they lead to slight derangements of digestion which manifest themselves clinically. There are of course the greatest individual differences in respect to these manifestations. One sometimes meets with persons more than fifty years of age in whom the bacterial conditions in the intestine do not differ materially from those observed during the period of normal adolescence. These persons are usually well nourished, muscularly strong, and capable of sustained mental and muscular work. They have a high percentage of hæmoglobin and their red blood cells approximate the upper physiological limit in regard to number. The volume of blood is large. These persons are remarkably free from nervous disturbances, even under trying conditions of work. They generally have at their disposal a surprising amount of reserve energy.

These are, however, exceptional instances of robust health. A large portion of the population in the United States presents different physical conditions by the time

they reach the fiftieth year of life, although they are in no sense in a state of invalidism. They are able to do large amounts of work without excessive fatigue and most of the time they feel well. While in such persons the fæcal flora shows nothing striking, it is usually not difficult to demonstrate that the number of putrefactive anaerobes in the intestine is larger than is the case with healthy individuals in the period of adolescence. The presence of *B. aerogenes capsulatus* can be determined by anaerobic plating in blood-agar, and the number of colonies will be found ordinarily to exceed the number obtainable from the fæces in the earlier period of life. *B. putrificus* may also be moderately abundant. The colon bacilli are well represented. The urine shows the presence of a slightly larger amount of ethereal sulphates than is ordinarily found in earlier life. The ratio of ethereal to preformed sulphates varies from 1 : 8 to 1 : 12 most of the time. A little indican is of frequent occurrence, and not rarely the reaction is strong with Obermeyer's reagent — not regularly strong, perhaps, but frequently so for a few days at a time. The phenol excretion may also be a little greater than that noted during earlier life.

In short, then, we find in middle life a large number of persons whose health is good or fair, but in whom the putrefactive processes in the intestine are distinctly more active than is the case with most younger persons who are representative of normal health. These persons, though in good health, are not robust. A period of sustained hard work is followed by considerable and per-

haps annoying mental and physical fatigue. Moreover, these persons have found by experience that they must be more careful than formerly in respect to food and drink, emotional and sexual excitement, etc. Dining out and the use of alcoholic drinks are indulgences quickly followed by unpleasant consequences. Physical exercise out of doors becomes more and more a necessity to this group of individuals. They are conscious that it requires careful living to keep them in a condition compatible with the performance of their duties.

Period of Senescence. — The age of an individual must be measured rather by the physiological potential of his cells than by the number of his years. There are men who at seventy have cells with functional capacities superior to those of other men who are little beyond forty, and who show their superiority in the ability to work without fatigue, to digest without any consciousness of the digestive processes, and to make large outputs of mental and muscular energy without ill effects. These persons retain soft arteries, are well nourished, and exhibit little atrophy of the subcutaneous areolar tissues, and hence show little wrinkling of the skin. They are, in short, candidates for an advanced age.

If we examine the intestinal bacteria and the urine of such people, we find conditions wholly in harmony with the unusual preservation of general functional powers and with the freedom from signs of disordered digestion. The fæces contain an abundance of viable bacilli of the *B. coli* group and the putrefactive anaerobes are few in number. Analyses show the presence of mere traces

of indol in the intestinal contents (perhaps one or two milligrams in one hundred grams fæces) a few milligrams of phenol, and no skatol. The urine is free from indican almost habitually, and only moderate quantities of phenol are excreted (twenty to forty milligrams in twenty-four hours). The ethereal sulphates are correspondingly low. The dimethylamidobenzaldehyde reaction is feeble or moderate in intensity.

Persons of this type are apt to die at an advanced age of some condition apparently quite distinct from disease of the digestive tract, especially thrombosis of cerebral vessels (from atheroma) or chronic myocarditis.

I have not had a wide experience in the careful study of the bacterial conditions in persons above sixty-five years of age, but believe it safe to say that a large majority of such persons (perhaps seventy per cent. of the population in the United States above this age) give evidence of distinct putrefactive processes in the digestive tract. These processes are in many instances characterized by their mildness. But if we compare these mild processes of putrefaction with those existing in the majority of persons under twenty years of age, we find a distinct difference between the two. I think it quite clear that the conditions in youth are much more close to the ideal physiological state of infancy and childhood than are those of senility. The difference lies mainly in the direction of the greater abundance of putrefactive bacteria in old age, but with this there is apt to be some diminution in the number of typical colon bacilli, if we may form a judgment on this point from the appear-

ance of the microscopical fields and the gas production in the fermentation tubes. These conditions of bacterial activity in the period of senility are like those which were just described as being common in people about the age of fifty. The main difference between the putrefactive conditions found at fifty and at seventy is that at the latter period they are a little more marked in their intensity and affect a much larger proportion of the population. The subjects in question at this later period of life are not ill, but in order to keep fairly well have to be very careful as to their habits of living. They are moderately anæmic and easily develop slight disorders of digestion. They weigh less than formerly, and though they may still be well nourished in appearance, are conscious of losing strength from year to year. They are undergoing what is usually regarded as normal involution.

Thus it is clear that there exists a distinct difference between normal childhood and adolescence on the one hand and normal old age on the other in respect to the intensity of the putrefactive processes that go on in the digestive tract. The origin and precise significance of this difference are at present not clear. It appears likely that the tendency to an increasing degree of putrefactive decomposition in the intestine is connected with the repeated but not necessarily severe derangements of intestinal function that are experienced from time to time by most individuals. In the course of these acute derangements, there is frequently an increased opportunity for the development of putrefactive anaerobes, and it is possible that owing to slight but persistent damage

to the epithelial and other structures of the intestinal mucous membrane, these anaerobes are gradually afforded more and more favorable opportunities for their growth.

As to the relation between the relatively active anaerobic life in the intestine during senility and the development of the involutional alterations in the tissues, it is difficult to formulate an opinion. Many factors may doubtless enter into the production of these cellular alterations, and it is difficult to assign to each of these its just position. One statement may, however, be confidently made: the onset of senility may be distinctly accelerated through the development of intestinal infections in which the putrefactive anaerobes are prominently represented. I have observed this in cases where it has appeared to me a certainty that other toxic causes of premature senility could be excluded.

The view that intestinal infections stand in a causative relation to old age is by no means new. It has been advanced by Metchnikoff on several occasions. It is probable that the "wild races" of bacteria of which he speaks as responsible for senile changes consist largely of putrefactive microörganisms. I do not know that Metchnikoff has singled out any definite group of micro-organisms as especially concerned. I am inclined to give prominence to *B. aerogenes capsulatus* as the most important factor in the production of the putrefactive decompositions of advanced age. It is extremely probable that persons with apparently equal numbers of gas-bacilli in the intestinal tract do not necessarily suffer an equal degree of harmful influence from their putrefactive

products. Some races of the gas-bacillus are much less pathogenic for animals than others, and these differences in pathogenicity may be of considerable significance to human beings harboring them.

CHARACTERS OF THE BACTERIAL FLORA OF CARNIVOROUS AND OF HERBIVOROUS ANIMALS

In the course of the study of anaerobes of the human intestine it appeared desirable to learn something about the characters of the bacterial flora inhabiting the large intestine of various domestic and wild animals. It was noticed that in the dog, which is frequently exclusively carnivorous, the intestinal contents often showed the presence of large numbers of spores, spore-bearing bacilli, and vegetative forms of anaerobes. The numbers present in the fæces were noted to be especially large in some animals which had been exclusively fed on meat. A study of a grown cat fed upon raw meat showed the presence of Gram-positive vegetative anaerobes from one end of the digestive tract to the other. Flora derived from the stomach, small intestine, and large intestine were inoculated and grown in bouillon flasks and showed an abundant production of methyl mercaptan as well as of hydrogen sulphide. The numbers of colon bacilli present in this case were relatively small as compared with the anaerobes. The study of the colonies obtained on anaerobic plates showed that a large portion of the organisms present in the intestinal tract were *B. aerogenes capsulatus*. Intravenous infusion of these organisms

into a rabbit which was afterwards killed and incubated showed in a high degree the typical gas formation.[1]

Observations on other cats showed the presence of considerable numbers of spore-holding bacilli and free spores, sometimes in chains, in addition to vegetative forms of anaerobes. The position of these spores and spore-holding bacilli has not been established in a bacteriological sense. Observations were also made upon the intestinal contents of the wolf, tiger, and lion. Several different tigers were studied, and the observations were not confined to the examination of one lion and one wolf. The material from the lion showed the presence of many free spores. It also showed the presence of considerable numbers of Gram-positive bacilli, suggesting *B. aerogenes capsulatus*. Gram-stained preparations from wolves showed pictures similar to those observed in the lion except that the spore-holding bacilli were more numerous. The findings in the case of supposedly healthy tigers were not essentially different from those in the case of the wolf and lion. In the case of one tiger, suffering from osteomalacia, greatly impaired nutrition, and loss of strength, the microscopical fields derived from several different samples of fæces revealed the presence of immense numbers of free spores and smaller numbers of immature Gram-negative spore-holding bacilli. These spores developed into organisms which possessed all the generally known cultural and biochemical characters of *B. aerogenes capsulatus*, including the ability to develop

[1] The method by which these incubation experiments were carried out is explained on p. 84.

a high grade of gas formation in rabbits injected and incubated.

It was found that bouillon cultures of the mixed fæcal flora from the lion, tiger, wolf, and cat all developed quickly a sufficient quantity of methyl mercaptan to give a prompt, strong reaction with isatin in sulphuric acid.

Experiments were made with the mixed fæcal flora from these carnivorous animals to determine their pathogenicity when injected into the subcutaneous connective tissue. It would have been better to have worked with pure cultures of the anaerobes in question, but opportunity has not yet arisen to isolate them. The result of the inoculations into guinea-pigs was the same in each instance. The animals died within twenty-four hours and usually in fifteen to eighteen hours. At autopsy the subcutaneous connective tissues were hæmorrhagic, œdematous, and showed necrotic changes which extended in some instances to the muscles. Gas formation was not usually noted as a prominent feature. These pathological alterations were not confined to the site of inoculation, but had extended to the subcutaneous connective tissues throughout the body and were especially pronounced in the axillæ and in the groin. It is unnecessary to enter here into the details as to the character of the organisms recovered from these lesions.

We may contrast with these findings the observations made upon herbivorous animals, including the buffalo, goat, horse, elephant, and camel. In the case of the camel, elephant, and horse the preponderant bacteria in the Gram-stained fields were small, Gram-negative

organisms which were regarded as special forms of *B. coli*. In the case of the goat the fields contained some Gram-positive bacteria, and of the Gram-negative ones a considerable number were of greater length than the dominant small forms which were regarded as belonging in the class of colon bacilli. In the case of the buffalo, mixed fields were found as regards the Gram-staining and many of the positive organisms were found to be small diplococci and small bacilli. In none of these animals were seen any organisms suggesting *B. aerogenes capsulatus*, excepting in the case of the buffalo, where the number of bacilli of this type was very small. Spore-holding organisms were not observed, but moderate numbers of free spores were noticed in all the fields except those from the elephant. In the fields showing the largest number of spores their occurrence was far less frequent than in the lion, tiger, wolf, or cat.

The mixed flora of these different herbivorous animals, grown upon peptone bouillon, failed to show the production of methyl mercaptan excepting in the case of the horse, where a moderate reaction was obtained.

Observations were also made upon the effect of suspensions of the mixed flora from herbivorous animals when injected subcutaneously. The quantities of suspension employed were usually about twice as great as in the case of the suspensions from the carnivorous animals. With the exception of the suspensions obtained from the horse, the pathogenicity of these suspensions was found to be slight, the guinea-pigs frequently living two or three days or entirely recovering. In the

animals injected with fæces from the horse were found hæmorrhagic and œdematous lesions with necrosis, similar to those found in the carnivorous animals. These lesions were, however, less pronounced than in the case of the suspensions from the carnivorous animals. In the case of the elephant a considerable quantity of fibrinous exudate was found about the point of inoculation. No œdema or necrotic change was observed in the subcutaneous tissues.

A further confirmation of the radical differences existing in the intestinal tracts of carnivora and herbivora is furnished by a series of observations with the Welch-Nuttall incubation test. Suspensions were made from the fæces of all the types of animals mentioned, and equal quantities of these suspensions were infused intravenously into a series of living rabbits. The rabbits were then quickly killed and incubated. On examination after twenty-four hours it was found that all the rabbits infused with suspensions from carnivora showed in an extreme degree the characteristic putrefactive changes in the liver, cellular tissues, etc., induced by pure cultures of *B. aerogenes capsulatus* or of the bacillus of symptomatic anthrax. The rabbits infused with suspensions made from the fæces of the herbivora showed similar but very much slighter changes in each case. The results for each group of animals separated the herbivora sharply from the carnivora. Examination of the livers showed the number of bacteria in the carnivorous series to be many times greater than in the herbivorous series. The microörganisms were regarded as being

almost certainly *B. aerogenes capsulatus* on account of their morphology and failure to sporulate. The bacilli of symptomatic anthrax readily sporulate in the incubated rabbits. The gas-bacillus (*B. aerogenes capsulatus*) does not sporulate under these circumstances.

These differences in the appearance and behavior of the bacteria derived from typical carnivora and herbivora suggest that the habit of living upon a diet consisting exclusively of raw meat entails differences in the types of bacteria that characterize the contents of the large intestine. The occurrence of considerable numbers of spore-bearing organisms in the carnivora points to the presence of anaerobic putrefactive forms in great numbers. The results of subcutaneous inoculations into guinea-pigs bear out this view and indicate that the numbers of organisms capable of producing a hæmorrhagic œdema with tissue necrosis, with or without gas production, are very considerable. Unfortunately the data pertaining to the biological properties of these pathogenic anaerobes are at present insufficient to permit us to classify them or to say more of their nature than that they are organisms representative of a definite group of putrefactive anaerobes which make butyric acid and hydrogen and exert a peptonizing action upon living tissues. Nevertheless the observations here recorded are of much interest in relation to the bacterial processes and nutrition of herbivorous [1] as distinguished from carnivorous animals, and are significant furthermore

[1] Many of the herbivora yielded mixed flora incapable of making gas on dextrose bouillon.

for the interpretation of bacterial conditions found in man. The question arises whether the abundant use of meat over a long period of time may not favor the development of much larger numbers of spore-bearing putrefactive anaerobes in the intestinal tract than would be the case were a different type of proteid substituted for meat.

Inquiries made of Dr. Blair, the pathologist in the New York Zoölogical Gardens, elicited the fact that while, upon the whole, the carnivorous animals are apt to live somewhat longer than the herbivorous animals of about equal size, the carnivora are much more likely to develop conditions of advanced anæmia in the later years of their lives than is the case with the herbivora. Dr. Blair states that it is usual in the later years of life for the carnivora to show a much diminished volume of blood and at least a moderate fall in the hæmoglobin. Instances are stated to be not uncommon in which a pernicious type of anæmia has developed in the carnivora. On the contrary, among the herbivora it is said that pronounced anæmias are very occasional. The examples of severe anæmia encountered among the herbivora were said by Dr. Blair to be in nearly all instances referable to gross animal parasites.

INFLUENCE OF FOOD ON HUMAN BACTERIAL FLORA OF THE DIGESTIVE TRACT

Surprisingly little is known about the influence of different classes of food upon the nature of the microorganisms in the digestive tract. It is evident that this subject is one that deserves the most careful kind

of study by means of modern methods. That a knowledge of the influence of different foods upon the flora in health and disease would not only be of great biological interest but would also give many indications of a practical sort for the use of different types of foods in pathological conditions, requires no argument.

Some observations have been recorded upon the influence of food which have led to the conclusion that there is little difference between a diet consisting of vegetable food on the one hand and a mixed diet with abundance of meat on the other. The methods employed for determining the presence of differences in the character of the flora were, however, so crude that the work in question cannot be regarded as in any manner conclusive. I have observed that the number of Gram-positive organisms in the fæcal fields was much increased when an adult subject who had previously been on a mixed diet began to live on a dietary consisting almost exclusively of meat. The microscopical appearances indicated that an increase in the number of putrefactive anaerobes was largely responsible for the change from a mixed fæcal field to one which was dominantly Gram-positive. In this connection I think the observation noteworthy that the intestinal contents of animals living on a diet of raw meat tend to give mixed or dominantly Gram-positive fields, whereas similar material from herbivorous animals tends to give fields in which Gram-negative organisms are predominant. As mentioned in connection with the discussion of the fæcal flora in herbivorous animals as compared with carnivorous animals, there is

a great difference in the numbers of anaerobes in the two groups, the number of these organisms being greater in the case of the carnivora.[1] Some observations of interest on the flora of dogs we owe to Lembke,[2] who found distinct differences between the flora after a diet of bread and after one of meat. Of especial interest is the fact that a bread diet rendered the fæces much richer in anaerobes than did a diet of meat. The bacteria observed upon the diet containing a great abundance of fat resembled closely those noted on a bread diet. The changes which are induced by alteration in the diet are said by Lembke to be generally of a temporary sort. On this subject he speaks in the following way: "If the diet is changed, there appear on the fæcal plates new colonies of the most varied sort. In the course of a few days these are materially reduced and the colonies of *B. coli* again gain the upper hand." It appears that with almost any change in diet new varieties of bacteria are introduced in large numbers and somewhat obscure the predominance of the colon bacilli in the fæcal fields. Very soon, however, the colon bacilli regain their original predominance, doubtless owing to the fact that they are better able to adapt themselves to the nutrient conditions than are the facultative forms which have been introduced. Lembke states that if one limits the entry of bacteria by sterilization of the diet, the foreign

[1] Escherich found in a young dog whose milk-fæces flora was very similar to that of normal nurslings, that after a pure meat diet there was only an extremely small proportion of colon bacilli, whereas there appeared large numbers of liquefying colonies.

[2] "Beitrag zur Bacterienflora des Darms," *Archiv f. Hyg.*, xxvi, p. 325, 1896.

types of microörganisms after a time disappear and the plates show exclusively colonies of the colon bacillus and *B. lactis aerogenes* in almost pure culture. On the other hand, there is good evidence that the Gram-negative bacilli of the fæces may be temporarily replaced by feeding Gram-positive acidophile bacteria.

It appears probable that in considering the influence of foods upon the flora of the intestinal tract one should take into account the factor of rapid digestion and absorption in the upper part of the digestive tract. For example, in cases where a patient takes daily a large quantity of meat which is imperfectly masticated, there is much more opportunity for the development of putrefactive anaerobes in the lower part of the intestine than if the same quantity of meat is thoroughly subdivided by mastication. I believe also that the influence of diet must be largely modified by the character of the dominant organisms in the intestinal tract and that this influence may come to the front in a telling way in cases of chronic infections of the large intestine.

THE REDUCING ACTION OF MEAT

In the study of the anaerobic conditions that prevail in the digestive tract it is important to recognize every factor that may enter into the production of anaerobic conditions there. It seems extremely probable that different articles of food have a different significance in respect to their influence on the presence or absence of oxygen in the digestive tract. It is known that fresh tissues of animals exert a considerable degree of reduc-

ing power and that this reducing power is much more active in general than the reducing action of vegetable cells. If one places in a cylinder or flask a quantity of finely divided fresh muscle of liver from a mammal and immerses this material in a dilute solution of methylene blue, under such conditions that oxygen from the air is largely excluded from the region occupied by the divided tissues, it is soon noticeable that the blue color begins to disappear, owing to the conversion of methylene blue into leucomethylene blue. The reducing action of fresh liver has been successfully employed by Professor Theobald Smith in rendering the closed arm of the fermentation tube more strictly anaerobic and thus facilitating the growth of certain strictly anaerobic bacteria. With these facts in mind one naturally asks whether the use of large quantities of raw muscle may not considerably aid in the production of anaerobic conditions in the digestive tract. In the case of carnivorous animals living on raw meat there seems little doubt that anaerobic conditions may exist throughout the digestive tract, and I think it probable that the reducing action of the meat in the upper part of the tract may materially contribute to diminish the quantity of oxygen carried into the digestive tract. Even in the case of man this factor is one which cannot be entirely ignored. Although human beings in civilized countries for the most part eat their meat after it has been subjected to cooking, there are many instances in which large quantities of raw or nearly raw meat are eaten. Moreover, meat that has been slightly cooked still retains considerable reducing power.

How far the excessive use of meat that is raw or slightly cooked may influence the production of anaerobic states in the digestive tract it is at present impossible to say, but it seems not unlikely that there are cases of excessive intestinal putrefaction dependent on the excessive activity of anaerobes in which the conditions of anaerobiosis are distinctly favored by excessive meat eating.

THE INFLUENCE OF THE EPITHELIAL CELLS LINING THE DIGESTIVE TRACT

We know at present little of the influence of changes in the epithelia lining the digestive tract upon the physiology and pathology of the human organism. There are, however, certain general considerations that require mention here. It may be safely assumed that every cell possesses a certain life potentiality; that is, has an inherent capacity if undisturbed by injurious agencies to live a certain period of time. Every epithelial cell of the digestive tract doubtless possesses a high capacity for reproduction. This power must, nevertheless, be a limited one, and if the cells of the digestive tract be injured by too many demands upon them, they may fail after a time to reproduce normally. Superficial cells which have under these circumstances undergone desquamation are thus not so easily replaced. It may happen that in some portions of the digestive tract the epithelial layer is much thinner than normal, owing to excessive desquamation as compared with their power of reproduction. For example,

we see in the case of Müller's superficial glossitis (geographical tongue) a condition in which varying areas of the tongue suffer a temporary or permanent loss of the superficial epithelial layers. In many cases of chronic disorder of digestion of very long standing, the papillæ of the entire dorsal surface of the tongue show excessive desquamation of the epithelium. In how far similar conditions occur in other parts of the digestive tract we do not know, but there is reason for believing that in some chronic derangements of digestion there is in the stomach and in the intestinal tract a similar thinning of the epithelial layer. How far conditions of the tongue may be regarded as an index to the conditions in other parts of the digestive tract is uncertain.

Of the pathological effects of excessive desquamation of epithelium we have at present little definite knowledge. It appears reasonable to suppose that where desquamation is excessive without corresponding reproduction of epithelial elements there must be diminished secretion, provided the cells in question take any part whatever in providing a secretion containing digestive enzymes. It seems likely also that the process involved in transudation may be abnormal under conditions in which the epithelial layer is greatly thinned. There are some patients in whom the tongue indicates an excessive desquamation of the epithelium and in whom cathartics such as salines no longer act promptly or efficiently. It seems to me possible that the not uncommon appearance of a loss of response to cathartics may be connected with the partial failure in function of the epithelium of the

intestinal tract. The correctness or incorrectness of this view can only be established by experimental methods. Still another influence of diminished function in consequence of a pathological thinning of the epithelial layers may be found in a diminished capacity on the part of the intestinal epithelium to act upon products of decomposition in the intestine. In experiments made many years ago with indol, it was found that the epithelium of the digestive tract possesses in a high degree the capacity to bind indol in such a way that this substance cannot be recovered by distillation. I think it likely that in cases where there is excessive production and absorption of indol in the intestinal tract the epithelium acts as a protective agency to the organism as a whole. This action of the epithelial cells is certainly not confined to indol. It seems not unreasonable to suppose that where the epithelium has been very much thinned, the organism suffers from the enfeeblement of this function incidental to the loss of epithelium. Here again experimental methods should throw more light upon this protective action of the intestinal epithelium.

THE PERMEABILITY OF THE MUCOUS MEMBRANE OF THE INTESTINAL TRACT FOR BACTERIA

Another function of the epithelial cells of the digestive tract has to do with the protection of the body from the invasion by bacteria within the tract. There exists some experimental evidence indicating that an intact, fully developed layer of epithelium is an important barrier to the entry of at least some kinds of bacteria

into the mucous membrane. How far the presence of mucus on the surface of the epithelial layer is a factor in rendering the mucous membrane impermeable is still a question. It appears from the work of Hilgermann[1] and others that the normal mucous membrane is much more permeable to some bacteria during the period of infancy than in later life. It was found by Hilgermann that when young rabbits and guinea-pigs are fed with tubercle bacilli, these organisms pass through the wall of the stomach and penetrate the digestive tract throughout its entire extent. It was noticed that the number of bacteria passing through the small intestine, especially in its upper third, was considerable, whereas the number which penetrated the wall of the large intestine was much smaller. In the vermiform process the conditions for the passage of tubercle bacilli appear to have been the same as in the case of the small intestine.

Hilgermann attempted to determine what factors are concerned in the passage of tubercle bacilli through the digestive tract in animals. He was at first inclined to regard the passage as accidental and due to small lesions, but this view was abandoned because it was found that the penetration did not occur at single points, but throughout the length of the gastro-enteric tract. It was found, moreover, that there is no evidence of penetration occurring in consequence of an irritation by a considerable number of bacteria acting locally. If this

[1] "Die Bakteriendurchlässigkeit der normalen Magendarmschleimhaut im Säulingsalter," *Archiv f. Hyg.*, liv, p. 335, 1905.

had really been the case, the bacteria must have passed much more abundantly than they did, instead of in the typical manner and in the definite stages observed by Hilgermann, who is inclined to believe with Behring that the mucous membrane in early life is lacking in natural protective substances capable of hindering the penetration of bacteria.

Of considerable interest in connection with the study of the penetration of the digestive tract by bacteria are the observations of Ficker [1] on the influence of exhaustion and of hunger upon the passage of bacteria. He calls attention to the fact that states of inanition favor the occurrence of infections, especially those arising from the intestinal tract, but that on the other hand the best nourished and strongest organisms may show a high degree of sensitiveness toward such infections. The experiments of Ficker indicate that excessive exertion is a factor which at times leads to an exhaustion which is a predisposing condition to infection, especially in the case of typhoid fever. This influence of exhaustion on the development of infection has been to some extent studied by Charrin and Roget on white rats which were fatigued in a rotating drum. These animals were shown to be more sensitive to anthrax than the control animals. Ficker selected dogs for his experiments, inducing fatigue in them through work in a treadmill. He found that a combination of fasting and fatigue facilitated in an extraordinary way the passage of germs through

[1] "Ueber den Einfluss des Hungers auf die Bakteriendurchlässigkeit des Intestinaltraktus," *Archiv f. Hyg.*, liv, p. 354, 1905.

the intestinal tract. In a dog that had hungered eleven days, a period of three hours of exercise sufficed to permit the free passage of microörganisms. It appears that neither of these factors is capable of giving rise to this degree of permeability when operating separately. Individual peculiarities were, however, observed in respect to the permeability of the tract, and these must have their explanation in special conditions in the intestine.

Ficker does not maintain that a full understanding of the conditions favoring the permeability of the intestinal tract has been reached through his experiments. He holds very definitely that in fully grown dogs, in which the intestinal tract is ordinarily little permeable, it is possible through inanition or fatigue or a combination of the two, to facilitate a penetration of the intestinal mucous membrane which is analogous to that observed in the infantile tract and further resembles the conditions present in the dying organism. Ficker suggests that under the influence of powerful bodily action the volume of the gastric juice and of other digestive juices is greatly reduced and that peristalsis is diminished, while on the other hand the lymph and blood flow are actively accelerated, all these being factors which might favor the penetration of bacteria. He suggests that perhaps the increased leucocytosis induced by active muscular exertion is responsible for the increased extravascular bactericidal power of the serum which he observed, and thus that the leucocytes can be regarded as carriers of bacteria from the intestinal tract. Such a transportation of bacteria by leucocytes

would presumably be facilitated in a high degree by the accelerated movement of the blood serum during active exercise. Ficker thinks it probable, also, that at the time of greatly increased muscular exercise the body cells generally, including those of the intestinal tract, suffer a temporary check in their metabolism and in their ability to liberate energy. This temporary cell infirmity is supposed in some way to influence the intestinal epithelium so as to favor the passage of bacteria.

Ficker further suggests that through his experiments are explained the well-known observations that the flesh of animals that have been slaughtered after having been driven long distances very quickly decomposes after the killing, while, on the other hand, the flesh is much better preserved in animals that are allowed to rest for several days before being killed. He also raises the question whether the penetration of the intestinal tract by bacteria may not explain some of the phenomena that have been noted after great fatigue, for example, the so-called "fever of exhaustion," and also the state designated by the older physicians as "autotyphization."

PHYLOGENETIC SIGNIFICANCE OF THE LARGE INTESTINE

In the study of the physiology and pathology of the digestive tract it is necessary to realize the importance of certain functions in the development of the race which may be too readily overlooked because they no longer possess the significance for the maintenance of life that was formerly the case. There can be little doubt that one important function of the large intestine

is connected with the inspissation of the contents of this part of the tract. In some lower animals and in some human individuals the desiccation of intestinal contents reaches so high a grade that the fæces voided contain only a very low per cent. of water. By means of this absorption of moisture the organism is protected against a loss of water which under certain conditions might prove a detrimental condition in the struggle for existence. It is easily conceivable that under primitive conditions of life, where water is not always readily obtainable, those animals which were best able to conserve their water and salts would have a material advantage over animals less well able to prevent this waste. In many mammals urine of very low specific gravity is excreted, although in the glomeruli of the kidneys in these same animals the concentration of the urine is extremely high. If it were not for the powerful resorptive action of the epithelia of the convoluted and other tubules of the kidney, the organism would be subjected to an enormous loss of water. Under conditions where water is freely obtainable such a loss would, perhaps, have little significance, but where the water supply is uncertain such wastefulness on the part of the organism would soon lead to death. Thus in the large intestine as well as in the tubules of the kidney we have to recognize mechanisms protective to the life of the individual through provision against the waste of water. In the case of man this function is of relatively little significance at the present time since water may usually be obtained in abundance. It seems probable that in addition to the

function just mentioned the large intestine has often served as a reservoir for food, but this function certainly is superfluous in man. These considerations are of importance; for if it can be shown that the large intestine is not needed either for purposes of inspissation or as a reservoir or as a place of importance in carrying certain digestive processes to an end, there is no reason why the intestine should not, under certain conditions of disease, be shortened by the elimination of a large part of the colon. There is some difference of opinion as to whether animals can live without the large intestine, but the balance of evidence is in favor of their being able to maintain life even after the large intestine has been excluded. There are also instances in human beings that point to the same conclusion. Dr. Bryant of New York tells me that in one instance he practiced the insertion of the ileum into the rectum. The patient was a woman who lived a considerable period of time in this condition without suffering in nutrition.

THE IMPORTANCE OF PROMPT RESORPTION FROM THE SMALL INTESTINE

It is almost self-evident that the prompt resorption of food from the small intestine is one of the most important factors in preventing the occurrence of excessive putrefactive conditions in the digestive tract. The passage of large quantities of partially digested proteid material into the region of the intestine where anaerobic conditions prevail must necessarily greatly facilitate the bacterial decomposition of proteids in the digestive tract.

As will be pointed out in dealing with the methods of diminishing chronic excessive intestinal putrefaction, those measures which are designed to secure prompt digestion and prompt absorption from the small intestine are of the greatest significance in limiting bacterial decompositions.

The administration of a cathartic which acts imperfectly (that is, which carries partly digested food from the small intestine into the colon without, however, securing an evacuation) leads to conditions similar to those following an excessive meal. In some persons this miscarriage of a cathartic is followed by headache and flatulence, in others by more serious signs of intoxication such as weakness in the muscles. In normal persons these effects are relatively slight; in persons whose digestive tracts are the seat of infection with putrefactive anaerobes the evidence of excessive fermentation and putrefaction is much more pronounced.

THE PHENOMENON OF SUBSTITUTION

I am convinced that what may be termed the phenomenon of substitution of flora in the digestive tract is a common and significant occurrence in the course of many derangements of the digestive tract. By substitution is meant the temporary replacement of one type of microorganism normally abundantly present in the digestive tract by an allied form which, though perhaps normally present in small numbers, never assumes a dominant place during health. The substitution may be complete or incomplete and may be a temporary or a prolonged phe-

nomenon. After a time the substituting microörganism loses its prominent place and in its stead the obligate bacteria of the tract again become dominant.

The phenomenon of substitution is one that most frequently involves temporary replacements of the colon bacilli in the lower part of the large intestine and also in higher portions. An example of what is meant by such substitution is afforded by the following case. A woman of twenty-five years of age developed measles and soon after convalescence became ill with what was apparently a condition of mucous colitis, lasting about one month and attended by slight fever. Studies of the flora made toward the end of the period of mucous colitis showed that the fæcal fields contained an excessive number of diplococci, and the sediments of the fermentation tubes exhibited streptococcal growths in great abundance. On litmus gelatin plates made from fæcal suspensions it was found that the dominant organism resembled colon bacilli in appearance, but differed from them in forming acid very slowly. Many colonies were fished from these plates and studied, but none were found which could be called colon bacilli. The organisms obtained differed from colon bacilli in making no indol and in possessing only feeble powers of gas production and of acid formation on glucose. They irregularly fermented levulose and lactose. They coagulated milk only after a lapse of ten days, and then the coagulation was incomplete. They were actively motile Gram-negative bacteria. Two weeks later, during the progress of convalescence from the condition of mucous colitis, it was

found that the fæcal suspensions gave on the gelatin plates large numbers of bacteria of the type just mentioned and in addition contained considerable numbers of true colon bacilli. One month after the first examination the fæcal suspensions showed the presence of large numbers of colon bacilli, which had now become the dominant type. From the litmus gelatin plates a very small proportion of bacteria were isolated which were incapable of coagulating milk, made no indol, and had little effect upon the sugars. It was evident that the substituted bacteria were now in a small minority. The substituted microörganisms in this case resembled the bacilli of typhoid fever except in the fact that they were able to ferment glucose and possessed the ability to ferment lactose and levulose in an irregular manner.

I have in several instances met with cases of substitution in which the colon bacilli have disappeared from the stools and have been replaced temporarily or permanently by other types of bacteria. Dysentry bacilli may temporarily replace obligate colon bacilli.

THE PRESENCE OF PATHOGENIC BACTERIA IN THE DIGESTIVE TRACT IN HEALTH

Evidence is gradually accumulating which goes to show that pathogenic microörganisms may be present in moderate or even considerable numbers in the intestinal tract under some conditions without giving rise to clinical manifestations of deranged function. It is now well known that moderate numbers of typhoid bacilli may in some instances be found in the stools

of patients who have lately had typhoid fever. Here the explanation of the absence of symptoms is doubtless to be sought in local or local and general conditions of immunity. There appear, however, to be instances in which there is no evidence that typhoid fever has occurred but in which nevertheless moderate numbers of organisms may be obtained from the fæces. The same facts hold true for the bacilli of dysentery. Duval has shown that dysentery bacilli are present occasionally in small numbers in the stools of normal individuals, and this observation has been confirmed by others.[1] We have noted the presence of *B. pyocyaneus* occasionally in the digestive tract of persons apparently in the best of health. It is likely, however, that in all these cases the pathogenic organisms in question are held in check by the bacteria present in the digestive tract or by the bacteria and the intestinal secretions so that they are unable to multiply in a significant manner or to gain entry into the cells of the mucous membrane. It seems not unreasonable to suppose that errors in diet or depressed general conditions favor the multiplication and penetration of pathogenic bacteria that have for some time been present in a slumbering state. Irritant foods may possibly so alter the secretions of the digestive tract as to favor definite infection by the semi-parasitic bacteria that are present.

The considerations just mentioned as applying to the

[1] In some of the apparently normal children from whom Dr. Wollstein obtained dysentery bacilli there were subsequently developed the clinical indications of dysentery.

bacilli of typhoid fever, of dysentery, and of *B. pyocyaneus* probably hold equally true of the more saprophytic forms, such as the microörganisms concerned in chronic excessive intestinal putrefaction. It is certain that the intestine may harbor considerable numbers of *B. putrificus* and *B. aerogenes capsulatus* or both of these together without the development of clinical manifestations. A variety of conditions may be presumed to so favor the development of these anaerobes that their products, instead of being formed in such small amounts as to be harmless, begin to exert a detrimental effect upon the organism. Especially important in this connection are influences which alter the character of the secretions in the large intestine or bring into the large intestine unusually large quantities of partly digested proteid food.

There is an important practical aspect to the fact that pathogenic organisms inhabit the intestinal tract without giving obvious clinical signs of their presence. A good quality of milk or meat, free from pathogenic bacteria, may be blamed for bacterial decompositions of a harmful kind which are in reality due to abnormal bacterial conditions prevailing in the digestive tract before the use of the food under suspicion. In certain conditions of the digestive tract an excessive or even a moderate meal of proteid food will precipitate an intoxication or a seizure of vomiting or diarrhœa. There are cases classed as "ptomaine poisoning" in which the digestive tract, rather than the food, is responsible for the observed disorders.

CRITERIA EMPLOYED IN THE CLASSIFICATION OF BACTERIA OF THE GASTRO-ENTERIC TRACT

It appears not out of place to refer briefly to the criteria employed in classifying the bacteria of the gastro-enteric tract although these standards of judgment do not differ from those of bacteria generally. The cultural characters of the growths upon ordinary media must be regarded as relatively less important in forming a judgment as to the character of given organisms than was formerly the case, since in the early days of bacteriology such cultural characters constituted our chief reliance in identification. While the cultural characters of a microörganism derived from the digestive tract must in every instance be taken into account, these do not usually suffice to give more than a suspicion of the identity of the bacterium in question. In general it may be said that the cultural characters do not possess a high grade of permanence and are, moreover, often not specific within certain groups. For example, within the group of colon bacilli one finds many organisms which must be regarded as distinct but which nevertheless give practically undistinguishable growths on the ordinary culture media. Indeed, a strain of colon bacillus may in time come to vary quite widely from its original appearance upon a given medium. The cultural characters of microörganisms are often much influenced by physical

conditions pertaining to a culture medium. For example, Dunham obtained widely different appearances in growths of the same typhoid bacillus when grown in gelatin plates in which the gelatin possessed varying degrees of concentration and slightly different chemical characters.

The morphological characters of organisms are certainly important to consider and must be known as a matter of course in any study of bacteria, but within groups of closely related organisms morphology itself gives us little aid. The question of motility is one of a good deal of importance and should always be considered. In general within a group of colon-like organisms a high grade of motility suggests pathogenicity, but the absence of motility or a low grade of it does not point in the opposite direction, since pathogenic types of bacilli in the dysentery group presumably not distantly related to the colon bacilli are non-motile. The determination of the staining characters of the organism with the Gram method is most helpful provided a uniform method of procedure be employed. In the absence of a uniform technique the Gram method is less good than a simple stain, since it serves only to confuse. Of great importance for the classification of the bacteria of the gastro-enteric tract, as of bacteria generally, are the biochemical properties. These biochemical characters include the cleavage abilities of the organism with special reference to their action on the soluble carbohydrates[1] with the production of acid and gas, their ability to produce indol

[1] The great importance of this method of studying bacteria was first pointed out by Professor Theobald Smith in connection with the differentiation of typhoid and colon bacilli.

and skatol, and to liberate sulphur products such as hydrogen sulphide and methyl mercaptan. The high grade of permanence of the fermentative characters of bacteria give this test much value. This permanence is probably more marked within certain groups such as the colon-typhoid group than in certain others, as, for example, the group of streptococci. Strains of colon bacilli may be carried through a long series of sugar-bouillon fermentation tubes without appreciably modifying in a quantitative way their ability to form gas and acid. In a series of experiments in this direction which was carried out by Mr. H. C. Ward, it was found that strains of colon bacilli which had grown for a long time upon a succession of sugar-bouillon tubes grew less well upon peptone bouillon than had previously been the case. On being cultivated in peptone bouillon after a long period of growth on sugar bouillon it was found that they produced only about one-half as much indol as had previously been the case. This result might easily have been interpreted as an evidence that the organisms in question were losing their ability to make indol upon peptone bouillon. Careful examinations of the growths, however, showed that the diminished production of indol was to be referred rather to an impaired ability of the colon bacilli to grow upon peptone bouillon, than to a real inability to make indol. The formation of indol is a character of considerable importance in the classification of bacteria, but there is some evidence that it is not so permanent and invariable a characteristic as the ability of the bacteria to ferment certain sugars. Much more

work is required to establish the exact position of indol production as a criterion for the classification of bacteria. Unfortunately, nearly all that we know at the present time about the production of indol is based upon the nitroso-indol test, which possesses two grave disadvantages. First, this test is not highly sensitive in the detection of indol, and secondly, the test as usually practiced does not exclude the possibility of the presence of other substances which react with indol. I consider it desirable to replace the old nitroso-indol test by the dimethylamidobenzaldehyde reaction first described by Professor Ehrlich and which is much more delicate than the nitroso-indol test. For quantitative determinations of indol the β-naphthaquinone-sodium-monosulphonate method which I have described is unquestionably the best. There is little doubt that in the classification of the intestinal bacteria more and more stress will be laid on the biochemical characters of the organisms in question. The pathogenicity of an organism is, of course, a highly important character, but is far more variable in general than the biochemical characters just mentioned. Virulent bacteria may easily lose their virulence or have it greatly increased, according to the circumstances under which they grow. The character of the conditions under which an organism has been cultivated must, therefore, be carefully borne in mind in drawing conclusions as to this point. An organism which has long been grown in the laboratory may easily lose its virulence on ordinary media. On the other hand, some microörganisms, such as diphtheria bacilli, may be grown for years on

artificial culture media of the same composition and still remain strikingly constant with respect to the activity of the toxins produced. The aerobic or anaerobic characters of microörganisms are of great importance in the classification of intestinal bacteria, since the putrefactive processes in the digestive tract are carried on largely through the agency of strict anaerobes. No study of the bacteria of the gastro-enteric tract can be considered thorough which does not take the strict anaerobes into account. This, of course, involves the use of anaerobic technique. Some highly aerobic bacteria, like the microorganisms of cholera and the bacilli of tuberculosis, multiply very poorly under strictly anaerobic conditions. The majority of pathogenic microörganisms in man are, however, facultative anaerobes capable of growing under anaerobic as well as aerobic conditions, although the anaerobic growth may be much less active than the development in the presence of air. The ability of organisms to make spores is another feature which has to be taken into consideration in any classification of bacteria. Most of the strict anaerobes of the intestinal tract — possibly all of them — are capable of sporulating under certain conditions, and this is a feature of great significance for their persistence in the gastro-enteric tract, since in the absence of the ability to sporulate in the tract the vegetative forms might easily lose their hold and die out. Of late years much attention has been given to the agglutinative properties of bacteria growing in the intestinal tract, with results of great importance for the classification of these bacteria. It was at first supposed that the

agglutinative characters of certain intestinal bacteria such as typhoid and dysentery organisms with human or experimentally induced sera possess a high degree of specificity. There was also at first an inclination to believe that two bacteria of different origin possessing the same agglutinative characters with one serum were brought very close together by virtue of this fact. It is now clear that the failure to distinguish between group or common agglutinins and specific agglutinins may lead one to think bacteria are identical when this is really not so. The group agglutinins may to a considerable extent be removed by the procedure of absorption which has lately come into use. By means of absorption methods it becomes possible to largely remove the common agglutinins from a serum which contains them by bringing this serum into contact with bouillon cultures of the bacteria in question and after a certain period of contact filtering off the bacteria, which have absorbed from the serum a large part of the common agglutinins. The specific agglutinative properties of the serum now come very clearly into play and may be a great aid in determining the relationship of two microörganisms suspected of standing in close relation to each other. The value of this absorption test is somewhat in question in some laboratories, but it has given a high degree of satisfaction in the laboratory of the Board of Health in New York City, where it has been employed extensively and systematically by Dr. Park and Dr. Collins.[1]

[1] The absorption method has also been successfully employed by Dr. E. K. Dunham in his careful study of the agglutinins of the meningococcus.

The agglutinative characters of many pathogenic bacteria are remarkably fixed properties. Dr. Theobald Smith attempted to induce variations in the agglutinative characters of hog-cholera bacilli and colon bacilli by treating a series of sensitive experimental animals with the bacteria to be tested. He met with no success. Dr. Smith tells me, however, that he thinks the chances for success in modifying the agglutinative characters of the bacteria would have been greater had he tried more resistent animals than were used, since this would have afforded more opportunity to excite antagonism on the part of the microörganisms, and with this, some alteration in agglutinative properties. It is stated that the agglutinative characters of some streptococci are more subject to variation than those of the *B. coli* group.

METHODS OF INVESTIGATION

It is desirable to speak briefly here of the methods that have been employed in some of the investigations with which this volume deals. Some of these methods are well known, others are new. They relate in part to the study of the morphological and cultural characters of the bacterial organisms found in the digestive tract under different conditions, but they have to do mainly with the products of the life activities of these bacteria upon different nutrient media.

Character of the Microscopical Fields. — By far the most helpful method of studying the microscopical fields is with the aid of the Gram stain. The routine

examination of the intestinal contents by means of this stain is a real aid in forming a judgment as regards the presence or absence of certain types of bacteria in the digestive tract.[1] There are many conditions in which, as must be obvious, it can be of little service. There are instances in which Gram-stained microscopical fields appear entirely normal, but after cultural and chemical studies it is evident that abnormal decompositions are at work and that pathological microörganisms are present. On the other hand, one may often form an opinion from the mere study of the fields as to whether the bacteria present are capable of initiating putrefactive processes or not. In order to make the staining of any value it is necessary that the method should be carried out in a uniform manner. The suspension of the flora used should not be too concentrated, as the pictures may be confused through an excessive number of bacteria. Suspensions of fæcal material in the proportion of one

[1] In studying the bacteriology of the intestinal tract it is greatly to be desired that very fresh specimens of the intestinal contents be employed, since it may happen that certain delicate forms of organisms may otherwise quickly die. In the case of colon bacilli, of many coccal forms, and of most of the anaerobes a period of delay appears to make little difference. In some of our investigations we have worked with strictly fresh material, but it has necessarily often happened that there was a delay of a few hours and sometimes of even greater time before cultures were made. The danger in these cases is simply the danger of losing some types which might otherwise be represented in the plates and fermentation tubes. It is stated by some writers that *B. bifidus* is sensitive to a depression of temperature, but we have not always found this to be the case with the temperature of the ice box, since we have been able to obtain growths of that organism in sugar bouillon even after several days' residence of the material upon ice.

part by weight to ten parts of normal salt solution answer very well as a material for making the smears. The following technique serves satisfactorily for routine work. One stains for three minutes with the Gram solution of gentian violet. The solution is then allowed to run off the slide, but the latter is not washed. Lugol's solution of iodine is then permitted to act for two minutes. The slide is now rinsed with water and decolorization is practiced for one-half minute by means of absolute alcohol. The smear is then restained with a fuchsin solution. Unless one employs approximately the same technique, varying and confusing results are obtained which make it almost impossible to say with confidence which organisms are Gram-negative and which are Gram-positive.

From the use of the Gram method one obtains some idea as to the numbers of microörganisms resembling the colon bacilli in morphology; one may form a judgment as to the state of their preservation, as to the presence or absence of slender, long, Gram-negative organisms of the type of *B. liquefaciens ilei;* a judgment may be formed as to the numbers of Gram-positive diplococci and other coccal forms; an opinion may be formed as to the probability of the presence of *B. bifidus*, especially in its unbranched form; and finally it is possible, after one has had experience, to make an estimate as to whether free spores and spore-holding organisms and vegetative anaerobic forms are present in excessive numbers. None of these microscopical appearances can be regarded as positive evidence of the identity of the dominant bacteria

present until this has been determined by cultural methods. It may, however, aid one in forming conclusions that are valuable on account of their high degree of probability. In the case of a given individual, isolation by careful plating and identification by suitable methods of any dominant organism becomes a great aid to subsequent interpretation of the Gram-stained field, even though one may not have the opportunity to make further isolations and identification in the same patient.

There are many applications of the Gram stain that may advantageously be made in the study of intestinal disorders, quite aside from the direct study of the fæces and intestinal material obtained through the use of cathartics. A study of the types of bacteria that are dominant at different levels of the intestinal tract in persons dying of various diseases is greatly facilitated by the use of Gram staining. This method of study of the bacteria at different levels has not yet received the attention it deserves. I consider it especially important to determine the distribution of the anaerobes in the small intestine in numbers sufficiently great to enable them to put their stamp on the character of the intestinal decomposition. The Gram stain further has proved highly useful in the study of the sediments in all the fermentation tubes inoculated with the mixed fæcal flora. Moreover, the same method has given considerable information in the study of the sediments obtained in various media on which certain mixed flora have been grown from seven to ten days. It is sometimes desirable to make use of a staining method for spores in order

to obtain an idea of the numbers of free spores present. Without this procedure one is apt to underestimate their number. The fæcal flora may also be stained directly for capsules by Welch's acetic acid method.

I shall not attempt here to discuss fully methods of isolation and identification of individual bacteria. The methods generally in use for making plate cultures are those which have been employed. In the case of the bacilli of typhoid fever, dysentery, and allied organisms this subject has already been fully developed by highly trained workers. The study of the diplococci and other coccal forms is one that has been relatively neglected (except in the case of acute infections) and is deserving of much fuller development. On the subject of the anaerobes of the large intestine almost nothing has been done, partly, perhaps, on account of the difficulties of anaerobic technique. Some acute infections with anaerobes have indeed been studied, notably by Tissier and by Klein, but until recently the chronic processes dependent on excessive development of putrefactive anaerobes have escaped attention. The necessity of furnishing anaerobic conditions for the growth of some of these bacteria considerably increases the difficulties of identification, but they are by no means insuperable. Where a high degree of anaerobiosis has been desired, we have found it helpful to make use of a stream of compressed hydrogen instead of using hydrogen generated in the laboratory. This is followed by the employment of the pyrogallic method for removing the last trace of oxygen. Where a somewhat less high degree of anaerobiosis suffices it

is very convenient to make use of the simple method, recently described by Hans Zinsser [1] from the Department of Pathology in Columbia University. Here two crystallizing dishes are made to do service for obtaining anaerobic growths. The smaller dish containing the inoculated agar is inverted inside the larger dish, the oxygen being exhausted by the pyrogallic method. Water, on the surface of which oil is placed, serves as a seal. This method should serve to remove some of the obstacles to the study of anaerobes.

The identification of the anaerobes calls for a good deal of patience and care. The anaerobic life of the large intestine (especially in disease) having been so little studied, it is not unlikely that new varieties will be found which have heretofore escaped notice. A growth of *B. bifidus* and other acidophiles occurs under anaerobic conditions in both glucose and plain agar, if one takes the precaution to make up the media so that they contain 0.5 per cent. of acetic acid.[2] In the study of *B. aerogenes capsulatus* it is important to use blood agar in order to obtain the conditions proper for the growth of these bacilli. The use of one cubic centimeter of defibrinated rabbit's blood to eight cubic centimeters of sugar agar answers well.

In studying the characters of the organisms for the purpose of identification the fermentation tubes are extremely helpful, as first pointed out by Professor Theo-

[1] "A Simple Method for the Plating of Anaerobic Organisms," *Journ. of Exper. Med.*, viii, p. 542, 1906.

[2] A beer-wort medium may be conveniently used.

bald Smith. Anaerobic organisms in general grow much better in the closed limb of the tube on sugar bouillon in the presence of bits of sterile tissue (such as liver from a guinea-pig or rabbit), and in some cases, as in that of *B. aerogenes capsulatus*, they do not grow in the absence of this aid. The identification of the anaerobes involves the study of their cultural characteristics, of their ability to form gas on sugar media, and the determination of their gas formula. The estimation of the ratio between the hydrogen and the carbon dioxide formed is an important point in identification first suggested by Theobald Smith. It is easy to determine this point by adding caustic potash to the contents of the fermentation tube, this being followed by absorption of the carbon dioxide.

The influence of the growth of anaerobes upon milk, especially litmus milk, is easily studied in the fermentation tubes and gives considerable aid in identification. For example, *B. aerogenes capsulatus* quickly makes acid on milk and sets up a stormy fermentation due to the very rapid production of gas from milk-sugar. It also acts proteoclastically on the casein, and the coagulated milk is partly digested and broken into small masses. This behavior is in marked contrast to that of the bacillus of malignant œdema. Very rapid and abundant gas production from milk occurs through the agency of *B. cloacæ*, an organism not rarely found in the human intestine.

The pathogenicity of the isolated anaerobes is also a point of importance in the establishment of their

identity. There is a considerable group of strict anaerobes whose members are able to induce in guinea-pigs (when subcutaneously injected) certain characteristic lesions, including hæmorrhagic œdema, necrosis of cells due to proteoclastic enzymes, and in certain cases evolution of gas. Finally, the introduction of cultures of putrefactive anaerobes into the circulation of a living rabbit which is killed in the course of a few minutes and then incubated is sometimes a method very helpful for the establishment of the identity of an anaerobic microorganism. This method, which was first employed by Welch and Nuttall,[1] must be regarded as an extremely important means of revealing not only the gas-forming properties of a pure culture of anaerobes, but also of showing those of a suspension of the mixed fæcal flora. Here the blood and tissues of the rabbit act as a peculiarly favorable culture medium for the growth of the gas-bacillus (*B. aerogenes capsulatus*), the bacteria having been thoroughly spread by the blood through the body and the conditions of growth being highly anaerobic. Welch and Nuttall made use of their procedure to isolate the gas-bacillus and to demonstrate its ability to make gas on a proteid medium containing little sugar. The bacillus of rauschbrand (symptomatic anthrax) also induces the conditions obtained by the gas-bacillus, but unlike the gas-bacillus, sporulates under these conditions. The bacilli of botulism and of malignant œdema do not

[1] "A Gas-producing Bacillus (*B. aerogenes capsulatus*, nov. spec.) Capable of Rapid Development in the Blood Vessels after Death," *Bull. of the Johns Hopkins Hosp.*, iii, p. 81, 1892.

form gas in the incubated rabbit, but induce other evidences of putrefaction.

It seems singular that almost no use has been made by subsequent investigators of this ingenious and extremely valuable method of studying the gas-bacillus. Acting on the suggestions carried by the paper of Welch and Nuttall, we have used the method not merely as an aid in the identification of *B. aerogenes capsulatus*, but also (with somewhat unexpected success) as a means of determining whether the gas-bacillus is present in considerable numbers in the fæces.

The conditions found at autopsy after twenty-four hours' incubation of a rabbit previously injected intravenously with a pure culture of *B. aerogenes capsulatus* have been so fully described by Professor Welch that it is unnecessary to add anything to his description aside from emphasizing the fact that the almost intolerable odor of putrefaction which is developed during the incubation experiment is dependent in part on the production of butyric or a closely allied acid.[1] It should also be added that an odor very similar to that characteristic of the incubation experiment can be observed in the fæces of some persons with chronic disturbances of digestion, and frequently in persons with advanced anæmias associated with irregular diarrhœal conditions.

The incubation method of Welch and Nuttall has apparently never been employed in connection with the

[1] I do not know of another equally impressive example of the ability of microörganisms to induce rapid putrefactive decomposition.

study of the human fæces, but I believe it has here an important clinical application. Although it is true that *B. aerogenes capsulatus* can be isolated from the fæces of a majority of adult individuals, including very many who are in excellent health, it is also true that there are wide differences in the number of *capsulati* habitually present in the case of different individuals. There are some young persons between the ages of five and twenty years from whom it is either very difficult to obtain *B. aerogenes capsulatus* by plating or whose movements give no evidence whatever of its presence. If we prepare a suspension of the fæces from such individuals by grinding one gram of the fresh material with nine cubic centimeters of 0.85 per cent. salt solution and filtering through absorbent cotton, we can inject intravenously one or two cubic centimeters of this suspension into a rabbit and then incubate the quickly killed rabbit for five hours at 37° C. without obtaining evidence of the abundant presence of the gas-bacillus. On opening a rabbit which has been thus incubated, one finds none of the signs of the activity of the gas-bacillus — no accumulation of gas in the liver or vessels or in the connective tissues or serous cavities. Moreover, smears made from the liver blood or the auricular blood either do not show the presence of *capsulatus* at all, or these organisms are present only in small numbers. If, however, the foregoing experiment be made with the fæcal material derived from a patient with pernicious anæmia or from a person suffering from a *capsulatus* diarrhœa, one generally gets an entirely different result. At the end of five

hours the liver is soft and friable, crepitates between the fingers, and on section shows the presence of many bubbles of gas. There may also be a small accumulation of gas in the peritoneal cavity. Smears from the hepatic blood and from the auricular blood swarm with organisms of the *capsulatus* type. The spleen also contains such organisms in great numbers.

The following protocols are instructive in this connection:—

Experiment 1. One cubic centimeter of a filtered fæcal suspension, from a normal person sixteen years of age, almost free from putrefactive products in the urine, was injected into a rabbit which was immediately killed by a blow on the neck. After five hours' incubation, the animal was examined. Abdomen distended slightly from distension of large intestine; liver firm, slightly friable, and free from gas. A stained liver smear shows a few short bacilli (not *capsulatus*). Heart's blood shows short bacilli in abundance, rarely a bacillus of *capsulatus* type.

Experiment 2. Two cubic centimeters of filtered fæcal suspension from a healthy man (æt. 42) recently recovered from universal eczema, were injected intravenously into a rabbit which was then promptly killed by a blow on the neck. Animal incubated for five hours at 37° C. On exhibition, no odor of butyric decomposition; liver firm and without gas bubbles. Smear from heart's blood showed a few bacteria of *capsulatus* type.

Experiment 3. Two cubic centimeters of filtered fæcal suspension from a healthy breast-fed baby were infused intravenously into a rabbit which was then promptly killed. Examination after five hours' incubation at 37° C. reveals no odor of butyric putrefaction, and liver is firm and free from gas. Smears from the heart's blood show it to be free from bacteria of any kind.

These experiments may be contrasted with the following:—

Experiment 4. Two cubic centimeters of filtered fæcal suspension from an anæmic baby with irregular diarrhœa were in-

fused intravenously into a rabbit which was then promptly killed and incubated for five hours at 37° C. On examination the tissues gave a strong butyric acid odor. Liver soft, friable, and filled with bubbles of gas. Smears from heart's blood show a great abundance of bacteria of the *capsulatus* type.

EXPERIMENT 5. One cubic centimeter of filtered fæcal suspension from a patient with pernicious anæmia was infused intravenously into a rabbit which was then incubated for five hours at 37° C. On examination the abdomen was slightly distended. Characteristic butyric odor. Liver crepitant, contains a few obvious gas bubbles. Bacilli of *capsulatus* type abundant in heart's blood, in almost pure culture. *Capsulati* also abundant in liver.

EXPERIMENT 6. Two cubic centimeters of filtered fæcal suspension from a patient with pernicious anæmia whose fæces contained a great abundance of free (*capsulatus?*) spores were injected intravenously into a rabbit which was incubated at 37° C. for twenty-four hours. At autopsy the animal was greatly distended with gas, and bloody fluid was oozing freely from nose, mouth, etc. Gas escaping from abdominal cavity burns with blue flame. Extremely offensive odor of butyric decomposition. Tissues in advanced state of putrefactive liquefaction. Blood from heart shows bacilli of *capsulatus* type to be extremely abundant. Most of these were Gram-positive and occurred characteristically in diplobacillus form, but there were also many long threads which were doubtless *capsulati*. Gram-negative forms also occur and of these one variety was especially prominent. This was a long organism bearing a large spore near either end. It is probably to be regarded as a young form of *capsulatus* about to undergo division midway between the spores. This organism was Gram-negative.

The foregoing experiments are typical of a large group and show plainly enough the difference in *capsulatus* activity in the case of material derived from normal and pathological fæces. It appears to be true that a ten per cent. suspension of fæces does not excite an active formation of gas in the liver, if the material injected has been derived from persons free from intestinal derangements, and with scanty evidences of putrefactive pro-

ducts in the urine. On the other hand, material from persons whose stools contain an abundance of *capsulatus* (including a large proportion of persons suffering from "primary" pernicious anæmia) induces with great regularity the peculiar alterations in incubated rabbits which have been already described. The short period of time (four to six hours) which is required to bring about the liberation of gas in the liver is a point worthy of notice.

The distribution of *B. aerogenes capsulatus* in the bodies of rabbits that have been subjected to the incubation test is a matter of some interest. Where normal rabbits are employed for this test one finds large numbers of the gas-bacillus in the liver and often considerable numbers in the heart's blood. One finds also moderate or considerable numbers of the bacilli in the spleen, where they have doubtless been arrested owing to mechanical conditions. In the brain, in the suprarenals, in the kidneys, and in other organs, one finds very few gas-bacilli. One may experimentally change somewhat the distribution of these organisms if previous to the injection and killing of the rabbit the animal be fed on large quantities of dextrose — say twenty grams daily for three days. Under these conditions, if the inoculation test be carried out, it will be found that not only do the liver and blood contain much larger numbers of gas-bacilli than is ordinarily the case, but the spleen also contains great numbers of the same organism. Moreover, the spleen which in normal rabbits gives no gas, will be found to contain numerous bubbles of gas and therefore

to float on the surface of water. This greatly enhanced growth of the gas-bacillus in animals fed upon sugar is doubtless connected with the increased blood content of the organism in glucose. It is paralleled by the conditions which we find at autopsy in persons dying of diabetes. I have tried to rid the body of its carbohydrates by means of phlorhizin poisoning to such a point that the growth of the gas-bacillus is impaired in the incubation test, but have not succeeded in distinctly altering the habitual bacteria.

The selective action of the dead organism for *B. aerogenes capsulatus* is certainly a striking feature. The infusion of any fæcal suspension means the introduction of many varieties of living bacteria. Yet after a few hours of incubation in the dead rabbit the number of microörganisms in the blood has been narrowed either to *capsulatus* alone or to *capsulatus* and one of two companions — often positive diplococci, sometimes spore-bearing bacilli resembling *capsulatus*, but not positively identified. A longer period of incubation usually eliminates from the blood all organisms except those of the *capsulatus* type. The initial bactericidal power of the blood and cell juices may suffice to kill many of the bacteria of the fæces, while the strict anaerobic conditions, so necessary to the multiplication of *capsulatus*, in itself cuts out many varieties.

It is believed that the more refined application of this method to the study of the fæces will prove of clinical value in several directions. It may also prove of utility in the study of milk. If, as seems probable, *B. aerogenes*

capsulatus is really identical with the *granulo-bacillus immobilis liquefaciens* of Grassberger and Schattenfroh (as is claimed by some observers and denied by others, including Fraenkel), this method may prove helpful in connection with the study of some diarrhœal diseases which have been attributed to the use of infected milk.

There is reason to think that the fæcal suspensions from most persons in good health would induce gas production in incubated rabbits, since the fæces of most apparently normal persons contain moderate numbers of *B. aerogenes capsulatus*. The difference, as regards the outcome of the incubation experiment, between the flora of these persons and the flora of persons with *capsulatus* infections is probably one of degree and not of kind. Some idea of the relative numbers of *capsulati* present might perhaps be gained by determining the smallest volume of a given fæcal suspension that will just suffice to induce the distinctive gas production in incubated rabbits within a given time.

As it was obviously impracticable to isolate in every instance the dominant microörganisms of the fæces, it was resolved to study the action of the mixed fæcal flora. This was done in the hope of obtaining information which should be of service in determining what bacteria are really dominant in the intestine. The effort has not been wholly successful, but has, nevertheless, proved useful in several ways. A suspension of the mixed flora was prepared in normal salt solution in the proportion of about one part of material by weight to ten parts of the solution. From this suspension a series of

fermentation tubes was inoculated in a uniform manner. The fermentation tubes contained dextrose bouillon, levulose bouillon (made with Schering's diabetin), lactose bouillon, saccharose bouillon, peptone bouillon and plain bouillon, litmus milk, dextrose bouillon containing methylene blue, dextrose bouillon containing neutral red, and dextrose bouillon containing methyl violet. The tubes containing the dyes were employed to determine the reducing activity of the bacteria. As the information yielded by these color tubes did not appear especially helpful, the method was temporarily abandoned. Far more useful was the study of the gas production in the sugar-bouillon tubes. These tubes contained concentrations of the sugars already mentioned equal to two per cent. in each case. It was found that the least gas was usually obtained on the saccharose medium, and the most on the dextrose or lactose. The quantity of gas produced in conditions of health by the mixed flora is somewhat variable, but may be roughly stated as varying ordinarily from fifteen to thirty per cent. of the height of the anaerobic limb. This estimate is based not on any one sugar tube, but on the average of the four tubes, the gas production, as already stated, having been unequal in these different tubes. In normal children on a milk diet the gas production is often somewhat less than in adults and may be not more than ten to fifteen per cent. In conditions of disease the gas production was found to be usually considerably less than the average production in health. This is true both of adults and of children. In the case of well-

marked examples of saccharo-butyric putrefaction the quantity of gas produced may be one-half, one-third, one-quarter, or even one-fifth of the normal gas production. I am disposed to attribute this mainly or wholly to an elimination of the colon bacilli. The gas production in the sugar-bouillon tubes has in several instances been observed to correspond fairly closely to the volume of gas produced by the colon bacilli from the same case. It is known that different strains of colon bacilli liberate different quantities of gas on the same sugar-bouillon medium, and I am therefore disposed to attribute the fluctuations observed in health mainly to the different gas-producing character of organisms of the colon bacillus group. It is possible that *B. lactis aerogenes* plays a part, but I believe this organism to have a subordinate rôle. There are cases in which the intestine contains yeast organisms, and under these circumstances there is a very much larger gas production than is ordinarily the case. A very abundant gas production in the sugar-bouillon tubes inoculated from the fæcal flora should excite suspicion of the presence of yeasts. Sometimes, however, the excessive gas production is dependent on the growth of *B. lactis aerogenes*. As I have already stated, this organism in pure culture does not ordinarily grow in the closed limb of the fermentation tube, and in order to enable it to grow it has to be aided by the presence of blood or bits of sterile tissue. We have observed instances, however, in which *B. aerogenes capsulatus* has grown in the closed limb of the fermentation tube without the addition of tissue, and I attribute this

to the transference to the tube of some nutritive substance contained in the intestine.

By far the greatest value of the gas production in the fermentation tubes is in those cases in which the gas production is much below normal. This diminution in gas formation is not a variable occurrence, but in the case of the same individual is persistent so long as the conditions of diet and habits of life remain the same. It is, in fact, an individual peculiarity. As already stated, it appears to depend on the elimination of typical colon bacilli. This view has been supported repeatedly by the results of plating upon litmus gelatin. The disappearance of the colon bacilli is occasionally met with in persons apparently in good health, but I believe this to be a very exceptional occurrence. Ordinarily the inability to produce gas in normal abundance is a sign of a temporary or persistent alteration in the character of the intestinal flora. There are instances, indeed, in which we have been unable to obtain any gas whatever in some or all of the sugar-bouillon tubes. These have been usually instances of an extremely marked form of saccharo-butyric putrefaction and have not been very uncommon among cases of pernicious anæmia associated with an infection with *B. aerogenes capsulatus* and the disappearance of the colon bacilli. This disappearance has been noted also in some cases of mucous colitis in which a foreign race of colon bacilli (or organisms intermediate between colon bacilli and typhoid or paratyphoid bacilli) have been found to be the dominant representatives of the colon bacillus group.

The influence of diet must not be overlooked in connection with the question of gas production in the fermentation tubes by the mixed fæcal flora. It seems probable that a diet containing an abundance of carbohydrates leads to greater gas production than a diet in which carbohydrates are much restricted. A patient on a diet consisting chiefly of meat will harbor organisms making less gas than if he were on a mixed diet. The fall in gas on a meat diet may amount to forty or fifty per cent. of the total gas production on a mixed diet, perhaps in some cases to more than this. This factor is thus one which must be taken into consideration in interpreting the results of the gas production by the mixed fæcal flora.

From the peptone-bouillon tube it is possible by means of the Ehrlich dimethylamidobenzaldehyde reaction to form a rough estimate of the abundance of the indol formed. Some idea of the quantity of ammonia and other volatile bases produced may be obtained by the use of Nessler's reagent. By diluting the bouillon and using definite quantities of Nessler's reagent in making the tests, one may gain some idea as to quantitative differences in regard to the production of bases. The addition of strong hydrochloric acid to the peptone-bouillon tube causes in some cases a liberation of sulphureted hydrogen, which collects in the upper portion of the closed limb and may give an indication of an unusual formation of this gas. That it is mainly or wholly hydrogen sulphide that collects under these circumstances is shown by the fact that the addition of a solution of

an iron or cadmium salt leads to the reabsorption of the sulphur compound, giving rise to sulphides of the metals.

In some cases fermentation tubes containing bouillon (or sugar bouillon) have had added to them calcium or magnesium carbonate. By maintaining the neutrality of the culture medium the carbonates have influenced the character of the dominant organisms in the fermentation tubes and have thus modified the proportions of the products formed and even to some extent their character.

It has been found useful to examine regularly the sediments of the fermentation tubes which have been inoculated with the mixed fæcal flora. This has been done as a matter of routine in a large number of instances, the examination having been made especially in the case of the four sugar-bouillon tubes and the peptone-bouillon tube. The appearance of the Gram-stained flora gives as a rule, but not always, an indication of the dominant flora in the lower part of the intestine. In tubes inoculated from normal persons, Gram-negative organisms corresponding in size and form to bacteria of the *B. coli* type grow abundantly in all the tubes. As a rule they constitute the dominant flora in all the sediments. Generally one sees mixed with them moderate numbers of Gram-positive and Gram-negative diplococci. Moderate numbers of organisms morphologically like *B. aerogenes capsulatus* are seen, but these forms may be wholly absent. There is an aerobic, Gram-positive bacillus, resembling closely in its morphology

the anaerobe just mentioned, and the two organisms can be positively distinguished only by making blood-agar cultures under aerobic and anaerobic conditions. The growth of the organisms is usually slighter in a sugar-free tube than in the ones containing sugar. The diplococcal forms, especially, are apt to grow poorly in the peptone bouillon and may quite fail to multiply. In material containing large numbers of the plain form of *B. bifidus* one finds in the sugar tubes large numbers of this organism in its bifid form. Very beautiful branching forms, presenting considerable variety in their morphology, may be observed. One has this experience especially with the lactose and dextrose bouillon, and I think the growths of *bifidus* are apt to be especially luxuriant in the lactose-bouillon tube. The so-called punctate form already described is very common in the fermentation tubes.

Under pathological conditions one may meet with different bacterial elements in the sediments. In cases in which the intestine contains large numbers of positive diplococci or streptococci, there is an abundant growth of positive diplococcus forms and streptococcus forms in the sugar tubes. One may find streptococcus forms very abundant even in cases where the tubes have been inoculated from flora not containing a strikingly increased number of such bacteria. As a rule a very great preponderance of streptococcal and diplococcal forms in the fermentation tubes points to an excess of such organisms in the digestive tract. By the use of this method attention has sometimes been drawn to this type of bacterial

activity in the digestive tract where it would otherwise have been overlooked. In some instances, also, one sees a great development of anaerobes in the fermentation tubes. Sometimes one sees sedimentary fields which consist wholly of streptococcal forms and of forms which are probably *B. aerogenes capsulatus*. This has been repeatedly noticed in some cases of advanced chronic saccharo-butyric putrefaction with an extreme degree of anæmia. The tendency for the organisms of the *B. coli* type to be poorly represented in cases of chronic saccharo-butyric putrefaction is evident in the sediment of the fermentation tubes. In some cases one finds that the fermentation tubes develop a greenish color in the open aerobic arm of the tube and also a greenish pellicle. Culture shows this to be due to the *B. pyocyaneus*. This organism has been found in only a small number of cases and usually, but not always, in persons presenting signs of digestive disorder.

It cannot be claimed that we now know the full value or the limitations of the information to be derived from the careful study of the sediments of the fermentation tubes, but it may safely be said that this method of investigation has often been helpful in gaining a clew to the nature of the dominant organisms in the intestinal tract. One cannot rely upon it alone, but in connection with data derived from other methods it helps us to form a conception of the bacterial types present and active in the lower part of the digestive tract. One finds in the same case almost regularly the same kinds of sediments, and this gives a degree of confidence in the

value of the sediments, as an indication of the well-defined character of the bacterial conditions that give rise to these individual peculiarities. A highly interesting observation which has been repeatedly made is that the character of the sediments may be greatly altered as a patient grows better or develops a more intense degree of a condition from which he is suffering. The gradual reappearance of the colon bacilli in the sedimentary fields, with the corresponding disappearance of an excessive number of coccal and streptococcal forms and of anaerobes, has repeatedly been noticed as a concomitant of a pronounced improvement in clinical conditions. In addition to the study of the mixed fæcal flora (as grown upon fermentation tubes) many observations have now been made upon larger volumes of media in which the mixed flora has grown for relatively long periods of time, usually a week. As a routine procedure in the investigation of many normal persons and numerous pathological conditions, four flasks containing about five hundred cubic centimeters each of medium have been inoculated with the suspensions of the mixed flora. The media employed have been peptone bouillon, peptone bouillon with calcium carbonate, sugar bouillon and sugar bouillon with calcium carbonate. The organisms have been permitted to grow for a period of seven days. Under the conditions prevailing in these flasks a large part of the growth has been anaerobic and a high degree of anaerobiosis has been maintained, in part owing to the formation of reducing products such as hydrogen, which are incidental to the development of fermentative and

putrefactive cleavages. Two objects have been served through the employment of these flask cultures: first, the study of the bacterial sediments after considerable periods of growth; and, secondly, the study of the products formed on a representative medium. As regards the characters of the organisms found in the sediments (at the end of seven days) it is not possible at present to state definitely to what extent the biochemistry of the digestive tract can be furthered by the mere study of the Gram-stained fields, without resort to cultural methods permitting positive identification of the dominant forms. The appearances in the flasks usually vary considerably with the different media employed and also according to the origin of the suspensions employed for inoculation. It has been found, in general, that the anaerobes grow more abundantly in the flasks in which a neutral reaction is maintained, owing to the presence of calcium carbonate. Whether the presence of calcium ions as such influences the growth of the organisms in a specific way is not known. It is certain that in some cases of excessive intestinal putrefaction attention has been called to the prolific growth of certain anaerobes which have been much less prominent in the intestinal contents themselves and in the sediments of the fermentation tubes (in which the suspended bacteria have been permitted to grow for a considerably shorter time than in the flasks). In several instances microörganisms of the morphological type of *B. putrificus* have been extremely abundant in the sediments of the calcium-carbonate flasks. Later it has been noticed in some of

these cases that the growth of what we may assume to have been *B. putrificus* is no longer evident, and this change in the character of the growths in the flasks has corresponded to a clinical improvement in the condition of the patients concerned. As regards *B. aerogenes capsulatus*, it cannot be said that we have learned more from the seven-day flasks than from the fermentation tube sediments already mentioned. In a few instances it has been observed that the peptone-bouillon flask has contained enormous numbers of spores when the corresponding calcium-carbonate flask has shown large numbers of vegetative forms of putrefactive microörganisms and relatively few spores. Further studies are necessary to determine the value of the seven-day-flask method and to show how the bacterial fields are to be interpreted.

The chemical examination of the seven-day flasks has included two different series of procedures. In the case of the peptone-bouillon flask and the peptone-bouillon flask containing calcium carbonate, the contents have been examined with respect to hydrogen sulphide, methyl mercaptan, volatile fatty acids, ammonia, indol, skatol, and phenol.[1] Quantitative deter-

[1] A number of observations have been made upon the bouillon and bouillon-carbonate flasks inoculated from the mixed flora with a view to determining whether indol acetic acid is ever formed. The method consisted in applying the three tests of Salkowski to the material which remains behind after the distillation of indol and skatol. In one case only has this residual material given reactions with the three tests of Salkowski. This was in the case of flasks prepared from a patient with peripheral neuritis and pronounced psychosis resembling that of alcoholic intoxication.

minations have regularly been made in the case of the volatile fatty acids, ammonia, indol, skatol, and phenol. Alcohol and acetone have also been tested for in these flasks. In the case of the sugar-bouillon flasks the contents have been examined for alcohol, acetone, the volatile fatty acids, and the non-volatile organic acids. The molecular weight of the barium salts of the volatile fatty acids has regularly been determined in order to obtain a conception of the chemical nature and quantity of the chief fatty acids produced. The interesting observation has been made that in the flasks containing calcium carbonate the molecular weight obtained for the volatile fatty acids has nearly always been somewhat higher than in the case of the molecular weight obtained from the volatile fatty acids of the sugar-bouillon flasks. This fact corresponds to the appearances in the smears (already noted) that putrefactive anaerobes are found in greater abundance in the neutral flasks than in the sugar-bouillon flasks to which calcium carbonate has not been added.

The fact that acetone has been found in many instances in the flasks containing peptone bouillon and peptone bouillon with the addition of calcium carbonate will be referred to in reviewing the facts regarding the nature of the products of fermentative putrefactive decomposition.

The method employed to determine the presence of

In this case, however, alcoholic intoxication could be almost certainly excluded. There were various other indications that the neuritis and psychosis were due to intestinal intoxications.

methyl mercaptan is as follows: One hundred cubic centimeters of the culture to be examined are transferred to a flask communicating through a calcium chloride tube with an Erlenmeyer flask containing isatin dissolved in concentrated sulphuric acid. A current of air is then drawn through both flasks so that any mercaptan given off from the culture flask will enter the isatin-sulphuric-acid flask. The presence of mercaptan is indicated under these conditions by a gradual change of the isatin solution from red to olive-green or grass-green.[1] In order to save isatin the reaction may be carried out substituting a test-tube containing about ten cubic centimeters of the isatin-sulphuric-acid mixture for the Erlenmeyer flask. The method is not adapted for quantitative determinations, but some idea can be gained through it of the quantity of mercaptan present in the culture, and it further serves to indicate differences in the amount formed in different cultures. Twenty-five milligrams of a one per cent. solution of methyl mercaptan suffice gradually to alter the isatin red solution (about fifty cubic centimeters) to a deep green in the course of ten minutes. Reactions as strong as this are occasionally obtained from one hundred cubic centimeters of a bacterial culture in the course of five or ten minutes. Frequently

[1] This method has been used by Niemann, ("Ueber die Abspaltung von Kohlensäure, Mercaptan und Schwefelwasserstoff beim Kochen einiger animalischen und vegetabilischen Nahrungsmittel," *Archiv f. Hyg.*, xix, p. 126, 1893); also by Bauer, ("Ueber die Einwirkung gespannter Wasserdampfe auf Keratin," *Zeitschr. f. physiol. Chem.*, xxxv, p. 346, 1902).

one obtains after the lapse of fifteen minutes only a browning of the isatin-sulphuric-acid solution with no definite appearance of a green tint. This appearance is probably to be interpreted as dependent on a mere trace of methyl mercaptan.

The method used for the determination of indol obtained from distillation of putrefactive mixtures is that based on the formation of a color compound due to the combination of two molecules of indol with one molecule of β-napthaquinone-sodium-monosulphonate. This method has proved in a high degree serviceable and may be recommended on account of its accuracy and quickness. The main features of this method are as follows:—

The method is dependent upon the almost quantitative precipitation of indol in alkaline solution by a dilute solution of β-naphthaquinone-sodium-monosulphonate. A dilute solution of indol (1 : 500,000 parts of water) made slightly alkaline with potassium hydroxide, gives with one drop of a two per cent. solution of β-naphthaquinone-sodium-monosulphonate a blue or green-blue color. A more concentrated solution of indol will give a precipitate which upon examination will be found to consist of well-defined acicular needles, bluish in color and closely felted together. The compound, evident either as precipitate or as coloration, is completely soluble in chloroform, with the production of a red color. From this extract the indol may be determined quantitatively either by evaporating the chloroform or by matching the color to a standard in the Duboscq colorimeter.

A slight blue precipitate forms in solutions containing one part of indol to 256,000 parts of water; in greater dilution the coloration is green, and fails entirely when the dilution is 1 : 1,024,000 parts. Even in this extreme dilution chloroform will indicate the presence of the di-indyl-di-hydronaphthaline-keto-sodium-monosulphonate by its faintly pink color. The reaction is essentially a time reaction, and even in dilute solution it has been found desirable to allow a period of not less than ten minutes to elapse before shaking out the condensation product with chloroform. The naphthaquinone solution should be added in sufficient excess to tinge the filtrate slightly yellow.

As indol is sometimes associated in the course of putrefaction with skatol (and this is not uncommonly so in the case of the contents of the large intestine), it becomes important to have a method for the separation of indol and skatol. These putrefactive decomposition products may be separated by means of their picrates, but the method involves so much time as to make it unfit for clinical or ordinary chemical investigations. It is believed that the method described by Herter and Foster [1] will prove useful in effecting the quick and nearly complete separation of indol from skatol. Moreover, this method serves also for the determination of the quantity of skatol present.

The procedure is based on the fact that by means of the naphthaquinone method for indol which has just

[1] "On the Separation of Indol from Skatol and their Quantitative Determination," *Journ. Biol. Chem.*, II, p. 267, 1906.

been described, it is possible to remove almost completely the indol from a solution containing both indol and skatol and that the skatol remaining after the removal of the indol can be distilled and recognized by means of the dimethylamidobenzaldehyde reaction described by Ehrlich. If one takes a putrefactive mixture containing both indol and skatol, these bases should first be distilled either in acid or alkaline solution — preferably with the aid of steam if the quantities are considerable. In the distillate the skatol passes over earlier than the indol, as can easily be shown by means of the blue color which it gives on boiling with Ehrlich's aldehyde. To the distillate containing the indol enough sodium or potassium hydroxide is added to render it slightly alkaline. An excess of the β-naphthaquinone-sodium-monosulphonate is now added to this solution. As already described, this substance in the course of a few minutes reacts almost completely with the indol present, but not with the skatol. The blue or purplish-blue precipitate of the newly formed indol-naphthaquinone compound is now removed by filtration. In cases where the concentration of indol is too small to give rise to a precipitate when treated with the naphthaquinone compound the solution simply develops a green or greenish-blue color. The solution is now acidified and subjected to distillation, with or without the use of steam. The skatol passes over into the distillate, whereas the indol is held back in the form of the indol-naphthaquinone compound, with the exception of a very small uncombined portion, which passes over with the skatol. The

amount of indol, however, which passes over after treatment with the β-naphthaquinone-sodium-monosulphonate is so small that it is practically negligible, although its presence is detectable through the red color which it gives when acted upon by dimethylamidobenzaldehyde. The distillate containing skatol is boiled with a solution of Ehrlich's aldehyde in sulphuric acid.[1] A slight amount of dilute hydrochloric acid is now added and has the effect of intensifying the blue color produced by boiling the skatol with the Ehrlich aldehyde solution. A little experience is required to find the amount of hydrochloric acid which gives the maximal intensification of the reaction. An excess of hydrochloric acid causes the blue color to fade. It is important to use an excess of the Ehrlich aldehyde solution in order to develop fully the color reaction with skatol. The color obtained through the action of Ehrlich's aldehyde upon skatol is purple-blue rather than blue so long as the solution is hot. On cooling it under the tap, the blue color asserts itself more strongly, and the solution may become somewhat opalescent from the separation of uncombined dimethylamidobenzaldehyde. Chloroform is now added to the solution containing the blue product. On agitation with the solution this carries out the blue color, and the chloroform assumes a pure blue tint. By means of a good colorimeter the quantity of skatol present in the original solution may be approximated by the intensity of the color reaction.

On evaporating the chloroform containing the blue

[1] Five per cent. aldehyde in ten per cent. sulphuric acid.

color resulting from the action of the dimethylamidobenzaldehyde on skatol, one obtains an amorphous blue material which can be partially purified from the admixture with Ehrlich's aldehyde by the use of petroleum ether. The nature of this compound is not at present known. The melting point of our preparation lay between 65° C. and 66° C.

The method of separating indol and skatol here described has been used in a routine way during the past year in connection with the study of the fæces and has given satisfaction. To twenty-five grams of the material have been added twenty cubic centimeters of water and one to two cubic centimeters of a ten per cent. sodium hydroxide solution. The suspension is then subjected to distillation with the aid of steam, until the distillate no longer gives a color reaction when boiled with the dimethylamidobenzaldehyde solution. Ordinarily the indol and skatol present go over completely within an hour, but where the material develops frothing a more prolonged distillation may be necessary. When the distillate has been obtained, it is treated in the manner above outlined for the separation of indol and skatol. In some cases one obtains only skatol from the fæces, but as a rule indol is also present. In the presence of indol the chloroform extract, instead of being a pure blue, may have a slightly purplish tinge, owing to unavoidable admixture with a slight amount of indol.

It is important in making the colorimetric estimations of the quantity of skatol present to employ a standard color solution for comparison with the color obtained

from the distillate containing the skatol. Various dyes have been tried with a view to obtaining a standard solution which will retain its color unchanged. Experience has shown that the best standard color solution is one obtained from a solution of skatol. Although such a solution fades after a few days, especially when exposed to the light, and may assume a greenish tint, in the dark it may last several weeks without undergoing appreciable change. Moreover, as the skatol standard solution is readily prepared, there is little disadvantage from being compelled to renew the color solution from time to time. This solution may be conveniently prepared by dissolving five milligrams of skatol in water and acting upon it with an excess of dimethylamidobenzaldehyde. It commonly requires from one hundred to one hundred and fifty cubic centimeters of chloroform to extract completely the blue coloring matter which has already been described. The quantity of coloring matter present is sufficient to impart a deep blue color to this volume of chloroform. Ten cubic centimeters of this solution are placed in the receptacle of the Duboscq colorimeter and used as a standard for comparison with the chloroform color solution obtained from the distillate to be tested. The matching of the colors can usually be made very closely. In cases where the quantity of skatol is so small that the trace of indol present influences the color of the chloroform solution, changing it to violet or even purple, it is more difficult to obtain a satisfactory matching of colors. In this case is may be necessary to add a small quantity of indol

to the skatol employed in making the standard color solution. This then imparts to the standard color solution a violet tint like that obtained from the distillate to be matched. It seems unnecessary to give further details. After some experience with the method of matching colors it is possible to employ the method so that it will give satisfactory quantitative results.

It is not desirable here to enter into a description of the chemical methods of studying the fæces and the urine. The methods ordinarily employed for this purpose are fully described in text-books relating to these subjects. It may be stated, however, that numerous observations have been made upon the acids and bases present in human fæces in health and disease and the intestinal contents have been studied for hydrogen sulphide and for the presence of mercaptan. The reaction of the fæces with concentrated solution of mercuric chloride, according to the manner first described by Schmidt,[1] is of much value in determining the presence of a substance supposed to be hydrobilirubin. The marked red color with yellowish fluorescence which is a characteristic of Schmidt's reaction is supposed to depend on the occurrence of a combination between the mercuric salt and hydrobilirubin. An analogous combination exists in Jaffe's zinc-chloride-urobilin compound. Both substances give the same spectroscopic picture; namely, a band between the lines *b* and *E*. An intensification of the red color obtained in the Schmidt reaction may often be secured by transferring a portion of the tested

[1] "Verhandl. d. Congresses f. inn. Medicin," xiii, p. 320, 1895.

material to a beaker of distilled water. Under these circumstances the color compound diffuses out into the water and imparts to it a distinct and often strong red color which is frequently more pronounced than one would have predicted from the appearance of the material which has developed the red color under the influence of the Schmidt procedure as ordinarily carried out.

In the course of a systematic examination of the fæces from normal individuals and from many pathological series it was noted that the mercuric chloride reaction was strongest in persons suffering from intestinal disorders, especially in those with intestinal putrefaction. The weakest reactions were found in the case of alcoholic stools and in those of children and young adults presenting only slight indications of intestinal putrefactive decomposition (low ethereal sulphates, absence of indican, and low phenol).

The Dimethylamidobenzaldehyde Reaction of the Fæces. — If one extracts human fæces with an aqueous sodium chloride solution (two grams of fæces to twenty grams of 0.85 per cent. sodium chloride solution), the extract will usually yield a color reaction with a suitably prepared acid solution of Ehrlich's aldehyde.[1] In the case of healthy children or adolescents on a mixed diet the color obtained is usually a light rose, and may be very faint. Between this light tint and a very deep cherry-red, all transitions are met.[2]

[1] Water, 270 c.c.; concentrated H_2SO_4, 30 c.c.; Ehrlich's aldehyde, 15 grams.

[2] We have employed a graded color scale in order to record our results with some degree of accuracy.

The explanation of the chemical basis of the Ehrlich aldehyde reaction of the fæces is not yet wholly satisfactory. Baumstark [1] thought it could be ascribed to the indol of the fæces and based a quantitative method for indol on the reaction. Bauer,[2] however, showed that the fæces contain another substance which reacts with the aldehyde, and claims that this substance is urobilinogen. I reached a similar conclusion independently, after noticing that the fæces in one instance gave an intense red reaction after the indol had been distilled off. In other cases a strong reaction was obtained in spite of the fact that the fæces were free from indol from the outset. That the reaction from this non-volatile part of the fæces depends wholly on urobilinogen does not appear to me to have been convincingly shown. It is true, however, that one may reduce urobilin (Schuchardt's) with alkali and zinc dust and thus obtain a substance which gives a stronger and more characteristic Erhlich aldehyde reaction than the urobilin itself. Probably both urobilinogen and a skatol derivative are implicated in the Ehrlich

[1] "Bestimmungen der Fäulniss Produkte im Urin und in dem Fäces mit Benützung der Ehrlichschen Aldehydreaktion," *Münch. med. Wochenschr.*, 1, p. 722, 1903; also *Arch. f. Verdauungskrankh.*, ix, p. 201, 1903.

[2] This distillation should be conducted in an atmosphere of carbon dioxide in order to prevent oxidation of the reacting substance, which is sensitive both to air and to the action of sunlight.

Bauer fell into error in claiming that the reaction from the fæces is not due to indol and that the method does not serve for the detection of this substance. I am confirmed in this view by Ury ("Die Ehrlich'sche Reaction im Stuhl," *Zentralbl. f. innere Medizin*, xxvii, p. 41, 1906).

aldehyde reaction of the urine, but it is possible that other substances are also concerned.

The relation of the hydrobilirubin reaction to the urobilinogen reaction of the fæces with Ehrlich's aldehyde is a point of interest on which one cannot at present express an opinion. There are instances in which the fæces, after distillation of all the indol present (as shown by the aldehyde reaction, which is very delicate), still give a reaction *in the cold* with a dimethylamidobenzaldehyde solution. This reaction is believed to depend on the presence of urobilinogen. It has been fairly well marked in some of our anæmia cases, but there is as yet no evidence that the reaction bears any definite relation to the hydrobilirubin reaction, although the two substances urobilinogen and urobilin (hydrobilirubin?) are closely related chemically.

The Dimethylamidobenzaldehyde Reaction of the Urine. — It is well known that on the addition of acid solution of dimethylamidobenzaldehyde to certain urines a red color is obtained, sometimes in the cold, more often only on the addition of heat. The explanation of the reaction has been the occasion of considerable discussion. Ehrlich [1] was inclined to attribute it to glycosamin; Neubauer [2] and Bauer [3] referred it to

[1] "Ueber die Dimethylamidobenzaldehydreaction," *Med. Woche*, p. 151, 1901.

[2] "Ueber die neue Ehrlich'sche Reaction mit Dimethylamidobenzaldehyd," *Sitzungsber. d. Gesellsch. f. Morphol. u. Physiol.*, ii, p. 32, 1903.

[3] "Die Ehrlich'sche Aldehydreaktion im Harn und Stuhl," *Zentralbl. f. inn. Med.*, xxvi, p. 833, 1905.

urobilogen, which has passed from the intestine into the urine. I have found that the administration of skatol to men and to monkeys is followed by some intensification of the Ehrlich aldehyde reaction, and believe that where skatol is produced in the intestine it may contribute to intensify the reaction.[1] Dr. Adolf Meyer, Director of the Pathological Institute of the State Hospitals for the Insane, tells me that in some observations made under his direction the administration of skatol to patients has been followed by a similar though slight intensification of the dimethylamidobenzaldehyde reaction of the urine.

This reaction is seldom found in its most intense form except in the urines from persons suffering from pathological conditions. It was noticed by Ehrlich that the intensity of the reaction is apt to be great in the case of patients with phthisis, typhoid fever, and chronic enteritis, and Clemens observed that persons with digestive disorders are among those whose urines are likely to give a strong reaction. It has appeared to me that the urines of persons with the urinary evidences of excessive chronic intestinal putrefaction (excessive indican and phenol and high ethereal sulphates) are especially liable to exhibit the deepest cherry-color tints on being treated with the reagent. The urine of normal children, in whom the processes of intestinal putrefaction are apt to be mild, usually give negative or almost negative

[1] "On a Relation between Skatol and he Dimethylamidobenzaldehyde Reaction of the Urine," *Journ. Biol. Chem.*, i, p. 251, 1905.

results. Although I have come to associate an intense Ehrlich aldehyde reaction of the urine with the occurrence of a high degree of intestinal putrefaction, it must be admitted that not every case of excessive intestinal putrefaction shows this reaction in its very marked form.[1]

[1] A good discussion of Ehrlich's dimethylamidobenzaldehyde reaction in the urine by C. E. Simon may be found in the *American Journal of the Medical Sciences*, cxxvi, p. 471, 1903. Bauer (*loc. cit.*) brings forward evidence to support the view that the Ehrlich reaction depends on urobilinogen. This view appears to me not irreconcilable with the fact that the administration of skatol intensifies or even gives rise to this reaction, since more than one factor may be operative in certain instances. I am not certain of the identity of the reactions obtained by Bauer and those dependent on the administration of skatol, as I have failed to make use of spectroscopic methods of study.

COMMON BACTERIAL INFECTIONS OF THE DIGESTIVE TRACT, CONSIDERED FROM THE STANDPOINT OF THE MICROÖRGANISMS

THE COLON-TYPHOID-DYSENTERY GROUP

A SURVEY of the bacteria which we may include in the colon-typhoid-dysentery group is beset with many difficulties with respect to satisfactory classification, arising from an incomplete knowledge of the biological characters of these bacteria. We know enough, however, to see that we are dealing with microörganisms not separated from one another by unbridged gaps, but rather linked in series by most intimate affinities.

Colon Bacilli. — If we ask the question, what pathological consequences do the colon bacilli of the human intestine bring to the human species, the answer is by no means simple. Many conditions formerly attributed to the colon bacilli were given their position without adequate reason. To-day we have the right to insist on knowing the fermentative and agglutinative as well as the cultural characters of the colon organisms which are stated to be the causes of disease, and too often this information is not given.

At the present time the view is generally held by pathologists that colon bacilli are capable of doing damage in two ways, as infective agents outside the

digestive tract and as infective agents inside the tract. We are not concerned here with those examples of colon-bacillus infection in which the organisms find their way into the urinary tract from the exterior and set up a cystitis or pyelitis. Nor shall I do more than refer to those infections in which colon bacilli have passed during life through some local spot of injury in the digestive tract into the blood or the peritoneal cavity, setting up in the former case a septicæmia or pyæmia, and in the latter a peritonitis. With less serious consequences than these, the colon organisms probably often pass temporarily from the digestive tract by way of the blood, to the kidney, with a resulting pyelonephritis or pyelitis, or at least a bacteriuria. It is noteworthy that the localization of colon bacilli under such pathological conditions as these is often attended by a heightened virulence for small animals as compared with the virulence of the original bacteria of the intestine.

The question which does especially interest us here is whether the colon bacilli of an individual ever do injury to that individual while still confined to the digestive tract. In attempting to answer it, I must divide the question into two — one relating to acute effects of the colon bacilli, the other relating to prolonged pathological activities.

If we go back to the time when Escherich first described *B. coli commune* as an obligate inhabitant of the human digestive tract, we find that there was a disposition to regard it as the cause of various diarrhœal affections. A close examination of facts relating to the

identity of the organisms in many instances makes it clear that affections attributed to colon bacilli were thus classed without sufficient justification. For example, many instances of supposedly colon-bacillus diarrhœa must almost certainly have been due to the dysentery bacillus. Remembering the cultural similarities of certain colon bacilli and dysentery bacilli, it is easy to understand how confusion might have occurred when the fermentative characters were left out of account, as was usually the case. Even an organism so different from *B. coli* as the bacillus of typhoid fever was confounded with it in the early days of bacteriology, when limited cultural criteria alone were employed. Thus progress in methods of identification has step by step circumscribed the conception of the colon bacillus and given it sharper definition. There are indications that this process of circumscription has not reached its final stage.

It seems established that organisms having the cultural fermentative and agglutinative characters of *B. coli* at times are endowed with a degree of virulence for small animals which does not pertain to colon bacilli derived from healthy human individuals. Such pathogenic bacilli have been found in great abundance in some diarrhœal conditions and appear to have been the cause of these acute derangements. It is not clear whether in such cases the dominant normal organisms have acquired increased irritant properties or whether there has occurred a process of substitution by which organisms present normally in small numbers or introduced from

outside temporarily gain the numerical ascendency in consequence of alterations in the nutrient media which favor this race while repressing the ordinary dominant one. In addition to a colon-bacillus diarrhœa associated with colon bacilli of more than ordinary virulence, it is probable that we must recognize a similar derangement in which there is no increase of virulence on the part of the bacilli, but simply an enormous physiological increase which verges on the pathological. Almost any healthy child who has eaten a largely excessive quantity of ripe fruit, rich in sugar, will develop loose movements in which colon bacilli are present in very large numbers. I do not know whether these organisms are any more virulent than the type dominant previous to the excessive indulgence, but it seems extremely unlikely that they suffer any appreciable change in the course of the short interval of excessive fermentative activity. Acetic, lactic, and other organic acids are formed in the course of the fermentative process rendered possible by the sugar-holding fruit, and it is to the irritant action of these acids, and perhaps also to an excessive liberation of gas, that the symptoms must be ascribed. Very likely, too, in a case like this, bacilli of the group of *B. lactis aerogenes* aid in the fermentation. In this instance we are dealing clearly with a process on the borderland between the physiological and the pathological.

Of the influence exerted by colon bacilli in the course of chronic processes within the digestive tract we have as yet little knowledge, partly because etiological relations are very difficult to establish in the case of obligate

bacteria. Attention has lately been called by Dr. Turck [1] of Chicago to the fact that he has experimentally induced in dogs the formation of ulcers of the stomach in many respects comparable to those occurring in man. The fact is in itself one of considerable interest to pathologists, although its bearing on the etiology of human gastric ulcers is obscure. The reported experimental results were obtained by feeding dogs, during considerable periods, bouillon cultures of bacilli derived from patients with gastric ulcer. The bacilli are stated to have been colon bacilli, but it is regretable that the cultural and biochemical characters of the bacteria are not mentioned. Moreover, while it seems likely that human colon bacilli may in disease migrate to the gastric region and multiply freely there, this is not now known to be the case in ulcer.[2]

In considering the part played by the colon bacilli in chronic disorders one fact stands out distinctly and gives us a clew to the nature of the activity of these bacilli. Human colon bacilli have practically no power to dissolve and peptonize native proteids, such as casein or egg albumen. I conclude from experiments which I

[1] "Ulcer of the Stomach; Pathogenesis, Pathology," *Journ. Amer. Med. Assoc.*, xlvi, p. 1753, 1906.

[2] The association of colon-bacillus infection of the stomach with gastric ulcer would not of course prove an etiological relation, as the infection might be a secondary phenomenon. Moreover, it must be remembered that the production of ulcers in dogs by means of human colon bacilli involves the action of foreign organisms. The presence of the obligate and homologous colon bacilli of man in the human stomach is thus not strictly comparable with the conditions induced in Turck's experiments.

have made on milk that the peptonizing action of these bacilli is extremely slight, though not absolutely lacking. On the other hand, the colon bacilli are able to cleave energetically peptones prepared by other microörganisms. Then they give rise to familiar products of putrefaction such as ammonia, volatile fatty acids, phenol and indol, and hydrogen sulphide. These facts have an important bearing on the significance of these bacteria within the digestive tract, which may be summarized as follows. If there is good absorption of proteids above the lower ileum, that is, above the level where there is a predominance of colon bacilli, little proteid material finds its way into the colon. Hence whatever may be the nature of the bacterial inhabitants of the large intestine, but little putrefactive decomposition occurs there. But if, owing to a superabundance of proteid food considerable native proteid finds its way into the colon, its fate depends on the character of the flora there. In a normal intestine, containing few peptonizing bacteria, there will be little putrefaction, because the dominant races, members of the coli group, cannot initiate active putrefaction of native proteids. But if there be present also putrefactive anaerobes capable of peptonizing proteids, the colon bacilli take an active part in breaking down the hydrolyzed proteids with which they are thus supplied. In this way the colon bacilli frequently become active participants in excessive intestinal putrefaction. I shall recur to this subject in the discussion of the putrefactive anaerobes.

The disappearance of typical colon bacilli from the

fæces of persons in whom such bacteria have previously been present is a phenomenon of much interest. It is observed in a variety of pathological conditions: in cases of excessive saccharo-butyric putrefaction due to infection with *B. aerogenes capsulatus*, in some cases of mucous colitis, in certain instances of dysentery due to the Shiga bacillus. Probably it occurs also in cholera and typhoid fever.

The disappearance of *B. coli* from the contents of the lower bowel is in itself no proof that these organisms are not present higher up, but I think it presumptive evidence that the growth of these obligate bacteria is at least more restricted at higher levels than is normally the case. Frequently the failure to find living colon bacilli in the fæces is representative of the entire gastro-enteric tract, since in these cases diarrhœal movements (spontaneous or due to purgatives) also fail to contain them.

One is justified in suspecting a complete or partial suppression of *B. coli* if the mixed fæcal flora fails to form gas in sugar-bouillon fermentation tubes or makes gas in smaller quantities than is usual for normal flora. This suspicion, if correct, is confirmed by the failure of colon bacilli to appear on litmus gelatin plates or by their appearance in abnormally small numbers.

The possibility that some cases of mucous colitis are due to colon bacillus infection has been suggested by Sir A. E. Wright, who tells me that he has cured certain obstinate and extreme instances of this affection by inoculations made with killed colon bacilli. The bacilli

used for inoculation were obtained in one instance from the cystitic urine of the patient. The study of mucous colitis from this standpoint appears promising. It is very desirable that the biochemical characters of the bacilli concerned in this disease should be well established. The possibility has not been excluded, I think, that in the cases mentioned by Dr. Wright the organisms concerned were not typical colon bacilli, but closely related pathological varieties.

Typhoid Bacilli. — It has just been pointed out that organisms of the strict colon bacillus group are not at present known to be the cause of severe acute intestinal diseases and that their chief rôle in chronic disorders of the intestine depends on their participating in putrefactive decompositions. We may briefly consider a microörganism which, while closely affiliated to the colon bacillus, is morphologically and in cultural characters different from it in important biochemical properties and is immeasurably more pathogenic for the human race. This is the bacillus of typhoid fever discovered by Eberth and Gaffky independently in 1884. This organism as compared with the colon bacillus is far less hardy in its growth on ordinary culture media and requires much more special conditions in order to thrive — conditions which it often finds in the human body. We do not know in what relation it stands to the colon bacillus in the long process of natural evolution. The claim that colon bacilli can be experimentally transformed into typhoid bacilli has never been made good, and still we cannot deny that what bacteriologists cannot do in the laboratory

may possibly have been done by nature and may perhaps be still in progress. The existence of bacteria intermediate between colon bacilli and typhoid bacilli — the paratyphoid and paracolon bacteria — is now established, and the fact that there are these transitional forms suggests that they may have been descended from a common ancestor through successive slow modifications.[1]

[1] The studies of Doebert ("Die verwandtschaftlichen Beziehungen zwischen den Bacillus faecalis alkaligenes und dem Typhusbazillus," *Archiv f. Hyg.*, lii, p. 70, 1905) indicate that there exists a close resemblance between the bacillus of typhoid fever and Petruschky's *B. fæcalis alcaligenes*. A culture of the latter organism furnished by Petruschky rendered litmus milk blue in twenty-four hours, forming a pellicle on the surface of the milk, and on potato gave a yellowish brown growth in one day. With typhoid serum the organism agglutinated in 1:800. It is stated that it was easy to modify the organism of Petruschky by passing it through guinea-pigs so that it acquired the cultural characters of the true typhoid bacilli. The organism modified by Doebert experimentally by passage through guinea-pigs was not agglutinated by the serum from guinea-pigs in 1:100, but was agglutinated by typhoid serum in a dilution of 1:15,000. Another strain of *B. fæcalis alcaligenes* studied by Doebert could not be converted in this manner into an organism so closely resembling the typhoid bacillus by means of passage through animals.

There appears to be little doubt that *B. fæcalis alcaligenes* is sometimes responsible for the occurrence of a febrile disease at present indistinguishable from mild forms of typhoid fever on purely clinical grounds. Though closely related to the bacillus of typhoid fever, it appears proper to regard the bacillus of Petruschky as a distinct bacterium. (For details as to the distinction of *B. fæcalis alcaligenes* and *B. typhi* see Piorkowski, "Zur Differenzierung der Typhusbacillus und Bacillus fæcalis alkaligenes," *Centralbl. f. Bakt.*, I Abt., Orig., xl, p. 437, 1905–06.) It seems most reasonable to look upon *B. fæcalis alcaligenes* as a variant of the typhoid bacillus which has lost the capacity to act fermentatively on dextrose. Something might be said in favor of regarding

While colon bacilli possess in a marked degree certain commonplace properties such as the ability to coagulate milk, to reduce neutral red, to make indol, and to ferment sugars, with the liberation of gas and acids, these properties have been lost in the case of the typhoid bacilli, excepting only the capacity to form acid from sugars. In respect to these characters, the paracolon and paratyphoid bacilli occupy an intermediate position. The loss of these relatively banal properties has been associated with the development of pathogenic properties for man — properties which are doubtless dependent on substances chemically well defined but at present quite unknown. It has, for several years, been recognized by students of immunity that in respect to the formation of these toxic substances there is a striking inconsistency in the behavior of the typhoid bacilli in the body and in artificial culture media; for in the former case, while they make poisons often sufficiently active to kill, in the latter only feeble poisons are obtainable. In partial explanation of this the view was advanced that, unlike the bacilli of diphtheria, which make

it as a hog-cholera variant or even as an extreme modification of *B. coli*. Its active motility, however, places it nearest to the typhoid or paratyphoid groups. Brion and Kayser ("Die nosologische Stellung des Symptomkomplexes 'Abdominaltyphus,'" *Deutsches Archiv f. klin. Med.*, lxxxv, p. 552, 1905–06) have described an organism differing distinctly from typhoid bacillus, but which was obtained from a patient showing a fever clinically resembling a mild typhoid. From the blood of this patient an organism was cultivated which agglutinated with the patient's serum in a dilution of 1 : 500. It had the property of liquefying gelatin and formed yellow colonies. They gave to it the name of *Bacterium flavosepticum*.

soluble poisons diffusing readily from the bodies of the bacteria, the typhoid bacilli hold their poisons tenaciously in their bodies, and that these endotoxins may be liberated in the body of the host, when the bacterial bodies are dissolved, but are not so liberated in ordinary culture media. A more adequate hypothesis was formulated by Professor Welch. According to this, the typhoid bacilli are stimulated in the body by means of its defensive juices to form and liberate their poisons. Such a stimulus being wholly lacking in artificial culture media, where their existence is not actively threatened, the bacilli are not forced to make their poisons. It may be that the difference in the poisons formed in the body and in culture media is mainly one of quantity rather than of kind. There is at least some ground for thinking that the character of the immunity is the same, whether the poison comes from the bodies of bacteria in culture or from similar bacteria under the stimulus of the protective juices of an animal.

The word "aggressins" has been introduced by Kruse and Oscar Bail [1] to designate substances of unknown constitution which are formed by most pathogenic bacteria when in antagonism with the animal organism, as for example when introduced into the peritoneal cavity of a guinea-pig — substances which break down in some way the natural defenses of the body, so that the bacterial invaders, previously held in check, become reproductively and invasively active. The "aggressins"

[1] "Untersuchungen über Typhus und Cholera Immunität," *Archiv f. Hyg.*, lii, p. 272, 1905.

were obtained from the peritoneal exudate and carefully separated from cellular elements by the centrifuge. It was found that numbers of typhoid or cholera bacilli below the fatal dose become fatal when injected simultaneously with "aggressins," that it is possible by means of "aggressins" to inhibit the protective action of a bacterial immune serum in the peritoneal cavity of the guinea-pig, and, finally, that one may by suitable treatment with "aggressins" induce immunity to fatal doses of typhoid or cholera bacilli. Bail regards this "aggressin" immunity as something distinct from a bactericidal immunity because a bacterial immune serum does not protect against "aggressins," but very recent studies by Wassermann and Citron [1] indicate that "aggressins" are really bacterial extracts, and that their influence in promoting infection depends on their ability to bind defensive substances largely derived from leucocytes and designated complements in Ehrlich's terminology. It is not yet clear whether the "aggressins" really act through binding complements *in vivo* or not, and this is a crucial point.[2] Hence we cannot feel certain how to interpret Sir A. E. Wright's observations on the immunization of English soldiers against typhoid fever, in which he met with at least a measure of success by

[1] Wassermann and Citron, "Ueber die Bildungsstätten der Typhusimmunkörper," *Zeitschr. f. Hyg.*, l, p. 331, 1905; Citron, "Ueber natürliche und künstliche Aggressine," *Centralbl. f. Bakt.*, I Abt., Orig., xli, p. 230, 1906; "Die Immunisierung gegen die Bakterien der Hogcholera (Schweinpest) mit Hilfe von Bakterienextrakten," *Zeitschr. f. Hyg.*, liii, p. 515, 1906.

[2] Wolff-Eisner, "Die Aggressinlehre," *Centralbl. f. Bakt.*, I Abt., Ref., xxxviii, p. 641, 1906.

vaccinating with typhoid bacilli killed by heating to 60° to 65° C. — a procedure which he dared to practice after learning from Pfeiffer that he had in man obtained the specific agglutination reaction of typhoid fever after the subcutaneous inoculation of a heated culture of typhoid bacilli. Chantemesse in 1904 obtained apparently favorable results in the treatment of typhoid fever by the use of a serum that developed in horses treated with a filtrate from typhoid bacilli which had grown on a medium containing splenic pulp and human defibrinated blood. It is possible that this serum acts by binding the "aggressins" made by the typhoid bacilli as they grow, thus removing in some degree a condition favorable for the multiplication of the typhoid bacilli.

We may conveniently classify bacteria in a rough way according to the degree of parasitism they exhibit. Thus the plague bacillus in man and the anthrax bacillus in guinea-pigs are highly parasitic, for they find in their hosts the conditions that permit their rapid multiplication and invasion, although the original infection may have been established by very few bacteria, perhaps even by a single bacterium. Directly opposed to the parasites stand the saprophytic bacteria, which have the utmost difficulty in multiplying in the tissues of their host even when introduced in large numbers, but may prove injurious if inoculated in enormous quantities. Many of the bacteria of the intestinal tract are of this character. Midway between these groups stand the hemiparasites, microörganisms which are invasive only when inoculated in considerable numbers and are pathogenic only when

the way has been prepared by poisons which neutralize the defensive substances of the host; for example, by "aggressins." The bacilli of typhoid fever and of cholera may be classed as hemiparasites, and their virulence appears to stand in a close relation to the efficiency of their "aggressins."

The ordinarily accepted view that the portal of infection for the inciters of typhoid fever is the digestive tract is doubtless true in general and still it may not represent the whole truth. There is some evidence to support the idea that at times the bacilli enter by way of the throat, for the organisms have been found in the tonsil, and a patient may be in the early period of the disease without showing the presence of the bacilli in the stools even when modern methods of isolation are employed. It is conceivable that typhoid bacteria in the intestine do not necessarily point to an original intestinal invasion, as they may at times come mainly from ulcerated Peyer's patches in which the bacilli have accumulated as a result of the invasion of these lymphatic structures in the course of a general bacillary infection which strongly tends to implicate the lymphatics. It would be of special interest to know whether the axillary and other lymph nodes at a distance from the intestine are early invaded in typhoid fever. Even where the invasion is by the intestinal path the typhoid bacilli, which tend to be irregularly distributed in the intestinal contents, may perhaps come from ulcerated Peyer's patches which have become infiltrated and ulcerated, secondarily to the typhoidal bacillæmia. We should

then be dealing with an eliminative process for bacteria, such as pharmacologists have long known to exist for poisons like carbolic acid, corrosive sublimate, and urea, and such as Dr. Flexner has shown to exist for ricin poisoning and for intoxications arising from the autolyzed dysentery poison.

That the blood contains typhoid bacilli early in the course of the fever is now well established for a very large proportion of all cases appropriately examined, and is probably true of all cases of typhoidal type associated with infection by typhoid bacilli. This bacillæmia explains many of the complications and sequelæ of the disease, the passage of bacilli into the spleen with enlargement of the organ, their passage into the liver and hence into the gall-bladder (frequently with cholecystitis, with or without gall-stone formation) and the entry of the bacilli into the urine, where they may be found for some weeks after the subsidence of the fever. That these living bacilli should find their way abundantly into the urine after convalescence has begun shows that the microörganisms must still have a foothold in the host, and points to unnumbered possibilities in respect to relapses and the spread of the bacilli. It could hardly have been predicted that convalescence is possible while the living inciters of the disease still persist in the body of the host. The explanation of this apparent paradox must be sought in the development of protective powers on the part of the host, powers which accrue to the host in consequence of the specific excitation and reaction of many different types of cells through sub-

stances made by the bacilli. It is quite aside from the present design to discuss problems of immunity, but it is perhaps desirable to note certain facts relative to the protective action aroused by typhoid bacilli. The experimental inoculation of human beings with typhoid cultures exposed to heat has been shown by Wright to result in the following changes in the blood; (1) an increase in the bactericidal power of the blood; (2) an increase in bacteriolytic power of the blood; (3) a development of antitoxic properties as indicated by the mitigation of the severe constitutional signs of intoxication that ordinarily follow a first inoculation with typhoid vaccine; (4) an increase in the ability of the leucocytes to take up and digest typhoid bacilli; and (5) the development of specific agglutinating powers of the blood for typhoid bacilli. With the exception of the agglutinating action of the blood, which bears no definite relation to the immunity of the patient, conditions similar to these apparently constitute at least a part of the mechanism of defense in the case of typhoid fever. How far the bactericidal property of the blood as measured *in vitro* is an evidence of effective bactericidal activity within the body is an open question. Bail emphasizes the fact that an experimental immune serum which is active outside the body, does not cause an effective solution of typhoid bacteria when injected with such bacteria into the blood stream. The antitoxic power noted by Wright is very likely dependent on "anti-aggressins" that have arisen in response to "aggressins." Effective "anti-aggressin" action is probably extremely important

in the struggle between the organism and the bacteria; for if the latter cannot restrain the natural protective forces of the animal they are attacking, they cannot efficiently multiply, and they ultimately fall a victim to the action of the leucocytes. During convalescence, after the subsidence of fever, the typhoid bacilli found in the urine can hardly represent bacteria that are multiplying in the blood and tissues, but rather are microorganisms that have come from the intestine from certain foci in the tissues and have escaped the action of the leucocytes and bactericidal serum. A state of balance between opposing forces then exists, and this may be readily disturbed to the detriment of the host by factors favoring multiplication of the bacilli in the intestine (such as improper food) or checking the formation of "anti-aggressins." It is a noteworthy peculiarity that a patient may harbor the inciters of typhoid or cholera in the intestine and still not develop the specific disease. The blood in these persons need not contain an increase in bactericidal substances, and this suggests that the individual in question has acquired a local immunity implicating the intestinal structures, which prevents the invasion of typhoid bacilli. Wassermann and Citron have shown that immunity substances are made in connective tissues as well as in the lymphatic system, spleen, and bone marrow, and furnish evidence pointing to the possibility of inducing a local intestinal immunity.[1]

[1] For a review of the recent literature relating to typhoid fever, see Kolle and Wassermann's "Handbuch d. path. Mikroorganismen," Supplementary Volume I, 1906; Kutscher, "Abdominaltyphus," pp. 251, 255, 269, etc.

Paratyphoid and Allied Infections. — By means of careful biochemical studies, including especially the study of the fermentative action on sugars (first systematically employed by Professor Theobald Smith [1]) and the agglutination reactions and pathogenic properties, it has been found that there is an important group of pathological bacteria which according to their characters range themselves between the group of colon bacilli and the typhoid bacilli. The group is large and somewhat heterogeneous as regards certain properties, such as agglutinative behavior and pathogenic qualities. The group includes the inciters of hog-cholera,[2] of the spermophile disease, of mouse typhoid, of guinea-pig disease, of certain forms of pseudo-tuberculosis, of calves' diarrhœa, of psittacosis or parrot plague (which may cause an atypical pneumonia in man), and it includes the paracolon and paratyphoid bacilli, *B. enteritidis* and Sanarelli's bacillus of yellow fever.[3] A glance at this somewhat formidable list shows that many of the bacteria in question are the

[1] "Das Gährungskölbchen in der Bakteriologie," *Centralbl. f. Bakt.*, vii, p. 504, 1890; "Zur Unterscheidung zwischen Typhus- und Kolonbacillen," *ibid.*, xi, p. 367, 1892.

[2] The agglutinative characters of some of the bacteria in this group are very similar though probably not really identical. Thus Smith found the agglutinative behavior of certain hog-cholera bacilli to be indistinguishable from that of Sanarelli's bacilli of yellow fever. It is possible that common group agglutinins were so abundant in the sera used as to mask the action of very specific agglutinins that may have been present.

[3] Another group of aberrant colon-like bacilli includes the specific incitants of various infectious diseases of wild and domestic birds. These organisms retain the ability to coagulate milk and make indol, but differ from colon bacilli in showing weakened fermentative powers.

cause of animal diseases. There is little doubt that they have their normal habitat in the intestinal tract of domestic animals, and this accounts for their very wide distribution and for the occurrence of some of them in the human intestine. It is especially those members of the group that are pathogenic for man that interest us here, and of these we may select for brief consideration the bacteria grouped under the term "paratyphoid."

Only five years ago Schotmüller first maintained that there are cases of fever which run a clinical course indistinguishable from typhoid fever but differing from it in being incited by the paratyphoid bacilli. This position is now firmly established by numerous observations; for although some of the characteristic lesions of typhoid fever, such as ulcerated Peyer's patches, are relatively seldom found in paratyphoid infections, it is not clear that there are any essential differences in the lesions observed.[1] That the poisons made by one variety of paratyphoid bacilli are probably closely related to the poisons made by the typhoid bacteria is suggested by the very close agglutination relationships that have been discovered between these bacteria.

It became necessary to make a separation of the paratyphoid bacilli into two groups owing to agglutinative and cultural differences — paratyphoid *A* and *B*. The bacillus of paratyphoid *B* is probably widely distributed. It is the cause of many instances of disease running a

[1] "Weitere Mittheilungen über mehrere das Bild des Typhus bietende Krankheitsfälle, hervorgerufen durch typhusähnliche Bacillen (Paratyphus)," *Zeitschr. f. Hyg.*, xxxvi, p. 368, 1901.

course like that of typhoid fever, but as a rule milder — this milder course corresponding to the intermediate position of the bacilli between colon bacilli and typhoid bacilli. The justification for separating the paratyphoid bacilli from those of typhoid has been questioned by some, but the biochemical distinctions between them are definite, and as the recognition of these distinctions is serviceable to man, it is desirable to emphasize them.[1] With the recognition of these different etiological factors the unity of the condition we call typhoid fever is swept away. This unity is in fact still further broken by the fact that another bacillus of the colon group, *B. fæcalis alcaligenes*, has lately been shown to be the inciter of febrile states clinically inseparable from mild forms of the fever caused by the typhoid bacilli.

It is an inevitable event in the development of bacteriological science that different investigators should sometimes discover the same microörganism in its relation to different diseases and that under these conditions of discovery the organisms in question should come to bear different names. Thus it has lately been insisted that the bacillus of paratyphoid *B* is identical with the bacillus of mouse typhoid and with the well-known *B. enteritidis*[2] of Gartner. In this case the identity

[1] So long as the possibility remains that closer study may reveal some differences of a characteristic sort between the lesions and pathogenesis of the disease caused by typhoid bacilli and the disease caused by paratyphoid bacilli it is perhaps permissible to speak of the latter class of cases as paratyphoid fever.

[2] Kutscher u. Meinicke, "Vergleichende Untersuchungen über Paratyphus-, Enteritis-, und Mäusetyphusbakterien und ihre immunisatorischen Beziehungen," *Zeitschr. f. Hyg.*, lii, p. 301, 1906.

apparently does not extend to completely reciprocal pathogenicity, for mouse typhoid bacilli have never been known with certainty to cause the typhoidal disease in man, whereas the paratyphoid bacillus *B* is pathogenic for mice.[1] Of greater importance is the fact that the widely distributed bacilli of paratyphoid *B* are certainly the cause of many outbreaks of meat poisoning,[2] and that the bacilli of certain cases of meat poisoning are identical with some which cause typhoid fever. A highly instructive instance is one lately reported in detail, in which seven persons living in one house suffered from an infection by paratyphoid *B* bacillus.

All the members of a family fell acutely ill with vomiting, diarrhœa, and fever. At the end of three days the temperature had returned to normal in all the patients except one, and in this instance the illness ran the course of a moderately severe typhoid, the fever lasting eighteen days. The sera from all these patients gave positive agglutinations with the bacillus of paratyphoid *B*.

Why it is that some persons develop an acute gastroenteritis after infection with this bacillus while others have typhoid fever, is still somewhat obscure. In cases of meat poisioning, the infected meat contains not only the bacilli but certainly also poisons derived from them. The presence of these poisons accounts for the acute

[1] It is also possible that the use of the absorption methods which reveal common agglutinins would show that the agglutinations for these three organisms (now claimed to be identical) are not in reality absolutely identical.

[2] Levy and Fornet, "Nahrungsmittelvergiftung und Paratyphus," *Centralbl. f. Bakt.*, Orig., xli, p. 161, 1906.

onset of symptoms.[1] The number of bacilli ingested doubtless exerts an important influence on the character of the illness. If few bacilli are associated with the poison, they usually do not suffice to set up an infection, and the seizure is transitory. If many bacilli are present, they may establish themselves, freely multiply, and invade the organism, causing the phenomena of typhoid fever. Hence in a case where the poison present in the food is associated with many paratyphoid bacilli, the fever incidental to the intoxication may be directly succeeded by a period of fever due to the bacillary infection. Animal experiment teaches that in certain virulent bacterial infections the period of incubation is in a rough way inversely proportional to the number of bacteria taking part in the infections. The ingestion of very large numbers of paratyphoid bacilli may thus be followed by a short period of incubation, whereas many days may probably intervene before the onset of fever if the number of bacilli barely suffices for them to gain a foothold in the blood-vascular and lymphatic structures. Although we have no definite information on this point in the case of paratyphoid infection, it seems safe to assume that the character of the anti-bacterial defenses of the organism is a factor of equal importance with the number of bacilli in determining infection. These con-

[1] Dr. Holt tells me it is common for typhoid fever in young children to begin acutely. Probably the defenses of the digestive tract against the bacilli are much feebler during infancy than in later life, and an insult from an error in diet may injure these local defenses so as to permit rapid and free multiplication of the typhoid bacilli.

siderations regarding the influence of the number of the bacilli and the vigor of the defenses doubtless apply also to the case of infections with the typhoid bacilli, but here there is probably never any primary intoxication from the presence of toxins in food, for the reason that the typhoid bacilli, requiring such special conditions for multiplication, do not often find the requisite conditions for giving off their poisons except within the human body.

Dysentery Bacilli. — Very few years have passed since it became apparent that certain microörganisms which had previously been regarded as colon bacilli are in reality quite distinct from them and stand in a direct causative relation to the widespread intestinal disorders of temperate climates which are grouped under the term dysentery. Takati Shiga, a gifted young Japanese with a German training, was able to show that the intestine in dysentery contains large numbers of bacilli which, while resembling colon bacilli in some respects, yet differ from them in their fate, in their appearance on agar plates, in their lack of motility, in their failure to make acid from sugar media, in their failure to produce indol or make gas, in their power to set up in man a disease clinically like dysentery, and in their ability to confer on the human blood serum the property of specific agglutination in high degree. This discovery was soon followed by the researches of Flexner in Manila, which showed that there is another type of dysentery inciter allied to the Shiga bacillus but differing from it in the direction of retaining more fully some of the

characters of typical colon bacilli, especially the capacity to ferment sugars. Just as in the case of the typhoid and paratyphoid bacilli, it is evident that the bacilli farthest removed from colon bacilli (the typhoid bacilli) in their characters incite the severest form of the disease, so in the case of the dysentery bacilli it is true that the severest forms of disease are dependent on those bacteria farthest removed from the colon bacilli in their biochemical characteristics; namely, the Shiga bacilli. The Shiga bacilli are the cause of the severe epidemics of Japan and of Germany, but in the latter country bear the name of Kruse. The Flexner bacilli are apparently the cause of dysentery in young children much more frequently than the Shiga bacilli. The two types of bacilli are further distinguished by their agglutination reactions and somewhat less sharply by their behavior toward immune sera.[1] The Shiga and Flexner organisms, however, taken together do not quite cover the entire range of bacillary dysentery; for, as Park and Hiss have

[1] It has been proposed to group these types of dysentery bacilli under the name paradysentery. As it appears to have been conclusively shown that the lesions caused by these bacteria are identical with those caused by the Shiga bacilli, there is no reason why the "paradysentery" bacilli should not be regarded as dysentery bacilli equally with the Shiga organisms. To use the term "paradysentery" on the ground that the bacilli so designated stand between the Shiga bacilli and the *B. coli* group in cultural, fermentative, and other characters does not seem appropriate, for there are doubtless many bacteria intermediate between the Shiga and colon organisms which do not cause dysentery. For the present it seems best to employ the names "Flexner type" and "Park-Hiss type" in referring to the organisms in question. Doubtless subdivisions within these types will prove desirable from a bacteriological standpoint.

shown, there are pathogenic bacteria intermediate in type between the two. This further distinction of a third variety is justifiable on grounds of convenience. Indeed, Shiga[1] has lately adopted the classification of Hiss, which recognizes four groups based on fermentative characters and has added to this a fifth, intermediate between the acid bacilli and the non-acid bacilli.

Like the typhoid bacilli, the bacilli of dysentery are hemiparasites; that is, their invasive and destructive properties are developed only when the bacteria have been introduced into the organism in considerable numbers or have had an opportunity to multiply owing to feeble powers of resistance on the part of the infected individual. Like typhoid bacilli, the bacilli of dysentery form substances — the so-called "aggressins" — which are capable of inhibiting or even completely paralyzing the natural protective action of the body fluids and thus removing the most important obstacle to the invasion of the bacteria which up to the moment of this opportunity had led a saprophytic existence. It is not clear whether these "aggressins" are identical with the soluble products of the dysentery bacilli which one obtains on filtering an old bouillon culture or are bodies wholly or in part distinct. It is well established that guinea-pigs may be actively immunized against the severest intraperitoneal infection by means of two or more injections of sterile guinea-pig exudate containing "aggressins" derived from the peritoneal cavity of a suit-

[1] "Observations on the Epidemiology of Dysentery in Japan," *The Philippine Journ. of Sci.*, i, p. 485, 1906.

ably prepared animal. It has further been found that as the result of long preparation with such exudates guinea-pigs, rabbits, and sheep furnish a serum which in amounts of one-half cubic centimeter is able to protect animals from intraperitoneal infection. It is claimed by Kikuchi [1] that the immune serum obtained through treatment with "aggressins" does not show *in vitro* the well-known characteristics of a bacteriolytic serum and it is maintained that this "anti-aggressin" action is the basis of a new type of immunity. The validity of the latter claim cannot be successfully upheld without further research in this complex problem. There are now two well-known antidysenteric sera designed for human use: one prepared by Shiga by immunization with the bacilli of the originally discovered strain of dysentery; the other made at the instigation of Dr. Flexner by means of the variety of bacillus bearing his name. These sera are regarded as being essentially bactericidal, but there is room for doubts as to how far the bactericidal action exhibited *in vitro* is operative after the serum has been put into the dysenteric patient. That these sera, though not universally applicable, are quickly effective in some instances of severe dysentery is certain. The effect consists mainly in a diminution in the number of movements, alleviation of tenesmus, some lowering of the temperature, and a calming of the nervous system which is sometimes very striking. These effects may

[1] "Weitere Erfahrungen über Aggressinimmunität gegen den Shiga-Kruseschen Dysenteriebazillen," *Archiv f. Hyg.*, liv, p. 298, 1905.

depend in part on an antitoxic action, which is perhaps mainly anti-aggressive. It seems clear that if the soluble poisons formed by the dysentery bacilli in the body could be promptly neutralized, the bacilli would soon fall a prey to the natural bactericidal process of the serum and leucocytes. It is reasonable to believe that sera may yet be obtained which will better fulfill this need than do those at present in use. It must, however, be clearly stated that even a powerful antitoxic or anti-aggressive serum could not be counted upon to cure all cases of dysentery, for in this disease there is in many subjects a period when other bacteria than the bacilli of dysentery (especially streptococci) enter the field of action in a significant manner. A secondary infection of mixed character is then set up, and against this the specific sera of dysentery must necessarily be ineffective. Finally it may be mentioned that there is sometimes a therapeutic difficulty of a wholly different character, due to the fact that a serum prepared with one strain of dysentery bacillus is apt to be effective only for this strain and to have little action on other varieties of the bacillus. A serum may thus be worthless in a given case because it is not adapted to the variety which is acting. Shiga has recently proposed the use of a universal or polyvalent serum designed to forestall embarrassments of this sort. Shiga's advice is as follows. Two horses should be simultaneously immunized, one with the bacilli of Shiga's types I and II, the other with the bacilli of types I and IV. As soon as a high grade of immunity is attained serum should be

taken from both horses and mixed in equal volumes. "By this means the best universal serum can be obtained" (Shiga).

I have mentioned that the dysentery bacilli resemble those of typhoid fever in being hemiparasites capable of infecting a susceptible individual only if present in such considerable numbers that his anti-aggressive powers are neutralized, thus clearing the way for a free multiplication of the bacilli. There are, however, some apparently fundamental differences in the biological characters of these two important pathogenic forms. While the typhoid bacillus, an actively motile organism, is highly invasive and sets up a bacillæmia with consequences already noted, the dysentery bacillus, a non-motile organism, is only slightly invasive, remains almost limited to the intestinal mucous membrane, and leads to neither bacillæmia nor bacilluria. The typhoid bacilli produce soluble poisons with difficulty, at least in fluid cultures. Some dysentery bacilli, namely those of the original Shiga or promptly alkali-making variety, show a considerable capacity to yield soluble poisons in fluid media. They differ in this respect from the acid-making varieties, which yield their active toxic product only within the animal organism.[1] The chemical nature of the poison is at present wholly unknown, but significant facts in respect to its mode of action in animals, including probably the human species, have been lately brought to light, chiefly through the experimental studies of

[1] Dr. Flexner tells me he has lately succeeded in obtaining toxins from his dysentery bacilli by means of a special procedure.

Dr. Flexner [1] and his associates. Of these facts the most important have to do with the pathological alterations observed in the intestinal mucous membrane in the course of human dysentery and in the course of experimental intoxications. By intravenously injecting into rabbits the poison obtained by permitting the bacilli to undergo a process of autolysis or self-digestion, Dr. Flexner was able to reproduce intestinal lesions analogous to those observed in human dysentery; namely, inflammatory, sometimes diphtheritic processes, especially in the large intestine and often attended by hæmorrhagic changes. It is quite clear that these lesions are incidental to an elimination of the injected poison into the intestine and that the damage done to the intestinal structures is due primarily to the contact with the poison in the blood during the act of elimination or secretion. It seems probable that a secondary invasion of the damaged structure by intestinal bacteria forms an essential part of the dysenteric process. These primary eliminative lesions are, as pointed out by Flexner, analogous to those experimentally called forth by corrosive sublimate and by ricin. I observed, many years ago, hæmorrhagic and necrotic intestinal lesions in dogs after intravenous infusions of urea, and these injuries also belong to the group of eliminative manifestations. The special value of Flexner's studies lies, it appears to me, in the fact that they show for the first

[1] "The Pathogenesis of Experimental Colitis, and the Relation of Colitis in Animals and Man," *Journ. of Exper. Med.*, viii, p. 514, 1906.

time that the poison of human pathogenic bacteria have the power of breaking down the resistance of the intestinal structures in the eliminative process. It has always been customary to think of the inflammatory, necrotic, intestinal lesions of human dysentery as the result of strictly local conditions, and latterly the dysentery bacteria have fallen under suspicion as the inciters of the lesions, in conjunction with the gross mechanical insults which might be offered by the hard contents of the lower bowel. Objections to this hypothesis of purely local causation are the fairly even distribution of the dysentery bacilli through a much larger extent of the intestine than that occupied by the severe lesions, and, secondly, the frequent absence of any ascertainable local and auxiliary factors. The newly acquired experimental facts force us to look at the local dysentery lesions in a wholly different light; namely, as being perhaps in part the result of the action of soluble toxins which have found their way by the blood to the gut in a changed (perhaps autolyzed) form and in considerable concentration. Although this mode of origin cannot now be positively affirmed for these local human dysenteric lesions, it must be regarded as extremely probable. There is reason to believe that the dysentery toxins are excreted in the bile as well as through the intestinal mucous membrane. These toxins so excreted may thus take part in doing damage to the intestinal structures.

One further phase of the action of the dysentery poison calls for mention; namely, its effect upon the nervous

system. Dopter [1] has shown that the toxin prepared from the Shiga bacilli may cause paralysis in rabbits and that this paralysis is usually referable to acute lesions in the gray axis of the spinal cord or ponto-bulbar region. As the result of the action of a similar poison Flexner saw small hæmorrhages in the brain and softening in the gray substance of the spinal cord with or without hæmorrhage. Rabbits are unequally susceptible to this nervous poison or neurotoxin, and only a small proportion of them develop nervous lesions after treatment with the poison, whether this be autolyzed or not. The fact that such lesions may be sometimes induced — and perhaps regularly under suitable experimental conditions — has a possible bearing of great importance for human pathology. It has long been clear to clinicians that young children are susceptible to paralyses referable generally to lesions in the gray substance of the spinal cord and that these paralyses are apt to come on at a time of disordered intestinal digestion; often indeed at the time of teething, to which process the paralysis has often been somewhat vaguely referred. As we have only learned in recent years to see a specific bacillary dysentery in many cases of slight intestinal disorder in children, it is likely that many instances of infantile spinal paralysis have been associated with a true dysenteric infection. Of the numerous cases of dysentery that occur yearly in children, only a relatively small number are attended with paralyses, but in view of the experimental data now in our posses-

[1] "Effets expérimentaux de la toxine dysentérique sur le système nerveux," *Ann. de l'Inst. Pasteur*, xix, p. 353, 1905.

sion this small number assumes a new interest. It remains for the future to definitely prove or disprove the rôle of dysentery toxin in the causation of poliomyelitis.

I have endeavored to sketch with few words some of our leading acquisitions and problems in the colon-typhoid-dysentery group of bacteria while touching only lightly the realm of hypothesis. It is certain that research will distinguish further varieties possessing interest for human pathology within this large group. Nor can we doubt that the methods by which we now seek to combat the infections due to members of this group will gain in efficiency and precision. Our attention has, perhaps, been too exclusively fixed on the specific excitants, and the rôle played by associated bacteria must receive more study, for it is clear that they sometimes play a significant part in determining the outcome of an infection. The difference that decides whether a man will live or die must frequently be a slight one, looked at from the standpoint of the processes of battle within the body. To learn to recognize more clearly in what this difference consists and how to use such knowledge to turn the tide of warring forces to the advantage of the life that hovers on the verge of extinction is among the problems which intelligent, careful research will help us to solve.

LIQUEFYING BACTERIA

In the foregoing section has been considered very briefly the highly important group of Gram-negative microörganisms which do not liquefy gelatin and which are facultative anaerobes. It is desired here

to refer briefly to certain Gram-negative facultative anaerobes which liquefy gelatin and which are regarded as having the ability to induce putrefaction in proteid material. The most important member of this group is the organism called *B. vulgaris*, or *proteus vulgaris*, which is known to make a tryptic ferment and to peptonize casein and attack carbohydrates. It is said by A. E. Taylor to produce indol, skatol, and diamino-acids when grown on casein. The organism is one which is sometimes found in moderate numbers in the fæces of healthy individuals where, as a rule, liquefying aerobic organisms are not numerous. *B. proteus vulgaris* was formerly regarded as a very important agent in the production of putrefactive decompositions outside the body and it has been suspected of inducing active putrefaction within the human intestine. Recent investigations indicate clearly that other types of microörganisms belonging to the class of strict anaerobes are much more important factors in putrefaction generally than is the *B. proteus vulgaris*, which is sometimes, however, associated with these anaerobes. It appears that the ability of *B. proteus vulgaris* to attack native proteids has been overestimated. I have gained the impression from the study of the action of this organism on milk in fermentation tubes that its peptonizing action on casein is ordinarily not considerable during a period of four to five days' sojourn in the incubator at body temperature. That *B. proteus vulgaris* is capable of inducing acute disease of the human gastro-enteric tract appears to be well established. There are instances on record of severe

meat poisoning apparently due to the contamination of meat by this organism. A number of instances have been recorded in which the poisoning following the eating of sausages has been attributed to the action of *proteus vulgaris*.[1] It has been found in such cases that the filtrate of the bouillon culture of the *proteus* organism derived from the contaminated meat or from the intestine has been fatal to experimental animals. There are also recorded some instances of poisoning through cheese which had become contaminated by *B. proteus vulgaris*. In one instance a man ate abundantly of a soft cheese containing this organism and soon developed seizures of vomiting associated with great prostration and cardiac depression followed by death after five days. Other persons who ate of the same material more moderately were similarly though less violently ill and ultimately recovered.[2]

B. proteus vulgaris is an organism which in general shows little tendency to invade the circulation from the digestive tract although it may be the cause of the severest gastro-enteric symptoms.

Other Gram-negative liquefying facultative anaerobes are sometimes found in the human intestine in great abundance. Their relation to the production of disease is not yet clearly established. Possibly some varieties of liquefiers are harmless if present in moderate numbers. In a case of pernicious anæmia from which I was unable

[1] A case of this kind is that reported by Schumburg, *Zeitschr. f. Hyg.*, xli, p. 183, 1902.

[2] *Berl. Molkereizeitung*, xiii, p. 78.

to cultivate any colon bacilli, the gelatin plates indicated the presence of very large numbers of an organism corresponding closely to that described by Ford[1] as *B. entericus*. This organism liquefies gelatin with great rapidity.

Lubenau[2] has lately described a spore-bearing Gram-positive aerobic organism which liquefies gelatin and which he looks upon as the cause of an outbreak of severe diarrhœa among the inmates of an institution. This outbreak was characterized by persistent vomiting, great prostration, severe headache, and frequently by mental confusion. The diarrhœal seizures were in some instances as frequent as twenty or more in twenty-four hours and were attended by great abdominal pain. The general condition of the patients improved rapidly, but the vomiting and diarrhœa lasted for two or three days. The temperature was only slightly elevated and even this slight rise was observed only in a small number of the cases.

The organism described by Lubenau as the cause of this outbreak resembled the common hay bacillus in morphology. It was an actively motile aerobe which formed no gas on sugar-agar and attacked milk slowly, producing alkali and peptone. It sporulated readily and in its sporulating stage was non-pathogenic for animals. When obtained in large numbers in its vegetative form, through growth upon milk to which

[1] *Loc. cit.*, p. 40.

[2] "Bacillus peptonificans als Erreger einer Gastroenteritis Epidemie," *Centralbl. f. Bakt.*, etc., I Abt., Orig., xl, p. 433, 1905–06.

Witte's peptone had been added, it induced pronounced diarrhœa in young dogs, sometimes associated with blood.

STREPTOCOCCAL AND STAPHYLOCOCCAL INFECTIONS

The biological characters of the streptococcal and staphylococcal forms of bacteria met with in the human intestine in health and in disease have not yet received sufficient attention. There are indications that this subject is likely within a few years to undergo a much-needed development. In the meantime it is impossible to deal with the subject of streptococcal and staphylococcal infections in a really satisfactory way.

The normal intestine (both small and large) usually contains Gram-positive diplococci in moderate numbers. In culture media these may grow freely in chains, the individual organisms undergoing division in a plane transverse to the line of the chain development. In bouillon cultures streptococcal organisms are more likely to maintain any virulence they may possess than when grown in sugar media. Large quantities of bouillon cultures of the Gram-positive diplococci of the normal intestine (which may have developed into chains on the bouillon media) may be injected into guinea-pigs either intraperitoneally or subcutaneously without giving rise to any symptoms or lesions. The human intestinal tract under normal conditions is probably most of the time free from pathogenic streptococci and in normal adults such pathogenic bacteria introduced with milk or

water are ordinarily quickly destroyed in the upper part of the tract.

During infancy the digestive tract is much less resistant to streptococcus infections, and invasion of the mucous membrane by streptococci is a frequent occurrence and may be associated with disturbances of almost any grade of severity. It has been shown by Booker in this country and by Escherich in Germany that some of the severest forms of infantile ileocolitis are associated with streptococcus infections and are probably dependent upon them. As the digestive tract of the infant is readily damaged so as to become permeable by bacteria, it is not surprising that streptococcus septicæmia is of frequent occurrence. The condition is one which frequently but not necessarily ends fatally. A less common result of the penetration of the intestinal wall by streptococci is purulent peritonitis.

In children there are some instances of severe ileocolitis in which streptococci appear to be the primary inciters of disease. At least one may say that other bacteria which might fall under suspicion, such as dysentery bacilli and pathological types of colon bacilli have not been found in the cases in question. It is doubtful, however, whether the biochemical characters and virulence of certain colon-like forms have received sufficient attention in those cases which are regarded as instances of pure streptococcus infections. In many examples of infantile diarrhœa it appears clear that the streptococci which are prominent in the stools are really secondary invaders. Here the primary infection through colon

bacilli or atypical colon bacilli or dysentery bacilli appears to have prepared the way for such a secondary infection. In the case of the dysentery bacilli it is probable that they have the ability to make "aggressins" which inhibit the local or even the general defenses so as to permit the free multiplication of pathogenic streptococci. A similar relationship is seen in some instances of smallpox, scarlet fever, and measles. It seems clear that in many of these cases, in which the rôle of the streptococci is that of a secondary invader, this infection is responsible for severe exacerbations or death.

In adults the intestinal tract is not rarely the seat of invasion by streptococcal microörganisms. Where these are virulent, they are apt to be associated with diarrhœal disorders. There are, however, instances in which the streptococci are only slightly pathogenic for animals, such as guinea-pigs, but are present throughout considerable periods of time in excessive numbers in the stools. Their presence may be demonstrated in the Gram-stained microscopical fields, where they are seen as short chains or as diplococci. In such cases these organisms will be found to be abundantly represented in sugar-bouillon fermentation tubes which have been inoculated from the mixed fæcal flora. We have observed instances in which these Gram-positive streptococcal forms enormously preponderate in the sedimentary fields from the fermentation tubes. It has sometimes happened, also, that even though the Gram-positive fields fail to show the streptococcal forms in such abundance as to attract attention, they outgrow other

types of bacteria in the fermentation tubes. In one instance in which such an overgrowth of the fermentation tubes was regularly observed and in which the movements regularly showed the presence of excessive numbers of epithelial cells, it was found that the patient had for many months been infecting himself daily from an old abscess next a carious tooth. Although the abscess was treated surgically, the improvement in the clinical conditions was very slow, and after a period of six months under very favorable conditions of outdoor life evidences of streptococcal infection still persisted to some extent. In this case loss of weight and strength, mental depression, and moderate anæmia were the prominent clinical conditions. The movements contained in addition to streptococci somewhat excessive numbers of *B. aerogenes capsulatus*.

The presence of large numbers of intestinal streptococci and diplococci in the fæces is a prominent feature in some cases of pernicious anæmia. The significance of this is not yet clear, partly because the streptococcal infection has been associated in these cases with the presence of greatly excessive numbers of putrefactive anaerobes, especially *B. aerogenes capsulatus*. It seems probable that a streptococcus infection of the intestinal tract may greatly contribute to bring about a deterioration in the physical condition of a patient already infected with the gas-bacillus. In several instances of severe anæmia — some of them of the pernicious type — a prolonged streptococcal diarrhœa has been observed to precede the onset of

more grave manifestations of alterations in the blood and loss of muscular power.

It is now well known that there are occasional instances of phlegmonous gastritis due to streptococcal infections. I have observed instances of extreme hyperchlorhydria which showed in the vomited material during a seizure of protracted vomiting large numbers of Gram-positive streptococci and many leucocytes.

The relation of streptococcus infections to appendicitis is still unsettled despite the considerable work that has been done on the etiology of this affection. In many instances the dominant flora in the diseased appendix are streptococci, but the question arises whether they are primarily or secondarily concerned. I have observations on two fatal cases of appendicitis in which the intestine between the appendix and the anus contained enormous numbers of streptococci and diplococci at all points. It appears to me probable that the careful microscopical and cultural study of the intestinal contents will prove an aid to diagnosis in some obscure forms of local intestinal infection by streptococci.

Of the position which should be assigned to infections of the intestinal tract by staphylococci there is little to be said at present. One sometimes observes in the sediments of the fermentation tubes or bouillon flasks which have been inoculated from mixed fæcal flora numerous clumps of Gram-positive cocci. The biochemical characters of these organisms have not been studied and their classification is at present uncertain. It is noteworthy, however, that in two cases of severe staphylococcus acne

in which the opsonic index was low for these organisms, the mixed fæcal flora regularly showed staphylococcal forms in great abundance in the sedimentary fields from the fermentation tubes. Such organisms, however, have been detected in great abundance in the flora from some persons suffering from excessive intestinal putrefaction in which acne was not a feature. The very free growth of organisms of this type in bouillon inoculated with the mixed flora has been observed especially in the case of material derived from persons suffering from disorders of digestion, but it is uncertain what significance should be attached to these observations.

The possibility of controlling streptococcal and staphylococcal infections of the digestive tract by means of the vaccination methods employed by Wright is worthy of serious consideration. There are cases of advanced anæmia in which it appears likely that streptococcus infection is an important etiological factor, perhaps through the agency of hæmolytic poisons and certainly through the causation of exhausting diarrhœa. If in such cases the streptococcal element in the infection could be controlled, it seems clear that the outlook for the patients would be improved.

BACILLUS BIFIDUS

It has already been mentioned that the intestinal tract in childhood contains large numbers of the not extremely anaerobic microörganism described under the name of *B. bifidus*. Many if not all adolescents and adults harbor *B. bifidus*, although in smaller numbers than in early

life—a fact not widely known. I have met with exceptionally robust adults in whom *bifidus* was very prominently represented, if one may trust the microscopical fields and the primary [1] fermentation-tube sediments. Whether *bifidus* ever assumes a pathological character in man is uncertain. I may refer here to the observation that a Gram-positive bacterium indistinguishable from typical *bifidus* in its microscopical characters is sometimes almost the exclusive inhabitant of the lower digestive tract in children with chronic intestinal indigestion characterized by abdominal distention and retarded growth. In sugar bouillon these fæcal bacteria show the typical varied morphological characters of *B. bifidus* of nurslings, but whether the organisms from these pathological cases are really identical with those obtained from the intestine of normal infants is uncertain because no adequate biochemical observations have been made. I think the possibility should be borne in mind that a bacterial form which is entirely physiological when dominant during infancy may not be equally physiological when it constitutes the dominant type in later years. The mere persistence of *B. bifidus* as the leading large-intestinal microörganism may carry with it certain physiological disadvantages, such as relatively feeble powers of defense against some harmful bacteria.

INFECTIONS THROUGH ANAEROBIC BACTERIA

The anaerobes of the human intestine have received far less attention than the aerobic and optionally anaerobic

[1] Made from direct inoculation of the fæces.

bacteria. This neglect may be ascribed to the technical difficulties connected with the study of microörganisms whose growth is checked by minute traces of oxygen, and not to any belief that such microörganisms are unimportant.[1] There is in fact growing evidence that the strict anaerobes play an important part in some pathological processes which have their seat in the digestive tract, and one may hazard the prediction that careful studies of them will give us wholly new light on some obscure forms of disease.

Bacillus Putrificus. — The organism described by Bienstock as *B. putrificus* is a spore-forming strict anaerobe, capable of actively attacking and hydrolyzing native proteids, and like many such anaerobes able to give rise to characteristic products of putrefaction, including butyric acid and hydrogen. It forms hydrogen sulphide and mercaptan but not indol. Considerable difference of opinion exists as to whether *B. putrificus* is or is not a regular inhabitant of the normal human intestine.[2] This question is bound up with the question of the identity of the bacterium. Bienstock, it should be noted, has lately brought forward evidence

[1] Tarozzi ("Ueber ein Leicht in aërober Weisse ausführbares Kulturmittel von einigen bis jetzt für strenge Anaëroben gehaltenen Keimen," *Centralbl. f. Bakt.*, xxxviii, I Abt., Orig., p. 619, 1905) has lately described a method by which it is possible to obtain aerobic growths of organisms which have hitherto been regarded as strict anaerobes, including *B. aerogenes capsulatus*, *B. botulinus*, etc.

[2] Passini holds that he can generally obtain it; Bienstock states that he cannot find it in normal persons.

B. putrificus is widespread and may readily become a contamination as it is common in laboratory dust.

to show that two distinct bacteria are included under the name of *B. putrificus*.[1] One of these, the true *B. putrificus*, attacks native proteids such as serum albumin; the other, though morphologically indistinguishable from *B. putrificus* fails to attack native proteids but decomposes sugars. Bienstock calls this organism *B. paraputrificus*. He states that he has found it not infrequently in the normal human intestine and believes it is *paraputrificus* and not *putrificus* that has been found in the contents of the lower bowel. As *paraputrificus* makes acid from sugars and does not cleave proteids, Bienstock inclines to the belief that the organism is antagonistic to the true putrefactive microörganisms of the intestine and thus supports the colon bacilli in this function. But it is not clear that acid-forming bacteria necessarily act as a check to the development of putrefactive bacteria except under special conditions, and it appears questionable whether *B. paraputrificus* is really a factor in checking the multiplication of harmful anaerobes.

The rôle of the putrefactive type of *B. putrificus* in pathological processes has not yet been determined. That it plays a part in certain putrefactive disorders appears to me probable on the following grounds. An organism corresponding to the cultural and morphological characters of *B. putrificus* appears in great abundance in week-old cultures of the mixed flora from some persons suffering from subacute or chronic intestinal symptoms at present not satisfactorily definable. As these organisms regularly appear after inoculation with the

[1] "Bacillus Putrificus," *Ann. de l'Inst. Pasteur*, xx, p. 407, 1906.

flora of these people, it seems fair to assume that they are in these instances regular inhabitants of the digestive tract, although they may not appear in their characteristic sporulating form in the microscopical fields. The flora of persons free from symptoms of intestinal derangement has not abundantly given us *B. putrificus* in our bouillon-calcium-carbonate flasks. This is in harmony with the statement of Bienstock that he failed to find *B. putrificus* in normal persons. It is true also of the intestinal flora of persons to whom the spores of *B. putrificus* had been given by the mouth.

One reason why it is difficult to define with confidence the part of *B. putrificus* in intestinal processes of putrefaction is that it is apt to be associated with other microörganisms which may be concerned with the processes in question, such as *B. aerogenes capsulatus* and some coccal infections. The association of excessive numbers of these three organisms in the large intestine is not uncommon in cases of chronic excessive intestinal putrefaction.

In every instance in which *B. putrificus* is present in bouillon flasks prepared by growing the mixed fæcal flora from cases of intestinal putrefaction, there is found also methyl mercaptan. This observation corresponds to the fact that *B. putrificus* in pure culture in peptone bouillon is capable of making mercaptan. It has not always been possible to grow *B. putrificus* from cases in which a methyl mercaptan reaction was obtained, and for this reason and others I believe that methyl mercaptan may be produced by other intestinal organ-

isms than *B. putrificus*. Nevertheless in my experience the strongest methyl mercaptan reactions have been obtained from those cases in which *B. putrificus* was present. *B. putrificus* grown in peptone bouillon makes more mercaptan than any other anaerobe with which we have experimented in my laboratory. The mercaptan reactions obtained from bouillon cultures made with the mixed flora from some healthy young children I am disposed to attribute to *B. putrificus*. This organism is very widespread and is present in most samples of dust. It is non-hæmolytic and probably makes no toxins. Considerable numbers of the typical bacillus may be injected intraperitoneally into guinea-pigs without causing noteworthy damage.

I believe *B. putrificus* to be the most energetic anaerobe now known in respect to the ability to proteolyze native proteids. Bienstock's organism is probably representative of a group whose members vary considerably in biochemical properties. We have in our laboratory a strain of *B. putrificus* of intestinal origin which makes very large quantities of indol in peptone bouillon. It is evident that the presence of such an organism in the intestine might contribute in an important way to the production of indol.

In a recent study of the anaerobic bacteria of the mouth, Dr. Antonio Rodella [1] has ascribed importance to *B. putrificus* as a cause of caries of the teeth and inflammation of the pulp. He finds the substance of the

[1] "Ueber anaërobe Mundbakterien und ihre Bedeutung," *Archiv f. Hyg.*, liii, p. 329, 1905.

teeth in caries to be infiltrated by this organism apparently in almost pure culture. Moreover, he has been able to induce a putrefactive decomposition in the substance of teeth previously decalcified by the use of acid. While the evidence thus far brought forward in regard to the rôle of *B. putrificus* in causing disease of the teeth is not complete (owing to the difficulty in excluding some symbiotic action of other kinds of bacteria), it appears in the highest degree probable that *B. putrificus* is an important factor in setting up caries. As this organism is an anaerobe, it is easy to see how scrupulous cleanliness of the teeth and gums must operate to prevent it from gaining a foothold in the mouth.

Bacillus aerogenes capsulatus. — *B. aerogenes capsulatus* is a strictly anaerobic, difficultly spore-bearing, Gram-positive, gas-making bacillus. It is found in small numbers relatively to other bacteria in the intestines of the majority of normal adults and in still smaller numbers in normal children. There are many healthy breast-fed and bottle-fed children in whom one fails to find evidence of the presence of this microörganism in the intestinal tract, either through careful study of the Gram-stained microscopical fæcal field or through ordinary plating methods. I will not say that the organism is actually absent from the intestine in these cases, but it is certain that if present it is in such small numbers as to play no appreciable part in the biochemical processes that occur in the digestive tract. Our knowledge of the part played by this anaerobe in human pathology has until very recently been limited to certain

surgical and obstetrical diseases. Some confusion has arisen from the fact that the organism is known in different countries under different names. In Germany it is known as the gas-phlegmon bacillus (Fraenkel); in France, as *B. perfringens* (Veillon and Zuber); and Dr. Welch was able to obtain the organism first described by him as *B. aerogenes capsulatus* from a culture of *B. enteritidis sporogenes* sent to him by Dr. Klein. The same organism as *B. aerogenes capsulatus* (but in a non-virulent form) has been described by Grassberger and Schattenfroh under the name of *granulo-bacillus immobilis liquefaciens*.[1] It occurs commonly in market milk.

Opinions differ rather widely as to the pathogenic properties of *B. aerogenes capsulatus* for man and animals. Professor Welch,[2] who first discovered the organism in connection with human surgical infections, was disposed to regard it as not being ordinarily highly pathogenic. In reference to healthy rabbits he says we cannot regard this bacillus as being pathogenic under ordinary conditions. Dunham,[3] who, in the course of his clever investigations on *B. aerogenes capsulatus*, discovered

[1] Professor Welch and some other bacteriologists regard the bacillus of Grassberger and Schattenfroh as identical with *B. aerogenes capsulatus* in everything except pathogenicity. Other writers dissent from their view. The two anaerobes are surely very closely related if not identical.

[2] "A Gas-producing Bacillus (Bacillus Aerogenes Capsulatus, Nov. Spec.) capable of Rapid Development in the Blood-vessels after Death," by Wm. H. Welch and Geo. A. F. Nuttall, *Bull. of the Johns Hopkins Hosp.*, No. 24, July-August, 1892.

[3] "Report of Five Cases of Infection by the Bacillus Aerogenes Capsulatus (Welch)," *Bull. of the Johns Hopkins Hosp.*, No. 73, April, 1897.

its ability to make spores, ascribes a greater degree of pathogenic action to the microörganism with which he worked — these bacteria also having been derived from cases of surgical infection. Grassberger and Schattenfroh, whose *granulo-bacillus saccharo-butyricus immobilis liquefaciens* was derived from milk and is regarded by Welch, Kamen, and others as identical with *B. aerogenes capsulatus*, found it to be non-toxic for guinea-pigs. Kamen,[1] although unable to obtain powerful toxins on ordinary culture media, regards *B. aerogenes capsulatus* as capable, by itself, of exciting inflammatory purulent processes. It is certain that there are various strains of *B. aerogenes capsulatus* as regards pathogenicity and that the different results obtained by different investigators with respect to that feature are due to this fact. Cultures of *B. aerogenes capsulatus* made by Mr. Ward from the fæces of a young man with slight digestive derangement and from material derived from a case of pernicious anæmia were injected into the breast muscles of pigeons (according to a suggestion from Dr. Flexner, who had found these animals to be especially susceptible). The cultures set up a localized necrotic inflammation with gas production in the connective tissues. Death occurred, apparently from toxæmia, within twenty-four hours. The application of these facts to *capsulatus* infection of the intestine is not now clear. It is certain that the pathogenicity of *B. aerogenes capsulatus* is different for different strains isolated from the human digestive tract. Thus

[1] "Zur Aetiologie der Gasphlegmone," *Centralbl. f. Bakt.*, I Abt., Orig., xxxv, pp. 554, 686, 1904.

Professor Theobald Smith sent us an organism (anaerobe xxxi) which he isolated from the stool of a patient with pernicious anæmia and which proved to be much less pathogenic for guinea-pigs than the typical form of *B. aerogenes capsulatus*. This organism differed but slightly from the typical bacillus in its morphology, but showed the important peculiarity of being non-hæmolytic. It fermented the various sugars, but the gas production was less abundant than in the case of the typical gas-bacillus. The gas production and decomposition induced in an incubated rabbit were also less pronounced than in the case of the typical *B. aerogenes capsulatus*.

A single observation was made by Mr. Ward on the blood serum from a patient with pernicious anæmia (and *capsulatus* infection of the intestine) with respect to a possible agglutinative action. The results were entirely negative.[1] It is interesting to note in this connection that Kamen [2] obtained no agglutinative action from the serum of rabbits which had been immunized with the gas-bacillus. Positive results have, however, been recently obtained by Werner,[3] who employed a special technical procedure in the immunization of the rabbits which served as experimental animals. The immune serum caused agglutination of the homologous gas-phlegmon bacilli (derived from a gas liver found in a fatal case of wound infection) in a dilution of 1 : 1000.

[1] Professor Theobald Smith tells me that he obtained negative results in agglutinative tests made with the blood of a patient with pernicious anæmia who showed large numbers of *B. aerogenes capsulatus* in the fæces.

[2] *Loc. cit.*

[3] "Die Agglutination bei Gasphlegmonbacillen," *Archiv f. Hyg.*, liii, p. 128, 1905.

Passini [1] obtained positive results in some of his cases not only with homologous strains but also with unrelated ones. In this case, however, the agglutinative action of the immune serum was less marked than in the case of the sera obtained by Werner. It is evident that there is still much to be learned in relation to the immunizing action of *B. aerogenes capsulatus* and that such an action in man may yet be discovered.

The organisms we have classed as belonging to the *B. aerogenes capsulatus* type are large, plump, usually straight bacilli, which as they occur in the fæces, can usually be shown to be provided with a capsule. Organisms which have developed in a living or dead rabbit always acquire capsules. The organisms occur very often in pairs, end to end, sometimes singly; sometimes in chains; sometimes as threads, which may be nearly straight or sharply bent on themselves. The ends of adjacent bacilli are slightly rounded or squared, though not so sharply squared as in the case of anthrax bacilli. They are immobile when viewed in hanging drops. Spore formation occurs with difficulty; *i.e.* chiefly under very special conditions, such as on a medium containing blood serum or within the body of an animal. Occasionally spore formation is seen in blood-agar colonies, the bacilli from which in other respects conform to the characters of *B. aerogenes capsulatus*. On sugar bouillon gas formation is abundant and rapid, twice as much gas (or more) being formed in twenty-four hours as is usually

[1] "Variabilität der Bakterien und Agglutinatinosphänomen," *Münch. med. Wochenschr.*, li, p. 1283, 1904.

formed by organisms of the *B. coli* group. The gas consists of from one-third to one-half carbon dioxide, the remaining gas consisting mainly of hydrogen.[1] The

[1] Professor Theobald Smith gives the following gas formula for *B. aerogenes capsulatus*: —

$$\frac{H}{CO_2} = \frac{2}{1} = \frac{3}{2}$$

The following table illustrates the approximate ratio of hydrogen and carbon dioxide: —

Source of Microörganism	Medium	Height of Gas Column in Millimeters (in 24 hours) Height of anaerobic limb 95 mm.	$\frac{H}{CO_2}$
1. Young man with slight digestive derangement	Milk	87	$\frac{2}{1}$
" "	Dextrose-bouillon-blood	7	$\frac{2}{1}$
2. Same case as No. 1	Milk	75	$\frac{2}{1}$
" " "	Dextrose-bouillon-blood	80	$\frac{2}{1}$
3. Pernicious anæmia	Milk	60	$\frac{3}{1}$
" "	Dextrose-bouillon-blood	75	$\frac{2}{1}$
4. Pernicious anæmia	Milk	70	$\frac{5}{2}$
5. Pernicious anæmia	Milk	100	$\frac{2}{1}$
" "	"	85	$\frac{5}{3}$
6. Pernicious anæmia	Milk	90	$\frac{2}{1}$
7. Same case	Milk	84	$\frac{5}{3}$
8. Aerobic capsulate bacillus	Milk	87	$\frac{5}{3}$
9. Milk	Milk	90	$\frac{2}{1}$
10. Milk	Milk	85	$\frac{5}{3}$

gas production in incubated rabbits is very rapid and is associated with a characteristic sweetish, sickening odor of butyric acid mixed with some unknown constituent or constituents. The gas obtained from the peritoneal cavity and connective tissues gives the hydrogen "bark" and burns with a blue flame. The liquefaction of muscles, liver, etc., is remarkably rapid in such incubated rabbits. Grown on pasteurized milk, the bacteria in question induce rapid gas formation ("stormy fermentation") with disruption of curds into small masses and peptonization of casein. They do not quickly produce hydrogen sulphide or methyl mercaptan on ordinary sugar-free media, but may perhaps make these sulphur compounds more readily on milk. Grown in fermentation tubes containing blood bouillon, they rapidly liberate hæmoglobin. The organisms are strictly anaerobic and many of their colonies on blood agar appear after two or three days as minute points which lie beneath the surface and develop into fuzzy spheres. These spherical colonies often have dark centers. The microörganisms induce inflammatory necrotic changes with gas formation when injected into susceptible animals, such as pigeons.

As first shown by Professor Theobald Smith, *B. aerogenes capsulatus* usually grows readily on bouillon in the closed arm of the fermentation tube provided small bits of sterile, fresh tissue are introduced into it. The liver of the guinea-pig may advantageously be used. The presence of the tissue probably favors the growth in two ways: by furnishing a constituent of the medium necessary for the growth of the organism, and by in-

ducing a more strict condition of anaerobiosis by the reducing activity of the cells.

If we cultivate *B. aerogenes capsulatus* on sugar bouillon we find that it is a large producer of gas (mainly hydrogen and carbon dioxide) and that it makes butyric and closely related acids in abundance, while the formation of lactic acid is small. On media which contain very little sugar but much proteid, the organism is still able to make gas in considerable amounts, though less freely than on a sugar medium, in which the liberation of gas is remarkably rapid. In nearly sugar-free media the gas bacillus produces butyric acid, and the quantity of this in old cultures may be surprisingly great. Ammonia is formed at the same time and serves to neutralize at least in part the acid which is simultaneously made. Most varieties of the organism are not indol producers when grown alone in blood bouillon. There are strains which make indol.

More important for the pathologist than any of these substances is the formation of a moderately hæmolytic substance (or substances) by the gas-bacillus. Evidence of such substances was obtained in a five-day culture of *capsulatus* in blood bouillon. One-half of one cubic centimeter of the filtrate from this culture induced hæmolysis in a suspension of rabbit's red cells prepared by Ehrlich's method, the filtrate having been carefully neutralized to the litmus point. The same result was obtained in the case of red cells from a large Rhesus monkey. Treatment of this filtrate in an exhaustion apparatus very slightly reduced the hæmolytic action;

heating to 70° C. for one hour reduced it still further; but even boiling did not wholly destroy it.

In order to determine whether this hæmolytic action was dependent in part on volatile ammonium compounds, the *capsulatus* filtrate was rendered distinctly alkaline with sodium carbonate and concentrated under reduced pressure at a low temperature for the removal of ammonia. The filtrate was then restored to its original volume. It was found that the hæmolytic action of the fluid was somewhat diminished but not lost.

The hæmolyzing action of *B. aerogenes capsulatus* is very clearly shown in fermentation tubes containing sugar-blood bouillon which have been inoculated with pure cultures. A free liberation of hæmoglobin occurs in twenty-four hours or less. A similar result is seen in the case of an aerobic organism closely resembling *B. aerogenes capsulatus* in morphology. The bacillus of malignant œdema does not exert a hæmolyzing action under similar conditions. *B. putrificus* was found to reduce hæmoglobin, but this change is much less marked in the case of *B. aerogenes capsulatus*.

There are other indications that *B. aerogenes capsulatus* makes a hæmolytic substance or hæmolytic substances. Rabbits injected with pure cultures of *B. aerogenes capsulatus* and then incubated at 37° C. soon show indications of hæmolysis, whereas control animals subjected to the same procedure do not exhibit an equal degree of hæmolytic change in the same period of time. This corresponds with the observation that advanced hæmolysis

is usually noted in persons who at autopsy show signs of general invasion of the gas-bacillus.

The capacity of *B. aerogenes capsulatus* to form poisons is apparently not limited to hæmolytic and proteoclastic substances. Kamen [1] states that when the sporogenic form [2] of the organism is grown on a suitable medium it forms soluble poisons (obtainable in the filtrate) which are capable of inducing in rabbits a state of nervous excitation followed by general convulsions and paralysis of respiration. He found that one-half to one and one-half cubic centimeters is fatal for one kilogram rabbit in one-half to one minute. Kamen likens the material studied by him to Faust's "sepsin," in that it acts as a respiratory poison and induces vomiting, bloody diarrhœa, tenesmus, and death. These gastro-enteric symptoms are associated with œdema and hyperæmia of the gastro-enteric tract. The poisonous substances in question are said to be dialyzable and not destroyed by heating for fifteen minutes at 60° C. I have been unable to confirm these observations by means of filtrates prepared from cultures of the vegetative form of *B. aerogenes capsulatus*, but it is distinctly stated by Passini that the products of the sporogenic and asporogenic forms of the organism are different.

I think it important to determine whether *B. aerogenes capsulatus* is able to produce substances injurious to the

[1] "Zur Etiologie der Gasphlegmone," *Centralbl. f. Bakt.*, Orig., xxxv, pp. 554, 686, 1904.

[2] This form of the organism was obtained by growing the common vegetative form on an egg-and-bouillon medium.

nervous system. Persons suffering from severe forms of chronic infection by this organism almost always show signs of intoxication of the nervous system, but such manifestations are very different in different individuals.

It is not yet clear whether the gas-bacillus makes a substance capable of exciting an acute inflammation of the ileum or colon or whether preceding mechanical or chemical irritation is necessary to enable the organism to multiply rapidly and excite further inflammation. Healthy monkeys may be fed considerable numbers of capsulati without developing signs of inflammation in the intestine, although such feeding is followed by an increase in these organisms in the fæces. Two monkeys fed on gas livers from incubated rabbits infused with pure cultures of *B. aerogenes capsulatus* developed soft stools temporarily. Such experiments are, however, quite different from the experiment of introducing capsulati into a digestive tract already somewhat inflamed and irritable in consequence of preceding infections. The ability of *B. aerogenes capsulatus* to cause an inflammatory necrotic process in the muscles of guinea-pigs and pigeons, which was noted by Dr. Flexner many years ago, is of interest in this relation. It appears probable that *B. aerogenes capsulatus* is often the cause of slight inflammatory or perhaps even necrotic changes in the mucous membrane of the intestine. Howard [1] has described instances of

[1] "Contributions to the Science of Medicine, Dedicated by his Pupils to William Henry Welch on the 25th Anniversary of his Doctorate," Baltimore, p. 461, 1900.

superficial necrosis of the mucous membrane of the stomach and intestine, associated with the presence of *capsulatus* in abundance. These necrotic areas most often lie beneath the folds of the valvulæ conniventes and may occur with gas cysts. It does not seem likely that *B. aerogenes capsulatus* is responsible for severe acute inflammatory lesions of the intestine, but it is probable that its activities will account for the subacute enteritis that is so often present in cases that show large numbers of the bacilli in the stools. It is certain that there are instances of acute diarrhœa associated with very free *capsulatus* multiplication and such diarrhœas are common in persons with severe primary anæmia.[1]

In 1905 Tissier [2] described an acute intestinal affection, especially frequent in nursling children, which he ascribed to the presence of almost pure cultures of *B. perfringens*. He claimed that in the treatment of these *perfringens* diarrhœas which ordinarily ran a mild course the most efficacious therapeutic agency was the employment of cultures of a lactic acid bacillus (*B. paralacticus*). I have in a few instances met with acute and subacute diarrhœal affections of children in which *B. aerogenes capsulatus* may perhaps have been the cause of the intestinal derangement, although in these cases pathogenic varieties of colon bacilli have not been absolutely excluded as causal agents.

[1] I am disposed to attribute this diarrhœa to an associated streptococcus infection in these cases. One may observe cases of advanced infection with the gas-bacillus without any diarrhœa; indeed obstinate constipation is found in some of them.

[2] "Étude d'une variété d'infection sur le nourrisson," *Ann. de l'Inst. Pasteur*, xix, p. 273, 1905.

In this connection it should not be forgotten that Klein isolated from the fæces in an outbreak of diarrhœa an organism which very probably is identical with *B. aerogenes capsulatus*. This organism was given the name *B. enteritidis sporogenes*. The position of the organism described by Klein is open to some suspicion because while it is stated by him to produce gas and butyric acid in the very characteristic manner observed by Welch and Nuttall for *B. aerogenes capsulatus*, it differs from the latter in having motility and apparently in sporulating much more readily. Cultures sent by Dr. Klein to Professor Welch contained bacilli which agreed in every detail with pure cultures of *B. aerogenes capsulatus*.[1] As, however, the fæces sometimes contain bacteria having the morphology of *B. aerogenes capsulatus* but differing from this organism in forming spores much more readily, it is possible that Klein was in reality dealing with impure cultures of *B. aerogenes capsulatus*. I am disposed to believe, after a careful review of the available evidence on the subject, that Klein had an organism distinct from *B. aerogenes capsulatus*, though he may have had this also.

B. aerogenes capsulatus belongs in the group of anaerobic putrefactive microörganisms capable of making butyric acid and hydrogen. If careful biochemical tests be made, there is no difficulty about distinguishing the gas-bacillus from other anaerobes that occur in the human digestive tract. The bacillus of malignant œdema is some-

[1] See "Manual of Bacteriology by Muir and Ritchie," American Edition, p. 354, 1904.

times found in the human intestine. Whether it is ever found in large numbers or ever possesses pathological significance is at present unknown. The bacillus of malignant œdema resembles the gas-bacillus in morphology, but sporulates more readily, is motile, and forms less gas on sugar media. Its behavior on milk is wholly different from that of the gas-bacillus. When injected into a living rabbit which is subsequently killed and incubated it is found that there has been little or no liberation of gas in the liver and elsewhere — a feature which easily distinguishes it from *B. aerogenes capsulatus*.[1] An organism which closely resembles *B. aerogenes capsulatus* in many respects is the bacillus of rauschbrand or symptomatic anthrax (the cause of the quarter-evil or black-leg). This organism is, however, frequently Gram-negative under conditions of staining in which the gas-bacillus is Gram-positive; it is also motile. It resembles *B. aerogenes capsulatus* in being an abundant gas producer, the gas formed showing a relation between hydrogen and carbon dioxide closely resembling that obtained from the gas-bacillus. This organism is the only one of the anaerobes which I have found to be able to give rise to a large production of gas in an incubated rabbit. It might be supposed that this organism could be mistaken for the gas-bacillus, but I think the difference in the conditions of sporulation would

[1] Ghon and Mucha gave a comparison of the gas-bacillus with the bacillus of malignant œdema and a third organism (derived from a case of peritonitis) closely related to these ("Beiträge zur Kentniss der anaëroben Bakterien des Menschen," *Centralbl. f. Bakt.*, Orig., xl, p. 37, 1905–06).

suffice to make the distinction. It was found that in the rabbit test the rauschbrand bacillus sent me by Dr. Theobald Smith sporulated very freely and characteristically in the blood and spleen and liver. We have never observed sporulation on the part of *B. aerogenes capsulatus* within the body of a rabbit incubated according to the Welch-Nuttall method. Whether the bacillus of rauschbrand ordinarily occurs in the human intestine is questionable. It seems unlikely that it should be found there except in the case of persons living in association with infected cattle.

Bacillus botulinus. — Botulism is a variety of meat poisoning in which the pathological effects are due to a powerful soluble poison made by a strict anaerobe, these effects being exerted mainly on the central nervous system. The organism in question resembles in some respects *B. aerogenes capsulatus*, and in others the bacillus of tetanus.[1] It was isolated in 1896 by Van Ermengen from a sample of ham, the eating of which in a raw state had caused many instances of poisoning and some with

[1] *B. botulinus* possesses a varied morphology, sporulates terminally, makes little gas on sugar-blood bouillon, and is motile. The specimen sent to me by Kral as Van Ermengen's *B. botulinus* was introduced into a rabbit and subjected to the incubation test. It was found that the liver was in an advanced state of putrefaction, but neither this organ nor any other part of the body showed even the slightest indications of gas formation. It is noteworthy that the vegetative form of the organism (which is the form in which the botulinus bacillus was introduced) showed evidence of sporulation in the spleen but not in the blood or the liver. An absorption test made by Mr. Ward showed that the gas formed by this bacillus on dextrose-blood bouillon consisted of four parts carbon dioxide to one part of hydrogen.

fatal ending. The symptoms closely resembled those of the so-called cases of sausage poisoning which had been reported from time to time. Very recently the interesting fact has developed in a Darmstadt outbreak that the same type of poisoning may occur from eating vegetable food; namely, in this instance, beans which were imperfectly canned.[1] It appears probable that the beans were infected through the use of manure obtained from pigs.

The following are the leading symptoms of classical botulism: first, disturbances of the external muscles of the eyeball, especially ptosis, abducens paralysis, and disturbances of associated movement with nystagmic contractions; second, disturbances of the internal muscles of the eye. In the majority of instances there have been paralyses of accommodation. In the Darmstadt outbreak from infected beans an enlargement and rigidity of the pupils was never observed, but in the outbreaks from meat poisoning these pupillary conditions have been usually noted. Third, there are usually derangements referable to the region of the pons and medulla oblongata, including disorders of the tongue, such as swelling and paralysis, pharyngeal and laryngeal paralyses and disturbances of the heart and respiration. Fourth, weakness and even paralysis of motion may appear. These disturbances have almost always been unaccompanied by changes in sensibility and

[1] Fischer, "Ueber eine Massenerkrankung an Botulismus infolge Genusses verdorbener Bohnenkonserven," *Zeitschr. f. klin. Med.*, lix, p. 58, 1906.

consciousness; fever has been absent and there has been an absence of disturbances of the stomach and intestine. The symptoms seldom come on before twelve to twenty-four hours after the ingestion of the poison. Very similar symptoms were produced by Van Ermengen in certain animals by giving them watery extracts of the infected ham or cultures either by the stomach or by subcutaneous injections. It is noteworthy that in these animal experiments there was observed a period of incubation of not less than six to twelve hours before the onset of symptoms. The characteristic paralytic effects can be induced by means of the filtered toxin, and it is therefore clear that the phenomena of botulism are to be ascribed, like the nervous phenomena of tetanus, to a powerful toxin. Indeed, the properties of the toxin of *B. botulinus* have been studied and have been shown to resemble closely the toxins of tetanus and diphtheria with respect to instability, conditions of precipitation, etc. An antitoxin[1] has been prepared by Kempner. It has the power of neutralizing the toxin and has considerable therapeutic effect when given a short time after the toxin.

It is practically very important to realize that meat may be extensively infected with the *B. botulinus* and may contain relatively large quantities of the toxins without showing the ordinary signs of decomposition. We

[1] For a discussion of the conditions under which antitoxin formation occurs see J. Forssman, "Studien über die Antitoxinbildung bei aktiver Immunisierung gegen Botulismus," *Centralbl. f. Bakt.*, Orig., xxxviii, p. 463, 1905.

have therefore in *B. botulinus*, as in the bacillus of tetanus, an example of strict anaerobes which have acquired the ability to make intense poisons but have apparently incidentally lost in a measure the common putrefactive properties ordinarily possessed by spore-bearing anaerobes. There is reason to think that isolated cases of botulism are not extremely rare in the United States. The recognition of these cases by purely clinical means may be very difficult when the typical symptoms are not fully developed. In foreign countries, where meat is distributed by a different system from that prevailing in the United States, the outbreaks of botulism are apt to involve many people in one locality, whereas in our country the cases are apt to occur in small numbers, owing to the greater opportunities for the consumption of portions of the carcass in different localities.

THE FERMENTATIVE AND PUTREFACTIVE PROCESSES FROM THE STANDPOINT OF THEIR PRODUCTS

It is helpful to review the fermentative and putrefactive processes in the digestive tract of man from the standpoint of their products although such a course may somewhat temper enthusiasm by revealing our ignorance in many important directions. I shall use the word "fermentative" to designate the decompositions of carbohydrate and fatty substances and the word "putrefactive" as applied to the cleavages of proteid and allied substances. The distinction is important, for while the products of fermentation are in themselves usually unimportant as agents of intoxication, the products of putrefaction include substances containing sulphur or nitrogen or both sulphur and nitrogen, and thus frequently derive a chemical basis for exerting toxic effects. While making this distinction, however, I do not lose sight of the fact that fermentative and putrefactive processes overlap in the sense that they furnish some products in common, such as carbon dioxide and volatile fatty acids; they are moreover closely linked by the fact that excessive fermentation in the digestive tract nearly always leads to excessive putrefaction, for reasons which I hope to make clear.

The products of fermentative decomposition in the

digestive tract may be dismissed with few words. The carbon dioxide which results from the breakdown of sugars is of interest mainly as a cause of gastric flatulence or small-intestinal flatulence. The alcohol formed is probably in too small amount to be toxically significant even when fermentation of sugars is greatly excessive. Of the acids formed we have to think of lactic, acetic, propionic, and butyric, but mainly of lactic and acetic. The higher volatile fatty acids come especially from spore-bearing anaerobes (although other types of bacteria may produce them) and therefore interest us also as putrefactive products. All these acids, however, are irritants by virtue of their acid properties. If present in considerable concentration in a healthy digestive tract or in more moderate concentration in the tract of a person with an irritable stomach or small intestine, they may be efficient factors in exciting vomiting or diarrhœa. Nervous disturbances resulting in delayed absorption may be responsible for the accumulation of considerable acid fluid in the stomach or small intestine. There is also a quite different aspect to the excessive production of acid; namely, the withdrawal of excessive amounts of alkali from the organism. All the acids mentioned are readily burned in the body, but if their absorption occurs with uncommon rapidity, they may be excreted unburned and combined with alkali. This robbing of the organism of alkali by acid is under some conditions a grave matter, leading to a definite form of intoxication, but probably the acids of fermentative origin are always associated with those of putrefactive origin in bringing about this

kind of intoxication, where this is of digestive rather than of metabolic origin. It appears to me very questionable whether one ever meets with cases of marked acid intoxication referable to the absorption of excessive quantities of organic acids formed in the digestive tract, but there is some reason to think that in young children a moderate degree of acidosis sometimes has its main origin in fermentative disturbances in the intestine.

Oxalic Acid and Oxaluria. — It is now well established that various molds and bacteria are capable of acting upon media containing sugar, in such a manner as to give rise to the formation of oxalic acid. The thought that considerable quantities of oxalic acid may arise in the digestive tract as the result of fermentative decomposition led Baldwin [1] to make studies with a view to learning whether oxaluria can be experimentally induced in dogs by feeding large amounts of sugar together with food (such as meat) which contains no oxalic acid. It was found in these experiments that oxaluria was not readily induced in this manner in healthy dogs, but that prolonged feeding of sugar in large quantities eventually led to a state of oxaluria. This experimental oxaluria was associated with a disturbance of function in the gastric mucous membrane during which large quantities of mucus were formed. The contents obtained from the stomach under these conditions failed to show the presence of hydrochloric acid. It seems clear that the

[1] "An Experimental Study of Oxaluria, with Special Reference to its Fermentative Origin," *Journ. of Exper. Med.*, v, p. 27, 1900.

oxaluria and the accompanying gastritis were referable to pathological fermentative processes induced by the excessive feeding with sugar. It was further found in these experiments that there had been a production of oxalic acid in the contents of the stomach.

It was further noticed in the experiments just mentioned that oxalic acid may be formed in a medium containing sugar and beef extract, when this medium is inoculated with material obtained from the stomach of human patients showing a marked grade of oxaluria.

Whether it is safe to conclude from the foregoing facts that oxaluria in human beings ever arises from the fermentation of carbohydrates in the digestive tract is not entirely clear. It appears probable that there are conditions of carbohydrate dyspepsia in which not inconsiderable quantities of oxalic acid are formed through fermentation of carbohydrates. The absorption of this oxalic acid would necessarily add to the quantity which must be burned in the organism in order to keep the excretion of oxalic acid within normal limits. That excessive excretion of oxalic acid by the urine is ever due solely to the production of oxalic acid in the digestive tract appears to me unlikely. Modern studies indicate that there are many ways in which oxalic acid can be formed in the organism, and the greatest significance that can be claimed for the oxalic acid produced within the digestive tract is that it may sometimes be formed in such amounts as to make it one factor in bringing about a pathological condition of oxaluria. Perhaps

the occurrence of carbohydrate dyspepsia which is so common in cases of oxaluria may influence the occurrence of oxaluria in an indirect manner through its effect upon proteid metabolism, but of such an influence we at present know nothing.

Of the intermediate stages by which sugars are decomposed with the production of oxalic acid we are at present in ignorance, but there is some reason to suspect that glyoxylic acid forms an intermediate product of decomposition. This, however, does not make it necessary to assume that any considerable quantity of glyoxylic acid is present at one time.

Propionic, butyric, and higher volatile fatty acids are common products of putrefaction. They are formed by putrefactive anaerobes not merely from sugar-holding media, but from proteid media in which there is no free sugar, although carbohydrates may be essential to initiate decomposition. Among the most important anaerobic bacteria capable of forming butyric acid during putrefaction are the motile organisms known as the amylobacteria, the *granulo-bacillus immobilis liquefaciens* of Grassberger and Schattenfroh (probably a non-pathogenic form of *B. aerogenes capsulatus*), the bacillus of symptomatic anthrax (rauschbrand), the bacillus of malignant œdema, and the *B. putrificus* of Bienstock (cadaver bacillus).

Acetone. — The interesting observation has been made that the mixed fæcal flora growing upon a peptone-bouillon medium for a period of a week at the body temperature in certain instances gives rise to the forma-

tion of acetone. This appears, however, to be an exceptional occurrence. Up to the present time it has been observed only in the case of material derived from two individuals. In one of these the bacteria with which the medium was inoculated came from a child suffering from well-marked chronic intestinal putrefaction of the marantic type with the development of a large abdomen.

The other instance in which the flora gave rise to the formation of acetone was one of advanced anæmia. Why acetone was obtained from a culture in dextrose bouillon and calcium carbonate but not in dextrose bouillon without calcium carbonate was not clear. The flora from this case also gave rise to the production of acetone in milk to which calcium carbonate had been added. The presence of sugar is not necessary to the production of acetone by intestinal flora, as is shown by the fact that it has been obtained from plain bouillon.

It is not at present clear what significance should be attached to the ability of the intestinal bacteria in certain cases to produce acetone. The observations heretofore made do not furnish us with any evidence that acetone is ever produced in the human organism in the intestinal tract through the activity of microörganisms. Nevertheless the finding of acetone under the conditions just mentioned suggests the possibility that under some conditions this ketone may be produced in the digestive tract. I do not know of any observations that have heretofore been made which have shown the formation

of acetone by intestinal bacteria. Schardinger[1] found an organism which he called *B. mascerans* which gave a mixture of acetone and alcohol during the fermentation of various carbohydrates.

An organism was also found by Bréaudat[2] which was able to produce acetone in a peptone solution. This bacterium was derived from the drinking water of a town in France, which came from a subterranean well. It was a Gram-negative facultative aerobe capable of liquefying gelatin. Under certain conditions this organism developed spores. On potato it gave rise to a violet color. The organism was described under the name of *B. violarius acetonicus.*

When we turn to the consideration of the nitrogen-holding and sulphur-holding products of putrefactive cleavage, the scantiness of our knowledge comes into view with almost discouraging clearness. That putrefactive processes are attended by the formation of bases, such as ammonia, amines, diamines (such as putrescin and cadaverin), cholin, neurin, sulphur compounds, and various aromatic bodies, has been known many years, and something has been learned, though by no means enough, about the media and the bacteria which determine the presence and proportions of these substances. When, however, we ask ourselves what we can safely say of the conditions under which such substances arise

[1] "Mitteilung aus der staatlichen Untersuchungsaustalt für Lebensmittel," *Wien. klin. Wochenschr.*, xvii, p. 207, 1904.

[2] "Sur un nouveau microbe producteur d'acetone," *Ann. de l'Inst. Pasteur*, xx, p. 874, 1906.

in the human intestine and of their pathological effects, we are able to give in most instances only very inadequate answers.

BASIC SUBSTANCES

In the course of putrefaction of proteids in the intestine ammonia is regularly formed. It does not appear, however, that it is formed in quantities or under conditions which render it toxic to the organism, although, as elsewhere stated, it is possible that ammonium butyrate acts as a local irritant. The organism is well adapted to care for moderate quantities of ammonia, which, as is well known, is united with carbon dioxide in the liver and elsewhere to form urea. The splitting-off of ammonia from amino-acids formed in the course of normal digestion is a physiological process of fundamental importance and it is probably owing to the physiological arrangements necessary for the disposal of ammonia in a relatively non-toxic form that the organism is able to tolerate the digestion of large quantities of ammonia-forming foods. Likewise we know nothing of any toxic action from methylamin or other alkyl amines. It has been supposed that guanidin[1] and methyl guanidin sometimes arise in the course of certain kinds of putrefaction. While it cannot be denied that this may be the case, we have at present no knowledge that it is the case, and are unable to correlate any clinical conditions with the formation and

[1] Kutcher showed that guanidin may be an end-product of prolonged pancreatic digestion. He found the base in the arginin fraction.

absorption of guanidin or methyl guanidin. Methyl guanidin arises from the oxidation of creatin (or creatinin), and it is conceivable that under some conditions of decomposition of meat these bodies are formed. Since, however, there is no definite evidence on this point in the case of man, I shall not enter into a discussion of mere possibilities.

In reference to the bases cholin and neurin the case is somewhat different. Of these bases neurin is much more toxic than cholin, its action being directed especially to the nervous system. That cholin is toxic when absorbed from the intestinal tract is not wholly clear although it is well known that the subcutaneous injection of cholin gives rise to nervous symptoms and a decline in blood pressure. The most definite results that have been obtained by experimental methods in respect to the production of cholin are those of Beattie Nesbit.[1] Nesbit started from the fact that lecithin may be decomposed with a yield of cholin. It has been assumed by Bócai that lecithin is decomposed during the process of digestion into glycerophosphoric acid and fatty acids and cholin, and that these products are individually absorbed. This view suggests that it might be dangerous to consume very large quantities of lecithin as, for example, in the form of eggs, as cholin cannot be regarded as a wholly harmless substance. It must be admitted, however, that there is evidence to support the idea that some

[1] "On the Presence of Cholin and Neurin in the Intestinal Canal during its Complete Obstruction. A Research on Autointoxication," *Journ. Exper. Med.*, iv, p. 1, 1899.

lecithin may be absorbed without decomposition.[1] It may thus be a matter of importance to the organism whether the lecithin introduced into the digestive tract is split through the decomposing action of putrefactive bacteria or whether it undergoes absorption. The mere quantity of lecithin introduced would therefore not be a measure of the quantity of cholin formed and absorbed.

In the experiments of Nesbit dogs which had been fed for several days upon the yolks of eggs were subjected to an operation in which a ligature was placed about the small intestine just above the ileocæcal valve. In experiments of this sort in which the animal lived for several days after operation the intestinal contents were removed and studied for the presence of cholin. On the basis of various reactions for cholin chloride it was determined that the intestinal contents of the animal contained this substance. Some evidence also was obtained to indicate that neurin was also formed. I think the evidence on this point is not quite convincing. Nesbit also found evidences of a ptomaine associated with cholin and neurin, the chemical nature of which was left in doubt. These observations render it probable that, provided the intestine contains any considerable quantity of lecithin, cholin, neurin, and perhaps other bases are formed as a result of intestinal obstruction. It must be stated, nevertheless, that Nesbit failed to obtain definite evidence that the bases found by him in the intestinal canal of the dog were really toxic. It is clear that much more work

[1] P. von Walter, "Zur Lehre von der Fettresorption," *Archiv f. Anat. u. Physiol.*, Physiol. Abt., p. 329, 1890.

needs to be done before the relation of cholin and neurin to intoxications from the digestive tract is placed on a satisfactory basis.

There are some instances of a remarkable idiosyncrasy on the part of certain persons toward eggs. I recall a child in whom the taking of an egg was regularly followed by prostration and slight fever and headache. Whether such phenomena as these bear any relation to the formation of cholin or neurin is of course an open question.

Putrescin and Cadaverin. — Putrescin (tetramethylendiamin) and cadaverin (pentamethylendiamin) are products of proteid decomposition which are formed at times in the human intestine under conditions at present obscure. It seems not improbable that peculiar states of bacterial activity may have an influence on the production of these bases, and it is very desirable that the bacterial flora should be studied with great care in those cases of cystinuria in which putrescin and cadaverin can be obtained from the intestinal contents. According to Brieger [1] the peculiar odor of cholera stools is due principally to pentamethylendiamin, but strict proof of this has not yet been furnished.

Baumann and Udranzky [2] found about 0.5 gram per

[1] Brieger and Stadthagen, "Ueber Cystinurie," *Berl. klin. Wochenschr.*, xxvi, p. 344, 1889.

[2] "Das Benzoylchlorid als Reagens," *Bericht. d. Chem. Gesellsch.*, xxi, p. 2744, 1888; "Ueber die Identität des Putrescins und des Tetramethylendiamin," *ibid.*, p. 2938; "Ueber das Vorkommen von Diaminen sogenannten Ptomainen bei Cystinurie," *Zeitschr. f. physiol. Chem.*, xiii, p. 562, 1899.

day of diamines (principally tetramethylendiamin) in the fæces of a cystinuria patient. This quantity is equal to the amounts found in the urine from the same case. In the urine, however, the cadaverin constituted about sixty per cent. of the diamines; in the fæces only from ten to fifteen per cent. of the bases consisted of cadaverin. Neither Brieger nor Baumann and Udranzky was able to find diamines in normal fæces. Moreover, Baumann and Udranzky were not able to find these bases in examples of disease other than cystinuria. Roos [1] in a case of combined malaria and dysentery with fever and enlargement of the spleen found in the fæces a small quantity of pentamethylendiamin. There was very little indican in the urine of this patient. He was unable to find diamines in cases of malaria alone. In a case of fever following gonorrhœa he was able to obtain small quantities of dibenzoylcadaverin.

Although the study of the conditions under which putrescin and cadaverin are formed in the intestinal tract is of much biological interest, there is at the present time little evidence that these diamines are ever formed in sufficient quantities in the human intestine to constitute in themselves factors in the production of states of intoxication. The association with cystinuria is a striking fact, and the further investigation of this disease will doubtless give us an explanation of the relationship between the production of diamines and the formation of cystin, if indeed there be any necessary relation.

[1] "Ueber das Vorkommen von Diaminen bei Krankheiten," *Zeitschr. f. physiol. Chem.*, xvi, p. 192, 1892.

Ellinger [1] found that putrescin may be obtained from the bacterial decomposition of ornithin. He found also that cadaverin results from the decomposition of lysin. Under the designation "ptomaines" Selmi [2] described a number of basic substances resembling plant alkaloids in their reactions and liable to be mistaken for morphine, conine, etc. He obtained them from cadavers. That such bodies are sometimes formed in the human digestive tract appears likely, but has not been satisfactorily established. In fact, the "ptomaines" are in most instances not so clearly defined in their chemical characters as could be desired. Moreover, their pharmacological characters are also insufficiently definite. For these reasons any discussion of these bodies in the present connection would be purely academic.

SULPHUR COMPOUNDS

Mercaptan. — The sulphur compounds resulting from putrefactive decomposition in the intestine have received little attention from the standpoint of their pharmacological action. It is therefore difficult at present to form a just estimate of their importance for intestinal intoxications. It was claimed by Nencki that methyl mercaptan is one of the gases formed in the intestine during putrefaction. He based this contention on a study of the fæces, but did not take the precaution to examine the material promptly after collection.

[1] "Die Constitution des Ornithins und des Lysins," *Zeitschr. f. physiol. Chem.*, xxix, p. 334, 1900.

[2] "Sulle ptomaine ed alcaloidi cadaverici e lore imputanza in toxicogia," *Bericht. d. deutsch. chem. Gesellsch.*, ii, p. 808, 1878.

It appears to me, therefore, not clear that the methyl mercaptan found by Nencki was not formed during putrefaction outside the body. My scepticism on this point rests partly on the fact that although I have made many examinations of the fresh fæces from persons with excessive intestinal putrefaction, I have never been able to find methyl mercaptan by means of the isatin-sulphuric-acid reaction. It appears improbable that methyl mercaptan is produced in the human large intestine in the course of ordinary putrefactive troubles, although it must be admitted that it may perhaps arise under some exceptional conditions. It may be stated before leaving the subject of mercaptan that experiments were made upon dogs and monkeys in which ethyl mercaptan in a solution of one part in one thousand parts of water failed, when injected into the large intestine, to give rise to definite evidence of intoxication unless very large quantities were employed. Thus during a period of ten days a dog of medium size received 20, 30, 50, 56, 110, 110, 110, and 110 c.c. of such a solution on successive days without exhibiting symptoms. The injection of 50 c.c. of a solution two and one-half parts per one thousand parts was not well retained. It was only when a dose of 120 c.c. of this concentration was introduced into the stomach that vomiting occurred. The rectal injection of an ethyl mercaptan solution of 1 : 1000 strength in quantities of 10 to 30 c.c. daily in a medium-sized monkey failed to give rise to symptoms.

Hydrogen Sulphide. — There is reason for thinking that the production of hydrogen sulphide in the digestive

tract is of more importance to the organism than the formation of mercaptan. That this gas is regularly formed in at least moderate quantities under normal conditions is apparent from the fact that the intestinal contents are rendered dark in color after the administration of iron salts owing to the formation of sulphide of iron. This occurs, as is well known, in well persons. Moreover, the presence of hydrogen sulphide in the freshly voided fæces can be readily demonstrated. It is also true that the mixed fæcal flora from the human intestine, both in health and disease, is capable of producing hydrogen sulphide upon a medium containing proteid that has been partially hydrolyzed. This is true, for example, of the growths upon bouillon. Hydrogen sulphide is produced early in the course of putrefaction, and its presence in flasks containing peptone bouillon inoculated with fæcal flora is regularly demonstrable within the first twenty-four hours. It is difficult to form a judgment as to how much hydrogen sulphide is produced through intestinal putrefaction in the course of a given period or how much is absorbed. Assuming that the production is the same in a given series of individuals, it is highly probable that the absorption would be variable, and it is of course upon the absorption of the gas that its importance to the organism chiefly depends.

It seems probable that in health the formation of hydrogen sulphide is limited to the large intestine and perhaps a short extent of the adjoining small intestine. Under pathological conditions, hydrogen sulphide is

formed in other parts of the digestive tract. There are cases of dilatation of the stomach from which one may remove material which is undergoing putrefaction with the formation of hydrogen sulphide. The production of hydrogen sulphide in this situation is certainly to be regarded as pathological. I am not clear, however, that it points to the presence of pathological bacteria, for it is easy to show that typical organisms of the *B. lactis aerogenes* type are able to form hydrogen sulphide when growing in bouillon containing cystin. Cystin is a decomposition product of proteids, and it is conceivable that in a stagnant stomach proteid digestion may go on to the formation of cystin among the cleavage products and that this is then decomposed through the action of *B. lactis aerogenes*. The ability to form hydrogen sulphide from cystin is widespread among bacteria. The colon bacilli liberate it abundantly when growing in a cystin medium.[1]

Among persons who take organic iron it is usual to note, after a little time, a dark brown color on the tongue which appears to be dependent on the formation of iron sulphide in this situation. The sulphide production is referable, doubtless, to the formation of hydrogen sulphide, but the conditions of its production are not known. It does not appear that the blackening of the tongue after taking iron can be regarded as pathological, as it may be observed in persons apparently free from disorders of digestion.

I have made some observations to determine at what

[1] This is true also of the bacilli of dysentery and of typhoid fever.

level of the digestive tract one finds bacteria capable of forming hydrogen sulphide when grown in peptone bouillon. These observations were made upon the digestive tract of infants. The mixed flora from various portions of the large intestine and lower part of the ileum regularly gave hydrogen sulphide. Where the inoculated material was derived from children dying of pneumonia or some condition unconnected with derangements of digestion, it was unusual to obtain hydrogen sulphide from bacteria derived from portions of the tract lying above the lower part of the ileum. It was frequently noted, however, in children dying from marasmus that the flora obtained from the stomach and from portions of the small intestine above the ileum gave hydrogen sulphide when grown on peptone bouillon. In some instances it was observed that more hydrogen sulphide was formed from the bacteria derived from the stomach of such children than from the bacteria obtained from the rectum.

We have at present very little satisfactory knowledge of the influence of hydrogen sulphide upon the organism in cases where the gas is liberated in the intestine. Frequent reference is made in works dealing with autointoxications to a publication by Senator,[1] concerning what he regarded as a case of pure hydrothionæmia. The patient in this instance was a man supposed to have been well previously and who after an error in diet be-

[1] "Ueber einen Fall von Hydrothionämie und über Selbstinfection durch abnorme Verdauungsvorgänge," *Berl. klin. Wochenschr.*, v, p. 254, 1868.

came ill with a gastro-enteric catarrh, presumably located mainly in the cæcum. On the third day after the illness there suddenly occurred a severe seizure of vomiting accompanied with an intense odor of hydrogen sulphide. Simultaneously the patient suffered from dizziness and a little later passed into a state of collapse without loss of consciousness, the eruction of gas continuing meantime. The first urine passed gave a definite reaction for hydrogen sulphide. On the following day the patient recovered. Other somewhat similar instances have been described by Betz,[1] Stefanio, and Emminghaus.[2] Among the symptoms that have been met with in such cases there have been prominent those pointing to disordered function of the central nervous system, including headache, dizziness, delirium, mental depression, drowsiness, stupor, and collapse. Somewhat similar manifestations have been observed in experimental poisoning by hydrogen sulphide in animals and men.[3] The nervous symptoms observed under these conditions have frequently been more severe than those arising in spontaneous hydrothionæmia, probably because the quantity of gas absorbed is greater in the former cases. Apparently somewhat at variance with the results of poisoning in dogs is the statement that many persons subjected

[1] "Ueber Hydrothionammoniaemie," *Memorabilien*, lx, p. 145, 1864.

[2] "Zwei Fälle von mehrfacher Perforation des Verdauungskanals und H_2S-Gehalt im Urin," *Berl. klin. Wochenschr.*, ix, pp. 477 and 491, 1872.

[3] See especially K. B. Lehmann, "Experimentalle Studie über den Einfluss technisch und hygienisch wichtiger Gase und Dämpfe auf den Organismus," *Archiv f. Hyg.*, xiv, p. 135, 1892.

to the Burgeon treatment for pulmonary phthisis have been able to take considerable quantities of hydrogen sulphide administered by the rectum without injurious effects. It appears to me extremely doubtful, however, from the results of experiments made upon dogs whether it is really possible to bring about the rapid absorption of more than small quantities of hydrogen sulphide in man without giving rise to collapse. In experiments conducted on dogs it is necessary to use the utmost caution in administering the gas by the rectum in order to prevent fatal collapse. Nevertheless, small quantities of the gas may almost certainly be absorbed from the human intestine without giving rise to noticeable disturbance of nervous function or to the presence of hydrogen sulphide in the urine. Friedrich Müller[1] showed that in various disorders of the intestine hydrogen sulphide is liberated in the lungs, but without simultaneous appearance of hydrogen sulphide in the urine, owing probably to the small quantity of gas formed. In some cases of extreme dilatation of the stomach the formation of hydrogen sulphide is so abundant as to make it seem strange that the patients escape without symptoms of intoxication. Their immunity in these cases appears to depend on the fact that there is an eruction of the gas when it accumulates in considerable volume.

It has come to be well recognized that local inflammatory conditions of the bladder may be associated with

[1] "Ueber Schwefelwasserstoff im Harn," *Berl. klin. Wochenschr.*, xxiv., p. 405, 1887.

a putrefaction of proteid material with liberation of hydrogen sulphide. This condition is, of course, one quite distinct from true hydrothionæmia, and it runs its course without symptoms of general intoxication.

The toxic character of hydrogen sulphide for dogs is plainly seen from a typical record of experiments in which a saturated aqueous solution of hydrogen sulphide was introduced into the rectum. Fifteen cubic centimeters of such a saturated solution were introduced daily into the rectum of a dog weighing sixteen and one-half pounds. No effects were observed. After one week the volume of the enema was increased to thirty cubic centimeters. After a daily administration of this amount during two weeks, the volume was increased to forty-five cubic centimeters. The animal immediately passed into a state of collapse. It was found that by injecting forty-five cubic centimeters very slowly the enema was tolerated, although it gave rise to prostration. After a period of five days, in which this quantity was daily administered, the volume was increased to sixty cubic centimeters, but with the result of bringing on collapse which proved nearly fatal. The volume of the enema was then reduced to thirty cubic centimeters. After a few days it became necessary to discontinue the injections of hydrogen sulphide, as the dog failed to eat. The nutrition of the animal suffered in the course of these infections. There appeared to be a slight reduction in hæmoglobin in the course of the experiment. A pronounced anæmia was, however, not induced.

Hydrogen Sulphide and its Relation to Enterogenic Cyanosis. — In 1902 Stokvis[1] described a peculiar and apparently new clinical state under the name of "autotoxic enterogenic cyanosis." His observation had reference to a man fifty-eight years of age who suffered from a severe enteritis associated with pronounced cyanosis of the skin and visible mucous membranes, together with a slight swelling of the terminal phalanges. On making a spectroscopic examination of the skin and mucous membranes of his patient, Stokvis was easily able to make out in addition to the two feeble oxyhæmoglobin bands a small absorption band in the red, corresponding to the absorption spectrum of methæmoglobin. It is of course well known that the experimental methæmoglobinuria which may be induced by a variety of poisons, such as potassium chlorate, nitrobenzol, and various derivatives of anilin, gives rise to a characteristic cyanosis of the skin and mucous membranes. Stokvis was therefore inclined to believe that the cyanotic discoloration of the skin of his patient could be ascribed to the methæmoglobin present in the blood. He assumed that some poisonous substances had been formed in the intestine which gave rise to a transformation of a portion of the hæmoglobin into methæmoglobin. Three similar cases were soon after reported by Talma,[2] who reached the same conclusions as Stokvis; namely, that the methæ-

[1] "Zur Casuistik der autotoxischen enterogenen Cyanosen (Methaemoglobinaemia (?) et enteritis parasitaria)," *Festschr. f. v. Leyden*, i, p. 597, 1902.

[2] *Tijdschrift voor Geneesk.*, ii, p. 721, 1902.

moglobin observed in his cases was the result of intoxication through the intestinal tract, due to poisonous substances formed there. In 1905 Hijmans van der Bergh [1] reported two cases of enterogenic cyanosis which at first sight appeared to have the same pathological basis as those reported by Stokvis and Talma. He found, however, on careful investigation that the substance contained in the blood of one of his patients could not have been methæmoglobin.[2] The absorption spectra observed by him showed the closest similarity to the spectrum of sulphhæmoglobin. Attempts were made to determine the presence of hydrogen sulphide or rather of sulphhæmoglobin in the blood, but these were not wholly successful, although he was able by means of the Caro-Fischer reagent (a solution of pure amidodimethylanilin with ferric chloride) to obtain a blue color due to the formation of methylene blue and indicative of the presence of hydrogen sulphide. Van der Bergh concludes that his patient suffered from the passage of hydrogen sulphide into the blood from the intestine.[3]

[1] "Enterogene Cyanose," *Deutsch. Archive f. klin. Med.*, lxxxiii, p. 86, 1905.

[2] This writer has lately reported one instance of enterogenic cyanosis associated with methæmoglobinæmia, in which the blood was shown to contain nitrites. This observation appears of much importance for the doctrine of intestinal intoxication, as it is well established that nitrites readily cause methæmoglobinæmia under experimental conditions. A. A. Hymans van der Bergh und A. Grutterink, "Enterogene Cyanose," Zweite Mittheilung, *Berl. klin. Wochenschr.*, No. 1, p. 7, 1906.

[3] Several of these cases of sulphhæmoglobinæmia have been associated with obstinate constipation, the relief of which has been followed by rapid improvement in the blood state.

The clinical conditions present in one of Van der Bergh's cases of enterogenic cyanosis deserve mention. This case was a child nine years of age in which there were marked digestive disturbances with diarrhœa. It had been observed by the mother that the child passed very little urine. This anuria was probably owing to the constant diarrhœa. For two years the mother had observed that her child readily became bluish on slight excitement. This condition gradually became more pronounced, and finally the child grew very cyanotic even when at rest. The ends of the fingers were also somewhat clubbed. When the child was three or four years of age the abdomen became much distended and this distension gradually increased. There was no evidence whatever of any cardiac affection. The urine was very much reduced in amount and contained no albumin and no sugar, but is said to have contained a little indol. The sediment contained many bacteria, leucocytes, and calcium oxylate. The movements were very thin, had a strongly alkaline reaction, and smelled of ammonia. The movements were sometimes yellow, sometimes dark brown or nearly black, and always became very dark on standing in the air. No protozoa were found. The author states that a fistula existed between the bladder and rectum, and this was probably the cause of the indol found in the urine. The child was not anæmic. An endeavor was made to control the cystitis by means of urotropin and the proteid food was greatly reduced in order to diminish the hydrogen sulphide formed in the intestine. This resulted in the disappearance of indol

from the urine. The development of hydrogen sulphide in the urine, however, could not be wholly checked. After a time, nevertheless, there was distinct improvement. The size of the abdomen became reduced and, strikingly enough, the blue color of the skin and mucous membranes in the course of a few weeks became much less marked and ultimately scarcely any cyanosis could be detected. Nevertheless by spectroscopic examination the characteristic sulphhæmoglobin band in the spectrum remained visible although apparently somewhat weakened.

AROMATIC PRODUCTS OF PUTREFACTIVE DECOMPOSITION

Phenol and Cresol. — Among the aromatic products of putrefaction are the phenols. In health the intestinal contents contain only small quantities of these substances. In some pathological conditions attended by excessive putrefaction in the intestine they are found in the intestinal contents in amounts considerably above the normal amount, which is always small. The quantities found are, however, never large — never so large, for example, as in the case of indol. What are the conditions of bacterial decomposition which lead to this production of phenol it is at present impossible to state with confidence. Phenol is doubtless derived from the breaking-down of tyrosin, and it is therefore in decompositions in which tyrosin-containing foodstuffs are involved that we would expect to find the phenols in greatest amount. I am not able to say whether the facts of experience correspond to this view. Of the common proteid foods, milk is one which yields considerable

quantities of tyrosin from its casein (the yield reaching as high as four and one-half per cent.).[1] Phenol is paired in the body, as is well known, with sulphuric acid and is excreted as phenol-potassium-sulphate.

Although the quantity of phenol found in the fæces at any one time is small, the quantity excreted in twenty-four hours in the urine may be fairly high. Cresols are included with phenol in these estimates. The reason for this considerable excretion is in part the fact that phenols are produced in the organism in the course of the metabolism of normal cells. The quantity thus formed is, however, small, and the excess above this amount which we find in certain putrefactive cases may therefore be properly attributed to the phenols produced by putrefaction in the intestine. I have found the highest values for phenols in the urine in cases of chronic intestinal indigestion characterized by marasmus and distension of the abdomen in children.

It does not appear that the phenols can be regarded as important toxic agents, since the maximal quantities which we find in the urine in cases of disease may be considerably exceeded during the prolonged therapeutic administration of phenols without giving rise to any symptoms whatever. Nevertheless, it is likely that the continued absorption of moderate quantities of phenols

[1] Among other substances which have been found to yield relatively large proportions of tyrosin are fibrin, 3.82 per cent.; zein from corn, 10.06 per cent.; glutenfibrin, 4.43 per cent.; conglutin from the seeds of lupins, 3.2 per cent.; horn, 4.58 per cent.; histone from thymus, 6.31 per cent.; protamin of the seehare, 8.4 per cent.

from the intestine over a long period of time may prove injurious to the cells of the liver and to other cells concerned with the pairing of phenol to phenol-sulphuric acid. This deleterious effect is probably of greater importance in persons in whom the cell protoplasm of the liver has been somewhat damaged than in the case of normal persons.

Skatol. — This substance is formed in very small quantities from day to day in some normal persons, and in persons suffering from excessive intestinal putrefaction it may be formed in larger quantities than is normally the case. As compared with the quantity of indol found in the intestinal tract, the quantity of skatol is almost always small. I have occasionally met with instances in which the fæces contain relatively large quantities of skatol, eight to ten milligrams in one hundred grams of fresh material. This has occurred only in persons with marked signs of intestinal or nervous disorder. Very rarely I have observed much skatol in the intestinal contents with only a mere trace of indol.[1]

Like indol, skatol is derived from tryptophan, but what are the conditions that determine its formation rather than the formation of indol we do not at present know. I have given this subject considerable attention, but without being able to determine the bacteria con-

[1] The most extreme instance of this sort was observed in a patient suffering from multiple neuritis and the psychosis characteristic of alcoholic intoxication. As this patient had not taken alcohol, the nervous condition was attributed to an obscure type of intestinal intoxication. A study of the urine and fæces gave some ground for this suspicion.

cerned in the formation of skatol rather than indol. Suspicion rests on a slender, curved, Gram-positive organism about four microns in length which I have several times found associated with the production of skatol, but which has not yet been obtained in pure culture.[1]

I found that the administration of skatol to monkeys by the mouth and by subcutaneous injections has been followed by the appearance of a substance in the urine giving the Ehrlich dimethylamidobenzaldehyde reaction of the urine, and the administration of 0.1 gm. of skatol to man has been followed by the appearance of a stronger Ehrlich reaction than was previously present.[2] I am inclined therefore to ascribe the intensification of the reaction in these cases to the administration of skatol. The direct evidence, however, is lacking to show that the reaction thus induced or accentuated is dependent on the presence of the same substance in the urine that gives rise ordinarily to the Ehrlich aldehyde reaction.

[1] The ground for this suspicion is that flasks containing putrefactive media inoculated with fæcal bacteria have shown the presence of this organism in very large numbers in a case where skatol has been present, whereas flasks prepared with the same media and the same bacteria plus magnesium carbonate have failed to show this microörganism and have also failed to show skatol. In these cases the difference between these flasks as regards other microörganisms than the one mentioned have been slight. The subject is difficult of investigation. One might suppose that the fæcal bacteria from a case in which the intestine contains considerable skatol would form skatol when grown in bouillon. I have never found this to be the case. Indol is formed, not skatol.

[2] "On a Relation between Skatol and the Dimethylamidobenzaldehyde Reaction of the Urine," *Journ. Biol. Chem.*, i, p. 251, 1906.

In most cases in which the fæces contain considerable skatol the urine gives a strong reaction with dimethyl-amidobenzaldehyde.

Skatol behaves in the organism much like indol as respects its toxic properties, although it is somewhat less poisonous. There is seldom reason to attribute to skatol any definite pathological effects, as it is formed in quantities too small to fall under suspicion. It is possible, however, that like phenol it may, under some conditions, play an auxiliary part in association with other substances in damaging living cells.[1]

Indol. — It has long been known that indol is a product of putrefactive decomposition of proteids, and Baumann showed many years ago that this substance is formed in the large intestine in the course of putrefactive processes occurring there. He showed, moreover, that the absorption of indol from the intestine is followed by the appearance of indican in the urine. Some observers have concluded that the indican of the urine may in part depend on the liberation of indol from the breaking down of body cells. I regard the evidence in favor of this view as unsatisfactory and believe that the indican of the urine depends exclusively on the resorption of indol from the intestinal tract excepting in those cases in which pathological processes such as a putrid abscess are associated with the formation of the base.

The observation has been repeatedly made by clini-

[1] There is an excellent discussion of the fate of skatol to be found in a recent paper of Porcher and Hervieux, entitled "Untersuchungen uber das Skatol," *Zeitschr. f. physiol. Chem.*, xlv, p. 487, 1905.

cians that persons in whom a very strong indican reaction can be obtained in the urine during a long period of time invariably suffer from nervous or dyspeptic disorders, and many careful physicians have believed that there is some causal connection between the absorption of indol from the intestine and the development of functional nervous or nutritional derangements. I believe that in recent years the grounds for this conviction have been considerably strengthened. Nevertheless, it can hardly be said that the relation between the absorption of indol and the symptoms of intoxication in man has ever been placed upon a firm scientific basis. I believe that I have myself underestimated the importance of indol as a toxic agent in man, and desire to present here evidence which has lately come to light in regard to the influence of indol on the animal organism. For this reason I shall discuss the subject of indol poisoning somewhat fully.

Indol is not a product of the tryptic digestion of proteids, and probably cannot be formed in the course of physiological processes without the intervention of organized ferments such as bacteria. In the human intestine the presence of indol is dependent on the action of living bacteria, although it is likely that the action of the digestive juices may prepare the way for the attack of bacteria on proteid material. The indol produced in the intestine is, like skatol, dependent on the production of a more complex substance known as tryptophan. A very careful study of the chemical constitution of tryptophan was lately made by Gowland Hopkins of Cambridge

University.[1] He ascribed to it the constitution of skatol-amido-acetic acid, but it has been shown by other workers that there is evidence for considering tryptophan as an isomer of skatol-amido-acetic acid; namely, indol-amido-propionic acid.

It has been known since the days of Claude Bernard that tryptophan arises at an early period in the putrefaction of proteids. It was further observed that while the tryptophan color reaction is almost always observed in the early stage of putrefaction, it later disappears. The cause of this disappearance is the further cleaving action of bacteria or unorganized ferments. Hopkins was able to show that the action of bacteria upon tryptophan may lead to the formation of indol, skatol, indol-acetic acid, and indol-propionic acid. An endeavor was made to determine the influence of individual types of bacteria upon the cleavage of tryptophan, and it was found that *B. coli* is capable of giving rise to considerable yields of indol at the same time that it produces indol-acetic acid. Observations with cultures of symptomatic anthrax representing a typical spore-bearing anaerobe, showed that this organism is able to make indol-propionic acid. At present, however, there is no evidence that more than a small proportion of tryptophan in a medium is converted into indol through the action of putrefactive anaerobes. I studied many years ago the influence

[1] Hopkins, F. Gowland, and Sydney W. Cole, "Contributions to the Chemistry of Proteids," Pt. II, "The Constitution of Tryptophane and the Action of Bacteria upon It," *Journ. of Physiol.*, xxix, p. 451, 1903.

of introducing large numbers of *B. coli communis*, *proteus vulgaris* of Hauser, and *B. acidolactici* into the intestinal tract of dogs, with a view to determining the effect upon indol production. It was found that the injection of pure cultures of colon bacilli into the jejunum of dogs was followed by an increase in the indican reaction of the urine and an increase in the output of the ethereal sulphates. In order to exclude the introduction of indol into the intestine in the course of these experiments large numbers of colon bacilli were grown on agar-plates and washed in salt solution. The introduction of the living bacteria was followed by an increase in the indican of the urine, whereas the introduction of sterile cultures prepared in the same way showed at most only a slight increase in the indican excretion. The inference was drawn that the small amount of indol introduced in feeding experiments made with colon bacilli does not account for the marked increase in indican which was noted in these experiments and that this increase is to be attributed to the action of the colon bacilli themselves growing upon a suitable medium. Similar experiments were made with pure cultures of *proteus vulgaris* with negative or slight effects as regards the excretion of indican. The lactic acid bacilli employed in similar experiments in which cultures were injected directly into the small intestine showed a tendency to cause a reduction in the excretion of indican and of the ethereal sulphates.[1]

[1] Herter, "On Certain Relations between Bacterial Activity in the Intestine and the Indican of the Urine," *Brit. Med. Jour.*, II. p., 1847, 1897.

There are many persons from whose intestines it is impossible to recover indol at all or in more than a mere trace. This is particularly the case with young children and young adults. There are, however, some older persons who, although suffering from disorders of digestion, do not form indol in the digestive tract. On the other hand, the production of considerable quantities of indol in the large intestine is a feature of many instances of intestinal putrefaction, and in some cases the quantity formed is large. One may find as much as fifty to sixty milligrams of indol by the naphthaquinone method in one hundred grams of the fresh stool. This probably approaches the maximal amount present at any one time. It is of course clear that such indol production is distinctly pathological in occurrence. Its production in smaller amounts permitting the recovery of five milligrams in one hunndred grams of moist fæces is no uncommon occurrence, and such indol production may or may not be associated with the development of intestinal or nervous or other disturbances. The significance of the indol produced during putrefaction in the intestine depends upon its absorption into the organism through the intestinal wall. That this occurs in considerable amounts is shown by the appearance of large quantities of indican in the urine of persons in whom the intestine contains large amounts of indol. A close relationship between the quantity of indican in the urine and of indol formation in the intestine is not always demonstrable. The fæces may contain little indol while the urine holds much indican. Conversely, the fæces

may contain a considerable quantity of indol, and owing to imperfect absorption the urine may contain only moderate quantities of indican. Upon the whole, however, if one considers the findings from day to day over a considerable period of time, it holds true that there is a rough relation between the indol formed in the intestine and the quantity of indican excreted. In conditions of health the absorption of moderate quantities of indol from the intestine is followed by the rapid oxidation of indol to indoxyl or some indoxyl compound, and this oxidation is associated with or followed by a synthesis with sulphuric acid, occurring mainly in the liver and partly in the muscles, and which results in the production of the indoxyl-potassium-sulphate of the urine. It is the indoxyl-potassium-sulphate (or indican) of the urine which on further oxidation yields indigo. Something is now known in regard to the fate of indol in the organism. While it is true that in general the aromatic compounds of putrefaction are resistant to oxidation, it is probable that indol when introduced in moderate quantities into the organisms of carnivora and omnivora suffers a break-down both in the pyrrol ring and the benzene ring. That is to say, a portion of the indol is burned completely in the organism. This view is based partly on what has been found to be the case for phenols and especially for cresol,[1] and partly as direct observations on the facts of ingested indol.

[1] Blumenthal, "Biochemische Untersuchungen über Vergiftung und Entgiftung bei der Lysolvergiftung," *Biochem. Zeitschr.*, i, p. 135, 1906. The author found that only from twenty to twenty-five per cent. of the cresol introduced into the animal

It was shown by Herter and Wakeman [1] that the living cells of the body, especially the hepatic and renal cells and the epithelial cells of the intestinal tract, have the power of absorbing considerable quantities of indol as well as of phenol and of tying them loosely in such a way that these bodies cannot be recovered by distillation. Owing to this property of the cells, by which they hold these aromatic bodies while subjecting them to oxidation and pairing, the nervous system is screened from their action. The importance of this screening action is considerable, for the presence of indol or phenol in very slight concentration in the blood of the carotid artery suffices to induce violent nervous excitation followed by a depression of nervous function. It is very noticeable that the capacity of different animals to remove indol from the circulation after intravenous injections differs greatly in individuals of the same species. What is particularly striking is the fact that in those animals which do not possess livers capable of promptly removing the greater part of the indol, the nervous system falls a prey to the action of the poison. Conversely, it is also true that in those animals in which nervous symptoms are very pronounced after such injections of indol, it is found that the blood and brain hold considerable indol, whereas the liver may be shown to have fallen far below its normal capacity in the removal of the poison from the blood.

organism could be found again in the urine when the doses are moderate in amount.

[1] Herter and Wakeman, "The Action of Hepatic, Renal, and Other Cells on Phenol and Indol," *Journ. of Exper. Med.*, iv, p. 307, 1899.

It may be regarded as settled that the liver, muscles, intestinal epithelium (and other cells) normally exert a protective action to the nervous system in screening it from the effects of an injurious percentage of indol in the blood by the ability of these structures to quickly bind any indol which comes to them. It may, moreover, be regarded as established that the same dose of indol administered to two human beings of about equal weight may regularly give rise to more pronounced nervous manifestations in one than in the other. While these inequalities may be due partly to differences in the rapidity of absorption, no striking differences due to this factor were noticeable in the excretion of indican in the urine, and it appears more probable that the differences in the observed toxic effects were dependent on inequalities in different persons in respect to their ability to oxidize indol and to pair it with sulphuric acid. The probability that individual differences in the oxidizing capacity of the tissues of different persons might play a part in determining the toxic effects of indol made it desirable to get experimental evidence as to the influence of the imperfect oxidizing action of the cells on the fate of indol in the body. Such experiments have been planned and made by Dr. A. N. Richards and Dr. J. Howland, and their results are of such interest for the general pathology of intoxications as well as for the question of indol poisoning, that I shall speak of them at some length.[1]

[1] The details of these observations have not yet been published, and I feel under obligations to Dr. Richards and Dr. Howland for permitting me to use their notes.

To reduce the oxidizing processes in the cells, animals were poisoned with hydrocyanic acid, a substance which possesses a high degree of power to depress the ability of animal cells to take up oxygen from the blood. The effect of dyspnœa from tracheotomized animals was also investigated to some extent and similar observations were made upon the influence of chloroform given to the point of narcosis upon the fate of indol.

In general it may be said that rats, mice, guinea-pigs, and dogs subjected to subcutaneous injections of potassium cyanide too small in themselves to cause marked symptoms, were later subjected to subcutaneous or peritoneal injections of indol or phenol in watery solution or in oil. The symptoms observed in such cases were compared with those obtained from the injection of indol or phenol without any preceding treatment with potassium cyanide. The result almost regularly observed was that the convulsive twitching which is characteristic of the action of the phenol and of indol was of greater intensity and longer duration in the animals subjected to potassium cyanide than in the case of the control animals. For example, into a guinea-pig weighing 370 gm. was injected 0.0003 gm. of indol per gram of body weight. The characteristic twitching came on in eight minutes and lasted ninety-seven minutes. It was mild in character. Into another guinea-pig, weighing 380 gm., 0.005 mg. of potassium cyanide per gram of body weight was injected together with 0.0003 gm. indol per gram of body weight. The twitching began in two minutes, was much more violent than in the

control animal, and lasted for five hours. Experiments were made which showed that the lengthening effect is not due to a decreased rate of absorption dependent on the depression of the circulation by potassium cyanide.

Experiments were also made to determine whether after subcutaneous injections of indol any uncombined indol may be excreted by the gastro-intestinal tract, and if so, under what conditions such excretion occurs. For example, in a dog weighing eleven kilos the small intestine in one foot of its extent (in the region just above the ileocæcal valve) was severed by Dr. Maury from the main gut and the ends sewed into the abdominal wall. The cut ends of the main gut were joined by end-to-end anastomoses. Complete recovery occurred and the health of the dog was apparently perfect. The mucous membrane at the fistulous openings was normal. One month later an experiment was made in which 0.5 gm. of indol dissolved in ten cubic centimeters of oil was injected subcutaneously. No symptoms developed. The loop of gut was washed out at intervals of one-half hour during the following nine hours, and no indol could be detected in the distillate by means of the naphthaquinone reaction. The urine obtained by catheter forty-five minutes after injection of indol gave an extremely intense indican reaction. Repeated examinations showed little falling off in the intensity of the reaction until the lapse of twenty-two hours. After twenty-eight hours the urine showed a normal indican reaction. After an interval of two days, in which the animal was apparently normal, he received successive injections of a two per

cent. solution of the potassium cyanide. The doses given sufficed to cause vomiting and some muscular weakness, but no convulsions. The injections were given during a period of two and one-half hours. At the end of this 0.5 gm. of indol was injected subcutaneously as in the first experiment. No further vomiting was noted, but twitching of the muscles of the face and legs was more marked than before. One and one-half hours after the indol injections the animal was lively and seemed to be recovering. Soon after he received more cyanide, in consequence of which there developed a few mild convulsions. There was much prostration and dyspnœa and some fall in temperature. The odor of indol was not detected at the fistulous opening. Washings made from the gut were distilled and gave a slight reaction for indol. It was found that the indican reaction in the urine was delayed in its appearance as compared with the previous experiment and that the quantity of indican as judged by the intensity of the color was considerably less developed in the Obermeyer reaction. The quantity of indol excreted was thus distinctly less than during the previous experiment. The most striking feature of this experiment is the fact that on the day following the administration of indol and potassium cyanide the animal remained all day in a condition of stupor interrupted by periods of frantic and poorly coördinated movements in which he recognized none of the attendants and did not respond to calls. These seizures could be brought about by local stimuli and resembled the behavior seen in dogs etherized

or chloroformed during the period of lessening consciousness which precedes that of complete anæsthesia. The animal refused food and water and showed signs of increased peristalsis. During the second day prostration and stupor were less marked, but uncoördinated movements were more frequent and associated with a state of delirium at times very violent. During the third day the condition was the same. During the fourth day the condition was the same, but the animal was weaker. Warm diluted milk given by a tube was instantly vomited. On the sixth day egg-water was retained. On the seventh day death occurred. The brain was found to be soft and congested. The cortical nerve cells were found to be œdematous and showed excessive chromatolysis. The mucous membranes of the loop and of the duodenum and jejunum were considerably congested. The ileum was slightly congested. The total amount of potassium cyanide given to this animal was 0.061 gm. in six doses in three hours during the first period, that is before giving the indol; after the indol, that is in the second period, 0.085 gm. was given in four doses in three-quarters of an hour.

The leading effects of the poisoning in this case were, first, a prolongation and intensification of the direct indol effects upon the nervous system, for example, the convulsive twitching; secondly, a delayed transformation of indol and a delayed excretion in the form of indican; third, gastro-intestinal symptoms; fourth, blindness; and lastly, various mental symptoms. Other similar experiments were performed which clearly showed

that when indol poisoning is preceded by the administration of physiological quantities of potassium cyanide the indol poisoning takes a wholly different course from that observed when indol alone is injected. As it is known that potassium cyanide depresses the ability of the cells to take up oxygen, it is reasonable to attribute to this depression in the oxidizing capacity of the body cells the failure of the organism normally to oxidize the injected indol. It is not unreasonable to suppose that in chronic disease in which the cells most concerned in oxidation, such as the liver cells, have been structurally damaged, that the ability to transform indol is less than in a state of health. Such alterations are common in human beings as a result of infections, alcoholism, and intoxications, etc., and the experiments just quoted enable us to understand how under such pathological conditions the absorption of a moderate quantity of indol from the intestine may be more harmful to the central nervous system than is the case in persons whose cells have suffered little deterioration as the result of disease.

As to the effects of absorbed indol upon the organism in disease it is necessary to speak with caution, since there is no evidence that indol is the only toxic substance absorbed in those cases where it enters the organism from the gut. Some light is thrown on the question by experiments made through administering indol to normal men by the mouth. In one of the cases, a robust young man whose urine had been free from indican, felt no effect from large doses given until after several days' adminis-

tration, when the influence on the nervous system became distinct and led to irritability, headache, flight of ideas, etc. It should be noted that the quantity of indol administered in this case was probably in excess of any amount that would be absorbed from the intestine, even in the most pronounced pathological condition. It would not, however, be safe to infer from this that smaller doses would have been harmless; for while such smaller doses might have produced little or no effect if given during a few days to a normal person, it is not unlikely that its long-continued administration would have led to symptoms. But here again it must be emphasized that the significance of indol absorption for the organism must depend largely on the ability of the organism to quickly oxidize and pair the indol to indoxyl-potassium-sulphate.

The idea that the circulation of free indol in the blood may act in a depressing manner upon muscular structures is suggested by the rapid muscle fatigue which comes on in some persons who have suffered for a long period of time from a high grade of indicanuria. It is also suggested by the observations which I have made that moderate doses of indol by the stomach may be followed in normal persons by a sense of muscle fatigue, which has worn off rapidly on prolonged exercise. Professor Fred. S. Lee of Columbia University was so kind as to undertake experiments upon the muscles of cold-blooded animals and mammals with a view to determining the influence of indol upon the onset of muscle fatigue. His results are expressed in the following notes:—

"Experiments have been performed on the muscles of frogs and cats. The method has been to irrigate corresponding muscles for a given length of time — the one with physiological-salt solution, the other with physiological-salt solution plus a small quantity of the drug, and then to stimulate the muscles and record the contraction and the amount of work done before exhaustion sets in.

"Indol in 0.05 per cent. solution — that is, one part to 2000 — causes an early fatigue of the muscles and a diminution in the amount of work of which the muscle is capable. In a specific experiment the indolized muscles performed 24,320 g. mm. of work before it became exhausted, while at the same time the corresponding normal muscle performed 44,000 g. mm. of work, and was still capable of doing more. The work actually accomplished by the two muscles was 100 (normal) : 55 (indol).

"Phenol in 0.05 per cent. solution — that is one part to 2000 — usually puts the muscle into a better working condition than that of the normal muscle. In a specific experiment the phenolized muscle performed 7550 g. mm. of work before it became exhausted, while at the same time the corresponding normal muscle performed only 4400 g. mm. of work. The work actually accomplished by the two muscles was 100 (normal) : 171 (phenol). This favorable action of phenol is followed, however, by the reverse effect, the phenolized muscle usually becoming exhausted before the normal muscle. In 0.1 per cent. solution — that is, one part to 1000 — the effect of phenol is like that of indol; in other words, the phenolized muscle performs less work and becomes fatigued more readily than the normal one."

Still more important than the foregoing experiments is the following observation by Dr. Lee: —

"The two muscles of a cat were irrigated for half an hour, the one with whipped blood, the other with whipped blood to which indol had been added in 0.004 per cent. solution; in other words, one part of indol to 25,000. The normal muscle then performed 34,400 g. mm. of work, while the indolized muscle performed only 12,880 g. mm. — the ratio of normal work to indolized work is 100 : 37."

This shows that indol in very minute quantity acting for a long time on the muscle diminishes its working

power, an observation of much significance, since it gives an experimental basis to the suspicion that indol is capable of acting as a depressant to the muscular mechanism. The most pronounced clinical indications of such action are perhaps those derived from some cases of myasthenia gravis. Dr. Tuttle of the Presbyterian Hospital tells me that he has lately had under observation a patient whose clinical history corresponds to that of myasthenia gravis and in whom the only objective sign of a pathological condition was an intense and persistent indicanuria which was not much modified by any treatment which was instituted. It is clear that cases of this type should be most carefully scrutinized from the standpoint of the possibility that the depression of muscle function is dependent on the action of aromatic products upon the muscles.[1]

The rapid onset of fatigue observed experimentally in muscles that have been irrigated with indol and the curves which such muscles show are presumably not specific for indol. They derive their significance for the human subject from the fact that indol is the only aromatic product which is known to be absorbed in quantities sufficient to render it probable that the indol has a toxic influence upon the neuro-muscular system. The absorption of skatol may act in an accessory manner to heighten the effect induced by indol poisoning, but the

[1] I am told that there are cases of myasthenia gravis in which the indican of the urine is not excessive. This fact is not necessarily a valid argument against the idea that indol acts as a muscle poison in myasthenia gravis, for it is true that the given derangements of function may arise from more than one poison.

absorption of considerable quantities of skatol must, I think, be an exceptional phenomenon in man. In some cases of excessive intestinal putrefaction in childhood associated with a retardation in growth and abdominal distension there is clearly a poisoning of the muscular system. These children show signs of fatigue very rapidly and in some cases, where the condition has come on in early life, they are slow in learning to walk. In such cases there is not only a large amount of indican in the urine but a considerable excretion of phenol. It is quite likely that the phenol in these cases plays a part in bringing about the depression of muscular action. Perhaps in some instances it is as much a factor in inducing fatigue as is indol.[1]

INDICANURIA

The term "indicanuria" is used to designate the presence of an abundance of indican in the urine as demonstrated by the presence of strong reactions (with the formation of indigo) on the use of Obermeyer's test or similar tests. It has already been mentioned that in normal childhood little or no indican appears in the urine. There are also many adults who seldom show the presence of indican in the urine. There are also many adults who show a

[1] The primary beneficial effect of phenol on muscle action observed by Dr. Lee does not speak against this view, as this effect is one that soon gives way to fatigue.

The very interesting observation has been made by Dr. Lee that a temporary beneficial action is exerted on muscle by all the common fatigue products studied by him.

moderately strong reaction for indican in the urine during a long period of time (that is, most of the time during many years) and still retain good health and do not suffer obtrusively from digestive disorders. This is true of many students who live sedentary lives. On the other hand, there are persons with digestive disorders who show little or no indican in the urine. Finally, there are not very rare instances in which during a short or a considerable period the urine contains very large quantities of indican so that the reaction with Obermeyer's reagent is intense. I think it safe to say that such persons are seldom free from clinical evidences of intestinal disorder and that often there are also in these cases some indications of intoxication.

Almost every physician who has observed considerable numbers of patients with reference to the presence or absence of indican in the urine, has been puzzled to interpret his results. For he has no sooner begun to attach importance to the presence of a strong indican reaction in certain cases than his faith in this as a sign of disease has been shaken by the fact that he has found a strong indican reaction in the urine of some person who is apparently well. I think it would greatly aid us in the interpretation of the meaning of indicanuria if we took into consideration the important factor of the ability of the organism to defend itself against the toxic action of indol by quickly disposing of it. It is of course difficult to fashion standards for the judgment of this factor of defense. But there are certain obvious considerations which bear on the judgment of the defensive

powers of the organism for oxidizing and pairing indol. It is certain that the oxidizing powers of the cells in early life are greater than in later life and that on this account organisms are likely to suffer more from the absorption of equal amounts of indol between the fiftieth and seventieth years of life than between the fifteenth and thirtieth. This is doubtless true quite aside from the degenerations that occur in organs which have been subjected to the action of disease. In cases where the element of disease has entered to injure the liver, kidney, intestinal epithelium, muscles, and other structures, the organism naturally becomes more sensitive to the action of indol than before its powers of oxidization and synthesis have become impaired. There are very many conditions in which the liver undergoes cirrhotic or fatty changes or in which both of these processes are marked or in which there are well-defined parenchymatous changes. It is certain that such persons will be especially sensitive to the action of all kinds of poisons, including that of indol. We have therefore in judging of the significance of any case of pronounced indicanuria to consider whether it occurs in a young person or in an older one; in a healthy individual or in one whose cells have been damaged by disease. In youth it is comparatively easy to find a diet and mode of living under which the indican can be made to disappear largely from the urine. In aged persons the task is usually more difficult, especially if the indicanuria has been of long duration.

Aside from cases of indol absorption from the presence

of a dead fœtus or an abscess, I believe that indicanuria always points to absorption of indol from the intestinal tract. There is no convincing evidence that the autolysis of organs during life yields indol, and I think that such a source may be regarded as a negligible factor in dealing with cases of indicanuria. Admitting, however, that indicanuria is of intestinal origin, it remains to be explained why it is present in some cases of intestinal disturbance and not in others. A wholly satisfactory answer to this question is not at the present time possible. Some facts which bear on the problem may be stated here. It has been claimed by Pizenti and others that the presence of pancreatic juice is essential to the production of indicanuria. This claim is based on the fact that indol is a product of proteolytic cleavage. The proteid food entering the intestine is supposed to be attacked vigorously by the abundant tryptic ferment of the pancreatic juice, and this decomposition is presumed to reach so advanced a stage as to lead to the indol production from which the indicanuria arises. While it is true that indol is a product of proteid cleavage, I think it extremely doubtful whether there is an opportunity for the process of cleavage to go so far as to yield indol, unless the quantity of proteid food is largely excessive and thus delays absorption. Ordinarily absorption occurs in the peptone and amino-acid stage of proteolytic digestion. It is not conceivable that in the absence of putrefactive bacteria from the small intestine a rapidly proteolyzed meal should lead to the formation of indol in the intestine, since indol does not arise from tryptic digestion. It has

been held that persons with pancreatic disease who are unable to secrete pancreatic ferments into the intestine do not develop indicanuria. This is surely a mistaken view. I have observed indican reactions to persist in dogs from which the pancreas had been removed, and I have met with some of the most marked instances of indicanuria in persons in whom autopsy or operation showed both the pancreatic and the biliary duct to be occluded.

Intestinal indicanuria must be regarded as an evidence of intestinal putrefaction. It is commonly easy to produce it experimentally in dogs by feeding a greatly excessive quantity of meat. Not only may an habitual indicanuria, such as is common among dogs, be increased by excessive feeding of meat, but the condition may be induced by excessive feeding in cases where the urine was previously quite free from indican. The explanation of this fact appears to me to depend on the presence of putrefactive bacteria in the ileum and large intestine of the dog. If we examine any portion of the large intestine or the lower ileum, we shall usually find there moderate or considerable numbers of anaerobic, spore-forming, butyric-acid-producing bacteria as well as colon bacilli. These anaerobes have the power of attacking native proteids which, under suitable conditions, they may energetically hydrolyze. Some anaerobes such as *B. aerogenes capsulatus* are usually unable to carry the decomposition as far as indol production. The colon bacilli normally present in the intestine, however, are able to make indol from peptones. It is only necessary, there-

fore, to feed the animal so excessive a quantity of meat that small masses of muscle fiber enter the lower ileum or the large intestine. Here they will be attacked by anaerobes and colon bacilli with a production of indol and subsequent development of indicanuria. Naturally any condition favoring stagnation in the small or large intestine will help to bring about this condition. It is possible that moderate quantities of hydrolyzed proteid reach the lower part of the ileum owing to impaired absorption. In this region both in man and in dogs the colon bacilli are numerous. Under these circumstances it is not necessary for anaerobes to take part in the attack on the already hydrolyzed proteid, since, as repeatedly stated, the colon bacillus itself suffices to do this. The effect of introducing partially hydrolyzed proteids into the large intestine was very plainly shown in the following experiment. A healthy dog which had been fed on meat in moderate quantities gave only a slight reaction for indican. Fifty cubic centimeters of a concentrated egg and meat mixture were subjected to short partial tryptic digestion (without the development of indol). This material was sterilized in the autoclave and then injected into the transverse and ascending colon and confined there by ligature. Two hours later the indican reaction in the urine was markedly increased and at the end of ten hours had become intense, this change doubtless having been due to the bacterial attack to which the material was subjected in the large intestine.

There are some persons in whom the imperfect action of a cathartic leads to the development of headache,

flatulence, and an increase of the indican in the urine. The explanation of this is to be sought in the fact that the cathartic causes the passage of native proteids and possibly of peptones from the small into the large intestine, where it is possible to attack the former if anaerobes be present. Presumably in persons harboring few anaerobic bacteria this effect would be slight, but in persons in whom organisms of the class of *B. putrificus* or *B. aerogenes capsulatus* are abundant one would expect the appearance of considerable indican in the urine.

The influence of constipation upon the development of indicanuria is very different in different persons. Most children and many adults may go without a movement for several days and still fail to develop indicanuria if this has previously been absent. There are other persons in whom constipation is followed by a marked increase in the output of indican. A wholly satisfactory explanation of this difference in the influence of constipation upon indol formation in the intestine is not at present possible. Sufficient bacteriological data are lacking. A probable explanation is the following. The formation of indol in the intestinal tract depends on the decomposition of proteids through the activity of microörganisms. If the colon bacilli have for any reason ascended in large numbers above their usual upper limit, namely the ileum, into the jejunum or duodenum, they will act there upon peptones formed in the course of the normal digestion of proteids. From such peptones they will make indol. Under these circumstances the aid of putrefactive anaerobes is not necessary for the production of

indol. If, however, there is no ascent of the colon bacilli to the region in which these organisms would find peptones to attack, the abnormal production of indol is not excluded, for, as already stated, the action of the anaerobes in the ileum and large intestine permits the hydrolyzing of proteids which then fall subject to the colon bacilli with a resulting production of indol. Both these methods of indol production may occur in the same individual, namely its production in the small intestine through the action of colon bacilli on peptones, and, secondly, the formation of indol in the ileum and large intestine through the combined action of putrefactive anaerobes and colon bacilli acting upon such native proteids as may have escaped digestion at higher levels. The occurrence of constipation may be considered as favoring both these methods of indol production, but only under abnormal conditions of digestion. The stagnation of food remnants and bacteria in the large intestine would cause no indol production according to the hypothesis here set forth unless masses of undigested proteid or hydrolyzed proteid material had entered the region of the large intestine. The stagnation of food in the small intestine would not lead to the production of indol there except in the presence of colon bacilli or other microörganisms capable of making indol from peptones or albumoses. Of the actual ascent of such bacteria from the upper level of their normal habitat into the higher levels of the small intestine we know as yet very little that is definite. The invasion of the gall-bladder by colon bacilli under conditions which make it improbable that there has been

any passage of colon bacilli from the intestine into the blood (and thus through the liver into the gall-bladder) makes it likely that there is in these cases an ascent of the colon bacilli to the level of the common bile duct. The colon bacilli travel so rapidly after death that it is only through very early autopsies that one could obtain evidence of the presence of these organisms at high levels of the intestinal tract. Reliable data relating to this point are at present lacking. There is, however, a probability that in altered states of secretion in the small intestine incidental to subacute or chronic enteritis the colon bacilli do ascend above the level of their normal habitat and thus become factors in the production of indol and hence of indicanuria.

That the conditions which lead to pathological indicanuria are not always the same is rendered probable by the marked differences in the amenability of this condition to treatment which have been observed by all clinicians who have attempted to modify this condition in a variety of patients. There are instances in which a reduction in proteid diet is in itself sufficient to greatly diminish the quantity of indican excreted. Reduction in the quantity of meat which is daily taken by a patient is often efficacious in effecting this end. The explanation of the improvement following a diminished use of meat perhaps lies in the fact that on the more limited diet smaller quantities of undigested meat pass into the region of the associated anaerobes and colon bacilli. A change from a diet containing much meat to one in which milk is substituted for meat sometimes leads to a prompt

reduction in the output of indican. The improvement is probably due to the more ready digestion and absorption of the milk. But there are instances in which the substitution of milk for meat does not materially influence the output of indican as judged by the Obermeyer reaction. The invasion of the small intestine by colon bacilli (and perhaps putrefactive anaerobes) would satisfactorily explain this phenomenon, since in such a case even the more rapid absorption incidental to a milk as compared with a meat diet would not suffice to elude the action of the colon bacilli upon peptones formed in the course of digestion. There are also cases in which no dietetic measures have a marked influence in reducing the excretion of indican. That is to say, the indicanuria remains well marked whether proteid food be employed in the form of meat or milk or eggs or cereals. Dr. George A. Tuttle of the Presbyterian Hospital has made the observation that there are cases of indicanuria which though persisting on a milk diet are susceptible of striking improvement or complete disappearance through the agency of potassium iodide. The cases of this type have always been cases of marked arterial sclerosis and some of them have had a history of syphilitic infection. The explanation of this noteworthy fact is obscure. The possibility occurs to one that the potassium iodide influences favorably the circulation in the intestine and this aids rapid absorption, but it is also possible that the iodide may in some way increase the oxidizing activity of the cells mainly concerned with the composition of indol in the body, notably the

liver cells.[1] The increased oxidation of indol would lead to a diminished excretion of indican and perhaps to its complete disappearance. Dr. Tuttle tells me also that he has had patients in whom the administration of iron in the form of Basham's mixture has soon been followed by a marked lessening of the indicanuria. As this has occurred in cases where the diet has been carefully studied and dietetic measures have failed to give the desired relief, Dr. Tuttle has attributed the therapeutic action to the iron.

It is worth noting that wherever there is a persistent indicanuria which has not yielded to a change in the character of the proteid food nor to a diminution of it nor to a restriction of the carbohydrates, one may rationally employ gelatin as a nitrogenous substance capable of replacing in some degree the proteids of the diet. Gelatin does not contain a tryptophan nucleus and hence cannot yield indol. It has been shown by physiologists that the metabolic needs of the organism for nitrogen may be in part met by the use of gelatin, although as is well known gelatin alone is incapable of maintaining the protoplasm of cells where proteids are wholly withdrawn from the dietary. I consider it entirely safe to replace a portion of the nitrogen of the food by gelatin (in the form of jellies) and believe I have observed a falling off in the indican of the urine in consequence of this substitution. Clinically, experience

[1] The presence of iodides in a urine containing indican interferes with the Obermeyer reaction, but this source of error appears to have been eliminated in the procedure followed by Dr. Tuttle.

shows plainly that in the treatment of indicanuria one has to consider not only the proteid food which is given, but also the carbohydrates. With many persons the excessive use of carbohydrates is habitual and the substitution of small quantities of quickly digested carbohydrates like rice for large quantities of bread or sugars may make a considerable difference in the amount of indican excreted. Diastatic enzymes can be made to render good service in this connection. Assuming that the persistent indicanuria in the cases in question is due at least in part to the upward extension of colon bacilli and certain putrefactive anaerobes into the small intestine, it seems reasonable to believe that the withdrawal of excessive carbohydrates is equivalent to a diminution in the pabulum on which the microörganisms multiply so readily with the production of gas and acid and indol.

As will be seen in speaking of the treatment of chronic excessive intestinal putrefaction, the use of cathartic medication is usually followed very promptly by a diminished absorption of indol from the intestine. Persons suffering from great indicanuria are apt to show a marked diminution in the excretion of indican after the administration of calomel or cascara or a saline or other cathartic. The indican may in rare instances entirely disappear temporarily. This effect is, however, only of very short duration. The diminished excretion of indican is due of course to the mechanical removal of indol-forming and indol-containing contents of the intestine. A diminished absorption of indol is associated

frequently with the relief of certain symptoms, such as headache and dizziness. It is not clear that these symptoms are due wholly to the excessive indol absorption. Unfortunately the relief is temporary and is followed by a continuance of the original putrefactive conditions and the resumption of indicanuria. Indeed, in some persons constipation following catharsis is associated with a still greater excess of indican in the urine than has been habitual. The repeated use of cathartics daily or at somewhat longer intervals for the purpose of diminishing the absorption of toxic substances is inadvisable, for it ultimately leads to a loss in weight and strength. It seems unnecessary to further discuss here the subject of indicanuria, which is, as a rule, linked with other intestinal conditions requiring consideration and which therefore it is not usually advisable to single out as the sole object of treatment.

THE POSSIBILITY OF THE OCCURRENCE OF INDOLÆMIA AND INDOLURIA

It naturally occurs to any one who considers the phenomenon of indicanuria and the conditions leading to it that in extreme instances of this phenomenon, where relatively large quantities of indol have to be oxidized, and paired, these preparatory processes may sometimes be insufficiently active to prevent the circulation of indol in the blood in such concentration as would lead to the appearance of free indol in the urine.

The occurrence of free indol in the urine is certainly a very exceptional phenomenon even in cases of experimental poisoning with indol. In the combined cyanide and indol experiments of Richards and Howland it was found that a trace of indol sometimes appeared in the urine. It is, however, noteworthy that in the experiments in which examinations were made for the detection of free indol in a loop of intestine (after poisoning with considerable quantities of indol) much more indol was found to be excreted by the intestinal loop than was excreted into the urine. This fact points decisively to a relatively difficult excretion of indol by the kidney. The tests employed for the identification of the indol included Ehrlich's dimethylamidobenzaldehyde and the β-napthaquinone-sodium-monosulphonate reactions. The latter reaction, as already explained, is absolutely distinctive for indol.

Although it is thus apparent that the conditions required to bring about the excretion of free indol in the urine must be such as seldom occur spontaneously in human beings, it cannot be denied that traces of indol may sometimes appear in the urine in the course of extreme indicanuria. In a few instances distillates from the urine contained a body which strongly suggested indol. But the actual proof of the occurrence of free indol in the urine in these cases of extreme indicanuria does not, I think, exist at present. It seems probable that a considerable concentration of indol in the blood is necessary to bring about the phenomenon of indoluria, and such a concentration as will insure this leakage into the

urine must be of very exceptional occurrence under clinical conditions. This fact does not seem to me to weaken the probability that it is not very uncommon for the blood to contain traces of indol in some cases of extreme intestinal putrefaction. The proof of the presence of indol in the blood in experimental poisoning cases is easily obtained, but I know of no instances of extreme indicanuria in which any attempt has been made to obtain free indol from the blood. The demonstration of the occurrence of indol in the blood even in the minutest trace would be of especial interest in those cases of indicanuria in which nervous symptoms are extremely pronounced.

In a recent paper dealing with the subject of indigouria, Porchet and Hervieux have reached the conclusion that indol is in itself devoid of toxic properties, but that indoxyl, its oxidation product, is a comparatively poisonous agent. They prepared their indoxyl from indoxyl carbonic acid and injected it into rabbits but fail to give the doses in which they found the indoxyl fatal. It does not appear to me that the opportunity for the occurrence of free indoxyl in the blood or tissues can ever be good, since the process of pairing with sulphuric acid is so intimately connected with the oxidation of indol to indoxyl that it is difficult to believe any significant amount of free indoxyl can ever be present at one time. One may, however, admit the possibility that in conditions of extremely abundant absorption of indol from the intestine there might be formed an amount of indoxyl larger than usual, within a time insufficient to secure

its complete pairing. Under such conditions it might conceivably be a factor in the production of symptoms. As compared, however, with the quantity of indol existing as such in the organism, it seems to me that the indoxyl must be insignificant. Evidence has already been offered to show that while indol is not a highly toxic substance for normal carnivorous or omnivorous animals, it becomes much more toxic when conditions arise under which there is defective oxidation. This aspect of the question of the toxicity of indol is one which has not been touched upon by any writers upon this subject.

Indigouria. — It has long been known that there occasionally occur cases of extreme indicanuria in which the urine upon standing in contact with the air gradually becomes blue and may liberate a pelicle of indigo upon the surface of the urine at the same time that a precipitate of fine particles of indigo occurs in the urine. Such cases are, I believe, very exceptional and do not arise except where the indicanuria has been of long standing. The subjects of this indigouria are almost invariably badly nourished and in poor, often precarious, health.

Procher and Hervieux [1] have lately studied experimental indigouria, which they found to arise when larger quantities than one or two grams of indol are administered by the stomach to dogs. The phenomena as they appear in the urine of these experimental cases are essentially the same as those observed in man. In the majority

[1] "Recherches experimentales sur les chromogènes urinaires du Groupe de l'Indol' (5e Mémoire) "De l'Indigourie," *Journ. de Physiol. et de Path. gen.*, viii, p. 841, 1906.

of cases the pigment which is separated from the urine is blue in color. In the case of rabbits the substance separated from the urine is red in color and represents the presence of indirubin, a substance closely related to indigo. It often happens in indigouria that both these substances are present together. The onset of the indigouria in dogs has been observed after three or four hours from the time of the administration of the indol, but the phenomenon is at its height after six or eight hours have gone by.

Porcher and Hervieux claim to have detected the presence of indoxyl in the urine of dogs which had been given experimental indigouria. It is thought by them that the indoxyl-potassium-sulphate (which they call the indigouria chromogen) is decomposed with the liberation of indoxyl. In herbivorous animals, such as the horse and the rabbit, the phenomenon of indigouria is said not to be a rare occurrence. In the horse the pigment consists of a mixture of indigo blue and indigo red, or indirubin, whereas in the rabbit the coloring matter is indigo red. It is stated that the occurrence of the phenomenon of indigouria in these herbivorous animals is not necessarily connected with any evidence of illness. I believe there is at present not the slightest evidence that the phenomenon of indigouria occurs in man in a state of health. There seems to me no evidence that indigouria depends on the presence of free indoxyl in the blood and urine. It is much more likely that it is a phenomenon dependent on the spontaneous oxidation of indoxyl-potassium-sulphate in the urine in those cases in which

the quantity of this substance is very extreme. I believe also that the phenomenon of indigouria never arises except where indol is being produced in the intestine in uncommonly large amounts and is subsequently absorbed in uncommon abundance.

INDIVIDUAL SUSCEPTIBILITIES TO DIFFERENT ENTEROGENOUS POISONS AS POSSIBLE FACTORS IN DETERMINING CLINICAL TYPES

Instances are many in which clinical experience has made it clear that two persons of approximately the same weight react differently to the same drug, and do so regularly. This is true of commonly used drugs, such as strychnine, morphine, cocaine, digitalis, and antipyrin. A scientific explanation of these differences is for most cases not now possible. That they do not depend on gross differences in rapidity of absorption is shown in the case of some of these drugs by the fact that subcutaneous injections give rise to the same qualitative differences. The individual variations are to be attributed rather to idiosyncrasies of the neuro-muscular system — the mechanism through which functional derangement most readily finds expression in the form of symptoms. The nervous system may prove uncommonly susceptible either on account of inherent physico-chemical peculiarities of its constituent nervous elements or because it is not adequately protected from the action of poisons by hepatic and other cells which have the power to bind, or bind and oxidize, or bind and oxi-

dize and pair, many substances injurious to the nervous elements. An inherent susceptibility of the nervous elements themselves probably has its physical substratum in side chains or receptors (according to the conception of Ehrlich) possessing affinities for substances possessing a definite chemical constitution.

Of individual human susceptibilities and reactions to the action of enterogenous poisons almost nothing is now known. Nevertheless one cannot fail to recognize the possibility that such individual susceptibilities and reactions may play an important part in determining the clinical manifestations of intoxications. It is well known to clinicians that there are some persons who promptly develop a widespread urticaria after indulgence in certain foods or drinks, as shellfish or strawberries or champagne. Sir A. E. Wright tells me that a patient of his develops an urticarial eruption after ingesting organic acids. I had a patient who suffered frequent urticarial seizures attributable to somewhat injudicious living. She had marked indicanuria and excessive putrefactive decomposition in the intestinal tract. A rigid diet maintained more than a year led to a cessation of the urticarial seizures and to a diminution in the indican excreted. In a mood of experiment she decided to test the action of champagne and found that one glass was followed within three hours by a violent outbreak of general urticaria. Here the susceptibility was highly pronounced. The urticaria may be connected, as Wright has pointed out, with a diminished coagulability of the blood, but this is in itself not a full ex-

planation of the locally deranged function, since it fails to take account of the nervous mechanisms involved in the localization of the urticarial wheals.

In some persons the indulgence in a single glass of champagne is followed within twenty-four hours by manifestations of gout. In still others champagne causes headache and the excretion of increased amounts of uric acid. The explanation of these different effects is to be sought in the individual cellular reactions of the patient rather than in the poison. There are probably many similar examples of individual susceptibility, but when we come to study the question in relation to processes found in the digestive tract we cannot make close comparisons between different persons because we cannot say what substances are being absorbed. We may know that patients of a certain group are alike in having intense indicanuria, but we cannot say that the intoxications may not be different in these cases owing to differences with respect to the absorption of other substances than indol. Careful research may help us to get much closer to the resemblances and differences in such cases. In the meantime we must content ourselves with the suspicion that chronic intoxications through the absorption of similar quantities of the same poisons produce different effects in different persons. Among half a dozen persons suffering from extreme indicanuria, one suffers from headaches (sometimes migrain-like), another is prone to lumbago, another, perhaps, has epileptic seizures, another mental depression, another progressive muscular atrophy, and still another suffers

from cyclical vomiting. It would be well worth while to learn in how far these different manifestations of intoxications are dependent on common factors or in how far on different agencies. There is good reason for suspecting that very similar bacterial processes in the digestive tract lead in one case mainly to digestive disorders and in others (owing to a lesser sensitiveness of the digestive tract itself) to better absorption of poisons and the development of more remote consequences such as gout, arthritis, anæmia, or nervous disorders. While it is possible that these very different manifestations are always connected with different and perhaps specifically different types of gastro-enteric infection and intoxication, the possibility is not excluded that even such very different disorders may have much in common in their etiology.

That the mental and emotional peculiarities of individuals have a large part in fixing the type of nervous reaction that occurs in consequence of intoxications has become apparent to careful students of pathological conditions. It is not unreasonable to believe that it will well repay close observers of the phenomena of disease to take into consideration the individual nervous reactions much more than is at present customary.

TYPES OF CHRONIC EXCESSIVE INTESTINAL PUTREFACTION

The considerable variations in the clinical manifestations and pathological accompaniments of chronic excessive intestinal putrefaction suggest that the etiological conditions vary considerably in different cases. I shall endeavor in the following pages to show that there are grounds for the separation of different types of intestinal putrefactive processes and that these grounds are based on differences in the character of the putrefactive products and in the bacteria concerned with these processes. The classification which I offer is a tentative one which may in time have to give way to one founded on a fuller knowledge of the significant intestinal conditions than we at present possess. In any event it must undergo modification. It is, however, proposed here in the belief that it will serve a useful purpose in helping to classify cases of disease and in giving direction to systematic study.

It is a striking fact that while many cases of excessive intestinal putrefaction are associated with the presence of indican in the urine in large amount, there are other cases of considerable gravity clinically which fail to show much indican in the urine or may indeed be entirely free from indicanuria during a long period of observation. I propose to divide cases of chronic excessive

intestinal putrefaction into those which show an excessive indicanuria and those which do not. The proposed classification recognizes three types of putrefaction which are common: the first may be called the *Indolic Type*, and is characterized by indicanuria dependent chiefly upon excessive decomposition induced through the co-operation of members of the *B. coli* group (and probably *B. putrificus*); the second type of intestinal putrefaction may be designated the *Saccharo-butyric Type*, and is initiated especially by anaerobic organisms in the digestive tract. In its simplest form it is associated with very little production of indol in the intestinal tract and hence with slight indicanuria or even an entire absence of indican from the urine. In the third group of cases we find associated the characters of the indolic and the saccharo-butyric types of decomposition.[1] I will describe these types briefly according to my present conception of their etiology and pathological characters.

[1] I think it will become desirable to distinguish a skatolic type of putrefactive decomposition, *i.e.* a condition in which the intestinal bacteria persistently form skatol in amounts in excess of the indol made. As mentioned in speaking of skatol, I have found this product of decomposition abundant and dominant over indol only under conditions of clinical deviation from normal standards. But as I am still in doubt as to the bacterial conditions that determine skatol production, I hesitate at present to make skatol prominent in a plan of classification. Moreover, I do not yet know whether excessive skatol production corresponds to characteristic manifestations.

I. THE INDOLIC TYPE OF EXCESSIVE INTESTINAL PUTREFACTION

It has already been stated that there is some reason for believing that organisms of the *B. coli* type which are capable of forming indol abundantly may invade the small intestine and there give rise to bacterial cleavages which largely replace the normal tryptic digestion. The proof that the small intestine is invaded by some variety of indol-producing colon bacilli in cases of marked indicanuria is still lacking, for the reason that it is ordinarily impossible to obtain evidence on this point from a living subject, whereas postmortem studies with reference to this point are open to criticism on account of the rapid wandering of the colon bacilli which occurs after death. Of all the organisms of the digestive tract which we have individually studied, we have found none that will make a larger amount of indol than certain strains of colon bacilli when grown on peptone bouillon. There are, moreover, cases of excessive intestinal putrefaction in which the dominant organism in the intestinal tract (if we may judge from the material obtained through the use of cathartics) is some variety of colon bacilli. In such cases, the anaerobes of the intestine may be present in very small numbers, so that it seems safe to exclude them from a significant part in the formation of putrefactive products. It seems reasonable in these cases to attribute the formation of indol to an unusual activity on the part of the colon bacilli. There are, perhaps, also instances in which the presence of an indol-producing

proteus organism is a factor. But I am not inclined to ascribe great importance to the *proteus* organism in this connection, for aerobic liquefiers of gelatin have generally been lacking or few in number. If we assume that the indol produced in these cases of indicanuria is made largely though the action of colon bacilli,[1] we are brought to the question, In what part of the intestinal tract does the indol production chiefly occur? We know that the colon bacilli have only a very feeble action upon native proteids such as are used for food. We are therefore forced to the conclusion that the indol produced by the colon bacilli must have been formed through the coöperative agency of the digestive juices or putrefactive bacteria. The anaerobic putrefactive bacteria may be excluded from the present discussion, since the cases here in question are those in which these bacteria are present in only very small numbers in the digestive tract.[2] So we have to fall back on the proteolyzing action of the digestive juices to explain that preparation of the proteid food which is necessary for a successful attack by organisms of the *B. coli* type. Where only a moderate amount of food is being used, and this in a form readily digested and absorbed, such as egg-white or milk, it is unreasonable to suppose that the foodstuff

[1] I am confident that an indol-making strain of *B. putrificus* is sometimes an important agent in establishing indicanuria, but do not know any instance where the influence of colon bacilli could be excluded.

[2] I have noticed that certain Gram-positive diplococci from the small intestine peptonize casein with moderate rapidity, and it is possible that such organisms, when excessive, prepare the way for the putrefactive action of colon bacilli.

is attacked by the colon bacilli mainly in the large intestine. It seems more legitimate to assume that they are attacked in the small intestine in the manner already mentioned. It is, of course, likely that in some cases hydrolyzed proteids find their way into the ileum and large intestine, and here it is necessary to assume that the colon bacilli have migrated upward from their usual habitat in order to explain the phenomenon of excessive indican production. I may mention an illustration of the indolic type of excessive intestinal putrefaction. The patient was a lady seventy-five years of age who had the peculiarity of having lived since childhood on a diet consisting almost exclusively of potatoes, hominy, and bread, and from which meat had been rigorously excluded. She ate little food, but was well nourished and uncommonly robust. She ultimately fell ill from a subacute gastro-enteric disturbance in which there was loss of appetite, irregularity of the bowels, and some signs of gastritis. The fæces at this time showed fields consisting mainly of Gram-negative bacilli mostly of the *B. coli* type.[1] These fields contained extremely few putrefactive anaerobes. Nevertheless the urine contained exceptionally large quantities of indican, and this was apparently a persistent feature.

The indolic type of chronic excessive intestinal putrefaction is common in cases of complete or partial occlusion of the common bile duct with or without jaundice,

[1] Two kinds of colon bacilli and a positive diplococcus were isolated by Mr. Ward. One of these strains of colon bacilli was a strong indol maker.

and is probably a common association of functional pancreatic achylia,[1] a state which is probably not uncommon. Some of the most pronounced instances of indicanuria with which I have met have been in cases of organic structural occlusion of the common duct. There must be in these cases a great diminution in the proteolytic action of the intestinal digestive juice, and putrefactive cleavages are doubtless due very largely to the action of bacteria and especially the colon bacilli. I have noticed in several cases of jaundice that the fæces held mainly Gram-negative organisms of the *B. coli* type. In some autopsies such organisms have been dominant throughout the digestive tract. Bacteriological studies for the identification of the organisms and their biochemical characters in such cases have not yet been made. I mention these appearances, however, as being of interest in the present connection, since they were characterized by the absence of putrefactive anaerobes in more than very small numbers. In some instances cultural observations on the mixed fæcal flora have borne out the view that the colon bacilli were the dominant type and the putrefactive anaerobes were few in number.

Occlusions of the small intestine are almost invariably followed by intense indicanuria even when the occlusion is in the jejunal region. The bacteriological explanation in such cases is not clear. The reversal of peristalsis, above the level of obstruction, must serve to distribute colon bacilli from the ileum or jejunum

[1] A. Schmidt, "Functionelle Pankreasachylie," *Deutsch. Archiv f. klin. Med.*, lxxxvii, p. 456, 1906.

throughout the small intestine, between the stomach and the obstruction. This dissemination of colon bacilli above the occlusion may be an important factor in favoring indol production. Where the obstruction lies high, as in the upper jejunum, indicanuria is said not to occur.

The development of a complete obstruction of the small intestine is followed by obstinate constipation, and this may influence the putrefactive conditions below the obstruction. But I have already mentioned that mere constipation does not necessarily lead to indicanuria. On this account it is difficult to determine the part played by bacterial conditions below the obstruction, in contributing to the formation of indol. It appears, on the whole, that it is mainly the bacterial conditions above the obstruction that determine the presence or absence of an extreme indicanuria. This view is not inconsistent with the fact that indicanuria may persist after the small intestine has been emptied of food through repeated vomiting, for it is known that indol often continues to be found in the digestive tract of starving animals. Small quantities of proteid (perhaps derived mainly from desquamated epithelial cells) apparently suffice to insure indol formation when the bacterial conditions are favorable.

A well-marked group of cases which may provisionally be classed with the simple indolic type is that which may be characterized as the marantic, large-belly type of chronic intestinal indigestion in children. This condition has long been known to clinicians as an

extremely obstinate form of digestive derangement, manifesting itself in a distinct retardation of growth (usually without much mental retardation), in distension of the abdomen by gas, flatulence, intolerance of carbohydrates, and voluminous, light-colored, usually gray and fatty stools containing much gas. In many of these children there is excessive sweating of the head. I have studied with considerable care several children corresponding to this clinical type (most of them patients of Professor L. E. Holt, who has in every way aided me in the effort to learn something about the underlying conditions present in this striking derangement). It was found that it is a characteristic in these cases that the urine contains a large amount of indican and usually an excessive amount of phenol. The phenol may amount to more than one hundred milligrams in twenty-four hours, in the urine from a child not more than three or four years of age. The Ehrlich aldehyde reaction of the urine may or may not be excessive. After a long period of careful dieting in which carbohydrates are very much restricted the indican may disappear from the urine in such cases although the patients show but little gain in weight or strength. This falling off in indican of the urine is to be regarded as a favorable indication and often precedes by many months more obvious clinical indications of recovery. In some instances, however, there occurs a slow gain in strength and in weight and in the ability to digest carbohydrate food despite the fact that the improvement in the excretion of indican and phenol is only slight. Under the most favorable circumstances

improvement is very slow, and slight errors in diet are followed by prompt reappearance of flatulence and perhaps diarrhœa. Fats and proteids are well tolerated.

A feature in this form of chronic excessive intestinal putrefaction is the readiness with which fatigue comes on. A child of six or eight years of age will show fatigue after a very short walk and will manifest a want of interest in exercise and play. There is in fact a state of chronic fatigue, which I attribute to the absorption of putrefactive products. If the trouble comes on during infancy, there may be considerable delay in the development of the ability of the child to stand and walk without support, and a child of five or six years or over may be unable to go upstairs except by a kind of creeping motion. A certain emotional irritability is common in these children. As a rule they are not much retarded mentally except in so far as they have missed opportunities for conventional learning. On the contrary, one not infrequently is struck by the contrast between the sharp wits of these children and their physical retardation.

The ethereal sulphates are regularly increased in the urine in cases of the kind under consideration, that is to say, there is not merely a relative increase of the ethereal as compared with the preformed sulphates, but usually also an actual excess in the absolute amount of the ethereal sulphates. The proportion of ethereal to preformed sulphates may be one to seven, or one to five, or even one to four. It can usually be shown that the stools contain considerable quantities of indol and phenol.

A noteworthy peculiarity of these cases is the presence

of a very large proportion of Gram-positive bacilli corresponding closely to the morphology of the organism described by Tissier as *B. bifidus.* It has already been pointed out that the fæces of breast-fed children consist very largely of Gram-positive organisms of the *bifidus* kind. When children reach the age of three or four years, the *bifidus* bacilli are usually comparatively few in number and the dominant type is some form of colon bacillus. In children from four to ten years of age the Gram-negative bacilli of the colon type are much in excess normally as compared with the organisms representative of *bifidus*. In the cases of chronic intestinal indigestion which are under consideration I have met with a great preponderance of *bifidus*-like organisms in children as old as seven or eight years. The cultural proof that the organisms to which I refer are in reality the same as those normally present in the fæces in infancy is lacking, as I have been unable to adequately study the biochemical characters of these organisms. In sugar-bouillon fermentation tubes, however, the resemblance is a very close one; that is to say, the growths observed in lactose or dextrose bouillon show many instances of the bifid form of *bifidus* and also many instances of so-called punctate forms in which Gram-positive portions of the bacillus alternate with segments of the cell body which fail to take the Gram-stain. Associated with these *bifidus* forms in the fæces of the marantic cases one finds moderate numbers of colon bacilli, or colon bacilli may be wholly absent from the fields. In the lactose-bouillon fermentation tubes one

may find excessive numbers of positive diplococci. It should be especially noted that the common anaerobic putrefactive organisms are present in very small numbers. It is not certain that the *bifidus*-like organisms found in these cases of chronic indigestion in children represent the preponderance of a type which should, under normal conditions, gradually be replaced by colon bacilli, but the idea is one which forces itself strongly upon one. It is possible that the organism may have acquired other characters than those pertaining to the *bifidus* bacilli of the nursling.

Associated with the Gram-positive *bifidus*-like organisms in the fæces one may find moderate numbers of positive diplococci or short streptococcal chains. These diplococci may appear in considerable abundance in the fermentation tubes or may give rise there to an abundance of streptococcus forms. In one apparently typical case which was under observation for a long time, the child at the height of the disease (when indicanuria was very marked) showed in the fæcal fields considerable numbers of these Gram-positive diplococci. After a period of six months, upon a strict proteid and fat diet, the diplococci were no longer present in the fields. It was also noticeable that indican had disappeared from the urine although the patient had gained very little in weight and strength.

Whether in these cases the production of indol is due to the colon bacilli present in the upper part of the tract or whether it is due in part to other types of organisms is by no means clear. *B. bifidus* in these cases certainly

does not occupy the entire intestinal tract, as may be shown in diarrhœal stools which contain colon bacilli as well as *B. bifidus*. It is uncertain in how far the retardation in growth and the myasthenia which are characteristic of these cases are explicable through the intoxication induced by the absorption of excessive quantities of indol or phenolic substances. I am disposed to think these bodies are an important factor in determining the undue fatigue. The retardation in growth may have in part a similar origin, but it is probable that this is partly the effect of a failure of the organism to utilize food normally, owing to the excessive decompositions that occur in the intestinal tract. No matter how much food is given, the excessive bacterial changes rob the organism of enough caloric potential to make normal growth impossible.

In the treatment of these cases it is necessary to be patient. It is essential to improve the character of the digestive processes before one can reasonably hope to secure a gain in weight and strength. Carbohydrates should be permitted in only very small amount and chiefly in the form of well-cooked rice or hominy or Huntley and Palmer's breakfast biscuits. It may be advantageous for a time to peptonize the milk in order to secure early absorption. Moderate quantities of chicken or beef or mutton may be permitted, but all meat should be given in a finely divided form. It sometimes has seemed best, in children as old as five or six years of age, to allow only two meals in the course of the day, these being rather generous. It is helpful to practice high irri-

gation in order to avoid as far as possible putrefactive decompositions in the large intestine. A great deal of rest is necessary for these children and they should not be permitted to play or walk to the point of fatigue. Patients of the class under consideration often do badly in cold weather, probably because they cannot afford the caloric loss to which they are then subjected. Where this is the case a period of residence in a mild winter climate is helpful. Improvement is sometimes so slow that every one concerned with the case may become discouraged, but it is certain that a high degree of improvement can occur even after several years of extremely slow progress. I have several times known this to be the case. In order to secure this improvement, however, it is impossible to make concessions in regard to diet, and the policy of largely excluding carbohydrates must be enforced. It cannot be denied that even in those cases where the best results are obtained there is commonly a retardation in growth which is still manifest at the time of puberty. Moreover, there is no doubt that persons who have suffered from the conditions under consideration during the period of childhood become especially susceptible to intestinal disorders in later years and seldom attain robust health. There are probably many persons in the community whose digestive derangements date from early life, and in many of these cases the less pronounced forms of the type of chronic intestinal indigestion which has just been described have doubtless constituted the foundation of invalidism in adult life.

II. THE SACCHARO-BUTYRIC TYPE OF CHRONIC EXCESSIVE INTESTINAL PUTREFACTION

This form of intestinal derangement is characterized by a chronic putrefactive process (having its seat mainly in the large intestine and lower ileum) and due to the action of very large numbers of strictly anaerobic butyric-acid-producing bacteria capable of multiplying by means of spore formation. A thorough study of the anaerobes concerned with this type of decomposition has not yet been made, but it may confidently be stated that the organism most prominently concerned in at least a large number of the cases is *B. aerogenes capsulatus* (*B. Welchii*). The characters of this organism have already been elsewhere described. Associated with *B. aerogenes capsulatus* may be found *B. putrificus* and possibly in some cases the bacillus of malignant œdema. In many cases, however, *B. putrificus* has not been found in the fermentation tubes or in the flasks containing various media into which suspensions of the mixed fæcal flora have been inoculated. Anaerobic plates made from the suspensions, heated for twenty minutes to 80° C., have shown *B. aerogenes capsulatus* to be the dominant or even the exclusive strict anaerobe growing upon sugar-blood agar or blood agar. In a certain proportion of cases the fæcal fields have shown *B. aerogenes capsulatus* to be associated with large numbers of Gram-positive diplococci, and these Gram-positive diplococci have been grown very abundantly in the fermentation tubes; that is, they have assumed a

prominence there which is not often observed in the case of persons in good health. It seems not improbable that the presence of these diplococci are of importance to the process, but their precise relation to it cannot be at present confidently stated. In some instances these Gram-positive diplococci appear in the fermentation tubes in great abundance, although they are not seen in the fæcal fields in such number as to attract special attention.

A very common phenomenon in cases of the saccharo-butyric type of chronic excessive intestinal putrefaction is the formation of only small volumes of gas in sugar-bouillon fermentation tubes after these have been inoculated with the mixed fæcal flora. As already explained, it has been our custom in routine work to study the gas production on four sugar-bouillon tubes containing dextrose bouillon, levulose bouillon, saccharose bouillon, and lactose bouillon. The gas production in these tubes has often fallen to one-half that observed ordinarily in health, and the volume may even fall to one-quarter or one-fifth the total gas production observed under normal conditions. In a few cases some of the fermentation tubes have contained no measurable amount of gas. This is, however, an exceptional condition. The explanation of this phenomenon of small gas production in the fermentation tubes appears to lie in the diminution in the number of living organisms of the *B. coli* type that are present in the fæces. That the colon bacilli are much reduced in numbers is evident in some of the cases of saccharo-butyric putrefaction from the

microscopical examination of the Gram-stained fæcal fields, even in fields from diarrhœal stools. It is possible that in some of the cases that have come under observation the typical colon bacilli have been replaced by some allied form of Gram-negative organism capable of forming less gas than is ordinarily found as the product of normal colon bacilli on sugar bouillon. We have not yet had an opportunity to make a careful study of our cases of saccharo-butyric putrefaction from the standpoint of the characters of the colon bacilli present.

While the reduction in the total number of living colon bacilli has been shown to exist in many of our cases of saccharo-butyric putrefaction by means of plating on litmus gelatin, there are exceptional instances in which there is no evidence that the number of colon bacilli has been reduced. The evidence, however, in these cases is confined to the appearance of acid-forming colonies on litmus gelatin and agar and to the occurrence of normal gas production in the fermentation tubes.

The presence of large numbers of putrefactive anaerobes in the intestinal tract and especially of *B. aerogenes capsulatus*, gives to the intestinal contents a peculiar character. These organisms, as already mentioned, are capable, under anaerobic conditions, of attacking carbohydrates and proteids in a vigorous manner. In either case there is a formation of butyric acid, often in considerable amount. This may be associated with the production of propionic, caproic, or valeric acids. The odor of the movements from patients in whom this

form of putrefaction is pronounced is often intense and characteristic, suggesting butyric or caproic acid. When proteids are attacked by *B. aerogenes capsulatus* there is not only a production of butyric acid, but also a liberation of gas, although there is less gas liberated than when the organisms attack carbohydrates. The gas liberated consists in part of hydrogen and in part of carbon dioxide. Perhaps some methane is also formed. As a result of the excessive production of gas the fæces have a low specific gravity and float on the surface of water. Frequently small bubbles of gas may be seen to be liberated. The presence of considerable quantities of gas in the form of small bubbles in the intestinal contents contributes to give them a light color. Another factor which enters into the light color often observed in the fæces in cases of saccharo-butyric putrefaction is a reduction of bilirubin and other coloring matters by the anaerobic bacteria. Reducing processes normally go on in the intestinal tract but they are usually of moderate intensity in conditions of health. In excessive saccharo-butyric putrefaction, on the contrary, the processes of reduction are extremely active, owing doubtless largely to the liberation of free hydrogen. The fæces of cases of saccharo-butyric putrefaction when subjected to an appropriate oxidizing agent, such as mercuric chloride in concentrated solution, usually give a strong pink or red color which is rendered more striking when the material which has already been reddened in this way is thrown into water, which then dissolves out the newly formed coloring matter and reveals its intensity. This

reaction is probably due to the presence of an excessive amount of hydrobilirubin.

In consequence of the presence of the higher fatty acids in the fæces in considerable amount, there may be imparted to the contents of the lower bowel a slight acid reaction. The reaction, however, may be neutral or even alkaline. The fatty acids in question are neutralized by bases formed in the course of putrefactive processes and especially by ammonia, ammonium butyrate sometimes being formed in considerable amount. It is probable that the production of ammonium butyrate in excessive amount acts as an irritant to the intestinal tract and may be the cause of a certain softness of the movements [1] in cases of excessive saccharo-butyric putrefaction or may even be responsible for a diarrhœal condition. It is, however, not clear that this is the sole cause of such evidence of irritation, as other irritative products may be associated with ammonium butyrate.

The fæces in the condition under consideration usually show the presence of little or no indol. Indol may be wholly absent. More commonly there is a small amount of indol, — perhaps two or three milligrams in one hundred grams of the intestinal contents. The quantity of phenol may be slightly in excess of that normally found, but this is, I think, not usually the case. In consequence of the small absorption of indol the urine con-

[1] The consistence of the stools may be influenced by the liquefying action of the anaerobes on proteids. A normal stool may stand in the laboratory for days without changing in consistency; a stool containing a great excess of putrefactive anaerobes may spontaneously liquefy in the course of a week.

tains little or no indican. The cases in which large quantities of indican are found in the urine must be regarded as belonging in a different category from the cases which are now under discussion. But it must be admitted that a patient may show considerable changes in regard to the indol production; a person who at one time produces little intestinal indol will at other times produce a considerable amount. Although the indican of the urine is usually small or may be wholly absent, the ethereal sulphates are often somewhat in excess. The explanation of this fact is not clear. It indicates that other putrefactive products than indol have been absorbed from the intestine and have paired with sulphuric acid. In some cases perhaps phenolic bodies are responsible for the increase in the ethereal sulphates, but I have observed cases in which the increase in the ethereal sulphates appeared to me to be out of proportion to the amount of phenol recoverable from the urine. It is quite possible that substances heretofore overlooked have been largely responsible for the increase of ethereal sulphates observed. There are, however, cases of excessive saccharo-butyric putrefaction in which one cannot say that the ethereal sulphates are in excess.

Of the formation of sulphur compounds in the intestinal tract during the process of saccharo-butyric putrefaction little is yet known. There is no evidence that hydrogen sulphide is produced in excessive amount in these cases. As regards the formation of mercaptan, the evidence is against the view that anything more than a trace of methyl mercaptan is liberated from the

putrefying material in the intestine, but it must be admitted that the facts bearing on this point are not conclusive. Outside the body the mixed fæcal flora from cases of excessive saccharo-butyric putrefaction is usually capable of forming mercaptan on peptone bouillon and in some cases the amount produced in twenty-four hours is sufficient to give a very strong reaction in the isatin-sulphuric-acid tube. This condition is unphysiological, as it is not observed in most adults in good health. A moderate mercaptan reaction is often obtained, as already stated, through the action of the mixed fæcal flora derived from apparently healthy young children. That the reaction is abnormal is shown not merely by the infrequency of its occurrence in healthy adults, but also by the fact that a patient when improving from conditions of marked saccharo-butyric putrefaction will usually lose the flora capable of making methyl mercaptan in peptone bouillon. A satisfactory explanation of the formation of methyl mercaptan by the mixed fæcal flora cannot yet be offered. It is easy to show that *B. putrificus* is able to form methyl mercaptan, and in some cases of saccharo-butyric putrefaction *B. putrificus* occurs abundantly, especially in peptone bouillon to which has been added magnesium or calcium carbonate. In such instances it would appear that the methyl mercaptan is formed as a result of the activity of *B. putrificus*. There are other cases, however, in which, although methyl mercaptan is formed by the mixed fæcal flora, one fails to obtain *B. putrificus*, and hence the reaction cannot confidently

be ascribed to the presence of this organism. I think it probable that *B. aerogenes capsulatus* is sometimes capable of making methyl mercaptan, but apparently it usually does not make this gas as abundantly as does *B. putrificus*, and there are strains which do not form it under the conditions in which the organism has been grown on blood bouillon.

Chronic excessive saccharo-butyric intestinal putrefaction is a widespread condition among adults. In children it is, I believe, a relatively uncommon occurrence. Although there is evidence that in children some acute disturbances of short duration are dependent upon this process, as people grow older they are in general more subject to the occurrence of this type of putrefactive disorder. The grade in which the disturbance is present varies within wide limits. *B. aerogenes capsulatus* is present in the intestine of nearly all adults, and there are probably few persons who from time to time do not suffer slight temporary derangements of intestinal digestion connected with the temporarily excessive multiplication of these anaerobes. There are probably persons who during an entire lifetime have digestive disturbances, usually slight but sometimes more marked, which are dependent on this form of putrefaction. Under such circumstances the duration of life may not be appreciably shortened. Persons who have long had disturbances of this sort may attain to seventy or seventy-five years of age. They, however, suffer in various ways a diminution in efficiency and are subject to various obvious disturbances. It sometimes

happens that people have a mild grade of saccharo-butyric putrefaction for a long period of time without being conscious of any distinct impairment of powers and without being conscious of anything approaching invalidism. On the other hand, there are some persons who develop this type of putrefactive disorder in a high degree. In them the evidences of intestinal indigestion are pronounced, and there is, after a time, a decline in the capacity of the organism to perform work. Such persons develop a condition of distinct invalidism, and life may be considerably shortened in consequence of the intoxications arising from this condition. As a rule, however, the severest cases of chronic saccharo-butyric putrefaction are not of the simple type at present under discussion, but are rather of that form which is associated with the production of an excess of indol in the intestinal tract.

Some of the immediate and remote consequences of chronic excessive saccharo-butyric intestinal putrefaction may be briefly enumerated. As already stated, the excessive production of ammonium butyrate is apt to lead to irritability of the digestive tract, and this is the more pronounced if there is also an abundant indol production. There is a tendency to desquamation of the epithelium of the digestive tract in the mouth and stomach and probably throughout the entire digestive tract. The indications of this desquamation in the mouth and on the tongue are often plain. A frequent sign of such desquamation in the intestinal tract is the presence of an excessive quantity of epithelium or

epithelial nuclei in the fæces. The patients who suffer from this condition usually do not tolerate well either carbohydrates or acids. The intolerance of carbohydrates is shown by the occurrence of excessive intestinal flatulence and sometimes slight diarrhœa after the use of considerable meals of cereals or starchy foods. Sugars, especially, are apt to be badly borne.[1] Probably this intolerance is due in part to the production of acids. There are cases in which the acid production from sugar begins very rapidly in the mouth, and a certain amount of butyric acid may be formed even in this situation, owing either to the presence of *B. aerogenes capsulatus* in the mouth or to the presence of aerobic butyric-acid producers. Probably the tendency to diarrhœa observed in some instances is due to the irritative action of the acids formed from the decomposition of the sugars in the small intestine. This intolerance is naturally most marked in those persons in whom evidences of epithelial desquamation in the mouth and elsewhere are most pronounced. The mucous membranes of the digestive tract are almost constantly in a state of excessive irritation, so that stimuli which in normal people would meet with little response give rise to an excessive peristalsis and diarrhœa. I think it not improbable that in advanced cases of saccharobutyric putrefaction *B. aerogenes capsulatus* invades the

[1] There are instances in which excessive indulgence in carbohydrates is followed by mental depression and muscular prostration. These symptoms appear to be simply a great exaggeration of what is often noticed in healthy persons after similar indulgences.

entire extent of the small intestine and is enabled there to decompose sugar with the formation of butyric and other acids. Such an opportunity to grow upon a carbohydrate medium might lead to a considerable increase in the number of anaerobes. These on passing into the large intestine would find an opportunity to set up their putrefactive activities on proteids after the carbohydrates had been exhausted.

The consequences of chronic excessive saccharobutyric intestinal putrefaction are by no means limited to disturbances of the digestive tract. The formation of an excessive quantity of the higher fatty acids may be presumed to lead in some instances to the absorption of an excessive quantity of these acids. As, however, the organism is able to oxidize the fatty acids with great readiness, one would hardly expect that so long as the oxidizing powers of the organism are unimpaired there would be any evidence of an increased excretion of organic acids in consequence of this excessive absorption. An experiment made at my suggestion by Dr. A. J. Wakeman bears out this view. A dog weighing about eighteen pounds was fed on a fixed amount of meat, namely one kilo daily, in order to study the influence of the feeding of ammonium butyrate upon the excretion of the nitrogen of ammonia. During the preparatory period of nine days determinations were made of the total nitrogen and of the nitrogen of ammonia excreted by the urine. The total nitrogen of ammonia excreted daily amounted to between one hundred and one hundred and fifty-six milligrams

during this period of preparation. During the five days on which the animal received daily four grams of ammonium butyrate the urine collected failed to show a distinct increase in the nitrogen of ammonia or in the proportion between the nitrogen of ammonia and the total nitrogen. Thus while one would hardly expect that in normal organisms on a moderate diet of meat the nitrogen of ammonia would be appreciably increased through the excessive absorption of fatty acids which are removed as compounds of ammonia, it is nevertheless conceivable that in conditions of disease in which the oxidizing powers of the organism are as a whole diminished (or taxed to the utmost) there might be an absorption of fatty acids in such excess as to somewhat increase the output of the nitrogen of ammonia. In other words, there would then be a slight degree of acid intoxication or acidosis resulting from the increased absorption of the volatile fatty acids. There are individual instances in which we have found indications from the urine that this is probably the case, but the evidence is not entirely convincing. The subject of the relation between excessive saccharo-butyric putrefaction and the excretion of an increased amount of nitrogen of ammonia calls for further and more careful observations, both experimental and clinical.

There is some evidence that the fæcal extracts obtained from persons with advanced chronic saccharo-butyric putrefaction may exhibit a decided hæmolytic action on the red blood cells of rabbits and monkeys, and in some instances such an action is pronounced.

The methods that have been employed in the study of the hæmolytic action of the fæcal extracts in such cases are not wholly beyond criticism. Nevertheless the indications seem sufficiently definite that there are substances formed in the intestine (in extreme cases of excessive saccharo-butyric putrefaction) that are capable of hæmolyzing more actively than is the case with material derived from healthy persons on similar diet. This hæmolytic action is perhaps in part referable to ammonium butyrate, but it has been found to persist even after the removal of the ammonium salts of the volatile fatty acids, and is probably due, in part at least, to some unknown substance. The fact already mentioned in speaking of the characters of *B. aerogenes capsulatus*, that this organism is able to produce hæmolytic substances, is worthy of notice at this point, since in many people who have long suffered from this type of putrefaction there are distinct evidences of anæmia.[1] The onset of anæmia in excessive saccharo-butyric putrefaction is usually very slow. It is first manifested by indications of a decreased volume of blood without any decided fall in the percentage of hæmoglobin or red blood cells. After a time, however, there may be a distinct fall in the hæmoglobin and later in the red blood cells, so that a moderate or considerable grade of secondary anæmia may be associated with the intestinal condition.

[1] I have discussed at some length the relation of anæmia to intestinal infections in a paper entitled: "On Bacterial Processes in the Intestinal Tract in Some Cases of Advanced Anæmia, with Especial Reference to Infection with *B. aerogenes capsulatus* (*B. Welchii*)," *Journ. of Biol. Chem.*, ii, p. 1, 1906.

In some instances which I have observed this association has been apparently a very close one; that is to say, it has appeared as if the anæmia in question were secondary to the chronic intestinal process, since other causes of anæmia, such as malaria, syphilis, intestinal worms or parasites, or other infections, have been excluded with a very high degree of probability. In some instances the grade of anæmia is intense and the patients may present the blood picture and clinical characters of a progressive pernicious anæmia.

The occurrence of a considerable degree of anæmia in any case of advanced saccharo-butyric putrefaction must depend upon an excessive destruction of red blood cells as compared with the reproduction of such cells. Individuals are known to possess very different powers of regeneration, and it is doubtless true that the absorption of equal quantities of hæmolytic poison by two individuals might lead to very different results so far as the blood is concerned, owing to differences in regenerating power. The fact that a patient is not anæmic in the ordinary sense, that is, shows no reduction below what is considered a normal percentage of hæmoglobin and no fall in the number of red blood cells, is by no means a positive indication that there is not an excessive blood destruction going on. In health the ability to regenerate blood cells is probably much in excess of the ordinary requirements. This margin of excess may not be exhausted for some years, notwithstanding a considerably increased drain is being made upon the regenerative structures through the excessive destruction of red blood

cells. Sooner or later a definite and increasing disproportion arises between the destruction and reproduction of red blood cells, and under these circumstances there arises a slowly or more rapidly progressive anæmia.

In some instances which I have classed as falling into the category of the simple saccharo-butyric type of putrefaction, depressive mental symptoms have been noted. In some of these cases an abundant growth of Gram-positive diplococci or streptococci have been found in the fermentation tubes. Whether these microörganisms have any part in the production of neurotoxic substances is uncertain. It has been mentioned that *B. aerogenes capsulatus* is said to make poisons capable of acting on the nervous system, but the bearing of this fact upon human symptoms is still obscure. As a rule well marked nervous symptoms have not been a feature in the class of cases to which the term saccharo-butyric putrefaction has been applied.

In the course of time, in well-marked instances of the saccharo-butyric type of excessive chronic intestinal putrefaction, there has been observed a considerable loss in weight and a diminution in muscular powers. In spite of a fairly good appetite patients are unable to gain either in weight or in strength. Frequently the indications of premature senility are evident. This is noticeable in an atrophy of the subcutaneous fat in premature wrinkling of the skin. A loss of general vigor is apt to be associated with a loss in sexual power.

III. THE COMBINED INDOLIC AND SACCHARO-BUTYRIC TYPE OF CHRONIC EXCESSIVE INTESTINAL PUTREFACTION

The association of the features of the two types of putrefactive decomposition already described is common. In cases presenting the features characteristic of both types of putrefactive decomposition the two processes often vary somewhat independently. It is in a measure an arbitrary matter to determine what cases fall under the combined type and what cases belong under the indolic or saccharo-butyric type. I would include as falling under the combined type of putrefaction all cases in which putrefactive anaerobes of the intestine are regularly very abundant in the fæces at the same time that there is a persistent indicanuria of high grade — an indicanuria not immediately and markedly influenced by even the strictest precautions in regard to the dietary. In the combined type of saccharo-butyric putrefaction nervous symptoms are apt to be prominent relatively early in the course of the affection. These symptoms are excessive emotional irritability, an inclination to mental depression, and the early onset of muscular and mental fatigue. At a somewhat later period in the course of pronounced cases, the hæmic disturbances become noticeable. The patients grow gradually more and more anæmic. There may be periods of improvement, both as regards the anæmia and the nervous symptoms, but on the whole the tendency is a downward one, and from year to year the patients become a little

less robust and less capable of rapid recuperation under favorable hygienic conditions. After a time — commonly, however, not until the process has been a very marked one for ten or fifteen years — the patients lose so much strength as to be unable to attend to ordinary business occupations without very great effort. A period of carefully regulated living, free from anxieties, may be followed by a considerable degree of improvement, but this improvement is usually extremely slow. In certain cases the anæmia ultimately deepens, and the patient may present the picture of a progressive pernicious anæmia. In other instances it is especially the nervous symptoms which increase, and periods of depression become more and more marked and of longer duration. The mental depression may become so pronounced as to necessitate a residence of the patient in a sanitarium or asylum, the conditions being those of mild or pronounced melancholia. As already mentioned when discussing the character of the toxic effects of the various poisons absorbed from the intestine, it is highly probable that different individuals react differently to the same toxic agencies. If, for example, there be absorbed from the intestine substances capable of damaging both the nervous system and the red blood cells, it is conceivable (assuming the same proportions and amounts of these substances to be absorbed in each instance) that one individual would become invalided first through damage to the nervous system, whereas the invalidism of another might come first through damage to the blood. The condition of invalidism in either

case would lead to an enforced rest and perhaps also to improved conditions of diet. The result is that while the improved hygienic conditions may not lead to a restoration of normal function, there is for a time a cessation in the progress of the injurious action of the intestinal poisons upon those structures that are most susceptible. Even in cases where there is very little or no improvement in respect to the especially susceptible tissues, there is also a cessation of the progressive damage done to the less susceptible cellular elements. For example, a patient suffering from combined indolic and saccharo-butyric excessive intestinal putrefaction may succumb on the side of the nervous system and be compelled to enter an asylum on account of mental depression. Under the improved hygienic conditions there may be only a little improvement in the nervous manifestations, but there may nevertheless be a cessation of the slowly progressive loss of balance between the destruction and the formation of red blood cells. The patient remains moderately anæmic, but does not grow extremely so, because there has been a slight mitigation of the absorption of substances injurious to the blood. It is in fact true that high grades of anæmia do not develop in patients who suffer from the combined indolic and saccharo-butyric type of putrefaction and find their way into an asylum on account of nervous symptoms.

In general, one may say of the combined indolic and saccharo-butyric type of chronic excessive intestinal putrefaction that the persons in whom these processes are highly developed reach a state of chronic invalidism

more rapidly than where either condition is alone present. Not only do the nervous symptoms come on earlier, but there is a relatively early loss in weight and power. The indications of premature senility may be pronounced. It is probable that atrophic processes are not limited to the fatty, muscular, and blood tissues. It seems probable that the cells of the liver and of the kidney undergo premature degenerative atrophy in these cases. This is doubtless the result of chronic parenchymatous changes due to constant exposure of the cells to cytolytic toxic agents present in very small amounts at any one time but acting constantly. We are still far from a full understanding of the effects of enterogenic poisons on different types of protoplasm. One tends, for example, to assume that the destruction of red blood cells, in the course of chronic or acute infections of the intestine with the gas-bacillus, is due to the direct hæmolytic action of hæmolyzing agents formed in the intestine. It is possible, nevertheless, that the destruction of red cells is sometimes accomplished through a process of phagocytosis which is made possible by injuries to red cells inflicted by special opsonins.[1]

The derangements of the nervous system which have been mentioned as concomitants of the combined indolic and saccharo-butyric form of excessive intestinal putrefaction — mental depression and muscle fatigue —

[1] Sir A. E. Wright tells me that he has observed pronounced phagocytosis of red cells in a case of pneumococcus infection associated with a pernicious type of anæmia. The subject of phagocytosis of red cells has lately been investigated by Hektoen (*Journ. of Infect. Dis.*, iii, p. 721, 1906).

constitute only a small part of the nervous manifestations of this pathological process. Almost every known expression of functional disorder of the nervous system is noted in some of the subjects of this variety of intestinal putrefaction. But it would confuse rather than help us to make a list of these derangements, including as they do almost all the symptoms encountered in the pot-pourri of neuræsthenia. If we could distribute these symptoms with confidence into those that are clearly the result of intestinal intoxication and those that are due to derangements of circulation or other mechanical or dynamic departures from normal function, it would repay us to attempt their classification. At present such an attempt would be foolhardy and futile. Only the most thoughtfully and carefully conducted clinical and experimental studies can help us to determine the influence of enterogenic poisons on the protoplasm of nervous structures, and the opportunities for serious studies of this kind do not now exist anywhere in the world. Among the problems which must sooner or later be approached by modern methods are the action of enterogenic poisons on the motor structures of the central nervous system, on the sensory paths (both peripheral and central), on the sympathetic nervous system, and on the peripheral and central mechanisms that subserve the special senses. To what extent these various structures may be damaged in consequence of chronic excessive intestinal putrefaction is uncertain. There are instances of typical progressive muscular atrophy (due to lesions of the ganglion cells of the anterior horns) in which there

are also pronounced evidences of excessive intestinal putrefaction, but the relationship between the two series of phenomena is not now definable. There are cases of intestinal putrefaction in which *B. aerogenes capsulatus* is the prominent microörganism of the digestive tract and in which there occurs a slow muscular atrophy associated with some fibrillation of the wasting muscle bundles. Possibly in such instances the resistance of the motor ganglion cells of the spinal cord is distinctly below that of normal structures, thus rendering these cells especially vulnerable to neurotoxic substances made in the digestive tube. There are also cases of multiple neuritis, resembling alcoholic neuritis, in which alcohol can have no etiological part, but in which antecedent gastro-enteric derangements are very prominent. The probability that these instances of peripheral neuritis (with the associated psychosis) are in reality due to intoxications from enterogenic poisons appears to me considerable, although the data now at my command do not suffice to establish this view. There is at present no evidence that infection by *B. aerogenes capsulatus* is in itself capable of inducing this type of nervous disease.

It is essential to realize that the onset of severe clinical manifestations of excessive intestinal putrefaction may not coincide with the period of most extreme development of the gastro-enteric process. A short time after therapeutical measures have been commenced the bacterial conditions in the tract may have been much mitigated. It may thus be unsafe to base a conclusion

regarding the etiology of a disease upon the bacterial conditions that present themselves after the disease has become established. I observed that it sometimes happens in the course of a pernicious anæmia that the numbers of *B. aerogenes capsulatus* in the fæces undergo so great a decrease in the course of treatment that the microscopical fæcal fields present a nearly normal appearance with respect to this organism. Hence the failure to find the gas-bacillus in large excess after a patient has for some weeks or months had the benefit of treatment by diet and rest does not necessarily exclude a preceding infection.

I believe the most intense period of the saccharo-butyric process sometimes precedes the development of a pernicious anæmia by a considerable length of time. The explanation of this seemingly paradoxical view lies in the fact that the normal defenses of the organism must be greatly impaired before a pernicious type of anæmia can arise. The bacterial process involved in breaking down these defenses (which include the ability of cells to bind, oxidize, and pair with enterogenic poisons) are intensive and of long duration. After a time there develop symptoms of weakness which make it necessary for the subject of the gas-bacillus infection to live with much greater regard for hygienic conditions; for example, with more rest, more out-of-door life, and a more restricted dietary. In consequence of these changes the intensity of the putrefactive process is mitigated. The quantity of injurious products absorbed, including those that are hæmolytic, may become so

moderate that little harm could be accomplished by them in a person with normal defenses. But in a person with greatly impaired defenses and diminished hæmopoietic powers (correlative to parenchymatous changes in the hepatic and other cells, on alteration in the bone marrow) the absorbed poisons suffice ultimately to make blood destruction preponderate permanently over blood formation. A relatively slight putrefactive process in the intestine thus ushers in the signs of the severe anæmia.

METHODS RELATING TO THE MODIFICATION AND CONTROL OF BACTERIAL PROCESSES CONCERNED IN CHRONIC EXCESSIVE INTESTINAL PUTREFACTION

THE difficulty of formulating measures for the control of the bacterial processes concerned with the occurrence of chronic excessive intestinal putrefaction is obvious. No two cases that come under observation are quite alike; hence specific measures which appear appropriate in one case cannot be recommended without modification in another. It becomes, therefore, almost impossible to describe a method of treatment with all the detailed modifications that are necessary in order to cover adequately the specific cases with which one has to deal in practice. Our store of experience is as yet so incomplete that it is impossible to predict in many instances the outcome of the measures proposed. This is equivalent to the admission that the therapeutical side of the subject of chronic intestinal putrefaction is still in a largely experimental stage. It will require many years of careful observation associated with a large amount of experimental work to enable the practitioner to place his methods of treatment on a really scientific basis. Nevertheless there are certain general therapeutical guides which, though by no means adequate for the direction of the practitioner in individual cases, are

helpful in forming a conception of the principles that must enter into the treatment of chronic infections of the digestive tract by putrefactive anaerobes. It must be emphasized that the degree of success with which these general principles of treatment are applied in individual instances of disease will depend upon an actual knowledge of individual peculiarities pertaining to patients.

The slighter grades of chronic excessive intestinal putrefaction are as a rule quickly improved by suitable measures. It is helpful to think of the clinical manifestations in these cases as represented graphically by a curved line running at a variable distance below a straight horizontal line representing the separation of physiological from pathological manifestations. A line traced above this critical line indicates that a person has no symptoms of diminished function; and the greater the distance between the two lines, the greater is the reserve or margin of resisting power which has to be lost before symptoms of derangement set in. A line running below the critical line indicates that the defensive processes of the organism are no longer adequate to prevent clinical manifestations of disease. Where derangements are slight it may be assumed that there will be a prompt disappearance of symptoms as soon as there has been a restoration of the balance between the powers of resistance and the agencies underlying disordered function. The mere disappearance of symptoms, however, does not mean that a condition of normal health has been reëstablished, for a state of robust health would

be indicated by a line running at a considerable rather than a slight distance above the critical line. The mere crossing of this line in an upward direction connotes the disappearance of symptoms. But this does not necessarily mean health, because there may be a margin so small that it is quickly exhausted by a detrimental influence. On the other hand, very pronounced symptoms may exist (especially in cases of short duration) without these symptoms necessarily possessing a grave significance. A slight improvement in physiological conditions is sufficient to make the line indicative of derangement in function cross the critical line of demarcation between physiological and pathological conditions. This conception of the part played by symptoms as an indication of actual states of functional activity is helpful in explaining why some cases are so much more refractory than others. In long standing conditions, in which a high grade of intestinal putrefaction has for some time been present, improvement is extremely slow even under the most favorable hygienic conditions. There is a discouraging persistence of symptoms until the critical line is reached. The depression of function is, however, so considerable that there may be a real gain in function without an immediate and corresponding subjective improvement. In such cases the patient seldom accumulates an adequate functional reserve even after the disappearance of all obtrusive symptoms. The line representing his physiological condition must be traced close above the critical line, and a slight depression in function is promptly followed by a return of symptoms.

Thus it is that in these severe types of intestinal derangement relapses are common and discouragements frequent, even among persons who from time to time are quite free from definite symptoms of disease.

The principles entering into the management of cases of chronic excessive intestinal putrefaction may be divided into three groups. First, those that relate to the avoidance of putrefactive bacterial contamination of the food; second, those that relate to the promotion of prompt digestion in and absorption from the small intestine; third, those that relate to agencies designed to reduce the numbers of putrefactive anaerobes living in the intestinal tract. It seems hardly practicable to separate here the indolic and the saccharo-butyric types of decomposition from the standpoint of the practical measures to be employed in modifying them, especially as the subject of indolic putrefaction has been already considered in part.

The Avoidance of Putrefactive Contamination of the Food. — As regards the avoidance of bacterial contamination of the food with injurious organisms it is evident that care in regard to ordinary principles of cleanliness must be of importance. In a large proportion of cases of chronic excessive intestinal putrefaction the hydrochloric acid of the gastric juice is secreted in diminished amount and frequently no free hydrochloric acid can be detected in the stomach. Often there is also a moderate degree of dilatation of the stomach or at least some atony. This results in a delay in the emptying of the stomach, which in the absence of an

adequate secretion of hydrochloric acid is favorable to fermentative decompositions and even to the beginning of putrefaction, if putrefactive bacteria are abundantly present. It is clear that in such cases the greater the freedom of the food from putrefactive bacteria, the less will be the liability to putrefaction at lower levels, since the stomach in the condition under consideration is unable to extensively destroy such bacteria, even in their vegetative forms. In the present connection I lay especial stress on the avoidance of putrefactive bacteria, but the introduction of pathogenic organisms related to the colon type, including, for example, organisms capable of inducing dysentery, should also be avoided, since any injury to the mucous membrane of the tract tends to impair the physiological inhibitions to putrefactive decomposition. The use of cooked food goes a long way toward securing conditions of sterility within the stomach. It is, however, usually impracticable to live exclusively on food that is sterile. The use of milk, for example, means the introduction of very large numbers of bacteria. So long as these bacteria are lactic-acid formers, no harm is done, but in many samples of milk there are putrefactive organisms, and these may be present in large numbers. This is true of *B. putrificus* and of an organism closely resembling *B. aerogenes capsulatus*, but differing ordinarily from it in being less pathogenic for experimental animals. Cheese is another article of food which commonly contains large numbers of putrefactive bacteria. In normal stomachs these putrefactive bacteria are quickly disposed of; in

cases of chronic excessive intestinal putrefaction the gastric conditions are usually such as to make the stomach incapable of adequately coping with the putrefactive bacteria introduced with the cheese. Cheese is therefore an article which should generally be excluded from the dietary of nearly all cases of excessive chronic intestinal putrefaction, although some forms of cheese (especially those which are newly made from milk containing few putrefactive bacteria) may be permitted in moderate quantities. In eating uncooked fruit it is desirable to guard against the organisms found on the skin. Fruits therefore should be always peeled. *Bacillus putrificus* and the bacillus of malignant œdema are often found on the surface of fruits, and Dr. Rettger has found the latter abundant on the skin of bananas.

The question of the sterilization of milk may be briefly touched upon in its relation to the dietary of persons suffering from chronic excessive putrefaction. So far as the treatment of chronic putrefactive disorders is concerned, I think it preferable to avoid sterilization except where the milk contains considerable numbers of putrefactive bacteria. One can form some judgment of this point by permitting samples of milk to stand in fermentation tubes in the incubator after subjecting them to a single partial sterilization by the process of pasteurization. Under these conditions lactic-acid organisms which restrain putrefactive decomposition are killed; the surviving spore-bearing putrefactive organisms of the gas-bacillus type reveal their presence by setting up a stormy fermentation of the milk. Effective

sterilization involves the destruction of the spores of the putrefactive anaerobes and can only be accomplished with certainty by the use of the autoclave. It is evident from what has been just said, that the partial sterilization of milk containing putrefactive organisms in abundance is not a desirable procedure, since it destroys the lactic-acid bacteria which are normally present and which exert an antiputrefactive action. It is safer for patients to drink milk containing lactic-acid organisms together with putrefactive ones than to drink the latter alone, unless these are very few in number.

An important measure bearing upon the introduction of putrefactive bacteria into the intestine is the proper cleansing of the mouth. Putrefactive bacteria such as *putrificus* are not uncommon in the mouth, especially where there is dental caries. They find in cavities sufficiently anaerobic conditions for their development. Whether other forms of putrefactive organisms occur in the mouth in cases of chronic excessive putrefaction in the intestine is not clear. I have never certainly found *B. aerogenes capsulatus*. The growth of this organism and of allied strict anaerobes could easily be prevented or minimized by careful cleansing of the teeth and especially by the avoidance of the lodging of food between the teeth. The maintenance of the mouth in a relatively cleanly condition through the intelligent and free use of the toothbrush and through the employment of an oxidizing tooth paste, such as Pebeco,[1]

[1] This is a paste containing a high percentage of potassium chlorate.

is a material aid in restricting putrefactive conditions in the upper part of the digestive tract. These measures are of much more service if supplemented by the process of flossing the teeth. It is, however, easy to exaggerate the importance of the oral conditions especially in the direction of assuming that a really cleanly mouth will insure a disappearance of excessive putrefaction in the intestine. While it is evident that to swallow large numbers of putrefactive anaerobes which have grown in the mouth (in cases where the stomach is unable to destroy these organisms) must tend to increase the intensity of the putrefactive processes in the intestine, it does not follow that a patient will promptly convalesce if this additional increment of bacteria be removed. Some improvement is perhaps to be expected through the cleansing of a very offensive mouth, but the permanent eradication of the intestinal conditions (when this is possible) depends on a number of factors and not upon one. There are cases in which an oral butyric putrefaction occurs very rapidly after a meal. In such cases food which is lodged in the teeth will in the course of an hour develop an intense odor of butyric acid. It is not yet certain upon which organisms this putrefaction depends. It is not impossible that the decomposition may be due to aerobic producers of butyric acid, but it seems probable that anaerobes are at least to some extent concerned in many instances.

The suitable care of the stomach may also be a factor in controlling putrefactive decompositions in the intestines. In persons suffering from atony of the stomach

(with or without pronounced dilatation) putrefactive microörganisms may gain a hold and initiate high up in the digestive tract a process which normally begins only in the region of the lower ileum. All measures that act in the direction of stimulating the stomach to normal motility must favor the mechanical removal of the bacteria concerned in putrefaction. But hygienic measures and drugs may not suffice to free the stomach from putrefactive organisms. In such cases the practice of lavage may be very helpful and may give quick relief from some symptoms of intoxication, especially headache. Even in persons in whom dilatation is not marked the use of lavage before breakfast may be a very helpful measure, and the selection of this time has the advantage of not depriving the patient of undigested food.

The Promotion of Prompt Digestion and Absorption in the Small Intestine. — Generally speaking it is safe to say that all those measures which aid in securing prompt digestion in and absorption from the small intestine will operate to diminish intestinal putrefaction, since the outcome of prompt absorption in the region of the small intestine is equivalent to offering a diminished opportunity for the attack of putrefactive anaerobes located in the ileum and large intestine. Probably the restoration of normal gastric secretion and of pancreatic secretion is the most important physiological agency in securing rapid digestion and absorption of proteids from the small intestine. An improvement in the quantity of gastric juice secreted and in the quantity of the hydrochloric acid which it contains usually goes

hand in hand with the betterment of motility. The influence of imperfect pancreatic secretion may safely be assumed to be important, but in this case direct clinical observation is not possible, and one has to infer the absence or presence of a pancreatic achylia from the manner in which the proteid food in a test meal is attacked by the digestive juices. The use of small cheese-cloth bags containing meat in a state of moderate subdivision may be helpful in measuring the ability of the intestinal enzymes to carry on the digestion of meat proteids.[1] The use of this method as an index of pancreatic digestion can hardly be regarded as free from error.

The most important physiological factor in the partial or complete restoration of pancreatic secretion is probably rest. The influence of fatigue upon the digestive processes is often very clearly demonstrated in a given individual, and the removal of opportunity for fatigue is conversely a factor in determining recovery. The rest which should be secured in such cases is in part physical, in part mental and emotional and sexual. Many patients commit the error of exercising freely when rest is far more important. The effect of mental, emotional, and sexual fatigue upon the digestive tract has not been so carefully studied as it deserves, but there is on doubt that in each case the expenditure of nervous energy may lead to very similar results as regards the digestive organs. Rest from work of a taxing character may be an extremely important factor in the restoration

[1] This procedure was lately suggested by A. Schmidt.

of normal gastro-enteric secretions. In many persons emotional fatigue, such as often results from strenuous business activity or from some kinds of recreation, has a distinctly harmful influence. Even the interest attendant on free and animated conversation may be injurious. In the case of fatigue of sexual origin great individual differences in susceptibility are manifested by different persons. In all cases of long-standing chronic putrefactive disorder in the intestine it becomes important to take cognizance of any sexual factor that may lead to fatigue. This relationship is one of the utmost importance and is frequently overlooked by physicians in their regulation of the lives of patients suffering from chronic digestive disorders. It is a very important point in this connection to realize that an intelligent abstinence from sexual excitement should be practiced before such abstinence is forced by the onset of impotence. When this has occurred, the conditions are probably much less favorable for the restoration of normal secretory activity in the stomach and intestine.

It is customary in cases where the free hydrochloric acid of the gastric juice is regularly diminished to advise the use of hydrochloric acid after meals in order to compensate the diminished secretion of the acid.[1] In some instances the use of hydrochloric acid is distinctly

[1] The absence of free hydrochloric acid in the gastric juice is a feature of nearly all cases of advanced saccharo-butyric putrefaction. The influence of gastric secretion has recently been studied and discussed by D. van Tabora in its relation to intestinal putrefaction. "Ueber die Beziehungen zwischen Magensaftsecretion und Darmfäulniss," *Deutsche Archiv f. klin. Med.*, lxxxvii, p. 254, 1906.

beneficial if one may judge by the feelings of the patient and by the diminution in the excretion of indican which is noted. Among the long-standing cases of chronic excessive intestinal putrefaction, especially in cases of the combined indolic and saccharo-butyric type, the use of hydrochloric acid is often attended by little improvement. The stomach, indeed, often irritable or unduly sensitive, may not accept even moderate quantities of the acid without showing indications of slight gastritis or local discomfort. The use of digestive enzymes such as pepsin, trypsin, pancreatin, etc., has been much lauded. It is possible that they do good in some instances, but I have never been convinced that their use is really an important factor in determining the ultimate outcome in marked cases of chronic excessive intestinal putrefaction. The use of an efficient diastatic ferment gives, I think, more tangible results, and there are cases in which a better utilization of carbohydrates is noticeable in consequence of the use of an active diastase. The criterion in such cases has been the better tolerance for carbohydrates which is manifested by the reduction in habitual flatulence, especially in the upper part of the digestive tract. I know of no observations claiming to demonstrate a direct influence either of diastatic or proteoclastic ferments upon the excretion of the ethereal sulphates or indican or upon the numbers of putrefactive bacteria in the large intestine.

Careful mastication of the food is certainly an important factor in determining how it shall be utilized. But one may readily exaggerate the influence of this factor, and

this has, I think, been done in some recent researches. There is, however, no doubt that in cases where there is a failure of the stomach to secrete free hydrocholric acid the careful prolonged mastication of the food much diminishes the opportunity for stagnation in the stomach, while it subsequently makes it less likely that undigested proteid will appear in the large intestine to be attacked there by putrefactive anaerobes. The fine subdivision of the food before it is eaten is often very helpful, especially in persons whose teeth do not permit full mastication. I question, however, whether the comminution of meat outside the body is a real substitute for its equally fine subdivision through the mastication by the patient, since in the latter case there is the advantage of an intimate admixture with the enzymes of the saliva. In most cases benefit is derived both from a fine subdivision of meats outside of the body and from their further subdivision through prolonged mastication. The tolerance for meats as measured by clinical phenomena is often greatly increased by insisting that the patient give due attention to mastication.

The avoidance of chemical and mechanical irritants assumes much importance in some instances of chronic excessive intestinal putrefaction. Among the chemical irritants may be mentioned the use of condiments such as pepper and mustard, and the use of free acids such as vinegar and lemon juice, and indulgence in an excess of salt, this being an error often committed by neurasthenic patients suffering from putrefactive disorders. The excessive use of salt is stated by some investigators to be

operative in bringing about the removal of too much alkali from the body, thus favoring a degree of acidosis. Among mechanical factors one has to consider mainly the consistence of food. The use of raw apples, of uncooked celery, and of spinach, and the use of salads may be detrimental for purely mechanical reasons. Sometimes the detrimental effect shows itself in the production of colic, in other cases it is manifested by an undue retention of the food within the stomach, where it excites discomfort. It has been already stated that there are long-standing cases of excessive intestinal putrefaction in which there is evidence of an abnormal sensitiveness of the partially denuded mucous membrane. In cases where the stomach is sensitive even to the presence of moderate quantities of food that has been carefully masticated, it is desirable to use a demulcent (such as flaxseed tea) before meals. This is often a highly efficient measure in allaying gastric tenderness. It may be desirable to give a demulcent drink before each meal during a long period of time.

It is obvious that in order to secure the best possible conditions of resorption from the small intestine it is important not to permit the consumption of excessive quantities of food. This is especially important in the case of proteids, particularly those of meat and milk. The use of an excessive quantity of meat frequently goes hand in hand with imperfect mastication. The result is that many masses of muscle fiber find their way through the small intestine into the lower ileum and large intestine, where they are attacked by putrefac-

tive bacteria. The putrefactive bacteria find in meat proteid and casein good media for their support.

The occurrence of large numbers of fragments of meat or smaller aggregations of meat fibers in the fæces is an indication of the imperfect utilization of meat and should operate as a signal for its more cautious and intelligent use. It has already been mentioned that the intestinal contents of carnivora contain many more putrefactive, spore-bearing bacteria than is the case with the herbivora. The full meaning of this observation is not at the present time clear. As certain anaerobes, especially the gas-bacilli, are closely associated with the development of some putrefactive disturbances of high intensity, it seems worth while to investigate more carefully than has yet been done the influence of meat upon the growth of the putrefactive anaerobes. Until further knowledge has been obtained in regard to the influence of meat upon the development of putrefactive anaerobes, in cases of well-marked saccharobutyric putrefaction, it seems rational to advise the practice of moderation in the use of meats in the dietary of such patients. In practice I restrict the use of meat to one meal each day and keep its amount moderate. There may even be conditions in which there is something to be gained by eliminating meat entirely from the dietary for short periods of time. The desirability of this more extreme measure must be judged partly by the clinical effects. It may be found that in some persons who have long been accustomed to the use of large quantities of meat in the dietary, a complete deprivation

would be followed by loss of strength, and this would hardly be compensated for by a slight gain in the direction of a diminution of the anaerobes in the large intestine.

It is often a difficult matter to determine how much food it is safe to permit a patient with chronic excessive saccharo-butyric putrefaction. If the patient be weak, anæmic, and emaciated, or muscularly atrophic, there is often a strong temptation to feed generously or indeed excessively. I consider it bad practice to urge the use of large amounts of food at the beginning of a course of treatment, since this must result in at least some exaggeration of the putrefactive process. But if rest can be secured to the patient and a more thorough digestion and resorption of food can be achieved, it becomes safe to increase the food gradually. It is much better to devote a few weeks to this elevation of the digestive functions than to proceed hastily to inaugurate a generous regimen. Even under the most favorable conditions the effect of the diet must be carefully watched. A distinct increase in the ethereal sulphates, an increase in the numbers of gas-bacilli in the movements, an increase of intestinal flatulence or the development of emotional irritability or mental depression are signs that the food should be decreased in quantity. Intestinal flatulence calls for a diminution in carbohydrate food.

Methods designed to reduce the Numbers of Putrefactive Anaerobes. — Among the agents most used with a view to reducing fermentative and putrefactive decomposition in the gastro-enteric tract are the so-called

intestinal antiseptics. A considerable literature exists in regard to them. Opinion as to their efficacy is divergent. I shall not undertake to discuss here the literature bearing on the subject, for probably all of the experiments that have been conducted with a view to determining the value of intestinal antiseptics have left out of account a quantitative study of the putrefactive anaerobes of the fæces — a study which obviously is beset with great difficulties, and could only be undertaken under especially advantageous conditions for work.[1] In some of the experiments upon this subject observations have been made on the excretion of the ethereal sulphates, the indican, and the phenolic bodies of the urine. These unquestionably possess a certain value, and if the observations be sufficiently long continued and properly controlled, may be regarded as conclusive indications as to the intensity of putrefaction. One difficulty in measuring the action of many of the antiseptic substances which have been used depends on the fact that they either decompose with the liberation of aromatic substances capable of pairing with sulphuric acid and forming ethereal sulphates, or they themselves are aromatic bodies capable of pairing in this manner. A study of the indican is not open to this objection, but as already pointed out there are cases of intestinal decomposition in which the ethereal sulphates may be considerably above the normal, although little or no indican is excreted. Again, in such observations

[1] Much of the modern literature relative to this subject is given by von Tabora, *loc. cit.*

it is most important that the quantity of proteid food (especially of meat) should be regulated in an exact manner, since an increase of putrefaction in the intestine through the use of excessive quantities of meat can easily be demonstrated.

I have made some observations on the action of so-called intestinal antiseptics, and I have reached the conclusion that most of them do very little good in effecting a diminution of the putrefactive anaerobes of the intestine. Their ability to control fermentative processes in the stomach cannot, I think, be questioned, and in cases where such processes are excessive they may indirectly do good by diminishing the opportunities for putrefaction in the intestine by placing obstacles in the way of the development of the sugar-splitting gas-forming anaerobes. It cannot be denied that through the use of glutoid or other protective capsules antiseptics can be made to reach the intestine itself before they are liberated. The tendency of such antiseptics when used in practical doses to enter into combination with many substances outside the bodies of the living bacteria must be regarded as greatly diminishing their ideal efficiency, estimated on the supposition that they act simply to kill or arrest the growth of living microörganisms. I have found in certain instances that salicylates, aspirin, and salol have exerted some action in diminishing the output of indican, but beyond this I have not been able to satisfy myself that the effect of intestinal antiseptics is pronounced. In many cases they are not well tolerated by the stomach after they have been used

for a time, and may thus do harm to the structures on which normal secretion depends. I do not feel that the subject of intestinal antisepsis has been developed in a scientific manner, and very carefully planned experiments may yet teach us that certain substances possess considerable value when used under appropriate conditions. At present antiseptics are used in an empirical and usually unintelligent way. It seems to me on theoretical grounds that agents which exert an oxidizing action in the large intestine are among those worthy of careful study. Some of the oxides of manganese liberate their oxygen slowly and would appear on this account to be suitable for studies of this kind. There is, indeed, some evidence that the dioxide of manganese may act beneficially upon putrefactive processes in the large intestine, but a scientific demonstration of the fact is, I believe, lacking.

Experiments made by Dr. Wakeman at my suggestion in reference to the action of oxidizing substances upon the contents of the large intestine did not give results encouraging to the view that such agents are likely to be in a high degree efficient in mitigating the activities of putrefactive anaerobes. He experimented with dogs showing a high degree of indicanuria and injected into the large intestine at various points a solution of hydrogen peroxide. In other experiments ferric sulphate was used as a catalyzer in recognition of the fact that the presence of this salt in small quantities renders certain oxidations of organic material much more efficient. Different percentages, ranging from one-half of one per

cent. to about two per cent. of hydrogen peroxide, were employed in different experiments. The solution of hydrogen peroxide was slowly introduced and in large volume, half a liter or more being injected in the course of an experiment extending over many hours. In these experiments no distinct decline in the indican was observed. It is possible, of course, that different results may be obtainable with other oxidizing agents or with oxidizing agents brought into the intestine in a different way. The results might have been more conclusive had the peroxide been introduced into the ileum.

It is well known to clinicians that the use of laxatives is followed by great temporary benefit in many cases of excessive intestinal putrefaction associated with constipation, sometimes even if this is not pronounced. Some advise the use of salts, others of cascara preparations, and still others are partial to the employment of calomel. That there is an actual reduction in the excretion of ethereal sulphates and of various individual products of putrefaction after the use of a laxative is certain. The explanation is of course very simple. The reduction depends on diminished absorption from the intestinal tract owing to the acceleration of the contents of the digestive tract. A similar phenomenon is witnessed in cases of even severe diabetes in which the sugar may drop very much during the period of action of a dose of calomel, or some other cathartic. Notwithstanding the immediate benefit to be obtained from cathartics I believe they must be employed with much discrimination and caution. It is easy to render the digestive tract

excessively irritable through their use, and it often happens that patients fail in nutrition owing to the diminished absorption of foodstuffs. The long-continued and frequent use of cathartic remedies has in my experience nearly always resulted badly. I think laxatives should be employed mainly for the control of the disturbances of a subacute or acute character arising in the course of chronic derangements rather than for the treatment of the chronic conditions themselves. If it is possible to use food containing an abundance of cellulose, this may have a beneficial effect in the direction of preventing constipation, but it frequently happens that patients who suffer from constipation are also excessively sensitive to those foods containing much cellulose and show gastric disturbances from their use.

The idea that pathological bacterial processes in the intestinal tract may be favorably modified in a clinical sense by making use of the antagonistic properties of certain harmless microörganisms is by no means a new one. It has long seemed probable that the well-known beneficial action of fermented milks such as *Kefir* and *Kumyss* is in part due to the restraining action exerted by microörganisms contained in these drinks upon the harmful bacteria in the digestive tract. The action has been attributed in these cases largely to the lactic-acid-forming bacteria concerned in the fermentation. The fermented milk known as *Matzoon* is a similar preparation. Lately there has been placed on the market a fermented milk which has been named *Bacillac*. The chief organism in this fermented milk is a lactic-acid producer

which has been studied by Professor Metchnikoff.[1] It is capable of inducing a high grade of acidity in milk, but does not grow readily on other media. This lactic-acid producer is said by Professor Metchnikoff to be entirely harmless and has been used by him and his associates as a means of lowering the putrefactive processes in the digestive tract. One feature of superiority claimed for *Bacillac* is that it does not contain any yeasts. This is a point of some importance, since it is probable that some of the fermented milks do contain yeasts capable of exerting a detrimental action on the human organism. Whether the *Bacillac* preparation will show itself to be superior in a therapeutic way to other fermented milks from which undesirable yeasts have been excluded must be regarded as an open question and one that can be decided only by extensive and very careful experimentation. There is certainly much to recommend the view that putrefactive intestinal processes are favorably influenced in their clinical course by fermented milk containing an abundance of lactic acid and lactic-acid organisms. The subject is one, however, which is very far from resting on a scientific basis, and a large amount of most careful research is necessary to establish the exact position of this prin-

[1] Dr. Collins found the organism to be Gram-positive, similar to *B. aerogenes capsulatus* in morphology, but larger and incapable of making indol or hydrogen sulphide. The presence and growth of this organism in milk flasks inoculated with human fæces containing an excessive number of gas-bacilli appeared to exert a restraining effect on putrefaction. The maintenance of an acid reaction is essential to this restraint.

ciple of treatment. It is important to determine the influence exerted by the lactic-acid bacteria themselves. It is by no means certain that most of the beneficial influence exerted by the fermented milks is not dependent on the lactic acid existing preformed in the milk. Whether the lactic-acid bacilli in a thoroughly fermented milk go on producing lactic acid in the digestive tract is uncertain and probably depends on the presence or absence of fermentable carbohydrates. If it can be shown that any type of lactic-acid bacilli is capable of carrying on its activities in the ileum and large intestine under anaerobic conditions, with a restraining action upon anaerobic putrefactive spore bearers (such as *B. aerogenes capsulatus*), an important contribution will have been made to rational therapeutics.[1]

Another therapeutic method which has been systematically employed in recent years in many cases of intestinal disease is irrigation of the colon. It is even possible to introduce a tube through the sigmoid flexure into the descending colon, and in some instances the tube may be passed still further in the direction of the ileocæcal valve. By means of physiological salt solution the large intestine has in many cases been washed free of its contents. This procedure has now been frequently practiced with extremely beneficial results in the direction of diminishing excessive intestinal putrefaction. I have known it in several instances to be followed by a reduction in the output of ethereal

[1] The bacillus of *Bacillac* grows well in milk under anaerobic conditions.

sulphates and in the excretion of indican by the urine. Coincidentally there has been definite relief from pronounced symptoms such as headache, mental depression, irritability, intestinal flatulence, etc. In several instances of advanced anæmia (including some showing the blood changes of the pernicious form) there has been a very rapid improvement in the hæmoglobin and red blood cells, together with a prompt disappearance of megaloblastic and other pathological forms of red blood cells. There is no room for doubt that the intelligent practice of high intestinal lavage leads to considerable improvement in many cases of chronic saccharo-butyric putrefaction. The beneficial effects have been pronounced and prompt in some cases where an extreme indolic putrefaction was associated with the saccharo-butyric type. If the lavage be practiced cautiously with respect to the avoidance of an excessive volume of fluid, the method may be applied for a considerable period of time without discernible harmful effects. It is perhaps better to employ lavage two or three times in the week than to use it each day. Where excessive quantities of fluid are used, distension of the colon occurs and may be followed by troublesome atony.

In some persons who are victims of a chronic infection of the intestine with putrefactive anaerobes the intelligent application of the foregoing measures suffices to bring about a high grade of improvement. The chances of obtaining a rapid amelioration of symptoms are greatest in those persons in whom the process has not been one of long standing. The factor of duration of the

infection appears to me fully as important as its intensity. It is doubtless true that many patients suffering from neurasthenic symptoms with mental depression or moderate anæmia, have been benefited by ordinary therapeutic measures suggested by experience and common sense, but not consciously directed by the physician toward the control of the putrefactive conditions in the intestinal tract. The recoveries in these cases have often been temporary in character, for, as already mentioned, there is a strong tendency for relapses to occur. Considerable improvement may follow a second course of treatment, and a third or fourth, but after a time it is noticed both by the patient and his physician that relapses occur more readily than formerly; *i.e.* in response to apparently more trivial errors of living. It is also noticeable that although the correction of these errors in living is still followed by improvement, the improvement is not so rapid and the periods of rest and recreation and careful living are less beneficial than was formerly the case. This may lead to discouragement. It is certainly true that the physician must be extremely cautious about venturing a prognosis in those cases of chronic excessive intestinal putrefaction that are not merely of long standing but have had the benefit of intelligent medical treatment and long periods of exemption from depressing conditions of living. The prognosis is best in those persons whose symptoms have not only been of moderate duration but who have had little opportunity to care for themselves — persons who have continued actively at work and have

committed gross errors in diet, in sexual life, etc. The removal of the obviously injurious conditions in these cases is almost always followed by a quick and satisfactory improvement, provided the patient be not burdened by significant neurotic taints or has not developed a considerable degree of anæmia.[1] In persons who have had the benefit of much rest and recreation and have cared for themselves under luxurious conditions of living, the outlook is much less promising because in many of these cases most of the ordinary therapeutic measures have already been exhausted. It should be remembered, however, that even in such cases a period of rest of one or two years under favorable conditions may bring about a great change in physical state, while short periods of rest show very little effect on the patient. For those who find it very difficult to follow in a rational way the advice of a physician, owing to inability to exert the necessary self-control, a period of residence in a good sanitarium under the immediate supervision of a tactful and intelligent practitioner may do much more good than home treatment. Similarly a cure at Carlsbad under rigid conditions of diet and general hygiene may be of very distinct benefit in reducing the manifestations of a chronic anaerobic infection of the intestine.

In extreme cases of chronic excessive intestinal putrefaction in which nearly everything has been tried

[1] I regard the presence of a high degree of anæmia and the persistence of a high grade of infection with the gas-bacillus as bad prognostic signs, especially if associated with a persistently intense dimethylamidobenzaldehyde reaction of the urine.

that can be suggested by competent medical men, the question of surgical interference may arise. The past ten years have recorded a very large number of surgical triumphs in connection with the human digestive tract. Unfortunately in a majority of the instances of disease successfully relieved by surgical methods little or nothing is known of the bacterial conditions in the digestive tract and what modifications they may have undergone as a result of treatment. There can be no doubt, however, that in some of the cases that have been successfully subjected to operation a part at least of the improvement has come about through the mitigation of putrefactive processes in the large intestine. It is, I think, extremely desirable that much closer attention should be given to the study of cases from the bacterial standpoint than has ever been the case, for the knowledge which must surely be accumulated in this way must prove extremely helpful in deciding what shall be done with certain patients suffering from chronic anaerobic infections of a severe type. An operation which has been very often practiced in one or another form is that of gastro-enterostomy. In cases of dilatation of the stomach which have resisted ordinary forms of treatment, admirable results have many times been obtained in the relief of a great variety of digestive disturbances. It seems highly probable that the relief afforded in these cases has been due in most instances in part at least to the greatly improved conditions of bacterial activity which followed the removal of a source of stagnation and putrefaction in the upper part of the digestive tract.

The pyloroplastic operations have also frequently been followed by striking benefit in chronic conditions. Even in cases where an operation is done, not so much for dilatation of the stomach as for a spastic or hypertrophic condition at the pylorus, the improvement may have been associated with a diminished excretion of the ethereal sulphates. I recall one case in which the ethereal sulphates had regularly run far in excess of the normal but in which this excess promptly disappeared after a pyloroplastic operation done for the relief of a gradually increasing stenosis of the stomach associated with hyperchlorhydria and periodical seizures of vomiting. Here the relief from putrefactive decompositions was probably merely one incident of amelioration of more acute and pressing conditions. Looking at the pyloroplastic and similar operations from the standpoint of chronic putrefactive intestinal disorders, one has to admit that the indications for such operations are at present not entirely clear.

The operations to which reference has just been made are designed to secure the prompt emptying of the stomach into the small intestine. An entirely different type of operation and one which has as yet been seldom performed relates to the attempt to exclude a portion of the large intestine. The fact that the large intestine is the seat of some of the most distressing derangements associated with putrefaction strongly suggests the possibility that these putrefactive disorders may be mitigated by surgical procedures which have for their aim the elimination of a portion of the colon. Operations with

this end in view have in a number of instances been practiced, but not, so far as I am aware, with the primary object of greatly reducing the opportunities for intestinal putrefaction. The value of such procedures is therefore as yet largely a matter of conjecture. That the elimination of the large intestine would be followed by a very great reduction in the absorption and excretion of products of decomposition must be regarded as certain. The experiments which have been made by Baumann and others on dogs in which a fistula in the ileum had been established, leave no room for doubt that putrefaction is greatly decreased by such a procedure.[1] The closure of the fistula and the reëstablishment of the natural paths have been in such cases promptly followed by a renewed excretion of the ethereal sulphates. Aside from the surgical difficulties incidental to operations designed to shorten the large intestine there are two possibilities of a disadvantageous outcome which will have to be tested by future experience. One of these is the possible detrimental influence of such a shortening of the gut upon the processes of nutrition. It is claimed by some physiologists that the processes of peptonization and absorption continue to be carried on in no unimportant degree in the large intestine, and hence that an elimination of a considerable portion of this part of

[1] In a dog whose ileum was inserted a few inches above the rectum the ethereal sulphates were low but the indican reaction (on a meat diet) was intense. This animal was prepared for use by Dr. Carrel of the Rockefeller Institute. The fluid fæces contained indol. After the operation there was a considerable gain in weight on a strict meat diet.

the digestive tract must necessarily be followed by some failure in nutrition. I question whether the latter part of this contention is justified; for while some digestive action cannot be denied to the large intestine, there are many reasons for thinking that by far the larger part of the nutritive resorption necessary for the maintenance of a good state of nutrition occurs above the ileocæcal valve. Even, however, if it be ordinarily true that a significant amount of material is absorbed from the region beyond the ileocæcal valve, there is no reason why such absorption cannot under special conditions be relinquished without detriment to nutrition. Even the partial artificial peptonization of proteids would do much to secure an adequate absorption of such food materials above the ileocæcal valve and in cases of long-continued intestinal intoxication with a grave prognosis, the resort to permanent peptonization of the food materials would certainly be the substitution of a lesser for a greater evil. Although it must be admitted that our knowledge on this point is not at present decisive, I am strongly disposed to take the view that nutrition could be adequately maintained in the absence of the greater part of the large intestine.

Another possibility of an unfavorable sort relates to the inconvenience incidental to the rapid passage of semi-solid or fluid intestinal contents. It seems probable that if the conditions of diet were carefully studied, any serious difficulty from this source might be avoided. Moreover, the objection just mentioned would be greatly minimized by connecting the ileum, cæcum, or trans-

verse colon with the rectum instead of making a communication in the abdominal or lumbar region.

It is to be remembered that operations like those just discussed would seldom be undertaken except where life is in danger from the persistence of a process which has been unsuccessfully treated by the usual hygienic and medical resources of the physician. There are conditions of mental depression and conditions of anæmia in which I believe an operation involving the shortening of the intestine to be justified. There is much to be said in favor of urging operation before the establishment of extreme conditions of deranged function in the nervous system or the development of anæmia associated with greatly impaired regenerative capacity of the blood. It is easy to understand that by too long waiting a biological situation, susceptible of great improvement by timely operation, might be rendered so extreme that the recuperative powers of the damaged cells would not suffice to restore even a fair degree of health.

The operation of connecting the vermiform appendix with the outer world through a fistulous opening has been repeatedly performed in cases of chronic dysentery, for the purpose of giving patients the benefit of thorough irrigation of the large intestine. It is possible that this operation (which might entail fewer risks to the patient than operations of the class just mentioned) may be worthy of trial for the relief of chronic intoxications leading to extreme anæmia or to serious disorders of the nervous system.

The possibility of checking the progress of specific

bacterial processes in the intestinal tract by means of specific bacterial vaccines or through the use of specific bactericidal sera has come plainly into view during the past ten years. Mention has already been made of the methods that have been employed to check the development of infections from organisms of the *B. coli* group and closely related bacteria. The work of Sir A. E. Wright upon the elevation of the opsonic index in cases of local staphylococcal infections and in the case of streptococcus septicæmia makes it not improbable that in the case of intestinal affections clearly shown to be in part dependent on staphylococcus or streptococcus infection, the methods of vaccination employed by him may be effective in increasing the phagocytic defenses of the body against these infective agents. Whether similar methods are likely to be of service in relation to such cases as show the presence of well-defined chronic infections dependent on anaerobic putrefactive organisms such as *B. aerogenes capsulatus* it is impossible to predict. It has been already mentioned that we have not as yet observed any instance of chronic saccharobutyric putrefaction (due to an infection with *B. aerogenes capsulatus*) in which there has been a heightening of the agglutination of the patient's blood with the infecting organisms. This, however, does not prove that specific antibodies are never formed through the agency of these anaerobes in the intestine. There are in fact some experimental observations which indicate that it is possible to develop a specific agglutinating power for *B. aerogenes capsulatus*. Whether the ability of the

leucocytes to take up these organisms suffers any diminution in chronic infections is not yet known; hence speculations as to the beneficial influence of the use of vaccines prepared from *B. aerogenes capsulatus* are useless. It is likely that this phase of study will receive attention before long.

SOCIOLOGICAL CONSIDERATIONS

It is hardly satisfying to conclude this consideration of the infections of the digestive tract without some reference to their sociological bearings. The medical practitioner too often is forced to deal exclusively with damage done — damage of an irreparable character. He is unable to grapple with the weighty problem of preventing disease. In modern civilized communities the task of prevention has fallen into the hands of health boards. Where these have been guided by highly intelligent and trained men, unselfishly devoted to the public good (as has lately been the case in New York City), there has resulted a noteworthy diminution in the death rates. This is true of many kinds of disease, but applies with great distinctness in the case of the mortality of infants from diarrhœal disorders. The infant mortality from such disorders is due mainly to the bacterial contamination of milk, and some municipal authorities have lately been able to insist on the systematic rejection of bad milk. The decline in deaths from summer diarrhœas is a result of this improved milk sanitation.

The health boards have, however, only begun their task. It is certain that the beneficent work of prevention will in time grow so efficient as to make the fatal milk infections of infancy comparatively rare, provided something be also done to raise the intelligence of pa-

rents. Equally preventable are the majority of cases of typhoid fever. It is not because of any lack of hygienic knowledge that typhoid fever yearly claims its hundreds of victims in the United States. It is because of the semi-criminal indifference of the uneducated politicians who rule many of our great cities. Eradicate the sources of typhoid contamination of the water supplies of cities, and the deaths from typhoid fever will dwindle to insignificant numbers. One highly competent and determined publicist who, backed by public opinion, should force the rulers of our cities to give the people uncontaminated water, would do more for his country than the hundreds of physicians who are constantly engaged in the treatment of typhoid fever.

But the problem of prevention will have been only partially solved when the infections of cholera infantum, dysentery, and typhoid fever are broadly checked by measures based upon what modern medicine has taught us about the causes of these diseases. There will remain the more elusive task of preventing the occurrence of the severe chronic infections of the digestive tract. The acute infections like dysentery and typhoid are so numerous and obtrusive in their manifestations that it is impossible to overlook them; the chronic infections are insidious in their onset, obscure in their manifestations, and seldom involve groups of individuals; hence they pass relatively unnoticed. The injurious effects of the chronic infections are none the less real. They restrict the fullest development of a nation by shortening the lives of many useful individuals and by rendering

many distinctly less productive than if these persons attained physical vigor more nearly in accord with their inherent possibilities. These partial failures of performance entail a severe economic loss to the community in which they occur. But this loss is one that cannot be adequately measured in dollars and cents, for it must make a nation less efficient in art, in science, in letters, and in all intellectual pursuits. And, finally, it must not be forgotten that the chronic enterogenic intoxications of the nervous system may be extremely detrimental to character and contribute to complicate and mar the finer human relations.

It is tacitly assumed by many that these various morbid manifestations are inevitable, are inherited, or are in some other way an expression of the will of Providence. The conditions to which I refer are indeed recognized by few persons to be what they actually are — the effects of chronic poisoning of gastro-enteric origin. Nevertheless it is certain that these relatively obscure physical conditions are in large measure preventable. But their prevention is not simple; for it implies, first, the recognition of chronic infections of the digestive tract (in which the putrefactive anaerobes are prominently concerned) capable of slowly damaging the cell potentialities upon which health depends, and, secondly, it involves the recognition and control of a series of causative or favoring elements, such as habitual errors in diet, sedentary habits, excessive mental and physical work, emotional fatigue, errors in sexual life, etc.

The recognition of the presence of a chronic intestinal

infection (as by *B. aerogenes capsulatus*) is not in itself difficult, but necessitates patient inquiry and a knowledge of methods of investigation on the part of the physician. The methods of investigating the digestive tract which have been briefly outlined in this volume must prove valuable in enabling the practitioner to determine the presence of abnormal bacterial processes *before* the onset of the clinical signs of incurable or highly refractory states of intoxication.

The control of the causal and favoring factors in the establishment of chronic intoxications is partly the duty of the physician. It is, however, a duty which it is unreasonable to expect him to discharge fully under the conditions existing to-day, no matter how capable and willing he may be. It is not possible for any practitioner to undertake the entire education of his patients in respect to knowledge of hygiene, or to develop their will power to the point where such knowledge becomes practically efficient. Yet this is what he must do to-day if he would achieve a high degree of success in the prevention of the worst consequences of the chronic infections of the digestive tract. Before he can become ideally efficient as a physician he must have the intelligent coöperation of parents and educational institutions.

Education in the facts of hygiene and in the practice of intelligent self-control cannot be acquired in a few hours, but necessitates years of appropriate teaching in home and school. So long as the schools and universities almost wholly fail to fit their pupils to meet even the most obvious requirements of practical life, so long will

it be impossible to avoid great losses of energy and other unhappy consequences of the chronic intoxications. At present the schools look to parents to instruct their children in the supposedly simple matters of regulating eating and drinking, exercise, habits of work, and sexual habits, while the parents vaguely hope (if they think at all about such matters) to be relieved of these embarrassing duties through the schools. The truth is that neither parents nor schools are to-day able to give this much needed sort of education. The remedy must be provided by the schools, which in their eagerness to impart conventionalized facts are now quite blind to some of the most pressing needs of their pupils. Through the schools and universities (or other appropriate organizations) the parents of the future must be educated both as to the facts and the moral aspects of bodily hygiene. The physician will thus be enabled to do better work in the prevention of some of the most distressing human ailments. And it seems not unreasonable to hope that some of the lessons now learned only by bitter experience, after much that is best in life has been sacrificed to ignorance and uncurbed impulse, will be assimilated sufficiently early in life to mitigate materially the lot of a not inconsiderable part of mankind. I believe the lengthening of the span of human life to be among the attainable results of such teaching. Is it not likely that as men grow wiser an increasing number will deliberately strive so to regulate their lives as to improve the expectation of crowning well-spent days with the peculiarly fine satisfactions of old age?

INDEX

A MANUAL OF DETERMINATIVE BACTERIOLOGY

By FREDERICK D. CHESTER

Bacteriologist of the Delaware College Agricultural Experiment Station, and Director of the Laboratory of the State Board of Health of Delaware; Member of the Society of American Bacteriologists; of the Society for the Promotion of Agricultural Science; and of the American Public Health Association

---

Cloth *8vo* *$2.60 net*

"We believe that it will be, as the author hopes, a convenient guide for the student with cultures to identify, and as such will prove a valuable adjunct to the elaborate manuals of this microscopic science."

— *New York Post.*

"The student of botany, the hygienist, and the physician are the richer by the publication of this important work." — *Medical News.*

"It is preëminently as a laboratory guide in the identification of the various forms of bacteria unknown to the student that the work is to be commended, and as such it is indispensable." — *Medical Record.*

"Will no doubt find a place among the reference books of most bacteriological laboratories." — *Plant World.*

"The book can be recommended confidently to the laboratory workers in general." — *Journal of the American Medical Association.*

PUBLISHED BY

THE MACMILLAN COMPANY

64-66 FIFTH AVENUE, NEW YORK

MANUAL OF BACTERIOLOGY

BY

ROBERT MUIR, M.A., M.D., F.R.C.P. Ed.,

Professor of Pathology, University of Glasgow

AND

JAMES RITCHIE, M.A., M.D., B.Sc.

Reader in Pathology, University of Oxford

AMERICAN EDITION (with Additions)

Revised and Edited from the Third English Edition by

NORMAN MACLEOD HARRIS, M.B. (Tor.)

Associate in Bacteriology, the Johns Hopkins University, Baltimore

---

With One Hundred and Seventy Illustrations

Cloth 8vo 565 pages $3.75 net

"The American edition of this well-known manual is perhaps one of the best and most comprehensive, up-to-date handbooks for the student published in the English language. The treatment of the doubtful questions is to be commended. The investigations of each observer and the conclusions are stated with as little bias as possible. . . . An appendix furnishes a compact outline of the principal literature on the different subjects. This outline deals chiefly with the original works found in the foreign languages." — *Medical Record.*

"Like Gray's Anatomy, Green's Pathology, Parkes's Hygiene, and other classical text-books, this manual is destined to remain for years to come the favorite of both teacher and student, to whose needs it is so admirably adapted." — *Philadelphia Medical Journal.*

"A very useful work for the purpose intended."
— *International Medical Magazine.*

PUBLISHED BY

THE MACMILLAN COMPANY

64-66 FIFTH AVENUE, NEW YORK

teins not attacked by facultative anaerobes
attacked by obligate do
or sometimes only if a little sugar
is added.

154. not peptonised by Coli.
Mucous Colitis + B. Coli
Typhoid + Cholera semi-parasites
Ref. for aerobic technique for Welch.
5. Yeasts detrimental
4. Bacteria of Arctic animals almost nil

B. Acidophilus Aerobic

B. Bifidus Anaerobic.

Anaemia - carnivora (? anaerobes
Penetration of walls of A.T. by ? dyspepsia ? C)
bacteria in infancy

Indol + CNS. Protection by liver + A.T.
Suggested mode of action of exercise - increased
oxidation.