response to the needs of the organism. Even if *Planktomya* should eventually prove to be an immature Leptonoid, the fact of the mode of development of the ctenidium would remain of the greatest interest. The existence of this minute form, apparently confined to the open sea between the latitudes of Ascension Island and Bermuda, suggests that the earliest (Cambrian) bivalves may also have been pelagic, which would explain their rarity and minuteness. Of the various larval bivalves found in the Plankton, all are dimyarian and destitute of a pallial sinus, the oral palpi develop in advance of the gills, which appear either as a single lamella on each side or as a number of buds not exceeding ten, while the hinge is either edentulous or taxodont; in all these characters recalling the Paleoconcha.

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A STUDY OF THE COLON BACILLUS GROUP, AND ESPECIALLY OF ITS VARIABILITY IN FERMENTING POWER UNDER DIFFERENT CONDITIONS.

A RESEARCH on the colon bacillus group of bacteria has been in progress during the past two years in the Laboratory of Hygiene of the University of Pennsylvania, in accordance with a scheme prepared by Dr. Weir Mitchell and Dr. John S. Billings, the director of the laboratory, for an investigation on the variability of bacteria, under the auspices of the Bache fund. A resumé of the results of last year's work was published in this JOURNAL under the title 'The Influence of Certain Agents in Destroying the Vitality of the Typhoid and the Colon Bacillus,' and the following article records the experiments made since that time. The organisms which, owing to certain common characteristics, are usually included in the group under examination, the colon bacillus group, have excited much interest during the past few years because of their resemblance to the bacillus of typhoid fever and the difficulty attending the differentiation of certain varieties of the colon bacillus from this organism; and also because of the importance ascribed in recent years to the bacillus coli communis, the type of this group, as a cause of inflammation and abscess in man. This organism first isolated by Escherich, in 1886, has been found to be constantly present in the intestinal canal of man, and also in that of many of the lower animals. Different observers have noticed variations in the characteristics of cultures obtained from different sources, and some have been disposed to consider each of these varieties to be a separate species, but the similarity of many prominent features of these cultures finally led to a grouping of these organisms as varieties of a species. Different groups of colon bacilli have been described by several writers. Achard[1] and Renault in 1892 had observed five types of the colon bacillus, and Tavel[2] had isolated fifteen varieties. Stoeklin[3] distinguishes thirteen forms which he classifies by their motility and the number of flagella. Gilbert[4] finds five varieties. Fremlin[5] describes several varieties obtained from different animals. Refik[6] records five varieties found in water. But an article published by Dr. Th. Smith[7], entitled 'Notes on the Bacillus Coli Communis and its Related Forms,' in which several varieties of the colon bacillus and other similar organisms are tabulated, showing their differentiation by means of the fermentation test and also by the comparison of other distinguishing reactions, has been the means of making better known a number of the different varieties and of establishing a basis for further classification. Very little comparison can be made, however, between the varieties described by Smith and those by other writers because of the paucity of the tests recorded by the latter.

Cultures from the species designated by

Smith as the typical colon bacillus, the transitional forms of the colon bacillus, and the pseudo forms of the colon and the typhoid bacillus, having been furnished to Dr. Billings, and placed in the hands of the writer, these have been studied for the purpose of becoming able to identify the different members of the colon group met with in further research, and an effort has also been made to differentiate each species by the appearance of its colonies.

The colonies on gelatine plates were drawn at different stages of growth and compared as to variations in size, outline, color and surface-markings; but these differences were not found to be greater than have often been observed in the typical colon bacillus grown in an unfavorable medium, or at a low temperature, or under some other unusual condition. The atypical forms of the colon bacillus, found in water and sewage, may present changes in microscopic appearance due to their environment. Therefore this means has not been found to be of much positive value in determining a variety.

Cultures of each variety were then observed in an unfavorable medium. Bouillon containing subnitrate of bismuth in varying amounts, one per cent., one-half per cent., and one-tenth per cent., was first used. The bismuth subnitrate was rapidly reduced in the one-tenth solution; and more slowly in the others. After remaining for twenty days in this medium the colonies became irregular in shape, but this feature was not more prominent in any one variety than in another, and it is of constant occurrence whenever the bacillus is kept for a long time without change of medium, or in distilled water.

Salicylate of bismuth was also used in the same manner. The same grotesqueness in the shape of the colonies was observed in the one-tenth per cent. solution, which was the only one in which the cultures lived.

A study of the different members of this group in stroke cultures on the various media in general use showed this means of differentiation to be of less value than the appearance of the colonies on gelatine plates. On agar-agar the only variation observed in the different types was, that in some cultures the growth stained the medium a yellowish-brown color while in others no such staining occurred. On blood-serum the growth was scanty in every instance and there was a tendency to remain in distinctly separate colonies. On gelatine, every variety presented a tufted appearance to some degree. A slight clouding of the gelatine around the growth was seen in the most alkaline cultures. The so-called artificial potato made of Petermann's fluid, stiffened with rice powder, potato-starch and calcined magnesia was tried, but was found to be very unsatisfactory, because of its constant alteration in reaction, produced by the action of the acid on the phosphates and carbonates, which action continued until the composition of the substance was entirely changed.

The reaction of cultures of the colon bacillus group as found by testing a considerable number of each different type, is always alkaline in media which do not contain sugar. In sugar bouillon the reaction varies with the kind of sugar used. If the sugar is broken up by the ferment of the organism, enough acid may be formed to finally kill the culture, but if only a small amount of acid is formed, then the culture finally produces an alkaline reaction.

The indol reaction was found of much value in testing certain varieties of this group. Some difficulty was experienced in obtaining an accurate test in cultures in which only a slight trace was present. The well-known test as given by Kitasato was used. Notwithstanding the great care taken in testing, ten tests made upon each organism gave greatly varying results. The

peptone used was then tested for indol, and of five bottles tested each gave a distinct indol reaction. Sargent's peptone was then substituted for Witte's and uniform results were obtained.

All of the different varieties of this group were found to be motile. Specimens were stained in order to compare these types with Stoeklin's groups of the colon bacillus, which are differentiated by the number of flagella. The writer was never able to attribute a certain number of flagella to any one variety, and each specimen showed a much greater number than was found by Stoeklin in the cultures forming his group.

From a study of these types of the colon bacillus group, it does not appear that any one or two characteristics can be relied upon to distinguish a variety; for great variations have been found in all of the important functions of the specimens tested. One of the most marked types is that described as the pseudo-typhoid bacillus, which does not ferment sugars nor produce indol, two of the functions especially observed when determining the typical colon bacillus. Such widely varying characteristics seem to indicate the necessity of using all of the biological features for the differentiation of a member of this group.

The fermentation test as given by Smith was used by the writer as the initial step for distinguishing and classifying cultures obtained from many different sources. The greater proportion was taken from the dejections of patients or animals sick with intestinal diseases, and especially from typhoid dejections obtained from a number of patients.

Gelatine plates were made from the materials investigated and a series of cultures was taken from such colonies as seemed characteristic of the colon bacillus. Each series was carefully tested in regard to indol production, fermentation, motility, etc. The fermentation tests were made with dextrose, lactose and saccharose, in one per cent. solutions. Meat extract bouillon was used with glucose, but for lactose and saccharose a special medium free from muscle-sugar was required. An acid-albumin medium was found to be free from sugar and also from indol. This proteid solution was tested by Pére,[8] using muscle for its production, and was found to give a luxuriant growth of the colon bacillus. Ohlmacher[9] recommends peptone prepared from egg-albumin. The writer has prepared it as given in foot note.*

Two hundred cultures were tested regarding fermentation with the three varieties of sugar. The cultures obtained from typhoid dejections were found to give somewhat less gas with lactose and saccharose than the standard colon bacillus described by Smith, which gave with glucose a volume of one-half of the fermenting tube; with lactose the same amount; with saccharose two-thirds. No culture was obtained from human dejections in which there was complete absence of fermenting power for one or more of the sugars.

A series of cultures taken from the intestine of a dog with ulcerated intestines was quite typical in appearance and reaction.

Cultures taken from the intestine of a healthy rabbit gave only a small amount of gas with glucose, and none with saccharose. Fremlin found that the colon bacillus from rabbits varies from that found in man; but his fermentation tests were decided by glucose alone.

The fermentation test was also applied to cultures of the colon bacillus obtained from specimens attenuated by growth under very

* The whites of two eggs are well-beaten and mixed with 500 c. cm. of distilled water acidified with 2 c. cm. of concentrated hydrochloric acid. Two and a-half grammes of scale pepsin are used for the digestion of this mixture, which is kept at a temperature of 40° C. for six hours. It is then neutralized, one-half per cent. each of peptone and salt are added, and enough water to make one litre, when it is boiled and filtered.

unfavorable conditions. Typhoid fæces were kept in the glass-stoppered bottles in which they were received, and plates made from time to time to ascertain whether the fermenting function of the colon bacillus is affected by prolonged residence in such a medium.

Of twenty series tested, three, covering a period of 126 days, are tabulated (see table).

Series A: This series consists of cultures from fresh typhoid fæces. The gas formation with glucose is typical, but with lactose and saccharose the amount is considerably diminished.

Series B: These cultures were made from the same specimen of fæces when it had been kept for thirty days. They show a great reduction in the amount of gas produced with each variety of sugar. No. 2 does not form gas with either form of sugar, and does not coagulate milk, yet in indol-formation, growth on potato, motility and macroscopic and microscopic appearance it is like the typical colon bacillus. Number 4 gives merely a bubble of gas with lactose and saccharose.

Series C: This series was obtained from plates made when the same specimen of fæces had been kept for 126 days. Growth was slower than usual, but the colonies finally presented the irregular outline, the translucent border and relief-map surface-markings which characterize the colon bacillus. The branching appearance of the stroke cultures on gelatine did not appear for several days. Growth on agar-agar was typical and the fæcal odor distinct. Milk was not coagulated by these cultures, even when it was boiled. The indol-reaction was distinct in every case. A microscopical examination showed a short, motile bacillus in pairs, or sometimes in chains of two or three, which in very young cultures might be mistaken for cocci in the stained specimens; but in the hanging drop the shape was easily seen and the motility was nearly as great as that of the typical colon bacillus. The fermenting function was entirely lost with all the sugars used. An acid reaction in every tube showed that the sugars were broken up into acids, to some extent.

These cultures, which after careful study are considered as true colon bacilli attenuated by prolonged dwelling in undiluted fæces, correspond very closely to those described by Villinger[10] in his investigations upon the changes produced in the colon bacillus by environment. A tested culture of the colon bacillus was so changed by different chemical and physical influences that nearly all of the characteristics by which it is differentiated from other organisms were lost. Some of these functions could be again revived by repeated changes in a favorable medium. The indol-formation, however, was never regained.

An attempt was made to attenuate a tested culture of the colon bacillus by inoculating it into diluted, sterilized, typhoid fæces. It was put into this medium on December 20, 1895, where it

GAS REACTION SHOWING THE TOTAL AMOUNT OF GAS FORMED IN THE FERMENTING TUBE.

Series A.	No. of Culture.	Dextrose.	Lactose.	Saccharose.	Series B.	No. of Culture.	Dextrose.	Lactose.	Saccharose.	Series C.	No. of Culture.	Dex. trose.	Lac- tose.	Saccha- rose.
	1	$\frac{1}{2}$	$\frac{1}{2}$	$\frac{1}{7}$		1	$\frac{1}{4}$	$\frac{1}{2}$	$\frac{1}{6}$		1	0	0	0
	2	$\frac{1}{2}$	$\frac{7}{13}$	$\frac{1}{10}$		2	0	0	0		2	0	0	0
	3	$\frac{1}{2}$	$\frac{7}{13}$	$\frac{1}{6}$		3	$\frac{1}{3}$	$\frac{1}{2}$	$\frac{1}{6}$		3	0	0	0
	4	$\frac{1}{2}$	$\frac{2}{9}$	bubble		4	$\frac{1}{3}$	bub.	bubble		4	0	0	0
	5	$\frac{1}{2}$	$\frac{1}{3}$	bubble		5	$\frac{1}{3}$	$\frac{8}{13}$	$\frac{1}{9}$		5	0	0	0
	6	$\frac{1}{2}$	$\frac{1}{5}$	bubble		6					6	0	0	0

has been kept at room temperature until the present time, April 12, 1896. Plates made on April 9th show as large and as characteristic colonies as from the original culture. The gas production of the original culture was, with glucose, one-half the fermentation tube; with lactose, one-half, and with saccharose two-thirds. After growing in the fæcal fluid for 110 days the gas production for glucose is one-third; for lactose, one-third; for saccharose, a bubble.

A culture was inoculated into the same fluid in a flattened test-tube, and exposed to the light and sun. It lived only thirty days, giving nearly the same reduction in gas production as the one which was kept in fæcal fluid for 110 days.

The effect of the insolation of cultures on agar-agar plates was also tested in regard to the fermenting power. Each culture was insolated for two hours and then incubated until the next morning. A series of cultures was made, consisting of removes from the original culture, which was the one used for inoculation of the fæcal fluid. The amount of gas produced after six removes was, with glucose, one-third; with lactose, one-quarter; with saccharose, a bubble.

The decrease in the fermenting function observed in the series of cultures taken from fresh typhoid fæces suggested that the colon bacillus may lose this function in some degree, because of the change in the nature and amount of proteid materials in the contents of the intestine, resulting from the throwing off of cellular products from the intestinal walls.

Père[8] found that the colon bacillus gave great variation in the amount of lactic acid formed in fluids containing different proportions of proteid materials. With 10 grammes of glucose and 6 grammes of peptone in 250 c. cm. of fluid, the colon bacillus gave 2.37 gr. of lactic acid; but with the same amount of glucose and 6 grammes of peptones only 1.170 gr. was formed.

A tested culture was therefore inoculated intraperitoneally into a guinea pig, recovered again, and the cultures tested in sugar bouillon. The gas production was considerably diminished by one passage through an animal, as will be seen in the first series of the following table. These cultures were taken from the spleen of the guinea pig. In the second series the cultures were taken from the typhoid spleen of man, in which the typhoid bacillus was also very plentiful. The escape of the colon bacillus through the ulcerated intestinal wall into the organs of the body has been especially noted by Wathelet,[12] who considers that it is frequently found in the spleen.

TOTAL AMOUNT OF GAS IN FERMENTING TUBE.

Series 1.	Dextrose.	Lactose.	Saccharose.	Series 2.	Dextrose.	Lactose.	Saccharose.
Original Culture.	1/2	7/13	1/2				
a	1/3	1/4	1/3	a	1/3	1/4	1/7
b	1/3	1/3	1/3	b	2/3	1/4	1/5
c	1/3	1/4	1/0	c	3/8	1/4	1/7
d	1/3	1/3	1/9	d	1/4	2/7	1/6

It seems, therefore, that the fermenting power of the colon bacillus may be easily decreased, not only by unfavorable conditions of growth, but also by conditions which do not injure its growth and which may be favorable for and increase some of its other functions.

The colon bacillus, deprived of its fermenting power, would be difficult to differ-

entiate in sewage waters; but it is probably killed in those waters, by exposure to sunlight, before this power is entirely lost. The members of the colon bacillus group which have been obtained by the writer from contaminated waters have rarely possessed the fermenting power which is found in cultures obtained from the normal intestines of man; they may be in a 'transitional' state.

The greater loss of fermenting power with saccharose than with other sugars, may be attributed to the nature of the sugar, which is not directly fermentable, but must first be inverted by a ferment, dextrose and levulose being formed, in which the process of fermentation is easily carried on. The duration of the fermenting process of the colon bacillus with saccharose is much longer than with other sugars.

The diminution in fermenting power noted in cultures obtained from organs undergoing inflammation will be further studied in connection with the influence of proteid materials on the colon bacillus.

My thanks are due to Dr. J. S. Billings and Dr. A. C. Abbott for direction in this work, and assistance in obtaining cultures; and also to Dr. Henry W. Cattell, who has furnished me with much valuable material.

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SUPERHEATED STEAM IN STEAM ENGINES.

The writer has been, for many years, much interested in the now century-old problem of the application of superheated steam to use in the steam-engine with a view to the extinction of those internal thermal wastes which have, from the days of Watt, been recognized more or less clearly as the most formidable obstacles in the way of improvement of the efficiency of the steam-engine, as in fact, of all known heat-motors. The material which has been meantime collecting was recently collated and abstracted, and finally published in a paper read before the American Society of Mechanical Engineers at its St. Louis meeting of 1896.* The subject has more of scientific than practical interest at the moment; but it is not impossible that the resuscitation of this once accepted and now comparatively little-used process may yet prove to be the means, and the only practicable means, of continuing indefinitely the improvement of the steam-engine begun by Watt a century ago. The alternative seems, at the present time, to be the discovery of some commercially practicable method of

*Superheated steam; facts, data and principles relating to the problem. Trans. A. S. M. E., 1896, Vol. XVII.