Bacillus Coli Communis.

BY

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BACILLUS COLI COMMUNIS.[1]

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Some three years ago I was appointed chairman of a committee to report to this section on the relation of the varieties of the colon bacillus to public health. It seems desirable, after this lapse of time, that a statement should be made concerning the work, although this paper is not to be considered in any sense the findings of the committee, but simply a communication from an individual.

For a number of years I have been deeply interested in the colon group of bacteria. Consequently, when this committee was appointed I entered into its work with renewed zeal, hoping that in some way some one would succeed in finding a method for more accurately determining the significance of these organisms from a sanitary point of view. The first undertaking of the chairman was to correspond with each member of the committee concerning a plan of work. It was the unanimous opinion that our knowledge of this group was too meager to warrant, or even make possible, the preparation of a committee report. It was the belief that for the present individual investigations were most needed to furnish reliable data concerning the biology—the natural his-

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tory—of this species and its varieties. Since that time a number of important papers have been published on this group. However, instead of a tendency to unify the various opinions and theories already entertained, they have carried us into a confusion of results and opinions from which the forthcoming of system and harmony seems more difficult than before.

As each member of the committee took up the particular phase of the subject that appeared to him as most needy of research, or that from circumstances he could best carry out, my attention became somewhat centered on the boundaries of the species of B. coli communis and the extent and the cause of its varieties. At that time the literature of the subject was voluminous, and certain well marked differential characters of the colon bacillus had been formulated. It was known that wells and water-supplies were being condemned if the analyst found what he considered to be B. coli communis in the water. Pathologists were finding this organism apparently as the etiological factor in a great variety of lesions, and bacteriologists were trying to distinguish between extreme varieties of the typhoid and hog cholera bacteria and those of the colon bacillus. Some of the French investigators had gone so far as to suggest that typhoid bacteria were degenerated colon bacilli. More recently a number of investigators have been engaged in working out with great care the characters

and properties of certain varieties of this species, and adding many subgroups and subvarieties to the already almost overtaxed list. There has not to my knowledge, however, been recorded the results of extended investigations into the phylogeny of the species. It is in connection with this phase of the topic that I desire to approach what seems to me to be the first part of the question before us, namely: what is the colon bacillus? When this organism was first discovered by Emmerich and designated B. neapolitanus, bacteriological methods were not sufficiently developed to enable one at this time to make even an intelligent guess as to which of the many at present recognized varieties it belonged. Because of this it seems necessary for us to determine the habitat and to find the limit of the properties that distinguish the species.

As the colon bacillus was first described from the intestine, it is widely believed now that this is its normal abiding place. Because of its hardy nature, however, it is often found in soil and water previously contaminated with human or animal excrement. It has not to my knowledge been demonstrated in any substance where the possibility of tracing it to this source was precluded. From this point of view the question suggests itself at once, what about the relation of the numerous forms that have been isolated from various extraneous material and classed without challenge

among the varieties of the colon group? To my mind the answer to this inquiry involves a knowledge of the as yet undetermined connecting forms between the prototype of the species and the now recognized colon bacillus; or, if its evolutionary origin is not accepted, a better knowledge of the differential characters and properties of the closely allied, though distinct species. This brings us at once to the determination of the specific boundaries.

When one begins to fix the characters and properties that shall be considered both diagnostic and sufficient for specific determination there appears an almost endless number of them that claim recognition. However, if these claims, such, for instance, as the curdling of milk within twenty-four hours, the production of indol in certain quantities, or any of the many other designated differential properties, are carefully weighed, there seems to be good reason for not fixing such narrow limits. The fact will appear that if all such definite features are retained they will neutralize each other to such a degree that the species will escape detection. In this connection we are sadly in need of the results of investigations defining the conditions under which such restricted limitations are applicable, and under what conditions of life they may vary. That bacteria do change in reference to their cultural manifestations and virulence, if not pathogenesis, has been demonstrated in a number

of instances. With this fact, which seems to be applicable to all species, can we say that the properties which characterize some cultures, or individuals, shall be considered as diagnostic of the species? For one I protest with the botanists against the fixing of a species on individual characters.

Without entering into a discussion of the literature, it seems to the writer that the results thus far recorded justify the acceptance with slight modifications of the requirements suggested by Smith in 1895 as necessary to distinguish the species. These may be summarized as follows:

(A) *Morphology.*—Slender, motile rods without spores. The motility is often most pronounced and rarely appears only in the organisms from young colonies on gelatin plates.

(B) *Physiology.*—(1) The growth of colonies on the surface of neutral or slightly alkaline gelatin is in the form of a delicate bluish or more opaque whitish expansion with a well defined but irregular margin. The gelatin is not liquefied and the growth is firm. (2) Milk is coagulated in from one to several days. (3) The growth upon potato is either pale or of a brownish-yellow color, or merely as a glistening, barely recognizable grayish or whitish layer. The quantity and also the color of the growth depends somewhat upon the reaction of the potato. (4) A distinct, but

not always marked, indol reaction is manifest in cultures seventy-two hours old. For this sugar-free bouillon is preferable to Dunham's solution. (5) In bouillon and on agar the growth varies. In bouillon the liquid may become faintly or heavily clouded, with or without a surface membrane. On agar the growth may be sparing or more fleshy, and vary in color from a neutral gray to a grayish-white. (6) They are divided by their action upon sugars into two groups: first, those that ferment with the formation of gas, dextrose, lactose, and saccharose; secondly, those that ferment with the formation of gas, dextrose, and lactose only.

In one-per-cent solution in bouillon at 37° C. in fermentation tube:

1. Dextrose, total gas about one-half the capacity of the closed branch $\frac{H}{CO_2}=\frac{2}{1}$ reaction strongly acid.

2. Lactose, gas production, gas formula, and reaction practically the same as for dextrose.

3. Saccharose, total gas about two-thirds the capacity of the closed bulb $\frac{H}{CO_2}$ approximately $\frac{3}{2}$; the final reaction may be slightly acid or alkaline, depending upon the time required for the evolution of the gas.

The time required for the production of the maximum quantity of gas varies as a rule from one to three days in case of the dextrose

and lactose, and from five to twenty days in case of the saccharose.

(C) *Pathogenesis.*—(1) Many cultures of B. coli communis are fatal to guinea-pigs of 900 grammes weight when inoculated subcutaneously in doses of ¼ to ½ Cc. of a fresh (24-hour) bouillon culture. Other cultures require the peritoneal injection of a like quantity of the virus for fatal results. (2) Some cultures do not produce morbid changes when injected in doses of 1 Cc. of the culture into the peritoneal cavity. (3) Cultures that have been isolated from the intestines of dogs have, in the writer's experience, been more virulent than those obtained from the normal viscera of other animals. (4) Although the colon bacillus appears to have become localized in the digestive tract of living animals (including man) and to that extent become parasitic, it is not necessarily virulent as determined by animal inoculation.

If in the study of a culture there should be some deviation from these properties, such as the failure to give an indol reaction, or to coagulate milk, or to act differently on some one or more of the sugars, it does not seem necessary to exclude it from the species. It does not seem necessary either to grant it the distinction of a variety unless it is clearly shown that such deviations are constant. The greater number of variations seem to be found

in the effect on milk and sugars, including the gas formula, and in their virulence.

A search for the source of varieties which have been described shows that, with few exceptions, they have been isolated from polluted soil, water, or lesions of various kinds in man or in animals. It seems rational from this to assume as a working hypothesis that at least many of the varieties described from these abnormal habitats of the species are in some way the results of environmental influence. It is along these lines that our subject is begging for experimental results. My study of colon bacilli suggests that normally, within the digestive tract, there are not many well established varieties. Furthermore, it does not appear from the literature that there has been as close a discrimination as equity demands between B. coli communis and other species originally quite as clearly defined. There is a strong tendency to bring all such forms within the limits of the colon group. It is not unlikely that this is the better method, but at present the importance of the colon bacillus as an index to sewage pollution is so great that I am constrained to believe that until more definite and experimental knowledge is recorded we should hold its specific characters within or near the limits herein suggested. Forms that are undoubtedly specific varieties should rank in importance equal with the species. Those forms that are not distinctively

colon as determined by the above mentioned characters can usually be identified as other species, or, in the absence of such identification, they may be placed among the unidentified forms hypothetically standing between the prototype and the species.

APPENDIX.

The appended tables represent the action of colon bacilli, isolated from the intestines of various species of animals, on the sugars and milk. In all other respects they conform to the more generally accepted characters and properties of B. coli communis.

ACTION OF BACILLUS COLI COMMUNIS FROM THE INTESTINES OF DOGS ON THE SUGARS AND MILK.

Dog No.	Indol.	Dextrose.			Lactose.			Saccharose.			Milk.
		Quantity of Gas.	H / CO_2	Reaction.	Quantity of Gas.	H / CO_2	Reaction.	Quantity of Gas.	H / CO_2	Reaction.	Coagulated in
1	+	9 / 13	5 / 4	Acid.	8.5 / 13	6 / 2.5	Acid.	2.7 / 13	1.9 / 0.8	Acid.	3 days.
2	+	7 / 13	2 / 1	"	5 / 13	3.3 / 1.7	"	0	—	Alk.	3 days.
3	+	7 / 13	4.3 / 2.7	"	6.4 / 13	3.9 / 2.5	"	0	—	"	4 days.
4	+	5 / 12	3.4 / 2.3	"	1 / 2	2 / 1	"	1 / 12	1.1 / 4	Acid.	3 days.
5	Colonies of Bacillus coli communis were not found.										
6	+	1 / 2	2 / 1	Acid.	1 / 2	2 / 1	Acid.	1 / 10	5 / 2	Acid.	3 days.

ACTION OF BACILLUS COLI COMMUNIS FROM THE INTESTINES OF PIGS ON THE SUGARS AND MILK.

Pig No.	Indol.	Dextrose.			Lactose.			Saccharose.			Milk.
		Quantity of Gas. / H / CO_2		Reaction.	Quantity of Gas. / H / CO_2		Reaction.	Quantity of Gas. / H / CO_2		Reaction.	*Coagulated in
1	++	6.3 / 12	3.8 / 2.5	Acid.	4.8 / 12.5	3.3 / 1.5	Acid.	1 / 3	2 / 1	Acid.	Coagulated.
2	—	1 / 5	1.8 / .7	"	5.5 / 13	3.7 / 1.8	"	0	—	Alk.	No change.
3	++	5 / 12	3.2 / 1.8	"	5.2 / 12	3.4 / 1.8	"	1 / 5	1.6 / .9	Acid.	Coagulated.
4	++	4.5 / 12.5	2.7 / 1.8	"	1 / 2	4.2 / 2.3	"	6.7 / 12.5	4 / 2.7	"	Coagulated.
5	+	1 / 2	2 / 1	"	1 / 2	4.2 / 2.5	"	0	—	Alk.	No change.
6	++	1.5 / 2.5	2.2 / 2	"	3 / 8	2 / 1	"	1 / 3	4.5 / 2.5	Acid.	Coagulated in 4 days.
7	+	1 / 2	2.7 / 1.5	"	5.5 / 12	2.5 / 1.5	"	2 / 5	2 / 1	"	Coagulated in 3 days.

ACTION OF BACILLUS COLI COMMUNIS FROM THE INTESTINES OF CHICKENS ON THE SUGARS AND MILK.

Chicken No.	Dextrose.			Lactose.			Saccharose.			Milk.
	Quantity of Gas. / H / CO_2		Reaction.	Quantity of Gas. / H / CO_2		Reaction.	Quantity of Gas. / H / CO_2		Reaction.	Coagulated in
1	3 / 13	2.5 / 0.5	Acid.	3.3 / 13	2 / 1.3	Acid.	0	—	Acid.	2 days.
2	7 / 13	2 / 1	"	7 / 13	4.7 / 2.3	"	0	—	Alk.	3 days.
3	4.5 / 13	3.5 / 1	"	5 / 13	3.8 / 1.2	"	0	—	Acid.	3 days.

*Notes concerning the time required to produce the changes in the milk in the first five cases were inadvertently omitted.

ACTION OF BACILLUS COLI COMMUNIS FROM THE INTESTINES OF CATTLE ON THE SUGARS AND MILK.

Cow No.	Indol.	Dextrose.			Lactose.			Saccharose.			Milk.
		Quantity of Gas.	H / CO_2	Reaction.	Quantity of Gas.	H / CO_2	Reaction.	Quantity of Gas.	H / CO_2	Reaction.	Coagulated in
1	+	1/2	3.5/3	Acid.	7/13	2/1	Acid.	7/13	4/3	Acid.	3 days.
2	+	7.5/13	2/1	"	5/13	3/1	"	5.3/13	3.3/2	"	7 days.
3	+	1/2	2/1	"	5.5/13	4/1.5	"	0	—	Alk.	3 days.
4	+	5.5/13	3.5/2	"	5.5/13	3.5/2	"	5.5/13	3.2/2.3	Acid.	7 days.
5	++	5/12	3/2	"	5/12.5	3/2	"	0	—	Alk.	3 days.
6	+	5/12.5	3/2	"	1/2	3/2	"	0	—	"	2 days.
7	++	1/2	3.9/2.5	"	6/13	3.6/2.4	"	0	—	"	8 days.
8	+	1/2	3.3/2.5	"	6/13	3.8/2.2	"	0	—	"	7 days.
9	+	1/2	3.8/2.4	"	5.3/13	3/2.3	"	8/13	4.8/3.2	Acid.	5 days.
10	+	1/2	3.7/2.5	"	1/2	4/2.5	"	0	—	Alk.	8 days.
11	+	6/12.5	3.5/2.5	"	7/13	2/1	"	1/3	2.7/1.8	Acid.	8 days.

ACTION OF BACILLUS COLI COMMUNIS FROM THE INTESTINES OF HORSES ON THE SUGARS AND MILK.

Horse No.	Indol.	Dextrose.			Lactose.			Saccharose.			Milk.
		Quantity of Gas.	H / CO_2	Reaction.	Quantity of Gas.	H / CO_2	Reaction.	Quantity of Gas.	H / CO_2	Reaction.	Coagulated in
1	+	1/3	3.5/2.7	Acid.	1/6	3.6/2.1	Acid.	0	—	Alk.	8 days.
2	+	6/13	3.5/2.5	"	5/13	3.3/1.7	"	7/13	1/1	Acid.	2 days.
3	+	6/13	1/2	"	bubble.	—	"	0	—	Alk.	Acid, no coag.
4	+	1/2	4.5/2.5	"	6/13	2/1	"	0	—	"	7 days.
5	—	1/3	1.4/1	"	1/2	3.3/2	"	0	—	"	2 days.
6	+	5.5/13	3/2	"	1/2	4.1/2.6	"	4/13	1/1	Acid.	5 days.
7	+	1.1/2.5	3/2	"	1/2	2/1.1	"	7.5/13	4.7/2.8	"	2 days.
8	+	6/13	3.7/2.3	"	5.4/12.5	3.4/2	"	7.5/13	4.6/2.9	"	4 days.
9	+	1/2	—	.....	5/13	3/1	"	0	—	Alk.	No change noticed.

ACTION OF BACILLUS COLI COMMUNIS FROM THE INTESTINES OF SHEEP ON THE SUGARS AND MILK.

Sheep No.	Indol.	Dextrose.			Lactose.			Saccharose.			Milk.
		Quantity of Gas.	H / CO_2	Reaction.	Quantity of Gas.	H / CO_2	Reaction.	Quantity of Gas.	H / CO_2	Reaction.	Coagulated in
1	+	7.5 / 13	4 / 3.5	Acid.	1 / 2	4 / 2.5	Acid.	0	—	Alk.	2 days.
2	+	5.7 / 13	3.2 / 2.5	"	5.5 / 13	3.5 / 2	"	0	—	"	4 days.
3	+	1 / 3	2.7 / 2	"	1 / 2	4.1 / 2.4	"	1 / 2	3.9 / 2.8	Acid.	4 days.
4	+	4.8 / 12.5	2.8 / 2	"	5.3 / 12.5	3.4 / 1.9	"	6.5 / 12.5	3.5 / 3	"	4 days.
5	+	5.8 / 12.5	3.3 / 2.5	"	1 / 2	4 / 2.3	"	5.5 / 12.5	3.6 / 1.9	"	2 days.
6	+	1 / 2	4 / 2.6	"	7.5 / 12.5	4.7 / 2.8	"	5 / 12	3.1 / 1.9	"	2 days.
7	—	1 / 2	3.7 / 2.3	"	6 / 13	3.8 / 2.2	"	0	—	Alk.	Acid, slight ppt.
8	—	6.7 / 12	4.1 / 2.6	"	5.8 / 12.5	4.1 / 1.7	"	0	—	"	Acid, slight ppt.

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